Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Guidelines for Mass Rearing and Irradiation of *Drosophila suzukii* for Sterile Insect Technique Application

Version 1.0

Food and Agriculture Organization of the United Nations
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Introduction

The Sterile Insect Technique (SIT) is a species-specific and environment-friendly method that uses releases of sterile insects to suppress or eradicate pest populations. This approach is effectively employed against many crop pests and disease vectors, such as the New World screwworm fly, various tephritid flies, tsetse flies, mosquitoes and Lepidoptera (Vreysen 2001, Hendrichs *et al.* 2002, Scott *et al.* 2017, Marec and Vreysen 2019, Oliva *et al.* 2021).

The SIT is considered a promising method in regulating populations of the spotted wing *Drosophila* (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), in infested, confined areas. *Drosophila suzukii* is native to Southeast Asia and has invaded most parts of the world, except for most African countries, Oceania, Central America, the Northern Andes and several Central Eurasian countries. Unlike most other *Drosophila* species, *Drosophila suzukii* oviposits on ripening, undamaged fruits. Larvae feeding within the fruits cause massive reductions in yield for a variety of soft fruits, especially cherries and berries (Rota-Stabelli *et al.* 2013).

At the IPCL (Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture), a considerable research effort has been dedicated over the past years to the development of SIT adapted to SWD and has evaluated different oviposition systems for mass rearing (Sassù *et al.* 2019a), the investigation of sterilization and transport conditions (Enriquez *et al.* 2021, Sassù *et al.* 2019b), the description of quality control procedures (Sassù *et al.* 2021) and the evaluation of different microbial symbionts to improve mass rearing or sterilization steps (Nikolouli *et al.* 2020, Nikolouli *et al.* 2021).

The present document describes the standard mass rearing protocols and sterilization procedures currently used at the IPCL. In the first part of the document, we describe the mass rearing oviposition system used at the IPCL and detail the different steps necessary to maintain a SWD laboratory colony, in particular egg collection, larval rearing, and pupae collection. In the second part, we present the pupal irradiation procedure, as well as the method to measure adult sterility level. All research presented below have been developed using a single colony established in 2014 from pupae sent by the Agricultural Entomology Research Unit of the Edmund Mach Foundation (San Michele all'Adige, Trentino, Italy). An additional document will present the quality control procedures developed at the IPCL, including routinary testing and mating compatibility and competitiveness testing.

We are grateful to Yeudiel Gómez-Simuta and Rui Pereira for their precious contribution to this document. We would like to thank Stephanie Beckham for proofreading the document.

A. Mass rearing

A.1. Mass rearing oviposition system

SWD adults are held in raised, large cubic cages that present at least one flat wax-coated panel, an artificial oviposition system that allows egg collection. The wax panel approach has been evaluated and suggested for mass rearing of SWD by Sassù and colleagues (2019a) based on a procedure originally developed for the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) (Ahmad *et al.* 2016). The wax oviposition system allows an easy, external collection of high-quality eggs as well as the estimation of the colony's egg production. It also prevents the colony from contamination, from other SWD strains or related fly species such as *Drosophila melanogaster*, that often occurs when eggs are collected directly on larval diet substrate inside the cage. The wax oviposition system can feature diverse designs and sizes to adapt SWD production to the rearing facilities' needs, from pilot projects to large-scale programmes.

A.1.1. Characteristics of the holding cage

An aluminium angle frame cubic cage (45 x 45 x 45 cm) (Figure 1) with:

- A solid, transparent PLEXIGLAS® floor
- A solid, transparent PLEXIGLAS® lateral panel with a circular, netted opening (around 20 cm diameter) to introduce pupae, food, and water inside the cage
- Two lateral mesh panels for cage ventilation. These panels consist of one internal mesh net (mesh size: 1 x 1.5 mm) and one external black mesh net (0.22 x 0.22 mm)
- A fourth lateral side as a wax oviposition site. The exact procedure to build this panel is detailed below
- A top mesh panel consisting of one internal mesh net (1 x 1.5 mm) and one external black mesh net (0.22 x 0.22 mm)

Every single component of the cage panels is fixed to the cage frame using contact glue (Pattex Contact Liquid, Pattex, Germany) (except for the wax panel, see paragraph A.1.2).

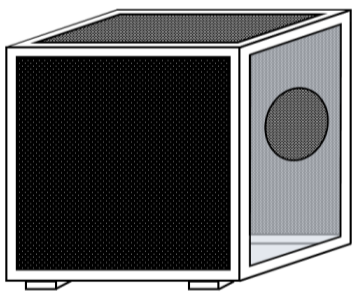

Figure 1. Example of a holding cage, inspired from Sassù et al. 2019a. The front panel represents the wax panel.

A.1.2. Creating the wax panel

The wax panel consists of two different mesh nets, an internal thick rigid net (mesh size: 1 x 1.5 mm or bigger, e.g. G-4005, Trevira CS, Germany) (Figure 2a) and a finer black nylon net (0.22 x 0.22 mm, e.g. Caracas, Heco Textilverlag, Germany) (Figure 2b), that are fixed to the cage frame and stretched together (Figure 2c) as follows:

1. A self-adhesive hook-and-loop tape (e.g. 150 cm 20 mm, fix-o-moll, Germany) is placed all along the metal frame
2. The thicker net is fixed to the hook and loop tape
3. The finer net is tightly fixed to the thicker net with a strong adhesive tape (e.g. Tartan TM Filament Tape 8954, 3M, USA)

An additional piece of adhesive tape is added along the bottom of the panel (Figure 2c) to provide a sharp edge that will facilitate future egg collection.

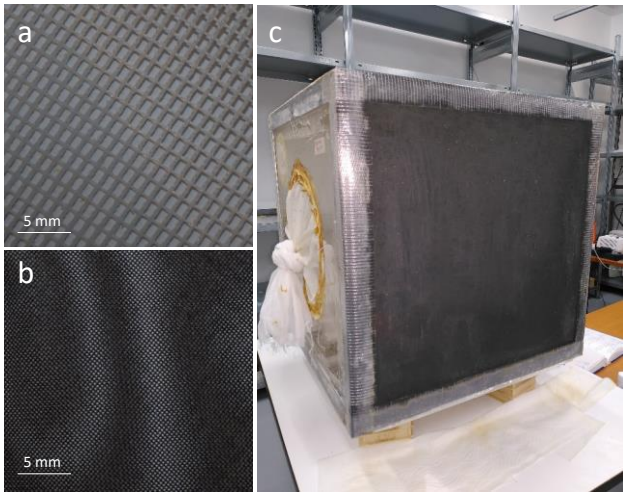

Figure 2. Structure of the oviposition panel. (a) Internal and (b) External mesh nets constituting the wax panel; (c) The mesh nets are fixed to the holding cage frame using a strong adhesive tape.

A hot liquid paraffin solution (solid paraffin 52-54) is applied to the surface of the panel using a brush saturated with wax to form a single sealed waterproof layer (Figure 3a). We recommend using a vulcanized, black bristle brush (1 ½″, i.e. 35 mm width) (e.g. black Chinese pig bristle window brush, ref. 1221535, Pinselfabrik Müller GmbH, Germany). The procedure is detailed hereafter:

1. First, the bottom of the panel is coated with wax in single, short bottom-up movements to seal any opening between the adhesive tape and the mesh net (Figure 3b)
2. The top of the wall must be brushed in the same way (Figure 3c)
3. The centre and the side edges of the wax panel are coated with wax in the same way (Figure 3d)
4. After a minute, coarse scraps of cooled wax are gently removed using a sharp piece of plastic (Figure 3e)

5. The very last remains are then removed using a hair dryer (blowing downwards) and paper towel (Figures 3f and g). Achieving this final step is controlled using a light source directed through the cage from the opposite side: most of the wax panel should appear quite clear (Figure 4)

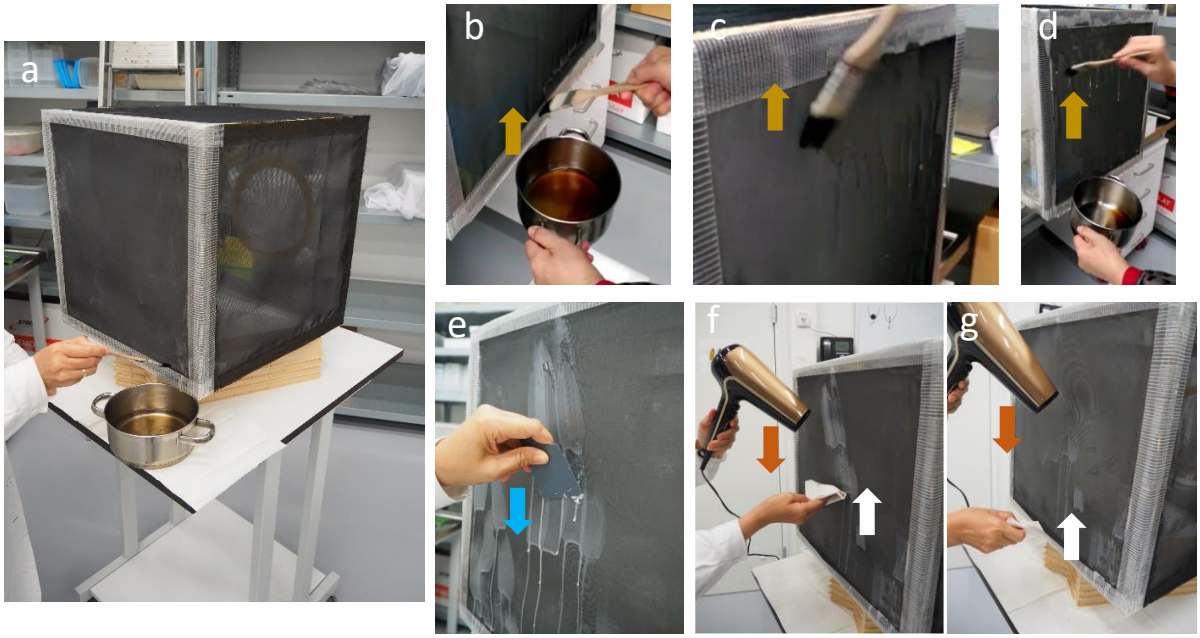

Figure 3. Creating the wax panel. (a) Applying the wax; (b) (c) (d) Processing the bottom, the top and the centre of the cage panel; (e) Removing excess wax with a sharp tool; (f) (g) Equalizing the panel surface with a hair dryer. The arrows symbolize the direction of the different movements.

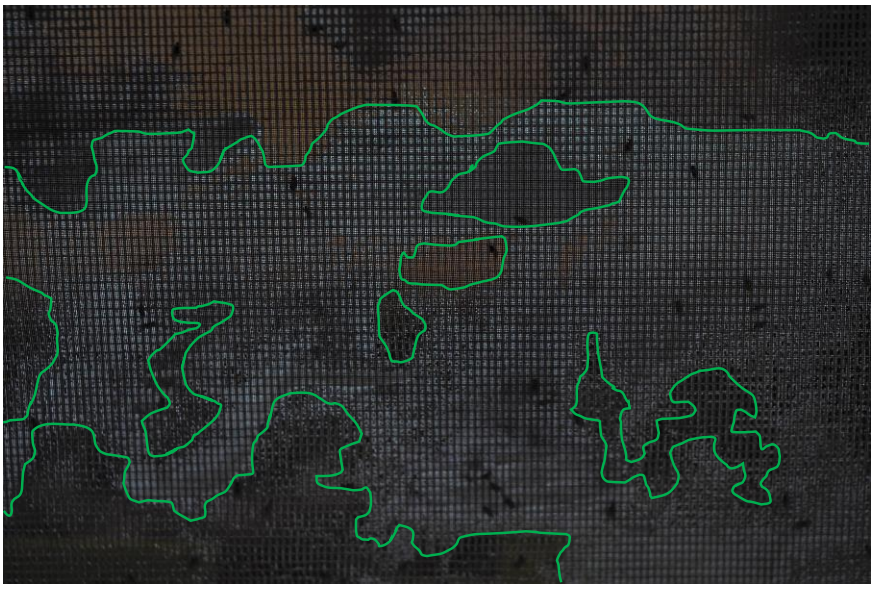

Figure 4. Aspect of the wax panel, using a light source directed through the cage from the opposite side. The large, bright area circled in green illustrates an adequate amount of wax. Darker areas indicate an excess of wax.

SWD females stand on the internal net and oviposit through the black external net. Eggs protrude from the wax panel and can be collected from outside. The wax panel is isolated from the outside with an aluminium angle frame PLEXIGLAS® protection (Figures 5a and b) between the different egg collections to avoid the oviposition of exogenous insects on the net. To stimulate female oviposition, water or fruit juice is slathered directly on the wax panel or on Petri dishes containing sponge cloth secured in the inner surface of the protection with hook-and-loop fasteners (Figure 5c). Several fruit juices, such as guava juice, greatly stimulate oviposition but are only suitable when eggs are collected daily, as juices usually promote the rapid appearance of fungal colonies on the wax panel.

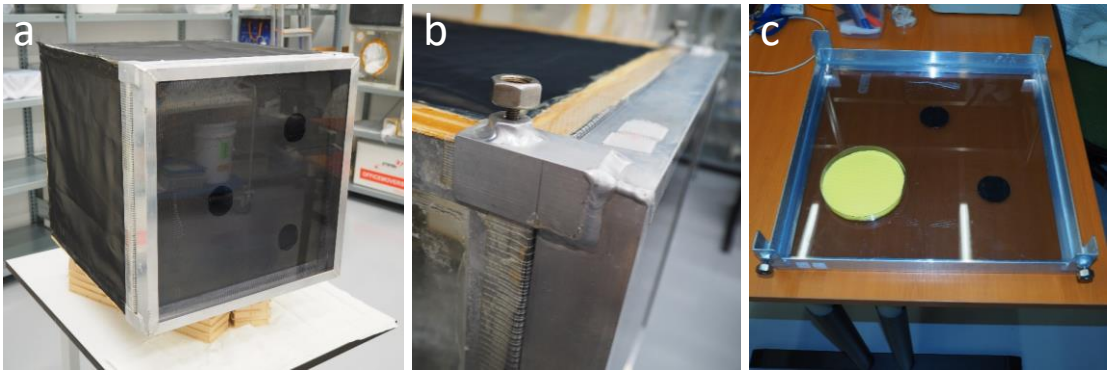

Figure 5. Protecting the oviposition panel. (a) (b) Wax panel protection; (c) Inner surface with wet sponge cloth

A.2. Mass rearing procedure

A.2.1. Setting up a holding cage

Approximately 30,000 pupae (placed on moistened paper towel) (Figure 6a) are necessary to set up a 45 x 45 x 45 cm cage (density of 2 flies/cm^2). As a fly diet, approximately 100 g of a mixture of sugar and enzymatic hydrolyzed yeast (3:1 ratio) contained in Petri dishes are placed inside the cage. Two to three 500 mL water reservoirs are placed along the inner face of the wax panel (Figure 6). Sponge cloth passing through the slit reservoir caps provides a hydration area for the flies. Felt or extra sponge cloth spread under the water containers will soak up any water passing through the wax panel while collecting the eggs (Figures 6b and c). The fly cage is kept for three weeks ± one week at 23 ± 2 °C, with 65 ± 5% humidity and a 14:10 h day-night cycle. Water should be replaced every two weeks.

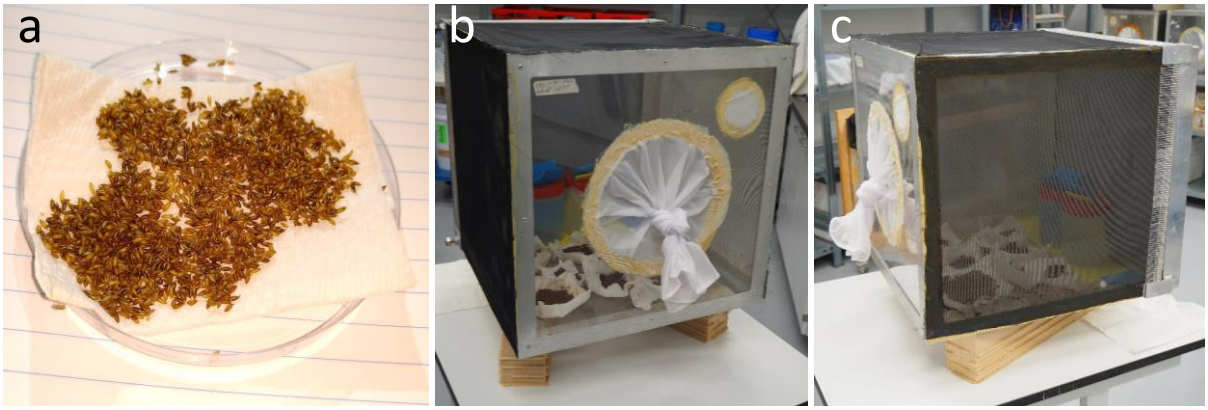

Figure 6. Setting up a holding cage. (a) Pupae container; (b) (c) Installation of a new cage.

A.2.2. Egg collection

Eggs are gently removed from the top to the bottom of the wax panel with a purified water pipette and collected in a container placed along the bottom of the cage (Figures 7a and b). As described previously, an additional, sharp piece of adhesive tape added along the bottom of the wax panel will facilitate egg collection (Figure 7b). Furthermore, the wax panel and the adhesive tape must be properly wax sealed to prevent water infiltration inside the cage. Eggs can also be collected with wet sponge cloth (gentle, short bottom-up movements) (Figure 7c), especially in panel corners.

Collection water is then filtered using a fine mesh sieve. Small piles of eggs are placed on wet pieces of black mesh net (4 x 4 cm; mesh size: 0.22 x 0.22 mm) (Figure 7d) and then gently spread out with soft forceps (Figures 7e and f).

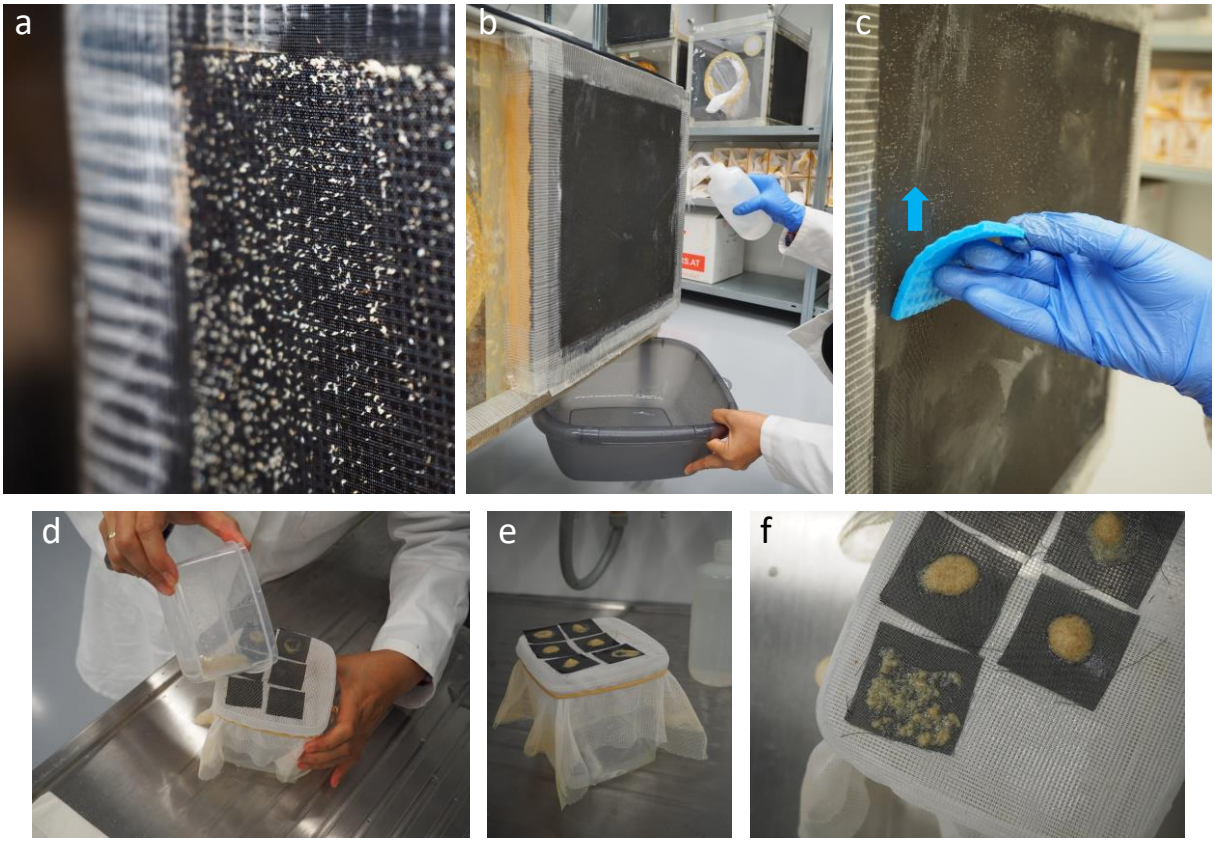

Figure 7. Egg collection. (a) (b) (c) Collecting eggs from the wax; (d) Constituting small piles of eggs; (e) (f) Spreading out egg piles with soft forceps.

Egg production through the wax panel begins approximately five days after the cage is set up. Maximum egg production is however reached approximately seven to ten days after the cage is set up. On average, at least 20,000 eggs (about 2 mL eggs) can be collected daily, preferably at noon. A total of 40 ± 10 mL eggs can be collected using a single cage based on one collection per day for two weeks (without using fruit juice as an oviposition stimulate). For experiments or quality control procedures requiring eggs, we recommend washing the wax panel in the early

morning (the eggs collected then can be used for routine rearing) and collecting eggs a second time two to three hours later for the experiments or quality control tests, as reducing the egg collection window might help to synchronize larval development and pupal production.

A.2.3. Larval rearing

Larvae are reared on a potato-based diet, a recipe first developed at Instituto de Sanidad y Calidad Agropecuaria de Mendoza (ISCAMEN, Argentina) that uses potato as a bulking agent. One kilogram of potato-based diet consists of 140 g potato flakes, 70 g inactive brewer's yeast (LBI 2245, Lallemand Bio-Ingredients, Canada), 65 g sugar, 3 g sodium benzoate, 3 g nipagin, 700 mL regular water, and is prepared as follows:

1. In a pan: dissolve the nipagin in 100 mL of boiling water
2. In the large bowl of a kitchen machine (e.g. Chef Robots, Kenwood Appliances, UK):
    a. Dissolve the sodium benzoate in 200 mL of water
    b. Add the dissolved nipagin
    c. Rinse the sides of the pan with the rest of the water and add it to the bowl
    d. Mix for a few seconds (medium speed)
    e. Add the sugar, yeast, and potato flakes, in that order
    f. Mix for 30-40 min (medium speed) (Figure 8a)

The recommended range for pH is 4.8 to 5.4. The diet can be conserved for a week in a hermetic container at ambient temperature.

Immediately after egg collection, pieces of black mesh net soaked with purified water and covered with eggs are placed on artificial larval diet (0.6-0.7 mL eggs – i.e. ± 6000-7000 eggs per 15 cm diameter rearing dish containing ~ 160 grams of larval diet) (Figure 8b). Infested rearing dishes are placed, individually or grouped per day of egg collection, in aerated plastic boxes (e.g. Figures 8c and d). Rearing dishes are maintained for ten days ± three days at 24 °C, with 65 ± 5% humidity and a 14:10 h day-night cycle. Eggs hatch within two days. After three days, rearing dishes must be checked to ensure the diet does not dry out; if it is, run some water along the edges of the dish. A thick, rigid net grid (mesh size: 1 x 1.5 mm) can be gently plated on the diet to improve larval penetration into the diet as well as to prevent future pupa from burying inside the diet in case of intense larval activity (Figure 8e).

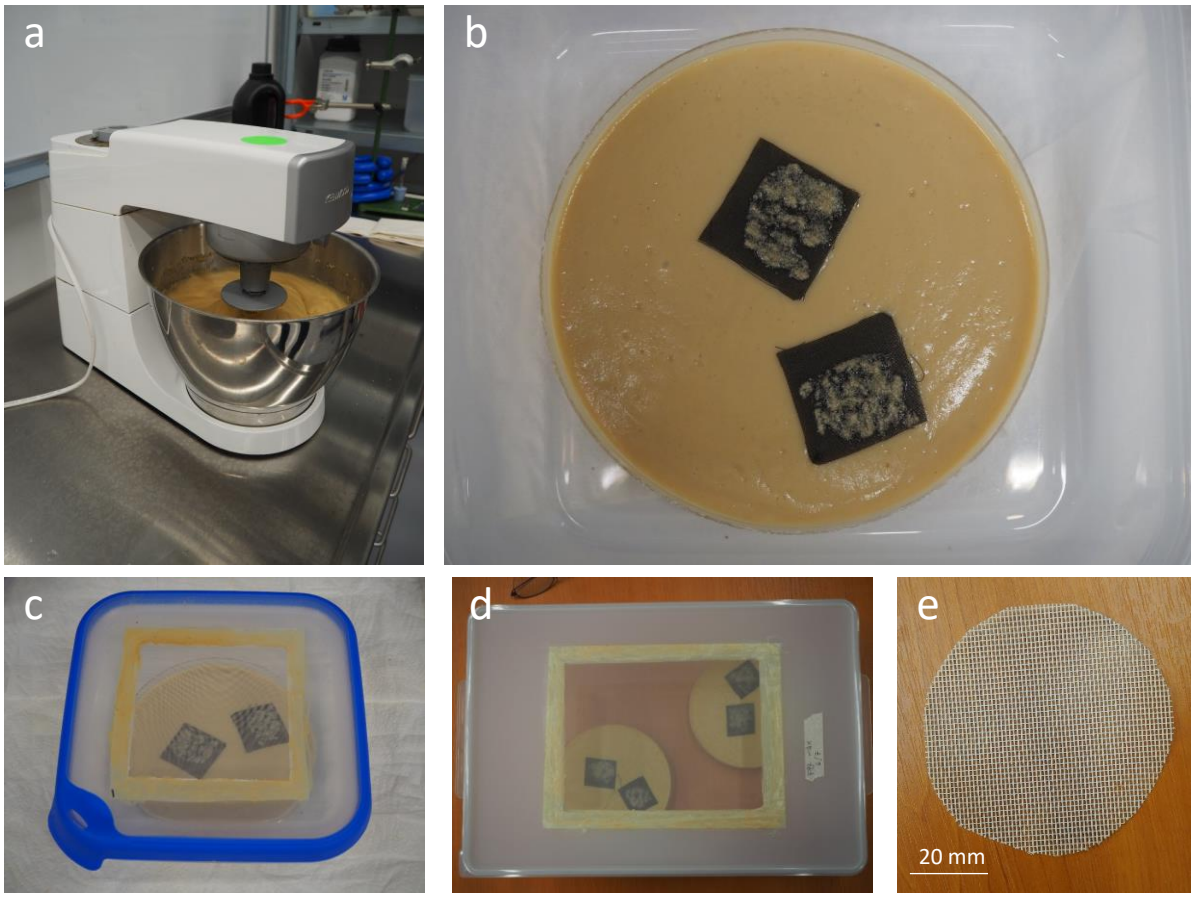

Figure 8. Larval rearing. (a) Preparing potato-based diet; (b) Infestation of a 15 cm diameter rearing dish; (c) (d) Larval development in aerated boxes; (e) Net grid that can be plated on the diet to improve larval and pupae survival.

Selecting artificial diets for insect mass rearing is achieved by considering production costs, production efficiency, and insect quality. The potato-based diet facilitates separation of pupae from the larval diet (it is indeed highly soluble in water), allows the production of large quantities of pupae, and is economically competitive. Using this diet, we observed that, from 1,000 eggs (i.e. 0.1 mL eggs) collected from a wax panel (n = 10 replicates), on average 897.8 eggs (51.99 SD) hatched, and 783.4 pupae (52.61 SD) were produced, i.e. about 3.5 mL pupae, 1 mL pupae being equal to 220 pupae under our conditions. From these – non-irradiated – pupae, on average 640.9 (47.21 SD) adults emerged, including 296.2 (24.87 SD) males and 344.7 (39.45 SD) females. The quality of the flies produced using the potato-based diet, and in particular the capacity of the males to compete sexually with wild males in an SIT context, must now be assessed. A few other diets have been proposed for the mass rearing of SWD (e.g. Aceituno-Medina *et al.* 2020). Additional ingredients can be added to the original potato-based recipe, such as carrot powder (ISCAMEN, Argentina).

N.b. A diet based on wheat bran has also been tested at the IPCL for the mass rearing of SWD. One kilogram of diet contains 280 g wheat bran, 130 g sugar, 70 g inactive brewer's yeast, 4.2 g sodium benzoate, 4.2 g nipagin, and 600 mL regular water. Quantities of pupae obtained from potato-based diet and wheat bran-based diet are comparable. Separating pupae from the larval diet is however difficult using wheat bran diet.

A.2.4. Pupae collection

Pupae are collected ten to eleven days after egg collection. About a fifth of the total number of pupae can be collected easily outside the larval diet using water, however they often produce adults earlier than pupae collected from the larval diet.

Larval diet is dissolved in a large container of water (about 20 °C) (Figure 9a) and pupae are separated manually from the larval diet residues by placing them on a fine mesh sieve, as used for egg collection, and rinsing them under running tap water (Figure 9b). For large amounts of larval diet, a machine with a wide, pierced, smoothly rotating arm can be used to separate the pupae and the rearing diet in a large container, since food components are heavier than pupae (Figure 9c).

Pupae separated from the diet must be collected from the sieve with soft forceps and placed on wet paper towel. The pupae collected can be used directly or stored in cooler conditions (around 15 °C, not lower) up to five days.

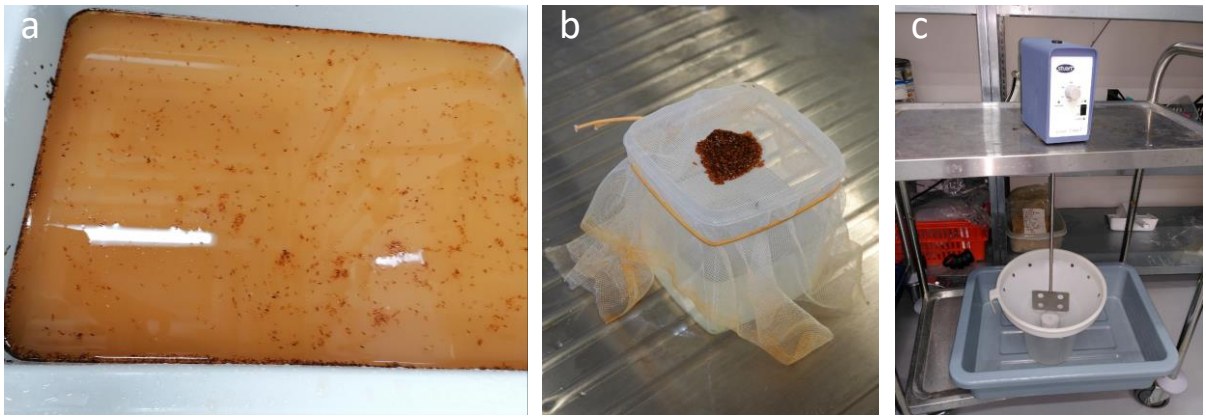

Figure 9. Pupae collection. (a) Dissolving larval diet; (b) Filtering pupae; (c) Example of medium-scale pupae collection device.

A.2.5. Ensuring continuous mass rearing

A new holding cage can be set up every three weeks or every month depending on the rearing facilities' needs. We recommend referring to Appendix 1 for examples of mass rearing schedules. About half of the 30,000 pupae necessary for a cage set-up can be collected several days in advance and stored in cooler conditions (see paragraph A.2.4), while others can be extracted from the larval diet on the same day as the set-up.

Old cages should be cleaned with cold water only, without detergent, within a week as follows: first, spray water through the opening. Flies will drop and succumb within a few hours, after which the insect carcasses are flushed out using water. Clean the cage panels with paper towel and let it dry. Clean the wax panel with water (no sponge) and replace any dirty pieces of the adhesive tape that secures it to the cage frame. Before using a cleaned cage, we recommend waiting at least one week and controlling the absence of unwanted insect species, in particular

dark-winged fungus gnats (Diptera: Sciaridae), and common vinegar flies such as *Drosophila melanogaster* or *D. simulans* (Diptera: Drosophilidae).

The same wax panel can be used for five or six different cages, after which the panel is coated with new wax following the procedure described in paragraph A.1.2.

B. Irradiation

The SIT classically relies on the sterilization of wild insect females following their mating with mass released males carrying, in their sperm, dominant lethal mutations induced by ionizing radiation (Robinson 2021).

Irradiation generally affects insect quality, with notable effects on longevity, flight ability, and mating competitiveness (Parker *et al.* 2021b). Irradiation as late as possible in the development pathway, ideally when adults are fully developed, minimizes damage to somatic cells and maximizes damage to germ cells (Robinson 2021). At the IPCL, SWD pupae are irradiated instead of adults, as it is often the case in tephritids for operational reasons (ease of mass rearing, handling, and shipping). However, the SWD pupal window for irradiation is short, a few hours prior to adult emergence. Sterilizing SWD adults rather than pupae in the future, a option currently addressed by scientific partners, should lead to higher male performances and should be more practical for large scale SIT programmes.

In this part, we detail the procedures to irradiate SWD pupae and the method to measure adult sterility level.

B.1. Monitoring pupal development for irradiation

SWD pupae are irradiated at the late pupal stage, when adults are fully formed – or almost – within the pupal chamber (i.e. pharate adults) and are close to emerging. Late pupal stage is characterized by pupae turning from light to dark brown, with adult wings visible through the pupal cuticle, and eyes turning from light to dark red (Figure 10a), as this is the case in tephritids (Resilva & Pereira 2014). Under the IPCL conditions, a group of pupae collected on the same day (Figure 10b), about ten days after egg collection, can be irradiated one to three days later. Reducing as much as possible the egg collection window, as well as the pupae collection window, will help to synchronize pupal development and will ensure most pupae will be at the ideal stage for irradiation, which can be difficult to achieve (Figure 10c).

Figure 10. Monitoring pupal development. (a) From left to right, intermediate pupal stage (one pupa) and late pupal stage (two pupae); (b) Freshly collected pupae; (c) Heterogeneous development within a pupal group.

In the absence of sex separation protocols adapted to the pupal stage that could be developed using near-infrared imaging, Mendelian genetics, or engineered sex-linked mutations as for other insects (Franz *et al.* 2021, Parker *et al.* 2021a), both males and females should be irradiated and released together. How exactly releasing sterile females impacts treated areas must be investigated, as sterile females could distract wild fertile males, acting as a 'sperm sink' (Parker *et al.* 2021a) but could also damage crops, potentially favouring insect and microbial infestations through egg-laying attempts.

B.2. Irradiation procedure

B.2.1. Irradiation dose

Optimal irradiation doses induce high adult sterility levels with little damage on insect performance while avoiding substerilization. In *Drosophila melanogaster*, unexpectedly high fertility can be measured days after irradiating males with sub-sterilizing doses (Lüning 1952), as immature sperm at the moment of irradiation is less mutable and radiosensitive than mature sperm (Lefevre & Jonsson 1964). It is possible to identify the sterilizing dose suitable for SIT based on the acceptable dose for males. As females are more sensitive than males to radiation sterilization, it will ensure all released females are fully sterile. Drosophilidae being among the least radiation-sensitive Diptera families (Bakri *et al.* 2021), irradiation doses necessary to sterilize SWD males are generally higher than ones used for other flies such as tephritids.

Several recent studies focused on radiation effects on SWD sterility, with a large range of irradiation doses tested at the pupal stage (e.g. Lanouette *et al.* 2017, Krüger *et al.* 2018a, Sassù *et al.* 2019b, Chen *et al.* 2022). These references are listed in Appendix 2. Based on egg hatch data, Sassù and colleagues (2019b) suggested that a dose of 170 Gy, inducing 97% male sterility, would be recommended in a suppression strategy while a higher dose of 220 Gy, inducing 99.8% male sterility, would be recommended in an eradication strategy. In both cases, females will be fully sterile, as they usually already are when exposed to much lower doses (Lanouette *et al.* 2017; Krüger *et al.* 2018a; Sassù *et al.* 2019b). Overall, a dose around 200 Gy should produce nearly fully sterile males (> 99% sterility) and could be proposed as the optimal dose for SIT releases (Sassù *et al.* 2020b), although there is no record on the performance of such irradiated insects under semi-natural conditions yet.

Reaching a certain sterility level might require choosing different irradiation doses depending on the irradiation conditions. For successful SIT programmes, it is essential to identify the optimal irradiation dose under local irradiation conditions, which necessitates establishing a dose-response curve (see Sassù *et al.* 2019b for an example), or by default reporting to the conclusions of a study performed under similar irradiation conditions (e.g. see Appendix 2).

B.2.2. Irradiation conditions

B.2.2.1. Atmospheric conditions

Irradiation in air generates free radicals, and therefore oxidative stress, that can be detrimental to insect performance. Sealing insect containers before irradiation allows the creation of a hypoxic environment (1-5% O_2): pupal metabolism quickly depletes the oxygen and produces carbon dioxide, resulting in radioprotection that would reduce SWD somatic damages, the same as for many insects (Nestel *et al.* 2007, Parker *et al.* 2021b). However, hypoxia would also reduce radiation damage on germ cells. Therefore, a higher irradiation dose might be needed under hypoxia than under normoxia (10-20.9% O_2) to achieve the same level of sterility (Yamada *et al.* 2019), as it has been reported for both SWD males and females (Sassù *et al.* 2019b).

Prolonged hypoxia without chilling may alter SWD flight ability, as for tephritids (FAO/IAEA 2017), long transportation of irradiated insects under these conditions should then be avoided (Enriquez *et al.* 2021).

B.2.2.1. Irradiation source and device

SIT programmes mostly rely on the use of ionizing radiation from isotopes (self-contained Gamma irradiators, using Cobalt-60 and Caesium-137) for insect sterilization (Mastrangelo *et al.* 2010). Safer alternatives to isotopic irradiators are electron accelerators and X-ray irradiators. In particular, low-energy X-ray irradiators are cheaper and more user-friendly than Gamma irradiators (Mastrangelo *et al.* 2010; Yamada *et al.* 2014; Bakri *et al.*, 2021).

Irradiation source and device could also influence the observed dose delivered, obtaining the optimal dose necessary to achieve a certain level of insect sterility then requires a dosimetry system. Before irradiating biological material, characterizing the irradiator is necessary to determine the dose rate (Gy/minutes) and the DUR (dose uniformity ratio). Dose mapping provides information on the dose distribution within the irradiation container (see FAO/IAEA (2020a) for more details). The dose rate will help to calculate the exposure time for the sterilization dose, and the DUR will determine the area of the irradiation container of the irradiator that can be used to achieve sterilization.

For gamma irradiation, the dose rate will depend on the activity of the source, the number of sources used, the distance from the source, and the shielding of the irradiation container

For X-ray irradiation, the dose rate will mainly depend on the energy (kV), the current (mA), and the shielding of the irradiation container

During the irradiation process of the biological material, we suggest using the same configuration used to determine the dose rate (e.g. number of sources, distance and type, position and size of the container).

For sterilization, SWD pupae are placed in an irradiation container for a certain amount of time that will depend on:

- the irradiator specificity, as the dose rate depends on the nature and the decay of the gamma radiation source. In addition, the nature of the energy transfer from radiation to the irradiated material influences the absorption rate of the radiation beam and therefore the dose distribution in the target material

- the placement of the pupal container into the irradiation chamber, as the distribution dose varies with the chamber volume

- the quantity/volume of biological material in the pupal container (attenuation of the radiation by absorption both in the sample material itself and the chamber and sample holder material)

B.2.3. Example of an irradiation procedure

We detail below a procedure for irradiating SWD pupae based on a protocol developed by Sassù and colleagues (2020b) at the IPCL, under hypoxic conditions, for the purpose of an experiment.

1. A group of pupae is placed in a polyethylene bag (VWR Polyethylene Tubing 89071-044, Radnor, PA, USA) sealed using a table-top sealer (Polystar 244, Rische + Herfurth GMBH, Hamburg, Germany) (Figure 11). Bags should be tied tightly enough to reduce excess air to the minimum. Overcrowded bags should be avoided, more information is needed to determine the ideal pupal density in a bag. Pupae must be collected at least a day before this step to avoid residual water from pupae collection

Figure 11. Example of a hypoxia bag.

2. Pupae are left in the bag for a minimum of 1 h at 24 °C, or longer at colder temperatures, to create hypoxia conditions. The oxygen level, measured with a gas-sensor device (CheckMate3, Dansesor A/S, Ringsted, Denmark), should be below 5% at the end of the treatment

3. Pupae could be irradiated using a self-contained Gamma irradiator (such as the Model 812 Co-60, Foss Therapy Services Inc., CA, USA) or an X-ray blood irradiator (such as the Raycell Mk2, Best Theratronics, ON, Canada)

    a. Using a Gamma irradiator (Model Gamma Foss 812): the high dose rate depends on radioactive decay. For this irradiator model, the three gamma sources should be activated, the canister containing pupae is placed on the turntable position 3 in the chamber, and the turntable is activated. These parameters should insure a uniform irradiation of the pupae.
    b. Using an X-ray irradiator (Model Raycell Mk2): the moderate dose rate is stable (7 Gy/min for this model). The canister containing pupae must be full of instant rice.

    For both technologies, if the canister is full of pupae, set the exposure time according to the minimal irradiation dose for the centre. If the pupae are only placed in the centre of the canister, with a polystyrene cylinder as a support, set the exposure time according to the dose needed for the centre.

    Dosimeters should be used to control the absorbed dose delivered to the pupae (see FAO/IAEA (2020a) for details about their characteristics and uses). Place three – four 10 x 10 mm Gafchromic^{TM} HD-V2 dosimetry films (Ashland Advanced Materials, NJ, USA) with the pupae to confirm irradiation dose. Read after 24 h using a Radiachromic reader (e.g. FWT-92D, Far West Technology Inc., CA, USA). The mean of these three values should be reasonably equal to the expected dose. Calculate lot homogeneity (mean and standard deviation from the mean). The films should be protected in paper envelopes (see FAO/IAEA (2020b) for details about the Gafchromic^{TM} system). Standard operating procedures for Gafchromic^{TM} film dosimetry system for Gamma radiation and for low energy X radiation are detailed in the documents FAO/IAEA (2022a, b).

B.3. Assessing sterility level

Assessing the sterility level of irradiated insects, necessary to establish a new dose-response curve or to confirm that the applied dose was correct, could be achieved following the method described below. To put it simply, male and female sterility are checked separately, and a fertility control is also performed. A minimum of six experimental blocks is required to obtain conclusive results. An experimental block is run as follows:

1. Within 12 h after pupal irradiation, young adults are collected, as well as non-irradiated ones.

   Sexes are quickly separated after a short anaesthesia using ice, CO_2, or ether. Anesthetizing *Drosophila* with ice is cheap and convenient; the process is detailed and illustrated in Barbato (2017). Young males and females might be difficult to differentiate with the presence/absence of a black spot on the wing (male: yes, female: no), and a quick examination of the body end's morphology is necessary (male: round, black end; female: sharp, light end with a line marking the ovipositor).

   Sexes are maintained separately in rearing vials for four days. Adding enzymatic hydrolyzed yeast to the nutritive medium should improve future female oviposition.
   At the end of the experiment, female vials should be examined, and the absence of pupae checked as a virginity control.

2. After four days, males and females are introduced in sex-mixed cages as following:

   - 'Male Sterility check' cage: 25 irradiated males + 25 non-irradiated females
   - 'Female Sterility check' cage: 25 irradiated females + 25 non-irradiated males
   - 'Control' cage: 25 non-irradiated males + 25 non-irradiated females

   All cages contain a small dish of enzymatic hydrolyzed yeast/sugar (1:3) and a small dish with artificial diet (e.g. wheat bran diet, see paragraph A.2.3) or fresh fruits (e.g. blueberries) to collect eggs.

3. Collect eggs the following day. If using wheat bran diet, the medium can be easily dissolved in some water upon a large piece of black mesh net, and the eggs collected. If using blueberries, report to Sassù *et al.* (2020b).
   Egg lines are arranged on a small piece of black mesh net, moistened with purified water and placed on the surface of a medium artificial diet Petri dish.
   Egg collection should be continued until a minimum of 500 eggs are collected within three days of collection.

   Count the number of eggs hatched after two complete days. Fertility is measured as the number of eggs hatched divided by the number of eggs collected. Fecundity is measured as the number of eggs collected divided by the number of eggs laid by non-irradiated females.

   Note the identity of the experimenter as well as the egg collection technique can deeply influence the results. Pursuing the experiment to assess the number of pupae and adults produced remains optional.

We recommend referring to Appendix 3 for an illustration of the experimental timeline.

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Appendixes

Appendix 1. Example of a mass rearing schedule, as used at the Insect Pest Control Laboratory

Day -7	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**
				Pupae collection (pupae are then stocked in cold conditions)		
Day 0	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**
Pupae collection Set-up of Cage 0 with about 30,000 pupae collected at D-3 (half) and D0 (half)						
+7	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**
Egg collection	Egg collection	Egg collection	Egg collection	Egg collection		
+14	**+15**	**+16**	**+17**	**+18**	**+19**	**+20**
			Pupae collection (pupae are then stocked in cold conditions)	Pupae collection (pupae are then stocked in cold conditions)		
+21	**+22**	**+23**	**+24**	**+25**	**+26**	**+27**
Pupae collection Set-up of Cage 1 with about 30,000 pupae collected at D+17/+18 (half) and D+21 (half)						

Table S1.1. Schedule example for mass rearing colony maintenance. Colours: Previous colony; Focal colony (Colony 0); Next colony (Colony 1)

Day -7	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**
				Pupae collection (pupae are then stocked in cold conditions)		
Day 0	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**
Pupae collection Set-up of Cage 0 with about 30,000 pupae collected at D-3 (half) and D0 (half)						
+7	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**
Egg collection	Egg collection	Egg collection	Egg collection	Egg collection		
+14	**+15**	**+16**	**+17**	**+18**	**+19**	**+20**
Egg collection	Egg collection	Egg collection	Pupae collection Egg collection	Pupae collection Egg collection		
+21	**+22**	**+23**	**+24**	**+25**	**+26**	**+27**
Pupae collection Egg collection	Egg collection		Pupae collection (pupae are then stocked in cold conditions)	Pupae collection (pupae are then stocked in cold conditions)		
+28	**+29**	**+30**	**+31**	**+32**	**+33**	**+34**
Pupae collection Set-up of Cage 1 with about 30,000 pupae collected at D+17/+18 (half) and D+21 (half)			Pupae collection	Pupae collection		

Table S1.2. Schedule example for mass rearing pupae production for both colony maintenance and experiments. Colours: Previous colony; Focal colony (Colony 0); Next colony (Colony 1); Collection for experiments

N.b. For experiments, reducing egg collection window is generally necessary to synchronize larval development and pupae production (see paragraph A.2.2).

Appendix 2. List of published research using irradiation for SWD control (SIT or phytosanitary treatment) (as of August 2022)

Gamma radiation					
cobalt-60					
Reference	***Irradiator***	***Dose(s) (Gy)***	***Irradiated insect stage***	***Conditions***	***Goals***
Enriquez *et al.* 2021	Gammacell 220 (Nordion, ON, Canada)	220	Pupae	Hypoxia and normoxia pre- and during irradiation	Effect of hypoxia and chilling on the quality of irradiated flies
Gutierrez-Palomares *et al.* 2019	Transelektro LGI-01 (Ganz Transelektro, Hungary)	60 – 70 – 80 – 90 – 180 – 200	Pupae	-	Effect of irradiation on sterility induction and quality
Gutierrez-Palomares *et al.* 2020	Unspecified	200	Pupae	-	Effect of irradiation on wing morphology
Kim *et al.* 2018	150 TBq capacity, ACEL (Nordion, Ottawa, Canada)	50 – 100 – 200 – 300 – 400	Eggs Larvae Pupae Adults	Normoxia	Development inhibition Effect of irradiation on sterility induction
Krüger *et al.* 2018a	Eldorado 78 (Foss Therapy Services, CA, USA)	75 – 150 – 200	Pupae	Normoxia	Effects of irradiation dose on sterility induction and quality
Krüger *et al.* 2018b	Eldorado 78 (Foss Therapy Services, CA, USA)	200	Pupae	Normoxia	Effects of irradiation on sexual behaviour
Krüger *et al.* 2021	Eldorado 78 (Foss Therapy Services, CA, USA)	200	Pupae	Normoxia	Effects of temperature and relative humidity on sexual behaviour and survival
Lanouette *et al.* 2017	Gammacell 220 (Nordion, ON, Canada)	30 – 50 – 70 – 80 – 90 – 100 – 120	Pupae	Normoxia	Establishing optimum irradiation dose
Nikolouli *et al.* 2020	Gammacell 220 (Nordion, ON, Canada)	45 – 60 – 90	Pupae	Normoxia	Combining SIT and Incompatible Insect Technique (IIT)
Sassù *et al.* 2019b	Gammacell 220 (Nordion, ON, Canada)	30 – 50 – 70 – 90 – 110 – 130 – 150 – 170 – 190 – 210 – 220 – 230 – 240	Pupae	Hypoxia and normoxia pre- and during irradiation	Effect of irradiation on sterility induction under hypoxia and normoxia

Sassù *et al.* 2021	Gammacell 220 (Nordion, ON, Canada)	220	Pupae	Hypoxia and normoxia pre- and during irradiation	Mating competitiveness of sterile males under different atmosphere conditions
caesium-137					
Reference	***Irradiator***	***Dose(s)***	***Irradiated insect stage***	***Conditions***	***Goals***
Chen *et al.* 2022	Unspecified	60 – 90 – 110 – 120 – 150 – 180	Pupae	Normoxia	Establishing optimum irradiation dose
Lanouette *et al.* 2017	Gammacell 3000 (Best Theratronics, ON, Canada)	30 – 50 – 70 – 80 – 90 – 100 – 120	Pupae	Normoxia	Establishing optimum irradiation dose
Lanouette *et al.* 2020	Gammacell 3000 (Best Theratronics, ON, Canada)	120	Pupae	Normoxia	Effect of irradiation on sexual behaviour
X-Ray radiation					
Reference	***Irradiator***	***Dose(s)***	***Irradiated insect stage***	***Conditions***	***Goals***
Follett *et al.* 2014	5 MeV, model TB-5/15 (Titan Corp., CA, USA)	20 – 30 – 40 – 50 – 60 – 80	Larvae Pupae	Normoxia	Development inhibition Effect of irradiation on sterility induction
Follett *et al.* 2018	5 MeV, model TB-5/15 (Titan Corp., CA, USA)	60	Larvae Pupae	Hypoxia and normoxia pre-, during, and post-irradiation	Effect of low-oxygen conditions on radiation tolerance in sweet cherries
Kim *et al.* 2016	7.5 MeV, UEL V10-10S	50 – 100 – 200 – 300	Eggs Larvae Pupae Adults	Normoxia	Development inhibition Effect of irradiation on sterility induction
Homem *et al.* 2022	Unspecified	Unspecified	Adults	Unspecified	Control of SWD using SIT in open polytunnels
Electron beam radiation					
Reference	***Irradiator***	***Dose(s)***		***Conditions***	***Goals***
Kim *et al.* 2018	10 MeV, MB10-8/635 (Mevex, ON, Canada)	50 – 100 – 200 – 300 – 400	Eggs Larvae Pupae Adults	Normoxia	Development inhibition Effect of irradiation on sterility induction

Table S2. List of published research using irradiation for SWD control (SIT or phytosanitary treatment) (as of August 2022)

Appendix 3. Suggested schedule for a sterility experiment under hypoxia conditions

Day 1	**Pupae irradiation under hypoxia conditions** • 9:00 am. Sealed bags for hypoxia • 1:00 pm. Irradiation • After irradiation, place pupae in emergence cages
Day 2	**Collection of freshly emerged adults** • 9:00 am. Separation of females and males • 2:00 pm. Second separation, if more adults needed • At the end of the day, discard the non-emerged pupae
Day 6	**Transferring adults in egg collection cages** • Insert a first egg collection device
Day 7	**Egg collection** • Collect eggs Nb. If more eggs are needed, change the egg collection device and collect eggs the next day
Day 9	**Assessing egg hatching rate**
Day X	**Assessing F1 adult emergence rate**

N.b. replicate at least six times per treatment tested. Add at least fertility controls.

Table S3. Suggested schedule for a sterility experiment under hypoxia conditions