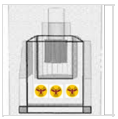

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Gamma radiation of *Drosophila suzukii* under hypoxia and normoxia atmosphere conditions.

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.76whrfe

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ABSTRACT

The sterile insect technique (SIT) is an environmentally friendly control tactic based on the sequential, inundative releases of sterile insects in a targeted area. Recently, this technique has been suggested as a potential complementary control method as part of an area-wide insect pest management program (AW-IPM) against *D. suzukii.* One of the key issues for the SIT application is to determine the radiation dose since the doses used to induce reproductive sterility can vary between sexes and among species. Treating insects with a lower oxygen atmosphere before and during exposure to radiation can mitigate some of the negative physiological effects due to the irradiation. The irradiation of pupae under the oxygen-reduced environments such as hypoxia is routinely used in the SIT operational programs of some tephritid species as it provides radiological protection. This treatment allows to have the sterile pupae already in sealed containers to facilitate their shipment. Therefore, it is crucial to study the effects of irradiation on the reproductive sterility in *D. suzukii* males and females under low-oxygen atmosphere (hypoxia) compare to normal oxygen level conditions (normoxia).

This protocol describes step-by-step the irradiation of *D. suzukii* pupae under hypoxia and normoxia atmosphere conditions. This protocol may be utilized for a further assessment concerning the quality of the sterile versus non-sterile *D. suzukii* adults such as laboratory and/or field behavioral studies. Additionally, the description of the hypoxia treatment in this protocol may allow for the evaluation of the handling and release processes of the sterile males. The adoption of this protocol by different laboratories should increase further studies for the applicability of SIT on *D. suzukii* or for continuing assessments to improve SIT technology and decrease the variability in data between different studies.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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GUIDELINES

This protocol has been developed at the Insect Pest Control Laboratory at the FAO/IAEA in Seibersdorf, Vienna.

SAFETY WARNINGS

All the radiation practices mentioned in this protocol must be conducted with the assistance of a registered radiation-exposed trained operator.

Pupae collection

1

Two days before adult emergence, *D. suzukii* pupae were carefully separated from the larva medium and placed on a moist paper ensuring that the pupae will not dry while completing their maturation.

Pupae of *D. suzukii* on a moist paper

22 °C 65% ± 5% RH and 14:10 (L: D)

Depending on the design of the experiment the number of pupae used may vary. In this protocol, a total of 6000 pupae were used (2000 irradiated pupae for each atmosphere treatment and 2000 non-irradiated).

Pupae-age identification

2 ⚠

The correct age of the insect pupae at the time of irradiation is of paramount importance for the success of the SIT. Not respecting this crucial “irradiation-age window” may cause variations in the resulting adults' sterility.
The pupae should be carefully checked and selected based on the description and figure below.

Five days post pupation, pupae are dark brown with visible red eyes and wings and they are about 24 hours before emergence.

Pupae 5 days post-pupation

This step is strongly influenced by the room temperature and humidity the pupae are held.

22 °C 65% ± 5% RH and 14:10 (L: D)

Pre-irradiation treatment

3 Six hours before the irradiation exposure, pupae were treated under two different atmospheres conditions: hypoxia and normoxia.

Hypoxia treatment

3.1 A group of pupae was placed in a polyethylene bag (VWR Polyethylene Tubing 89071-044, Radnor, PA) sealed using a table-top sealer (Rische + Herfurth GMBH, Polystar 244, Hamburg, Germany).

Pupae after 5 hours of hypoxia treatment

Pupae were left in the bag for 5 hours at 18 °C to allow them to metabolize the oxygen present in the bag and thus create hypoxic conditions.
To assess oxygen depletion, a gas-sensor device (CheckMate3, Dansesor A/S, Ringsted, Denmark) was used to measure the oxygen level inside the bags. At the end of the hypoxia treatment, the concentration of oxygen was approximately 0.3%.

18 °C

05:00:00

The size of the bags depends on the number of pupae. Bags should be tied tightly so as to reduce to the minimum excess air.

It is highly suggested to avoid creating overcrowded bags. That may cause damages to the pupae especially during the hypoxia treatment.

Normoxia treatment

3.2 The pupae of the normoxia group (*ca.* 2000 pupae) were maintained under normal laboratory conditions before irradiation (oxygen concentration of about 20.9%).

22 °C

05:00:00

Irradiation treatment

4 ⚠

Pupae were exposed to gamma rays using a ^{60}Co irradiator (Gamma Cell-220, Nordion, Canada).

Hypoxia treated pupae were kept sealed in bags during the irradiation exposure.
Pupae treated under normoxia were placed in a perforated bag allowing airflow inside.
Pupae from both treatments were located together in the middle of the irradiator chamber to ensure that all the pupae received the same dose.

Three 10 by 10 mm Gafchromic®HD-V2 dosimetry films (International Specialty Products, NJ, USA) were irradiated together with the pupae to confirm the irradiation dose. The optical density of the films was measured 24 hours after the treatment using a Radiochromic reader (FWT-92D, Far West Technology, Inc., Goleta, CA, USA).

The time of irradiation exposure is strictly depending on the source of the irradiator. Because of the source decay, each Gamma Cell requires specific calibration for a given dose. Therefore, no time details of the irradiation treatment will be provided.

The irradiated pupae from normoxia and hypoxia were removed from the bags and placed in separate cages (17 x 8 x 11.5 cm) until adult emergence.
The third group of non-irradiated pupae kept under laboratory conditions and without irradiation treatment was used as a control.

Irradiated adults

5

To ensure that the pupae had the appropriate and synchronized age at the time of irradiation, only adults that emerged within 24 hours after the irradiation were used for the experiments.

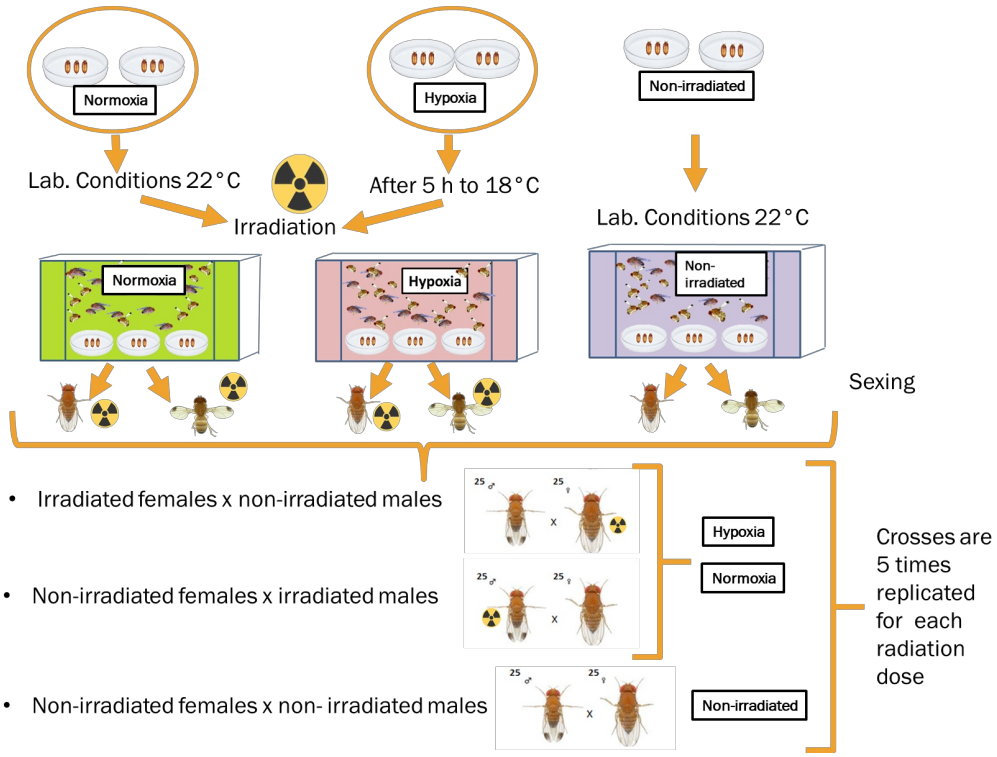
Emerging flies were sexed under a short period of CO_2 anesthesia.

The effect of the atmospheric conditions was tested on two types of crosses for each radiation dose to determine the lowest dose able to confer complete sterility under hypoxia and normoxia treatments.

- irradiated females x non-irradiated males
- non-irradiated females x irradiated males

A cross of non-irradiated females x non-irradiated males was used as a control.

All of the crosses were replicated five times for each irradiation dose.



Protocol scheme

Fecundity e fertility tests

6 Adults of each cross were kept together for four days to ensure sexual maturation and insemination of all females. Adults were provided with water and food containing a mixture of sugar and hydrolysate enzymatic yeast (MP Biomedicals) in a 3:1 ratio.

On the fifth day, three fresh blueberries were placed on top of each cage as oviposition sites.

The blueberries were daily replaced, and eggs were carefully collected using forceps.
The collections were continued until 500 eggs had been obtained per replicate, per cross and per irradiation dose or for a maximum of three days.

Infested blueberry

The eggs were placed on a wet black net in a Petri dish filled with larval diet and counted.

Fecundity was calculated as the total number of eggs collected per cage divided by the average number of eggs oviposited by the control cross.

Fertility was calculated as the number of eggs hatched divided by the number of eggs laid.