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Radiation effects on *Drosophila suzukii* (Diptera: Drosophilidae) reproductive behaviour

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Abstract

Female remating is a widespread behaviour, reported in several insect species. This behaviour can affect the efficiency of sterile insect technique (SIT); however, little is known about the postcopulatory behaviour of some pest species considered as candidates to be controlled by this technique, such as *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). In this study, we investigated the effects of male and female sterilization on mating and remating behaviour of *D. suzukii*. First, we tested the occurrence of multiple mating in different combinations between sterile and fertile males and females. Then, we tested the effects of male and female sterility on female propensity to mate and remate. We found an overall low remating rate by *D. suzukii* females. Male sterility did not influence mating and remating likelihood; however, copula duration of sterile males was shorter compared to fertile males. On the other hand, sterile females were less likely to mate. Our findings encourage further research regarding the use of SIT to control *D. suzukii*.

KEYWORDS
copula duration, female remating, mating, sexual receptivity, Spotted Wing Drosophila, sterile insect technique

1 | INTRODUCTION

Polyandry is a mating behaviour where females mate with multiple males. This behaviour is a widespread phenomenon and is considered a major component of mating systems (Arnqvist & Nilsson, 2000; Denis et al., 2017). However, the precise reasons for a female multiple mating are still unknown. The most obvious benefit associated with polyandry is the increasing diversity of genetic constituents of offspring and overall lifetime reproductive success (Arnqvist & Nilsson, 2000; Zeh & Zeh, 2001). However, fitness costs have also been associated with mating, such as decreasing female longevity and increasing of female death rate (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995; Rice, 2000).

The level of polyandry seems to result from the balance between costs and benefits and ranges from monogamy to high promiscuity (Torres-Vila, Rodriguez-Molina, & Jennions, 2004). Nonetheless,

several factors have been reported to affect female remating frequency, such as first copula duration (Farias, Cunningham, & Nakagawa, 1972; Saul, Tam, & McInnis, 1988), nutritional status (Blay & Yuval, 1997), strain (Vera, Wood, Cladera, & Gilburn, 2002) and male sterilization (Abraham, Cladera, Goane, & Vera, 2012; Gavriel, Gazit, & Yuval, 2009; Katiyar & Ramirez, 1970). In fact, products in seminal fluids may act as potent inhibitors of female remating (Radhakrishnan & Taylor, 2007).

Polyandry is common in many species of *Drosophila*, although a considerable variation in remating frequency occurs among the members of this genus (Singh, Singh, & Hoenigsberg, 2002). While some *Drosophila* species can achieve up to 96% of remating frequency, *Drosophila subobscura* (Collin, 1836) (Diptera: Drosophilidae) rarely remates a second time (Maynard-Smith, 1956; Singh et al., 2002). Experiments regarding multiple mating were performed on a series of drosophilids, and for certain species, we have a lack of

knowledge on whether females are monadrous or polyandrous, such is the case of the Spotted Wing Drosophila, *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae).

Drosophila suzukii is a widely distributed pest species, originally from Asia, and currently found in Europe (Calabria, Máca, Bächli, Serra, & Pascual, 2012; Cini, Ioriatti, & Anfora, 2012), North and South America (Bolda, Goodhue, & Zalom, 2010; Deprá, Poppe, Schmitz, De Toni, & Valente, 2014; Walsh et al., 2011) and able to invade new areas, where it has not been detected yet (Dos Santos et al., 2017). Recent studies reported the possibility of adopting the sterile insect technique (SIT) as a strategy to suppress *D. suzukii* populations, by itself or integrated to other techniques, such as biological control and *Wolbachia* (Garcia, Wollmann, Krüger, Schlesener, & Teixeira, 2017; Krüger et al., 2018; Lanouette et al., 2017; Nikolouli et al., 2017; Schetelig et al., 2017).

However, it is crucial to understand the remating behaviour of this species, to better apply SIT to control *D. suzukii*. SIT depends on the ability of mass-reared and sterilized insects to mate with wild ones and induce reproductive failure, reducing infestation levels in subsequent generations (Knipling, 1955). Although desirable, monogamy is not a mandatory feature for a species to be eligible for SIT, since polygamy is considered compatible with the SIT, as long as the mating is random (Barclay, 2005).

Irradiation can affect the quality of sterile males, including its ability to inhibit females from remating (Cayol, 2001; Landeta-Escamilla, Hernández, Arredondo, Díaz-Fleischer, & Pérez-Staples, 2016). Previous studies reported that irradiated males of *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), *Anastrepha serpentina* (Wiedemann, 1830) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) were less likely to suppress female remating (Abraham et al., 2012; Gavriel et al., 2009; Landeta-Escamilla et al., 2016; Mossinson & Yuval, 2003). In contrast, no evidence of irradiation effects on inhibition of female remating was observed in *Bactrocera cucurbitae* (Coquillett, 1899) (Diptera: Tephritidae) and *Bactrocera tryoni* (Froggatt, 1897) (Diptera: Tephritidae) (Collins, Pérez-Staples, & Taylor, 2012; Haq, Vreysen, Abd-Alla, & Hendrichs, 2013; Kuba & Ito, 1993; Radhakrishnan, Pérez-Staples, Weldon, & Taylor, 2009).

If sterile males fails in suppress female remating, mated females could remate with wild males, decreasing the efficiency of SIT (Landeta-Escamilla et al., 2016). As females can store sperm from different matings, they can use viable sperm from a wild male instead sterile sperm from irradiated male, and produce progeny (Bertin et al., 2010; Scolari et al., 2014).

Since there is no information on the female remating behaviour of *D. suzukii*, we aim to evaluate whether females remated, and if so, how long was the sexual refractory period. We also sought to determine the effects of male and female sterility on the remating behaviour of *D. suzukii*.

2 | MATERIALS AND METHODS

Flies were obtained from a colony established in the Laboratório de Ecologia de Insetos, in the Universidade Federal de Pelotas. The laboratory rearing originated from infested blackberries collected in January 2016, in Pelotas, Rio Grande do Sul, Brazil (31°38′20″S and 52°30′43″W), and the colony was maintained for over an year, when the experiments were conducted. Flies were reared on artificial diet, following Schlesener et al. (2017), at a temperature of 23 ± 2°C, 70 ± 10% relative humidity (RH) and 12:12 hr (L:D) photoperiod.

2.1 | General procedure

Sterile insects were obtained by irradiating pupae 24 hr before emergence at 200 Gy using an Eldorado 78 (Atomic Energy of Canada Ltd, cobalt-60 source), following conditions and procedure proposed by Krüger et al. (2018). Unirradiated pupae were retained as the control. Following irradiation, *D. suzukii* pupae were placed into plastic cups (700 ml) and allowed to freely emerge in a chamber at 23 ± 2°C and 12:12 hr (L:D) photoperiod. Newly emerged flies were separated by sex to prevent potential matings, and placed into plastic cups with water and a hydrated mixture of sugar (União®, São Paulo, SP, Brazil), wheat germ (Walmon®, São Paulo, SP, Brazil) and hydrolyzed yeast (Bionis YE NS and Bionis YE MF, Biorigen®, Lençóis Paulistas, SP, Brazil) in the proportion of 3:1:1 (adapted from Nunes et al., 2013).

To conduct the mating experiments, mating chambers were constructed from modified centrifuge tubes (50 ml; Synth®, Diadema, SP, Brazil). A rectangle hole was cut in the side of the tube (2 × 4 mm) and covered with voile fabric allow aeration. An orifice (10 mm diameter) was cut on the top, where a drilled microcentrifuge tube (1.5 ml; Eppendorf, São Paulo, SP, Brazil) was inserted to provide hydrated food (as described above). To avoid fermentation and contamination, the food was changed twice a week. The mating chamber was provided with an oviposition substrate consisting of a squared slice (10 × 10 × 10 mm) of agar (19 g; Vetec®, Duque de Caxias, RJ, Brazil), raspberry jelly (10 g; Neilar®, Rio do Sul, SC, Brazil), methyl paraben (0.8 g dissolved in 8 ml of 90% ethanol; Synth®, Diadema, SP, Brazil) and distilled water (850 ml; adapted from Salles, 1992). Besides providing a place to lay eggs, the oviposition substrate provided humidity, and it was changed every 2 days. Observations began at the onset of the lights (08:00 a.m.) and ended at 11:00 a.m., according to the period of higher mating activity (i.e., first 3 hr of light; Revadi et al., 2015). All the bioassays were performed under the temperature of 23 ± 2°C, 70 ± 10% relative humidity (RH) and 12:12 hr (L:D) photoperiod. The experimental design was completely randomized, and each female was considered as a repetition. In the bioassays performed to evaluate the effect of male and female sterility on reproductive behaviour, mating activity was evaluated when insects were 4 days old, as indicated by Revadi et al. (2015).

2.2 | Multiple mating of females

When one day old, fertile and sterile female flies were singly housed in mating chambers. In the next morning, two males, either fertile or sterile, were placed in each mating chamber. A total of 120 females were observed, 30 for each mating combination: fertile female × fertile males (F♀ × F♂), fertile female × sterile males (F♀ × S♂), sterile female × fertile males (S♀ × F♂) and sterile

female × sterile males (S♀ × S♂). Flies were observed continually to register the occurrence of copulation. Unsuccessful males were removed from the chambers to prevent disturbance of the copulating pairs. At the end of the copulation, the successful males were removed and discarded. If no copulation was observed during the observation period, both males were removed. This procedure of offering two virgin males to females was repeated every 2 days for 16 days, totalizing eight opportunities to mate. The males offered to females were from the same treatment (i.e., sterile or fertile) through all the mating opportunities. The readiness to mate (number of days from adult emergence until the first copula) and remating frequency were observed.

2.3 | Effect of male sterility on female remating behaviour

A total of 250 fertile female flies were placed individually in mating chambers. Then, 24 hr later, two males, either fertile or sterile, were assigned to each female. Once a copula occurred, time of initiation and cessation were recorded for determination of its duration. The unsuccessful male was removed from the chamber to avoid disturbance of the copulating pair. At the end of the observation period, all the males and the females that did not copulate were discarded. Following the initial mating, every 2 days, two virgin males, either a fertile or a sterile, were housed with each mated female, and copula observation occurred as described above. A total of seven opportunities of remating were given to each mated female. The latency period (time taken for copulation to commence in minutes), the sexual refractory period (number of days since first mating in days) and copula duration (in minutes) were evaluated ($N = 67$ for fertile males × fertile females; and $N = 60$ for sterile males × fertile females).

2.4 | Effect of female sterility on female remating behaviour

We placed 120 fertile and 120 sterile females individually in mating chambers. For each female, two males, either fertile or sterile, were offered (i.e., fertile male × fertile female—F♂ × F♀, fertile male × sterile female—F♂ × S♀, sterile male × sterile female—S♂ × S♀, sterile male × fertile female—S♂ × F♀). Copulations were observed as described above. After mating, males were removed and females were kept in the mating chambers. Every 2 days, during 14 days, two virgin fertile males were placed in each mating chamber. The latency period, the sexual refractory period and copula duration were observed ($N = 87$ for fertile females × males—either sterile or fertile; $N = 57$ for fertile females × males).

2.5 | Statistical analysis

The effect of male and female irradiation on probability of mating and remating were analyzed by Chi-square likelihood ratio tests. The readiness to mate, latency period, the sexual refractory period and copula duration were submitted for analysis of variance (ANOVA) through the F test ($p ≤ 0.05$). All the analyses were conducted using R Program (R Development Core Team 2011).

3 | RESULTS

3.1 | Multiple mating of females

Irradiation did not affect the readiness to mate ($F_{3,73} = 2.51$, $p = 0.0655$). When fertile females were 4-day old and coupled with fertile males, they were ready to mate $4.87 + 1.89$ days after emergence, and when coupled with sterile males, first mating occurred $4.31 ± 2.29$ days after emergence. Sterile females, when coupled with fertile males, start to mate at $6.38 ± 2.88$ days and sterile females, when coupled with sterile males, were ready to mate $5.60 ± 2.85$ days after emergence.

For the fertile female × fertile males treatment, from the 30 females analyzed, 21 mated once and two mated twice, resulting in 8.69% remating females. For the fertile female × sterile males treatment, from 30 females analyzed, 13 mated once, and none remated. From the 30 females analyzed of the sterile female × fertile males, 17 mated once, seven mated twice, one mated thrice and one mated five times, resulting in 34.61% remating females. From the 30 females of the sterile female × sterile males treatment, 17 mated once and only one mated twice yielding 5.55% remating females.

3.2 | Effect of male sterility on female remating behaviour

There is no evidence that male sterility have an effect on female likelihood to mate ($χ^2 = 1.07$, $df = 1$, $p = 0.3004$) nor on inhibition on female remating ($χ^2 = 1.72$, $df = 1$, $p = 0.1903$). In fact, only 7.29% of all the females remated. Sterility of males also did not affect the likelihood to be rejected in a second mate ($χ^2 = 3.03$, $df = 1$, $p = 0.08$).

Male condition (i.e., fertile or sterile) had no effect on latency ($F_{1,126} = 0.09$, $p = 0.7548$), and the average time taken to a copula to initiate was 98.98 min. Also, although sterile males had longer copula durations than fertile males ($F_{1,126} = 5.34$, $p = 0.0225$; Figure 1), copula duration did not affect female likelihood to remate ($χ^2 = 1.94$, $df = 1$, $p = 0.1640$).
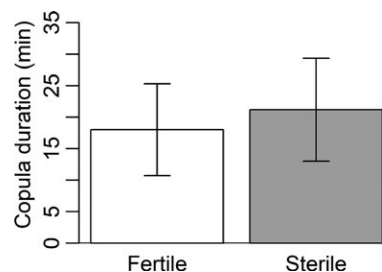

FIGURE 1 Average (±SD) copula duration (min) of fertile or sterile *Drosophila suzukii* males

3.3 | Effect of female sterility on female remating behaviour

The combination of male and female condition affected the mating probability, as well as female sterility itself; however, male condition per se had no effect (Combination: χ^2 = 25.76, df = 3, p < 0.0001; Female: χ^2 = 15.83, df = 1, p < 0.0001; Male: χ^2 = 0.28, df = 1, p = 0.5981; Figure 2). While 72.5% of fertile females mated, only 47.5% of sterile females mated. However, neither male nor female sterility had an effect on female remating (Combination: χ^2 = 1.51, df = 3, p = 0.6794; Female: χ^2 = 1.04, df = 1, p = 0.3074; Male: χ^2 = 0.0, df = 1, p = 1.0000). As a matter of fact, only 17.5% of females remated.

Female sterility had an effect on the time taken to a copulation to commence ($F_{1,142}$ = 45.90, p < 0.0001; Figure 3). However, female condition did not affect copula duration ($F_{1,142}$ = 1.57, p = 0.2116; Table 1), refractory period ($F_{1,40}$ = 0.06, p = 0.8033; Table 1), nor remating duration ($F_{1,40}$ = 1.56, p = 0.2191; Table 1). Average value ($\pm SD$) observed for copula duration was 23.76 ± 6.88 min, for refractory period, when occurred, was 7.95 ± 3.86 days, and for remating duration was 26.5 ± 11.82 min.

4 | DISCUSSION

There is little information about the reproductive behaviour of *D. suzukii* in literature. Our study revealed novel aspects of mating and remating behaviour of this species, as well as the influence of sterility on these aspects. In our study, flies were ready to mate, on average, around 4 days after emergence, being consistent with findings reported by Snellings et al. (2018). Comparing to tephritid fruit flies, frequently controlled using SIT, *D. suzukii* presents a much shorter time span to be sexual mature. To avoid field mortality, several tephritid SIT programmes keep sterile flies within the facility during sexual maturation period, resulting in higher costs of maintenance

(Bachmann et al., 2017). Thus, a short period for sexual maturation represents an asset for *D. suzukii* in SIT programmes.

For bisexual strains, the release of sterile females can decrease SIT efficiency, since they will compete with wild females for matings (Orozco, Hernández, Meza, & Quintero, 2013). However, sterile females of *D. suzukii* were less likely to mate than fertile females, representing another positive aspect for SIT. Similarly, Landeta-Escamilla et al. (2016) also reported that sterile females of *A. serpentina* were less inclined to mate, but the reason remains unknown. Additionally, in our study, male irradiation did not affect female likelihood to mate; this is consistent with *B. cucurbitae* and *B. tryoni* (Collins et al., 2012; Haq et al., 2013), but contrasting to results reported for *A. serpentina* (Landeta-Escamilla et al., 2016).

Understanding female postcopulatory behaviour is crucial when SIT is considered to control a pest. Although monogamy is not a requirement of the SIT, a differential rate of remating by females first mated with sterile males or a wild male will compromise the technique (Calkins & Parker, 2005; Radhakrishnan & Taylor, 2008). Species from the genus *Drosophila* are known for their extreme reproductive phenotypes, showing enormous variation in their mating and remating behaviour (Bundgaard & Barker, 2000). Despite many species of *Drosophila* display polyandry (Singh et al., 2002), most of the tested *D. suzukii* females were monandrous. It is possible that if observations for remating were carried over a larger period, a higher number of remated females would be observed. However, due to high levels of natural mortality added to a long refractory period, probably a small proportion of females would survive long enough to have the opportunity to remate (Abraham et al., 2011).

After mating, females experience a series of physiological and behavioural changes that result in a shift on female sexual receptivity (Avila, Sirot, LaFlamme, Rubinstein, & Wolfner, 2011). This receptivity is affected by short- and long- term factors. The short-term effect, known as copulation effect in *Drosophila*, is the decrease in receptivity due to seminal fluids components transferred by males during mating (Neubaum & Wolfner, 1999; Singh et al., 2002). Long-term effect is indirectly linked to sperm load, and is called the sperm effect (Manning, 1962; Singh et al., 2002). The lack of influence of male sterility on the female receptivity to remate, reported in this study, suggests that irradiation does not affect those factors; however, further studies should be developed to confirm this. The effects of male sterility on female remating
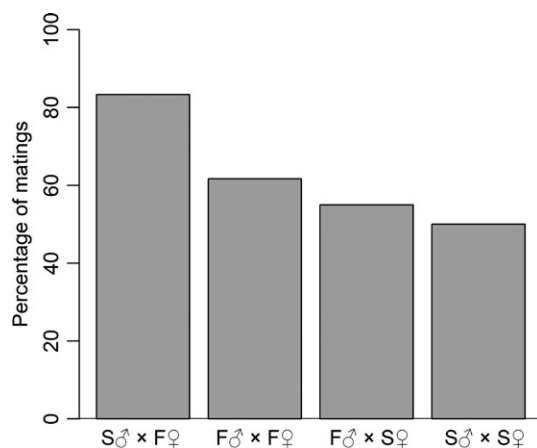

FIGURE 2 Absolute percentage of mating by sterile males × fertile females, fertile males × fertile females, fertile males × sterile females and sterile males × sterile females of *Drosophila suzukii*
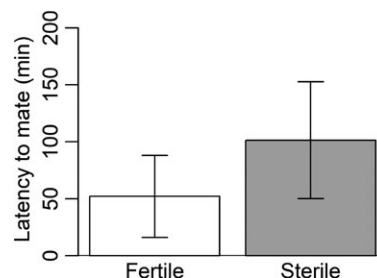

FIGURE 3 Average (±*SD*) latency period (min) of fertile or sterile *Drosophila suzukii* females

TABLE 1 Average values (±*SD*) observed for copula duration (min), refractory period (days), and for remating duration (min) of *Drosophila suzukii*

Treatment	Copula duration	Refractory period	Remating duration
F♂ × F♀	23.49 ± 7.21[ns]	8.25 ± 4.83[ns]	29.62 ± 13.30[ns]
F♂ × S♀	22.67 ± 7.33	7.85 ± 3.78	28.15 ± 8.05
S♂ × S♀	23.17 ± 7.78	8.36 ± 3.77	20.27 ± 10.06
S♂ × F♀	24.98 ± 5.82	7.40 ± 3.78	28.7 ± 15.31

Note. ns, Not significant.

vary among fruit fly species. Sterile males of *A. serpentina* and *C. capitata* are less likely to inhibit female remating (Gavriel et al., 2009; Landeta-Escamilla et al., 2016), while no difference was found for *A. fraterculus*, *Anastrepha ludens* (Loew, 1873), *B. cucurbitae* and *B. tryoni* (Abraham et al., 2013, 2016; Arredondo, Tejeda, Ruiz, Meza, & Pérez-Staples, 2017; Haq et al., 2013; Radhakrishnan et al., 2009).

Our data showed that male condition did not influence mating latency period; however, sterile females presented a longer mating latency compared to fertile females. Effects of irradiation on latency were previously reported in males of *B. cucurbitae*, *B. tryoni* and *C. capitata*, but not in females (Haq et al., 2013; Radhakrishnan et al., 2009; Virginio et al., 2017). According to Cayol, Vilardi, Rial, and Vera (1999), mating latency is controlled by females and not by males. In our study, *D. suzukii* fertile females showed the same receptivity to mate to either sterile or fertile males, while sterile females were less eager to mate. The absence of effects of *D. suzukii* male sterility on latency have significance to SIT, as both sterile and fertile males will initiate courtship at same time, competing fairly for females.

The female condition of *D. suzukii* did not have an effect on mating duration, but sterile males differ from fertile males in the duration of copula. Shorter copulation when sterile males are involved was already reported for *A. serpentina*, *A. fraterculus* and *C. capitata* (Allinghi et al., 2007; Cayol et al., 1999; Landeta-Escamilla et al., 2016; Virginio et al., 2017). Nonetheless, the importance of this effect on the efficiency of the SIT is not clear, since there is no relationship between copulation duration and the ability of males to transfer sperm (Allinghi et al., 2007; Collins et al., 2012; Harmer, Radhakrishnan, & Taylor, 2006). Collins et al. (2012) suggest that factors associated to copula duration, others than sperm abundance, play an important role for remating inhibition by tephritid flies, such as the components of the ejaculate. However, in our study, copula duration did not affect the probability of female remating.

Findings reported in this study encourage further research regarding the use of SIT to control *D. suzukii*. Previous studies reported that sterilization does not affect quality of *D. suzukii* (Krüger et al., 2018). In addition, it seems that radiation does not influence the ability of males to mate and inhibit remating in *D. suzukii* females. Although most of the tested females did not remate, it is important to verify the effects of remating in fecundity and fertility. Some *Drosophila* species are known to use sperm from some male partners (Davis, Castillo, & Moyle, 2016). If this is the case of *D. suzukii*, females previously mated with sterile male, could recover fertility after remating with a fertile male, and jeopardize the success of a SIT programme. Thus, it is necessary to verify the effects of female remating on the fertility recovery of *D. suzukii*.

AUTHOR CONTRIBUTION

Author 1, author 5 and author 6 conceived research. Author 1, author 2, author 3 and author 4 conducted experiments. Author 1 analyzed data and conducted statistical analyses. Author 1 wrote the manuscript. Author 1 and author 6 secured funding. All authors read and approved the manuscript.

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