

Mating Competitiveness of Sterile male *Drosophila suzukii* Under Different Atmosphere Conditions [†]

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Abstract: The implementation of the sterile insect technique (SIT) to control *Drosophila suzukii* requires the released sterile males to compete with their wild counterparts. We performed multiple-choice mating tests to assess the effect of irradiation and atmosphere treatments on the mating competitiveness of sterile males under laboratory conditions. Overall, irradiation and atmosphere treatments did not influence the sterile males' ability to compete with the untreated counterparts.

Keywords: Sterile insect technique; pest control; hypoxia; drosophila

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1. Introduction

The sterile insect technique (SIT) is an environmentally-friendly control method that has been used during the last decades in area-wide integrated pest management (AW-IPM) programmes to control, suppress and/or eradicate agricultural insect pests [1, 2] and vector insects [3, 4]. The concept of the SIT is that the males of the target species are sterilized using ionizing radiations and then released in a pre-established and delimited area. Systematic releases of a critical number of sterile males overcome the chances of wild-to-wild matings. On the other hand, successfully matings between sterile males and wild females prevent the offspring and so reduce the persistence of the wild population in the area [5]. In polygyny species, males must compete with other males to access the females and achieve reproductive opportunities [6]. Copulatory success is leading by a female choice primarily based on the courtship display by males which often involve long-lasting performance that might include visual, chemical, and/or acoustical signals demanding a large amount of energy [7, 8]. Radiation exposure for insects' sterilization can induce adverse effects on the physiology of the flies with consequences on the mating behavior and sexual performance of the released sterile males [9]. Studies on different fruit fly species have proven irradiation exposure to lessen the mating success of irradiated males and their ability to compete with the non-irradiated males once released. For example, in a review on the competitiveness of the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann, 1824), sterile males were found less competitive than their wild counterparts [10], which was later shown also in other sterilized and released species in SIT programmes [11–13]. Consequently, different strategies have been applied supplying for the reduced quality of the released sterile flies i.e. increasing the number of the male released, applying a lower irradiation dose, and/or reducing the levels of oxygen during the pupae irradiation [14]. In presence of atmospheric oxygen, ionization radiations generate free radicals that irreversibly damage organisms' cells [15, 16]. Therefore, retaining insects

under low-oxygen levels during irradiation abates the impact of the free radicals and overcomes some of the adverse effects on flies' life traits and sexual competitiveness [17–20]. In some of the currently-running SIT programmes [21], pupae are hermetically sealed in containers for a certain period prior and during the irradiation [22]. This practice allows the flies to exhaust most of the oxygen supply within the container by natural respiration and thus creating hypoxia or anoxia conditions [23]. The packaging of pupae in anoxia has a radioprotective outcome on the flies, but it is also a practical approach for handling the insects during irradiation and then transport them to the release area [24, 25].

Drosophila suzukii (Diptera, Drosophilidae) (Matsumura 1931) is an invasive insect pest worldwide [26–29]. Its spread has caused substantial yield losses of small-stone fruit crops, in particular of different species of berries, and grapes [30–33]. *Drosophila suzukii* females are capable of perforating the peel of ripening fruits to lay their eggs and the hatched larvae are then feeding on the fruit pulp causing its complete rottenness [28]. The technological package for the application of the SIT as an ecological economic viable method to control populations of *D. suzukii* in a confined environment such as greenhouses has recently developed [34–37]. In previous studies, the irradiation dose needed to induce reproduction sterility on *D. suzukii* males was determined under normal oxygen atmospheric level [38, 39] and low-atmospheric conditioning (Sassù et al., 2019b). In these studies, preliminary tests to control the quality of the sterile flies i.e., emergence rate, flight ability, and survival flies were measured, but no differences were found. Later, mating tests were also performed to assess the ability of *D. suzukii* sterile males to mate and re-mate with fertile females [40]. Likewise, *D. suzukii* sterile males did not show any adverse impact on the ability of mating and re-mating with the only exception for the copula duration of sterile males which was shorter compared to fertile once [40]. Despite the encouraging results, yet no evidence has been provided on the ability of the sterile males to compete with the non-irradiated counterparts neither in two-choice or multiple mating experiments. Furthermore, there is no study evaluating the effect of atmospheric conditioning on the competitiveness of the sterile *D. suzukii* males.

Here we studied the mating competitiveness of adults exposed prior and during irradiation under hypoxia atmosphere treatment and compared with flies irradiated under normoxia (normal oxygen atmospheric level) and non-irradiated.

2. Materials and Methods

2.1. Study population

Drosophila suzukii flies used for the study were produced at the Insect Pest Control Laboratory (IPCL), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Seibersdorf, Vienna. The colony was established in 2014 with flies received from the Agricultural Entomology Unit of the Edmund Mach Foundation in San Michele All'Adige, Trento Province, Italy. Flies were kept in our laboratory for about 50 generations at the beginning of the experiment. The colony was reared in cages (40 × 40 × 40 cm) and provided with water and a blend of sugar and yeast hydrolysate enzymatic (MP Biomedicals) (3:1) as adult diet (Deutscher et al. 2017). The pupae used for the irradiation were obtained using the colony rearing method applied at the IPCL [41]. The rearing colony was maintained at temperature of 22 ± 5 °C, 65 ± 5% relative humidity (RH) and under a 14:10 h light: dark (L:D) photoperiod.

2.2. Irradiation and atmosphere treatments

One day before the emergence, two groups of about 3000 pupae each were treated under hypoxia (approximately 0.3% of O₂) and normoxia (normal O₂ atmosphere level) atmosphere conditions following our previously published protocol (<http://dx.doi.org/10.17504/protocols.io.76whrfe>). Immediately after the atmosphere treatments, hypoxia and normoxia treated pupae were exposed at 220 Gy using a ⁶⁰Co source (Gamma Cell-220, Nordion, Canada) (Sassù et al., 2019b). A third group of approximately 3000 pupae kept under the same conditions of

the normoxia, but without the irradiation treatment, was used as a control group. Pupae were kept in separated cages until the adult emergence.

2.3. Marking

To discriminate between irradiated and non-irradiated flies, pupae were marked with a similar method currently used to mass-mark millions of sterilized tephritid fruit flies (Schroeder and Mitchell 1981). After irradiation, pharate pupae from the different treatments were placed in a plastic cup with either yellow or pink dust (DAY-GLO Color, Cleveland, OH). Pupae were gently “trundled” in the plastic cup until they were all coated with dust. At the emergence, the expanded ptilinum, a retroflex membrane used by dipteran to open the pupal case, contacted the dust on the pupae case and retained in the face of the adult head. Most of the dust adhering to the body of an emerging fly groomed off in the 4 days prior the experiment starts, but the dust enclosed in the ptilinum stays. The dust particles enclosed in the ptilinum were detected with a UV light dissecting microscope (Hagler and Jackson 2001). Because of the smaller size of *D. suzukii*, the formula applied to mark other species of mass reared Tephritidae fruit fly was adjusted as follows: 0.001 gr of dust powder for approximately 220 *D. suzukii* pupae. For each replicate, the colors of dust (yellow and pink) were inverted between treatments to avoid possible conditioning effects cause by the colors.

2.4. Mating tests

Immediately after emergence, adults were immobilized under a short term of CO₂-anesthesia and separated by sex using stereoscope. To avoid selecting different ages, only the adults that emerged within 24 hours after the irradiation were used for the experiments. Adults were kept with unlimited access to water and adult diet in cages for five days allowing sexual maturity. Experiments were conducted at the same controlled conditions as the rearing colony: 22 ± 5 °C, 65 ± 5% RH and a photoperiod of 14:10 h (L:D).

Five different crosses were tested: ten irradiated males and ten irradiated females hypoxia treated × ten non-irradiated males and ten non-irradiated females (hereafter “hypoxia cross”); ten irradiated males and ten irradiated females normoxia treated × ten non-irradiated males and ten non-irradiated females (hereafter “normoxia cross”); twenty non-irradiated males × twenty non-irradiated females (hereafter “control cross”); twenty irradiated males × twenty irradiated females hypoxia treated (hereafter “control-hypoxia cross”); twenty irradiated males × twenty irradiated females normoxia treated (hereafter “control-normoxia cross”). All cross types were performed at the same time, with three replicates each cross type. The experiment was repeated five times (blocks) except for the cross types: control-normoxia and control-hypoxia that were repeated three times.

2.5. Laboratory observations

To evaluate the mating competitiveness between treated and untreated males and females of *D. suzukii*, observations were carried for 6 hours (9 am to 3 pm) under laboratory conditioning (22 ± 5 °C, 65 ± 5% RH). Food and water were removed, and three blueberries were placed in each cage (400 mm long and 200 mm diameter) to stimulate the mating. During the mating tests, flies were continuously observed to identify mating. Once copula occurred, the time was recorded and the couple was gently collected from the cage and placed in a clear plastic vial of about 10-20 ml covered by a cotton lead. The mating pairs were left 1-2 minutes to allow the males to complete the females’ mount before moving them into a vial. The mated adults were neither replaced nor released back into the cages after mating. In case the couple splits during the collection, both female and male were reintroduced in the cage while cage type and time were annotated. All vials contained mating pairs collected from hypoxia and normoxia crosses were individually observed under fluorescence microscope (Leica M205 FA, Vienna, Austria) to identify the treatment of the female and male of a pair.

2.6. Studied parameters

The following parameters were recorded for each of the collected mating pair: cage type, female and male treatment/s, time of the beginning of the mating and time of the end of the mating. Consequently, we calculated: time to mating (time from the beginning of the experiment to the beginning of the copula); duration of copula (time from the beginning to the end of copula); and percentage of mating per treatment and cross type. Additionally, females collected from the pairs of the control cross were singularly placed in a petri dish filled with a medium consists of agar (1%), raspberry juice (44.5%), sugar (9%), yeast (0.5%) and water (45%) to provide oviposition surface. After 48 hours females were removed, and the number of eggs counted. Egg hatch was determinate 48 hours after the eggs count. This procedure was made to assess the possibility of a direct correlation between the time of mating, copulation duration and female fecundity.

2.7. Statistical analysis

All statistical analyzes were performed using R software (R Development Core Team 2008, URL <http://www.R-project.org/>). Shapiro and Bartlett tests were performed to test respectively the normality and the homoscedasticity of the data. Differences between types of mating as a function of time to mating, duration of copula and percentage of mating were analyzed using a Kruskal-Wallis rank-sum test [42]. A Gaussian linear mixed-effects model was used to test the effect of the duration of copula on the egg production and the egg hatch [43].

3. Results

3.1. Time to mating and duration of copula

The treatment and the type of cross of the mating pairs did not influence the time of mating (Kruskal-Wallis: $\chi^2 = 1.54$, $df = 4$, $p = 0.82$). Despite the treatment and the type of cross, the time of the first mating usually occurred within few minutes from the beginning of the tests (Fig.1). The duration of the copula was also not affected by the type of cross and the treatment of the mating couple (Kruskal-Wallis: $\chi^2 = 5.27$, $df = 4$, $p = 0.26$). The time average of copulation among all the different treatments and crosses was 24 ± 0.6 minutes (Fig. 2). The duration of copula did not influence egg production (z value = -0.182, $p > 0.05$) or egg hatch (z value = 1.489, $p > 0.05$).

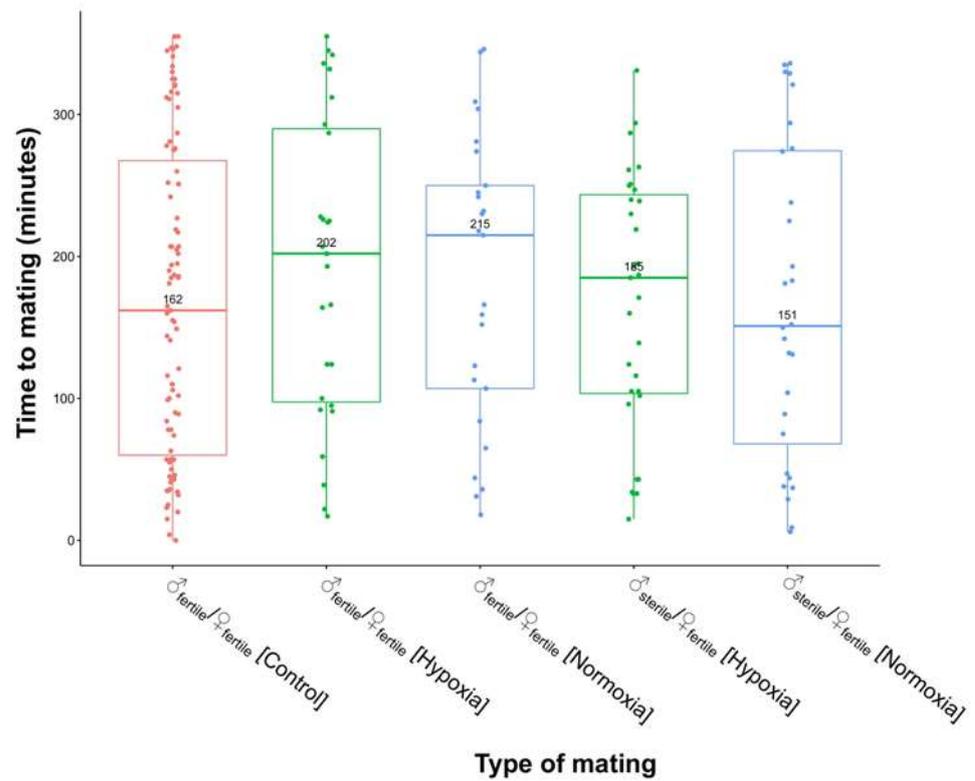


Figure 1. Box plots represent the time to mating of different mating pairs and cross types. Bold lines and numbers upon them represent 95th percentiles. Types of cross: control (red); hypoxia (green); and normoxia (blue).

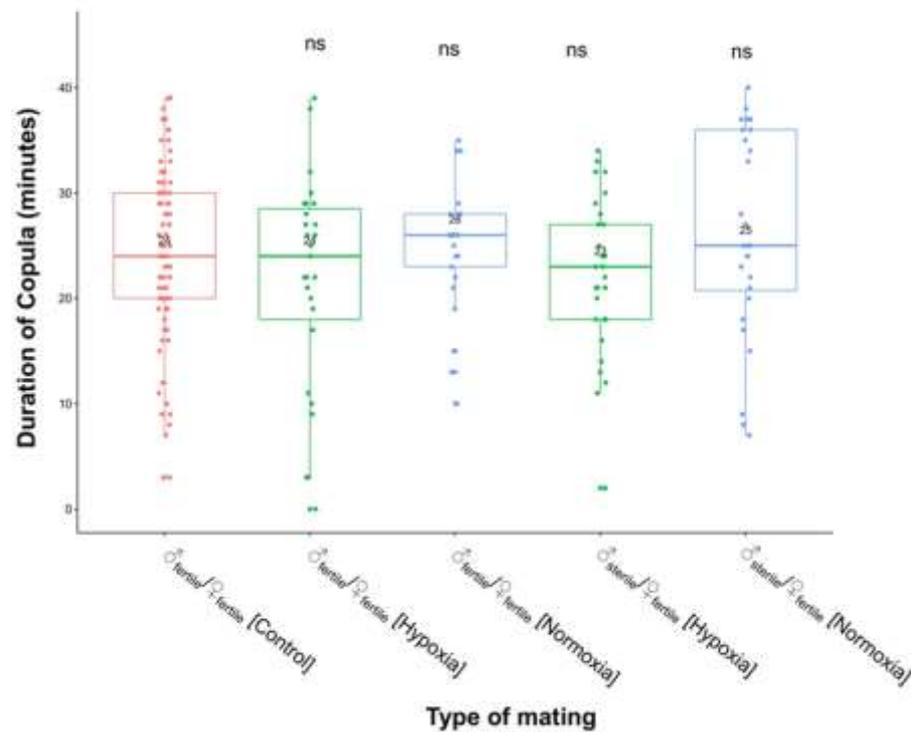


Figure 2. Box plots represent the duration of the copula for different mating pairs and cross type. Bold lines and number represent median values, dots represent observations, and horizontal bars indicate 95% percentiles. Control cross (red); hypoxia cross (green); and normoxia cross (blue).

3.2. Percentage of mating

There was no difference in the percentage of mating between sterile and fertile male in the both atmosphere conditions (Kruskal-Wallis: $\chi^2 = 0.55$, $df = 3$, $p = 0.91$) (Figure 3). The mean percentage mating between sterile males and fertile females were 20.67 ± 8.67 and 17.67 ± 4.33 for hypoxia and normoxia treatments, respectively.

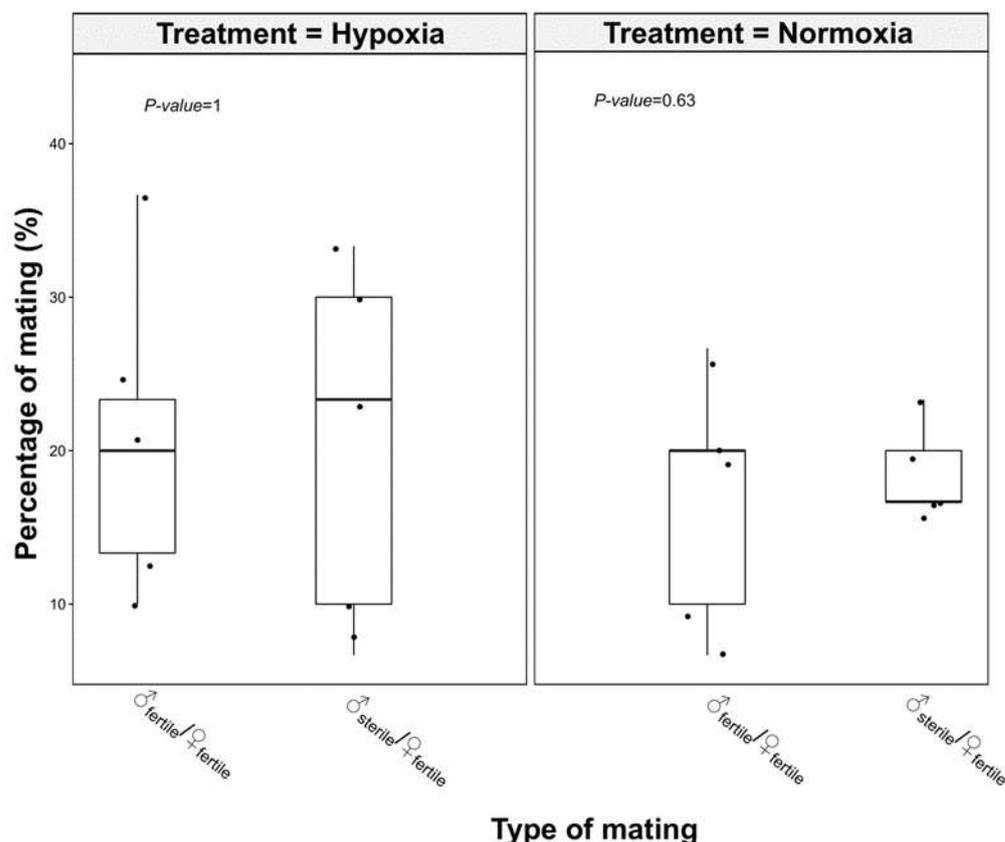


Figure 3. Box plots represent the percentage of mating in different mating pairs and cross type. Bold lines indicate median values, dots represent observations, and horizontal bars indicate 95% percentiles. Types of crosses for hypoxia (left) and normoxia (right) are reported in x-axis.

4. Discussion

The mating competitiveness of the released sterile males is one of the most important prerequisites for the success of SIT programme. The required condition is that the released males have similar competitiveness as the wild males for mating with the wild females. This will allow an increase of sterile mating in the targeted area and the gradual decline of the wild population during the following generations [44]. It has been largely proven that irradiation doses to achieve a sufficient level of sterility causes the subsequent decrease of competitiveness of the sterile males [45–48]. One method to preserve the insect quality is to treat the flies during irradiation with depletion of atmospheric oxygen. For the management of *D. sukukii* pest populations via SIT is therefore crucial to assess the biological quality and competitiveness of the sterile males. Reported studies showed that a dose of 200 Gy did not cause a significant decrease of quality of *D. sukukii* flies i.e., emergence rate, flight ability, and survival [38, 39]. These results echo those of a later study comparing the quality of non-irradiated with irradiated *D. sukukii* flies using different atmospheric treatments [35]. Likewise, positive results were also achieved when investigating the retained ability of the sterile *D. sukukii* males to mate and re-mate with fertile females [40]. Here, we studied the effect of irradiation and atmosphere treatments on mating competitiveness of *D. sukukii* males. The time of copula results differ from a previous study where *D. sukukii* sterile males presented shorter copula time compared with the

fertile males [49]. In our study, both parameters did not significantly differ between cross types nor among irradiation and atmosphere treatments. Sterile males were therefore competitive since they began mating with the females at the same time and as long as the fertile males. The competitiveness tests showed no difference in the percentage of mating between sterile and fertile males in both hypoxia and normoxia crosses. Overall, the results of this study showed that irradiation does not impact the males' competitiveness, and that the atmospheric treatments did not significantly improve the male performance. Encouraged by those findings, we believed that investigations should follow and that the mating competitiveness of the sterile *D. sukukii* males should be tested against the wild males under natural conditions.

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