

DISSERTATION

For the Degree of *Doctor rerum naturalium technicarum (Dr. nat. techn.)*

THE STERILE INSECT TECHNIQUE FOR THE BIOLOGICAL CONTROL OF *DROSOPHILA SUZUKII*

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Abstract

Drosophila suzukii is an invasive pest native to Asia. Since its first report, it has successfully established in America and Europe, where it has rapidly become a major pest of berries, grapes and stone fruits, causing great economic losses. The current strategy to limit the damage caused by *D. suzukii* on crops relies on insecticides. Among the existing biological control methods, the Sterile Insect Technique (SIT) represents a promising alternative to other management strategies, in order to control *D. suzukii* populations, particularly in confined areas, such as greenhouses. SIT is a species-specific method that has proven to successfully control several insect pests. Furthermore, SIT decreases management costs and reduces the impact on ecosystems. Driven by the idea of applying SIT on *D. suzukii*, this study 1) tested the suitable range of radiation doses that consequently will determine the optimized dose to reach an adequate sterility level for control measures. Radiological sensitivity of pupae treated under two atmospheric treatments, i.e., hypoxia and normoxia conditions, was tested and the effects on emergence rate and flight ability were analysed; 2) developed an optimal oviposition system allowing the production of a high number of flies for mass-rearing.

An optimal irradiation dose of 220 Gy was determined for males which induced 99.8% sterility, based on egg hatch data, while an irradiation dose of 170 Gy was found to induce 97% sterility. In both radiation doses, irradiated females were 100% sterile. Both radiation exposures, i.e., 170 and 220 Gy, did not reveal any negative effect on the emergence rate and flight ability of *D. suzukii* males and females under hypoxia or normoxia. Therefore, the hypoxia condition is recommended to facilitate the protocols of packing, irradiation, and shipping of sterile *D. suzukii* pupae.

For the second objective, two egg-collection systems were developed and compared with respect to the number of eggs produced, egg viability, pupal and adult recovery, adult emergence rate, and flight ability. *Drosophila suzukii* females kept in cages with a wax panel produced significantly more eggs with higher viability, higher adult emergence, and flight ability rate as compared to the netted oviposition system. Furthermore, the wax panel system proved to be a more practical and less laborious system as compared to the netted oviposition system, regarding the collection of eggs,

showing great promise as an effective system for the mass production of *D. suzukii* for SIT.

In conclusion, these results provide evidence that *D. suzukii* flies can be produced in large quantities, irradiated, and transported for SIT programmes, setting out the basic conditions for the effective use of the SIT as a control strategy for this invasive pest.

Kurzfassung

Die Kirschessigfliege *Drosophila suzukii* ist ein in Asien heimischer invasiver Schädling. *Drosophila suzukii* hat sich in Amerika und Europa aber auch anderen Kontinenten stark ausgebretet und ist hier zu einem der wesentlichen Schadinsekten an Beerenarten, aber auch an anderen Früchten wie Weintraube geworden. Die derzeitige Bekämpfungsstrategie von *D. suzukii* beruht vor allem auf der Anwendung von toxischen Insektiziden. Als Alternative gilt die Sterile Insect Technique (SIT), eine biologische, umweltfreundliche Methode, die in den letzten Jahren als potentielle Managementmethode für *D. suzukii* diskutiert wurde. Die Adaptierung von SIT auf eine Art wie die Kirschessigfliege ist aufwendig, doch in Folge sind eine Verminderung der schädlichen Auswirkungen auf das Ökosystem sowie eine Reduktion der Kosten im Vergleich zu Insektiziden zu erwarten.

Diese Arbeit konzentrierte sich auf zwei wichtige Aspekte der SIT bei *D. suzukii*: 1) Die Bestimmung der optimalen Strahlendosis, die zur Sterilisierung der Männchen führt. Diese Strahlendosis darf nicht zu hoch sein, um nicht die Fitness der frei gelassenen Männchen zu reduzieren, muss aber möglichst alle Individuen sterilisieren, damit sie effektiv ist, 2) die Optimierung der Massenzucht von *D. suzukii*. Die Arbeit hat sich bei diesem Aspekt auf die Optimierung des Eiablatesystems bei der Massenzucht konzentriert.

Im ersten Teil dieser Studie wurde die optimale Strahlungsdosis für die Sterilität von *D. suzukii* bei beiden Geschlechtern untersucht. Des Weiteren wurden die Auswirkungen von Hypoxia und Normoxia auf die radiologische Empfindlichkeit der Fliegen verglichen. Basierend auf der Schlupfrate der Eier wurde die Dosis von 220 Gy als Optimum gefunden. Diese Strahlendosis induziert bei Männchen eine Sterilität von 99,8% und wird daher für SIT empfohlen. Niedrigere Dosen, wie 170 Gy, hatten eine Sterilität von 97% zur Folge, ein noch ausreichender Wert für die Anwendung von SIT. Beide Strahlendosen induzierten 100% Sterilität bei Weibchen. Es wurden keine Unterschiede zwischen Hypoxia und Normoxia festgestellt. Daher kann Hypoxia in Folge auch für das Versenden der sterilen Fliegen verwendet werden, was die Umsetzung der SIT erleichtert.

Im zweiten Teil dieser Studie wurde das Eiablatesystem für die Massenzucht verbessert. Es wurden zwei verschiedene Systeme entwickelt und neben Eianzahl auch noch Fitnessparameter, wie Lebensfähigkeit der Eier, Puppen und Adulten untersucht. Weibchen von *D. suzukii* legten auf ein neuentwickeltes Wachspaneel signifikant mehr Eier, mit höherer Lebensfähigkeit, im Vergleich zum bisher verwendeten Netzsysten ab. Das Wachspaneelsystem erwies sich auch als praktischer, da es mit einem geringeren Arbeitsaufwand verbunden ist.

Zusammenfassend ist anzumerken, dass SIT bei *D. suzukii* erfolgreich angewandt werden kann. Man kann mit dem neuen Eiablatesystem große Mengen an Fliegen züchten, und die gezielte Strahlendosis unter Hypoxia garantiert einen einfachen Transport und eine effektive Anwendung dieser Technik bei diesem invasiven Schadinsekt.

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Grazie soprattutto al sostegno della mia famiglia e delle mie amiche. Una poesia cita:

“Se vuoi costruire una barca, non radunare uomini per tagliare la legna, dividere i compiti e impartire ordini, ma insegnala la nostalgia per il mare vasto e infinito.”

Antoine de Saint-Exupéry

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1. Introduction

1.1 *Drosophila suzukii*

1.1.1 BIOLOGICAL TRAITS

The spotted wing *Drosophila* (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera, Drosophilidae), is a species of the genus *Drosophila* and within this, a member of the *melanogaster* group within the *suzukii* subgroup (Van der Linde, & Houle, 2008). Adults are 2,0 to 3.5 mm length with females being slightly larger, with red eyes, light brown thorax and yellowish-brown abdomens with transversal medially uninterrupted dark bands (EPPO Global Database, 2019). Males are characterized by the presence of a dark spot near the wing tips and by two short sex combs on the first and second segment of fore tarsi. Females are characterized by the serrated and significantly enlarged ovipositor which is equipped with large bristles and a sharp tip (Atallah et al., 2014; Walsh et al., 2011) (Fig 1).

The complete life development is between eight and fourteen days depending on the temperature with an average development of ten days at 25°C (Calabria et al., 2012). Females can lay hundreds of eggs during their life, which hatch in one to three days and develop through three larval instars. Pupal stage is four to sixteen days, while adults can survive up to nine weeks and reach their maturity two days after emergence (Kanzawa, 1939; Lee et al., 2011, 2015; Walsh, 2011). Although the preferred temperature is between 20 and 25°C, adults of SWD have a large thermal plasticity (Enriquez & Colinet, 2017; Tochen et al., 2014) and can overwinter in a reproductive diapause in natural environments (Rossi-Stacconi et al., 2016; Stockton et al., 2019; Zhai et al.,

2016). Unlike most species in the family Drosophilidae, the ovipositor is optimized for piercing and gives SWD females the ability to perforate the peel of unripe fruits to lay their eggs (Mitsui et al., 2006). Larvae then feed on the fruit pulp, causing complete decomposition (Asplen et al., 2015; Kanzawa, 1939). *Drosophila suzukii* breed up to ten generations per year, wherefore a single colonizing female can generate billions of descendants by the end of her reproductive season (Hamby et al., 2016; Rota-Stabelli et al., 2013). Due to the damage caused by SWD larvae, secondary infections caused by pathogens can occur. Despite *D. suzukii* not belonging to the Tephritidae, its ability to oviposit in fresh unripe fruits and cause enormous damage means it is considered a ‘fruit-fly’ (Mohr, 2018).

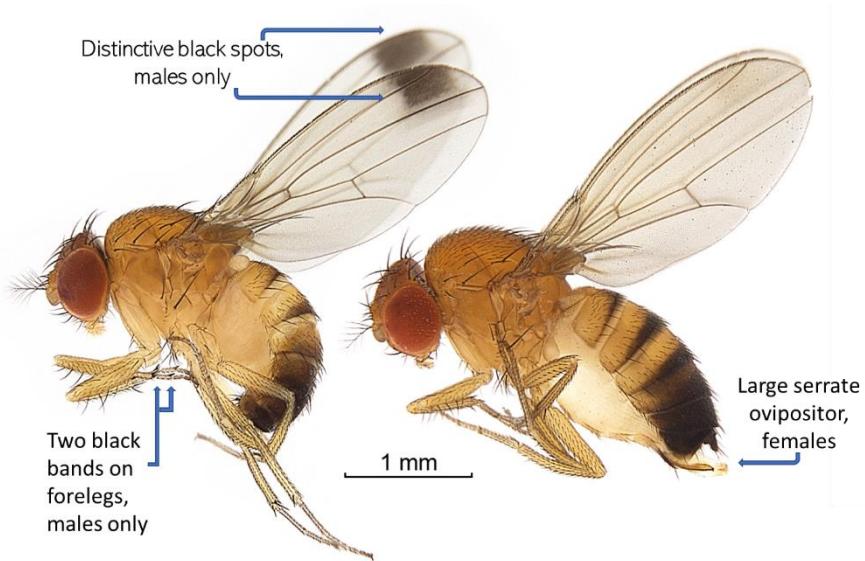


Figure 1 Male and female of *Drosophila suzukii*. Photo: McEvey S.F., Australian Museum, 2017.

1.1.2 DISTRIBUTION, INVASION AND ITS ECONOMIC IMPORTANCE

The origin of *D. suzukii* is south east Asia, however the exact country of origin is in question due to the uncertain chronology of some historical specimens (Calabria, 2012; Hauser, 2011). Records of its extensive presence in other east Asian countries is summarized by Hauser et al. (Hauser, 2011; Hauser et al., 2009). The biology with focus on the fruit's damage was done by Kanzawa in Japan (Kanzawa, 1939). In 1980, SWD was identified in Hawaii (Kaneshiro, 1983). In Europe and USA, SWD was detected for the first time in 2008 (Beers et al., 2011; Calabria, 2012). Since then, SWD has spread widely in Europe, USA, Canada, and has recently colonized parts of the South American continent (Asplen, 2015; Deprá et al., 2014; Lue et al., 2017; Morales, 2020). The polyphagous behaviour allows this pest to oviposit eggs in a large variety of cultivated soft-skinned fruits, such as small fruits, berries and stone fruits e.g. *Rubus* spp., *Fragaria ananassa*, *Prunus* spp., and *Sambucus* spp. (Briem et al., 2018; Lee, 2015). Its wide range of host fruits also includes wild and ornamental plants (Cini et al., 2012). In seasons in which the main host fruit crops are not available, SWD refuges in alternative landscapes, i.e. forest, where wild fruits provide alternative reproductive sites (Thistlewood et al., 2018). The wide niche distribution, high fecundity, relatively short life cycle, and high passively and active dispersal (Lavrinienko et al., 2017; Santoiemma et al., 2019; Tait, Grassi, et al., 2018) have facilitated the spread of SWD and its considerable damage on fruits. Studies of the economic impact have revealed the significant yield losses caused by SWD infestations (Benito et al., 2016; Bolda et al., 2010; De Ros et al., 2013; Farnsworth et al., 2017; Goodhue et al., 2011; Mazzi et al., 2017; Morales, 2020). However, the impact on yield losses might be even greater if it is considered that many of those studies are often limited to local scale and/or restricted in time (Benito, 2016).

1.1.3 CURRENT AND FUTURE MANAGEMENT TACTICS TO CONTROL SWD

Management tactics to control SWD can be separated into five categories:

1. Cultural practices and mechanical control are the non-chemical methods applied pre- and post-harvest to prevent the spread of SWD population. Cultural practices includes sanitation by pruning, removal and effective disposal of dropped and overripe fruits and/or ornamental hosts plants (Cini, 2012; Haye et al., 2016; Leach et al., 2018; Rendon & Walton, 2019), and shortened harvest intervals (Leach, 2018). Mechanical barriers such as net exclusion are also adopted methods for limiting SWD infestation that have demonstrated high effectiveness (Cormier et al., 2015; Leach et al., 2016; Rogers et al., 2016; Swoboda-Bhattarai & Burrack, 2014). Although those methods are not adequate on their own to achieve suppression, they are highly important to limit the pressure imposed by this pest.
2. Chemical control is the most applied method worldwide for preventing and controlling infestations of SWD. Among the tested insecticides, the registered spinosyns (chemical compound found in the bacteria *Saccaropolyspora spinosa*), the organic compounds pyrethroids and the phosphate esters organophosphates are the most effective ones against SWD (Beers, 2011; Diepenbrock et al., 2016; Haviland & Beers, 2012; Schetelig et al., 2018; Shawer et al., 2018; Van Timmeren & Isaacs, 2013). Although chemicals are often the most effective approach, the short generation time of SWD requires frequent applications. Moreover, spraying close to the pre-harvest of fruits, often important with SWD, limits the export market due to the discrepancies concerning restrictions on pesticide residues (Haviland, 2012). The many negative effects of chemical treatments, the high costs and the fast spread of insecticide resistance require alternative solutions (Chouinard et al., 2016).

3. Biological control agents include a vast range of environment-friendly control methods based on the use of natural enemies of the insect pest that can contribute to the reduction of pesticide usage, input costs and associated environmental risks (Whitehouse et al., 2018). Generalist and specialist parasitoids of SWD proved effective at controlling SWD during some of its life stages (Chabert et al., 2012; Kasuya et al., 2013; Mazzetto et al., 2016; Rossi-Stacconi et al., 2013, 2015, 2019). As alternative methods to the use of parasitoids, the effects of other biocontrol agents such as bacteria, fungi and virus on SWD have also been investigated (Carrau et al., 2018; Foye & Steffan, 2020; Naranjo-Lázaro et al., 2014; Renkema & Cuthbertson, 2018; Schetelig, 2018). In particular, the maternally inherited intracellular bacterium *Wolbachia* is largely present among insect taxa and induces a reproductive modification called cytoplasmic incompatibility (CI) (Werren et al., 2008). The mating between infected males and wild females that are infected with another *Wolbachia* strain causes cytoplasmatic incompatibility (CI) and consequent sterility of the matings (Bourtzis et al., 2003; Bourtzis & Miller, 2006; Zabalou et al., 2009; Zheng et al., 2019). The release of *Wolbachia*-infected males in the wild population induces sterility in the wild females which should cause the target population to decline. This strategy, called incompatibility insect technique (IIT), can be deployed for insect pests where an efficient method for sexing is available as *Wolbachia*-infected females can still reproduce with the wild males. If the exclusive release of males is not possible, IIT may be combined with the sterile insect technique (SIT) (Bourtzis et al., 2014; Kittayapong et al., 2019; Zhang et al., 2016; Zheng, 2019). The potential of *Wolbachia* has been recently exploited in *D. suzukii* and the SIT/IIT approach suggested as a potential population suppression tactic against this pest (Cattel et al., 2018; Nikolouli et al., 2018, 2020).

4. Behavioural control are species-specific and eco-sustainable methods used to disrupt the reproduction of the insect pest. Several behavioural control techniques rely on natural and/or synthetic lures to *attract and kill* adults, whereas others use synthetized pheromones to confuse the adult's recognition and consequently, avoid mating. A large amount of laboratory and field studies have explored novel and existing lures to be used for mass-trapping, *attract and kill* and *push and pull* strategies to reduce SWD infestation (Briem, 2018; Bueno et al., 2019; Burrack et al., 2015; Cha et al., 2013; Guedes et al., 2018; Iglesias et al., 2014; Lee et al., 2012, 2013; Snellings et al., 2018; Tait, Kaiser, et al., 2018; Wallingford et al., 2017). Recent studies about the oviposition cues of SWD contribute greatly to the understanding of its host selection behaviour, suggesting the possibility to use these cues as a disruption method for SWD management (Cloonan et al., 2018; Crava et al., 2019; Tait et al., 2020).

5. The sterile insect technique (SIT) is a biological control method based on interruption of reproduction caused by radiation exposure (Knipling, 1955). SIT has become a significant component to control insect pests worldwide (Enkerlin et al., 2017a; Marec & Vreysen, 2019; Pereira et al., 2013; M. J.B. Vreysen, Gerardo-Abaya, & Cayol, 2007). The use of SIT against *D. suzukii* has been proposed by many experts (Cattel, 2018; Cini, 2012; Follett et al., 2014; Krüger et al., 2018; Landi et al., 2015; Lanouette et al., 2017; Nikolouli, 2018; Schetelig, 2018), and it has constituted the main objective of international projects (Colinet & Stauffer, 2016; FAO/IAEA, 2017).

1.2 The sterile insect technique

1.2.1 GENERAL INTRODUCTION

The SIT is a biocontrol method used as a component of area-wide integrated pest management programs (AW-IPM). Its principle is based on the mass production of a large number of individuals from the targeted pest species which are sterilized by exposure to ionizing irradiation, and finally released into an infested area. Once in the field, sterile males will compete with wild males to mate with wild females, generating a *birth control* effect in the population, and thus reducing the pest population size and its effect on crops (Dyck, Hendrichs, & Robinson, 2005; Knippling, 1955).

The first pest eradication using SIT occurred in Curaçao Island in the 1950s against the cattle pest New World Screwworm, *Cochliomyia hominivorax* Coquerel (Baumhover, 1966; Klassen & Curtis, 2005); this was followed by the eradication of the melon fly, *Bactrocera cucurbitae* Coquillett in Japan (Kuba et al., 1996), and the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann in Mexico (Suckling et al., 2016). Pest management programmes that include SIT have suppressed, eradicated and/or contained damages caused by many of the world's most severe and difficult-to-control agriculture pests (Marec, 2019; Simmons et al., 2010; M. J. B. Vreysen et al., 2007; Marc J.B. Vreysen, 2006). The SIT is currently applied in 19 countries on more than 27 different species, not only pest of fruits and plants, but also on vector species such as mosquitoes and tsetse flies to reduce the spread of diseases (Alphey et al., 2010; De Beer et al., 2017; Enkerlin, 2017; IDIDAS, 2009; Lees et al., 2015). The SIT has many advantages because the technique is environment-friendly, species-specific and can easily be integrated with other biological control methods such as parasitoids, predators and pathogens (Enkerlin, 2005; Marc J.B. Vreysen, 2006). However, the establishment

of SIT on an insect pest requires an extensive knowledge of the target species for determining whether integration of the SIT into an AW-IPM programme is appropriate (Lance & McInnis, 2005). The SIT programme necessitates the 1) development and application of the irradiation sterility dose, 2) the mass-rearing techniques, 3) appropriate quality control guidelines and finally shipping, 4) holding and releasing of the sterile insect into the target areas (FAO/IAEA/USDA, 2014).

1.2.2 IRRADIATION

Insect reproduction sterility is induced by exposure to ionizing radiation such as gamma radiation, X-ray emitted from Cobalt-60 and Caesium-137 sources or by high-energy electrons produced from an electron accelerator. Doses of ionizing radiation cause chromosomal fragmentation in sperms and/or inactivation of germ cells, generating random dominant lethal mutations that can arrest the development of insects, inhibit reproduction or induce reproductive sterility (Henneberry & McGovern, 1963; Muller, 1954; Yanders, 1959). Data from the International Database on Insect Disinfestation and Sterilization shows that the sterilization doses are diverse among the insect genera (IDIDAS, 2009). Furthermore, distinct responses to the radiation are also observed between the sexes, with females usually more radiosensitive than males (Nation et al., 1995), although exceptions have been documented (Dyck, 2005). Hence, a large range of doses must be tested, resulting in a dose-response curve for establishing the optimal irradiation dose that prevents reproduction.

Insect radiation generates negative effects that are directly proportional to the absorbed dose. Consequently, the radiation doses to induce full sterility may influence biological and physiological traits of species such as reduced emergence rate, mating competitiveness and compatibility in the field (Mudavanhu et al., 2016). Previous

studies on fruit flies showed how abiotic factors such as atmosphere setting at the time of irradiation can minimize the negative effects on the quality of the released adults (Nestel et al., 2007). This is because the quality-reducing effects of ionizing radiation on insects are associated with the production of deleterious free radicals that are formed by the ionization of the intracellular water (Arthur et al., 2015; Wallace, 1998). Therefore, exposing insects to a lower oxygen atmosphere, i.e. hypoxia or even zero oxygen, i.e. anoxia, before and during exposure to irradiation can mitigate some of these negative effects (López-Martínez & Hahn, 2012; Mastrangelo & Walder, 2011; Nestel, 2007; Von Sonntag, 1991). The irradiation of pupae under hypoxia is routinely used in the SIT programme of tephritid species e.g. *C. capitata* and *Anastrepha ludens* by sealing the pupae in plastic bags in which the amount of oxygen is consumed by the insects' normal metabolism and respiration, thus creating hypoxia. Sealed pupae under hypoxia not only provide radiological protection to the insect, but it also means the sterile pupae is already in sealed containers which facilitates their packing and shipment to the final release destination (FAO/IAEA/USDA, 2003). Because hypoxia treatment abates the effects of the radiation exposure on insects, higher doses are needed to produce comparable reproductive sterility than in normoxia (Kingsley Fisher, 1997; Hallman & Hellmich, 2010; Yamada et al., 2019). For this reason, a dose-response curve under hypoxia must be established on the target species.

To overcome the quality-reducing effects of ionizing radiation, another solution can be to expose the insects to doses that induce a lower percentage of sterility: *sub-sterility* or *semi sterility* (Helinski et al., 2006). In this case, while a certain level of fertility is still introduced in the wild population, the reduced detrimental effects on flies enhances their performances in the field (Parker & Mehta, 2007). Increasing the sterile male's success once in the field can be also accomplished by increasing the amount and the

frequency with which the sterile insects are released (Madakacherry et al., 2014; M. J.B. Vreysen, 2005). The SIT/IIT combined approach can be an alternative strategy to remedy otherwise damaging high irradiation dosage (Bourtzis & Robinson, 2006; Kittayapong, 2019; Zhang, 2016; Zheng, 2019). SIT/IIT is used in species where both sexes are released because *Wolbachia*-infected females can still reproduce with the wild males and therefore they need to be sterilized through irradiation. The advantage is that female sterility usually requires lower irradiation doses than males which may abate the negative effects on the sterile males (Bourtzis, 2006; Zabalou, 2009; Zheng, 2019).

1.2.3 MASS-REARING

Mass-rearing techniques are essential components for the development of the SIT on a pest insect. To allow the inundative releases of sterile insects, the mass-rearing must provide a continuous and stable production of sufficient numbers of insects at an economically viable cost (Gast, 1968). Mass-rearing techniques include a long list of procedures that can permit a complete or partial artificial rearing of the target insect. Those procedures are specifically developed based on the biology and physiology of the target insect (Dyck, 2005), and they must be simple to construct, clean and operate (Balestrino et al., 2010). Because domestication and long rearing conditions have demonstrated their negative effect upon the fitness and mating behaviour of reared fruit flies (Aluja et al., 2009; Liedo et al., 2007; Rempoulakis et al., 2016), several studies have been focused on each aspect of the rearing, i.e. cage design, artificial adult and larval diets, oviposition substrates, separation systems for eggs, larvae and pupae, and insect handling procedures. Usually rearing cages are specifically designed to maintain a good density of the insect colony population and sex ratio to avoid the stress caused by overcrowded, intra competitiveness, high mortality as well as the alteration of sexual

behaviour (Liedo, 2007; Orozco-Dávila et al., 2014). Artificial diet also plays an essential role in the quality of developing flies (Rajabpour et al., 2018; Shelly & McInnis, 2003). Nutrient imbalance and microbial population contained in the diet can affect survival, the weight of immature individuals, time of development, body size, and fecundity as well as the fitness and competitiveness of the sterile males released in the field (Bourtzis, 2006; Rempoulakis et al., 2018). An efficient oviposition system is also specifically improved so as to ensure a high production rate and efficient recovery of the maximum number of eggs per number of females in the rearing cage. Egg-lay systems must also comprise of easy-to-handle methods to enable the collection and estimation of the cages' eggs production for continuous monitoring of the colony (Economopoulos & Judt, 1987; Jaldo et al., 2001). Important aspects of large-scale rearing facilities also include the maintenance of the insects' prior complete development. Large-scale facilities are often equipped with storage room allowing different temperatures to manipulate schedules of the adult's emergence time to enable practical and optimized organization of the transport, shipment, and release of the insects (Cáceres et al., 2007). Sex-separation systems are mechanical or genetic-based methods used for separating males and females' insects and therefore produce only male adults of the target species. Those methods have a subsequent impact on the applicability of the SIT programmes because male-only releases decrease the costs of the production, as well as boosting the efficiency of the field releases (Cáceres, 2002; Cáceres et al., 2002). Improving each aspect of the rearing methodologies has a significant positive effect on the fitness of insect strains and could significantly enhance the performance of mass-reared sterile males in the field, reducing the overall operational costs (Ami et al., 2010; Augustinos et al., 2015; Cáceres et al., 2014; Calkins & Ashley, 1989; K Fisher & Cáceres, 1998).

1.3 Research objectives

The rapid invasion of *D. suzukii* in diverse continents like Europe has encouraged research to find an effective and inexpensive but also eco-sustainable control method. The principles of the SIT comply with these criteria and therefore the use of this technique on SWD has been advised. The work of this thesis is part of an international project that aimed to determine the feasibility of using sterile insects that will be produced via SIT and IIT as an additional control tactic for the integrated pest management of *D. suzukii* populations in confined areas such as greenhouses. Specifically, the research objective of my thesis work focused on validating some of the SIT major components and their possible applicability on SWD. Prior requirements for the successful implementation of SIT programme is the establishment of protocols that will allow mass-production, sterilization and shipment of insects without detrimental effects on their biological quality parameters. Subsequently, 1) the effects of radiation under different atmosphere conditions for SWD and 2) a new artificial oviposition system for the mass-rearing of SWD were developed.

*1) Effects of radiation doses under different atmosphere conditions for the complete sterilization of *D. suzukii*.*

Responses to the radiations vary among species, therefore a comprehensive study on a large range of radiation doses was made to test the reproduction sterility of SWD, giving the dose and the optimal sterility level for the SIT application. The irradiation tests were also performed under hypoxia because it can provide radiological protection, and also facilitates the packing and shipment of pupae to the final release destination. Additionally, quality control parameters were assessed after irradiation exposure to

assess possible negative effects caused by the selected doses or the atmospheric conditions. The induced sterility on *D. suzukii* males and females on a large range of gamma radiation doses was determined. All doses were tested under hypoxia and normoxia atmosphere conditions and the differences in radiological sensitivity were assessed. Because irradiation under hypoxia reduces the sensitivity of insects to radiation exposure, higher doses were needed to produce comparable reproductive sterility than in normoxia conditions. Two irradiation doses were selected to perform emergence rate and flight ability testes and control for the detrimental effects on *D. suzukii* adults. There were no negative effects in both selected doses and atmosphere conditions compare to the untreated flies.

2) Artificial oviposition system for the mass-rearing of *D. suzukii*

For the applicability of the SIT on SWD, the establishment of mass-rearing methods is fundamental. In this study, rearing cages were designed for the development of an artificial oviposition system for the mass-rearing of *D. suzukii*. The two different egg collection systems, a netted panel and a waxed panel, were compared with respect to the number of eggs produced, egg viability, pupal and adult recoveries, adult emergence rate and adult flight ability. As a result, a suitable oviposition method to collect and measure a large number of SWD eggs was established, bringing promising results for the fast development of the future mass production techniques for this pest.

2. Results

2.1 Irradiation dose response under hypoxia for the application of the sterile insect technique in *Drosophila suzukii*

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Irradiation dose response under hypoxia for the application of the sterile insect technique in *Drosophila suzukii*

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Abstract

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 oxygen-reduced environment such as hypoxia or anoxia is routinely used in the sterile insect technique (SIT) of some tephritid species as it provides radiological protection. This treatment allows to have the sterile pupae already in sealed containers facilitating the shipment. SIT is an environment friendly control tactic that could be used to manage populations of *Drosophila suzukii* in confined areas such as greenhouses. The objectives of this study were to assess the effect of irradiation on the reproductive sterility in *D. suzukii* males and females under low-oxygen atmosphere (hypoxia) and atmosphere conditions (normoxia). Additionally, we assessed the differences in radiological sensitivity of pupae treated under hypoxia and normoxia conditions. Finally, the effect on emergence rate and flight ability of the irradiated *D. suzukii* adults exposed to doses that induced >99% of sterility were assessed. Pupae needed a 220 Gy irradiation dose to achieve >99% of egg hatch sterility in males irrespective of the atmosphere condition. For females the same level of sterility was achieved already at 75 Gy and 90 Gy for the normoxia and hypoxia treatments, respectively. Radiation exposure at 170 and 220 Gy under the two atmosphere treatments did not have any effect on the emergence rate and flight ability of *D. suzukii* males and females. Therefore, hypoxia conditions can be used as part of an area-wide insect pest management program applying SIT to facilitate the protocols of packing, irradiation and shipment of sterile *D. suzukii* pupae.

Introduction

The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), is an invasive and polyphagous pest of Eastern Asian origin attacking several commercial soft-skinned fruits and berries in Europe and the Americas [1,2]. The female is

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equipped with a long-serrated ovipositor that can penetrate the epicarp of ripe soft-skinned fruits/berries [3]. The damage is caused by the feeding of the larvae on the fruit flesh and subsequently by infecting microorganisms resulting in reduced fruit quality [4,5] and economic losses [6–11].

The host fruits are infested close to the harvesting time making the control with broad-spectrum insecticides difficult [12]. Therefore, several approaches have been proposed to manage *D. suzukii* infestations like biological control tactics [13], cultural control practices [14], use of biorational pesticides [11], and mating disruption [15]. However, most of them appear to be partly effective and/or unsustainable [11,16,17].

Recently, the sterile insect technique (SIT) has been suggested as a potential complementary control method against *D. suzukii* [16,18–20]. The SIT is an environmentally friendly method based on the sequential, inundative releases of sterile insects in a targeted area. The released sterile males compete with wild males to mate with wild females [21]. A mating of a sterile male with a virgin wild female results in the production of non-fertile eggs and the reduction of offspring results in suppression or even, in specific situations, the local eradication of the wild population [22]. One of the key issues for the SIT application is to determine the radiation dose since the doses used to induce reproductive sterility may vary between sexes and among species [23,24]. As a sex separation system for *D. suzukii* is not available yet, both males and females need to be released in an SIT programme. Thus, it is crucial to study the impact of irradiation on *D. suzukii* female fertility and male mating competitiveness.

One of the crucial requirements for the successful implementation of an SIT programme is the establishment of protocols that will allow mass-production, sterilization and shipment of insects without detrimental effects on their biological quality parameters including male competitiveness during mating and longevity [25–27]. The negative effects of the radiation increase with increasing doses [28], and therefore, exposure to high levels of radiation may influence the physiology and behavior traits of the species [29]. The quality-reducing effects of ionizing radiation on insects are associated with the production of deleterious free radicals that are formed by the ionization of the intracellular water [30]. Exposing insects to a lower oxygen atmosphere such as hypoxia (low oxygen condition) or anoxia (zero oxygen condition) before and during exposure to radiation can mitigate some of these negative effects [31–34]. The irradiation of pupae under hypoxia is routinely used in the SIT programmes of tephritid species e.g. *Ceratitis capitata* and *Anastrepha ludens*, not only because it provides radiological protection, but because it also allows to have the sterile pupae already in sealed containers (e.g. plastic bags) which facilitates the packing and shipment of pupae to the final release destination [35]. However, irradiation under hypoxia atmosphere reduces the sensitivity of insects to radiation exposure, thus higher doses are needed to produce comparable reproductive sterility than in normoxia conditions [36,37].

The objectives of this study were: 1) to assess the effects on the induced sterility of a long-range of different radiation doses for *D. suzukii* males and females; 2) to compare irradiation effects under normoxia and hypoxia when pupae are irradiated under these conditions; and 3) to determine the effects of irradiation under normoxia and hypoxia on adult emergence rate and flight ability of *D. suzukii*.

Materials and methods

Drosophila suzukii colony

All flies used in this study were obtained from a colony maintained at the Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria. The colony was established in 2014 with pupae from the Agricultural

Entomology Unit of the Edmund Mach Foundation in San Michele All'Adige, Trento Province, Italy. Flies were kept in cages and were provided with water and adult diet containing a mixture of sugar and hydrolysate enzymatic yeast (MP Biomedicals) in a 3:1 ratio [38]. Controlled environment conditions of $22 \pm 5^\circ\text{C}$, $65\% \pm 5\%$ RH and a 14:10 (L:D) photoperiod were maintained. Artificial diet (brewer yeast 5%, sugar 11.15%, carrot powder 13.70%, sodium benzoate 0.19%, nipagin 0.15%, water 69.81%) served as egg-laying substrate and larval diet. The diet was changed daily and kept in the laboratory until larval development and pupation had been completed. After 11 days, pupae were separated from the diet and kept under the regular laboratory conditions.

Pre-irradiation treatments

One day before adult emergence, two groups of 2000 pupae each were treated under normoxia or hypoxia, respectively. At this age, the pupae are dark brown with visible red eyes and wings and they are about 24 hours before emergence. The pupae of the normoxia group were maintained under normal laboratory conditions before irradiation, while the ones of the hypoxia group were sealed in a hermetic polyethylene bag for 5 hours at 18°C to allow them metabolize the oxygen present in the bag. To assess O_2 depletion, a gas-sensor device (CheckMate3, Dansensor 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% compared to 20.9% in the normoxia group. Both groups were irradiated the same day immediately after the end of the normoxia and hypoxia treatments.

Irradiation treatment

Pupae of the hypoxia and normoxia treatments were kept in sealed and perforated polyethylene bags (10 x 7 cm), respectively, and exposed to gamma rays using a ^{60}Co irradiator (Gamma Cell-220, Nordion, Canada). To ensure that the pupae received always the same dose, the bags were placed in the middle of the chamber by using a polystyrene cylinder as a support. Three 10 by 10 mm Gafchromic® HD-V2 dosimetry films (International Specialty Products, NJ, USA) were irradiated together with the pupae of each treatment 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) [39]. The following irradiation doses were applied: 30, 50, 70, 90, 110, 130, 150, 170, 190, 210, 220, 230 and 240 Gy.

The irradiated pupae were placed in a cage until adult emergence. Only adults that emerged within 24 hours after the irradiation were used for the experiments while those that emerged later were discarded. The emerged flies were sexed under a short period of CO_2 anesthesia. The effect of the irradiation on sterility was tested for each radiation dose on two types of crosses: 25 irradiated females x 25 non-irradiated males and 25 non-irradiated females x 25 irradiated males. Three additional doses (75, 80, 85 Gy) were tested on cross: irradiated females x non-irradiated males to determine the lowest dose able to confer complete sterility under hypoxia and normoxia treatments. All doses were tested using pupae from different generations. For each dose a control cross (0 Gy) of 25 non-irradiated females x 25 non-irradiated males was performed. All crosses were replicated five times per dose. More details of the described protocol at: <http://dx.doi.org/10.17504/protocols.io.76whrfc>.

Fecundity, fertility and reproduction index (RI)

Males and females from each cross were transferred to a cage (17 x 8 x 11.5 cm) and kept together for four days to ensure sexual maturation and complete insemination of all females.

Adults were provided with water and diet *ad libitum*. On the fifth day, three fresh blueberries were placed on top of each cage to serve as oviposition sites. The blueberries were daily replaced, and eggs were carefully collected using forceps. The eggs were placed on a wet black net in a Petri dish filled with larval diet and counted. 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. The hatching rate was recorded 48 hours after egg collection. All the experiments were carried out under the standard laboratory conditions.

Fecundity was assessed for the irradiated females x non-irradiated males cross and calculated as the total number of eggs collected per cage divided by the average number of eggs oviposited by the control cross. The hatching rate was assessed for all cross types. The Reproduction Index (RI) was used to determine the total direct radiation effect on female reproduction. The RI was developed as an index to express the female reproduction by combining fecundity and egg hatch after the irradiation exposure. The RI is calculated as [(fecundity * egg hatch) / 100].

The effect of radiation dose and atmosphere treatments on F1 progeny was also assessed for all cross types by counting the number of pupae and adult emergence rate. The emerged flies were sexed, and the sex ratio was calculated as the proportion of male and female adults.

Effect of irradiation dose on emergence rate and flight ability

For the quality control tests, pupae were irradiated either with a dose at 170 Gy or 220 Gy. Five replicates containing 25 pupae each were used for each atmosphere treatment and control (0 Gy). The experiment was repeated twice (hereafter: “blocks”). To ensure the proper age of the pupae at the time of irradiation, they were observed under a stereoscope and only the dark-colored ones with visible red eyes and wings were selected [40].

For the emergence rate and flight ability tests the pupae were irradiated with the same procedures described above and immediately placed inside a paper ring in a Petri dish and covered it with a black PVC cylinder (8.4 cm internal diameter and 10 cm height) for 48 hours. The PVC cylinder was coated internally with talcum powder to prevent the emerging adults from crawling out of the cylinder instead of flying. The emergence rate of these pupae was estimated by subtracting non-emerged pupae and partially emerged flies from the total number of emerged flies. Fully emerged flies with and without body deformities were separately scored. Flies found inside the cylinder, but without any deformities were considered not able to fly and thus recorded as “no fliers”. The index of flight ability was calculated as the number of emerged flies, subtracted by the number of “no fliers”, and divided by the number of emerged flies as described in the “Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies” manual [41]. Flies outside the tubes were removed every hour to avoid re-entry in the cylinders. No food or water was provided during the experiments.

Data analysis

All statistical analyses were carried out using R 3.6 [42]. All data were binomially distributed and analyzed using the generalized linear models. Fecundity rate was analyzed using a generalized linear model with Poisson distribution [43]. To assess differences between hypoxia and normoxia and between irradiated and non-irradiated flies, irradiation doses, atmosphere treatments, their interaction and replicates were modeled as a fixed effect. The quality control parameters (adult emergence and flight ability) were analyzed using a generalized linear mixed models (binomial family) where the irradiation doses, atmosphere treatments, and replicates were included as fixed effects and blocks as a random effect. Tukey’s HSD corrections were used for multiple comparisons. For all data significance was set at $\alpha = 0.05$. The dose-response

male and female fertility under normoxia and hypoxia atmospheres were corrected against the fertility of the control cages and transformed using the normal equivalent deviate (N.E.D.). In all cases, the mean \pm SD is reported.

Results

Fecundity

Crosses between non-irradiated females and irradiated males. The average number of eggs laid by the fertile females of the untreated control groups (376.7 ± 143.2) did not differ compared to the hypoxia groups in all irradiation doses (328.1 ± 142.2) (z -value = -1.838, $P = 0.157$). However, there was a difference in the number of eggs laid by the females of normoxia crosses, and the untreated control group (303.2 ± 153.4 eggs) (z -value = -2.949, $P = 0.008$). No difference was detected in fecundity between the hypoxia and normoxia atmosphere conditions (z -value = -1.133, $P = 0.493$), but there was an effect of the interaction (all comparisons $P < 0.002$, data pooled for all irradiation doses and atmosphere treatments).

Crosses between irradiated females and non-irradiated males. The irradiation exposure decreased the fecundity of females irradiated under hypoxia (z -value = -43.41, $P < 0.0001$) and normoxia (z -value = -46.23, $P < 0.0001$) compared to control females. At 30 Gy the fecundity of the irradiated females was 25.9% under hypoxia and 10.4% under normoxia compared to the control group. In all irradiation doses, there was difference in the fecundity between hypoxia and normoxia atmosphere conditions (z -value = -14.08, $P < 0.0001$). The interaction between atmosphere conditions and irradiation doses had an effect on fecundity of females at dose from 30 Gy to 70 Gy ($P < 0.01$, data pooled). At 90 Gy, only one egg was laid by the irradiated females treated under hypoxia and 3 eggs from the irradiated females treated under normoxia (0.05% and 0.19% of the fecundity of the respective control groups) (S2 Fig).

Fertility

Crosses between non-irradiated females and irradiated males. The irradiation exposure had an effect on the male reproductive fertility compared to the control males (hypoxia: z -value = -13.921, $P = 0.001$; normoxia: z -value = -14.809, $P = 0.001$). In all irradiation doses, the reproductive fertility of irradiated males under hypoxia was different from that of the normoxia treated males (z -value = 2.443, $P = 0.0387$). On average, the dose necessary to obtain the same sterility level under hypoxia was 15 Gy higher than normoxia (Fig 1).

Male irradiated under normoxia conditions reached more than 99% sterility levels when irradiated at 190 Gy while the same sterility level required 210 Gy under hypoxia conditions. Pupae irradiated at 220 Gy induced 99.8% of sterility under both normoxia and hypoxia conditions. A dose of 170 Gy induced 97% of sterility when males were treated under hypoxia (Fig 2). These two doses were selected to further study the effect of irradiation dose on the adult emergence and flight ability.

In all irradiation doses, the number of pupae and adults produced by the non-irradiated females and irradiated males cross was lower under hypoxia (pupae: z -value = 5.629, $P < 0.0001$; adult: z -value = 4.731, $P < 0.0001$) and normoxia (pupae: z -value = 5.483, $P < 0.0001$; adult: z -value = -4.524, $P < 0.0001$). There were not differences in the number of pupae and adults produced when irradiated males mated with non-irradiated females under hypoxia and normoxia (pupae: z -value = 0.437, $P = 0.899$; adult: z -value = 0.57, $P = 0.568$). At the lowest dose (30 Gy), the percentage of pupae and adults was less than 50% compared to the control regardless of the atmosphere treatment. Above 150 Gy, less than 1% of the F1 pupae completed the metamorphosis under either hypoxia or normoxia treatments. No F1 pupae were produced at 220 and 240 Gy under hypoxia and normoxia, although at 230 Gy there was

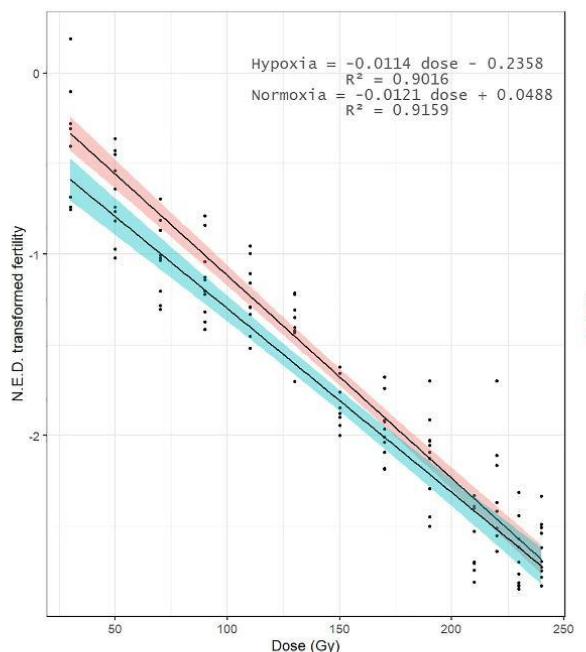


Fig 1. Linear regression of the transformed fertility of non-irradiated females and irradiated males. Linear regressions (bold lines) of the dose response fertility value (full dots) under hypoxia ("h") and normoxia ("o") atmosphere in a cross of irradiated males with non-irradiated females. The y-axis represents the irradiation doses (Gy); the x-axis represents the normal equivalent deviate transformation (N.E.D.) of the corrected fertility. Blue and red shaded areas represent the 95% confidence level interval for predictions from the linear model for the normoxia and hypoxia atmosphere, respectively.

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a 0.06% and 0.09% pupae recovery under hypoxia and normoxia, respectively. No adults emerged when males were treated with 190 Gy under hypoxia and with 210 Gy under normoxia. Irradiation doses and atmosphere conditions did not affect the sex ratio of the emerged F1 adults (hypoxia: $F_{1,90} = 1.628$, $P = 0.20$; normoxia: $F_{1,88} = 0.5509$, $P = 0.46$) ([S1 Table](#)).

Crosses between irradiated females and non-irradiated males. Irrespectively of the irradiation dose, fertility from irradiated females and non-irradiated males crosses was lower compared to the untreated control (hypoxia: z -value = 13.987, $P < 0.001$; normoxia: z -value = 14.267, $P < 0.001$). Except for the 30 Gy treatment group, fertility was higher under hypoxia conditions than normoxia ($F_{1,32} = 7.3635$, $P = 0.01063$). On average, the dose necessary to obtain the same sterility level under hypoxia was 10 Gy higher than normoxia ([S1 Fig](#)). Full sterility was obtained when females were treated at 75 and 85 Gy under normoxia and hypoxia conditions, respectively ([Fig 3](#)).

The females reproductive index (RI) at 70 Gy under normoxia was reduced to 0.12% compared to 90% of the control females. Similar data were obtained with females irradiated at 80 Gy under hypoxia conditions (0.13%) ([S2 Table](#)).

Females irradiated at 75 and 85 Gy under normoxia or hypoxia produced either no pupae or there was a failure in adult emergence. Atmosphere conditions did not affect the sex ratio of the emerged F1 adults (hypoxia: $F_{1,24} = 0.347$, $P = 0.56$; normoxia: $F_{1,19} = 1.906$, $P = 0.18$).

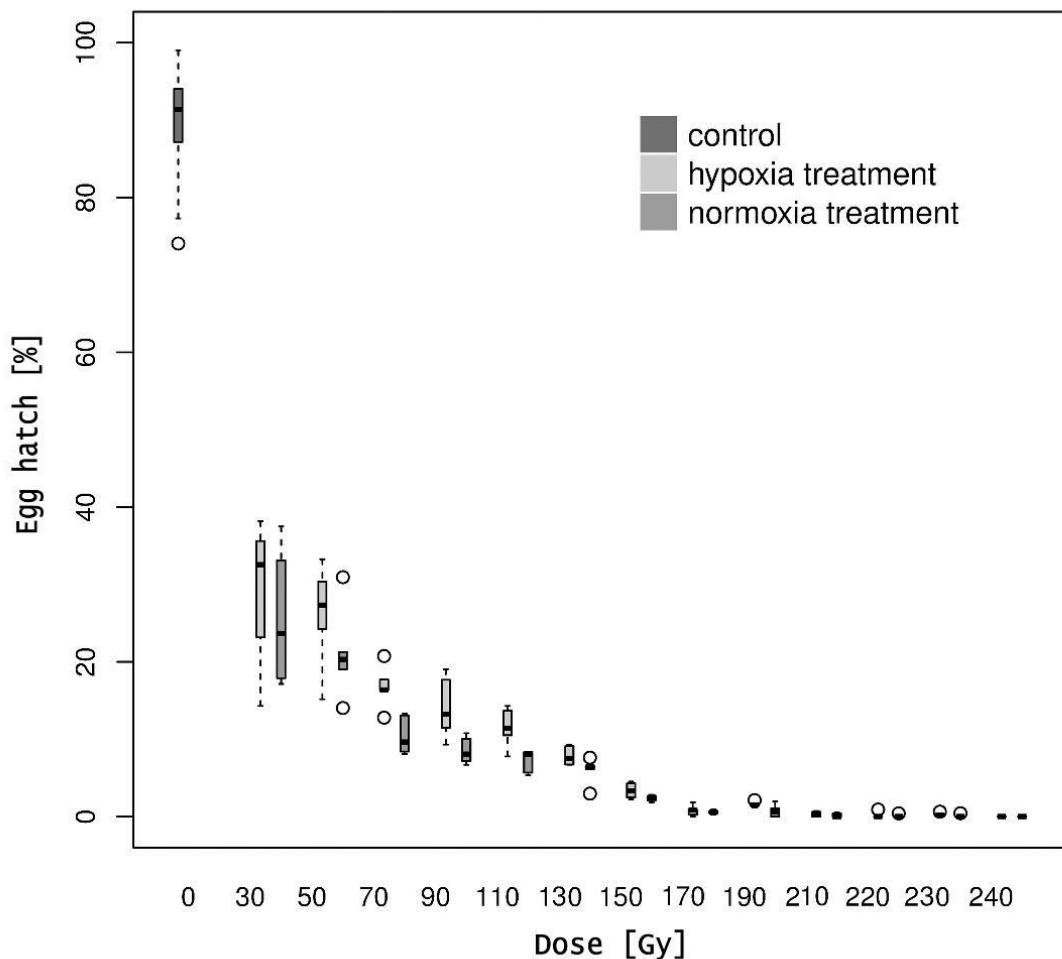


Fig 2. Percentage of egg hatch of non-irradiated females and irradiated males at different irradiation doses under hypoxia and normoxia atmosphere treatments. The effect of different irradiation doses on *D. suzukii* egg hatch under hypoxia and normoxia atmosphere conditions in a cross of irradiated males with non-irradiated females. Bold lines represent medians, dashed lines represent upper and lower whiskers and circles represent outliers.

<https://doi.org/10.1371/journal.pone.0226582.g002>

Effect of irradiation dose on emergence rate and flight ability

Male and female emergence rate and flight ability were assessed for pupae irradiated under normoxia or hypoxia at 170 Gy allowing a residual fertility of >3% [44] or at 220 Gy which induced a sterility of >99%. Emergence rate was independent of dose (z -value = 0.576, P = 0.565), atmosphere condition (z -value = 0.225, P = 0.972), and irradiation exposure (hypoxia: z -value = 0.221, P = 0.973; normoxia: z -value = 0.446, P = 0.896). Adult emergence rate was 92.0% and 94.4% for the dose of 170 Gy, while 93.6% and 92.8% for the dose of 220 Gy under hypoxia and normoxia, respectively (Fig 4 and S3 Table). Flight ability was likewise independent of dose (z -value = 1.852, P = 0.064), atmosphere condition (z -value = 1.263, P = 0.416),

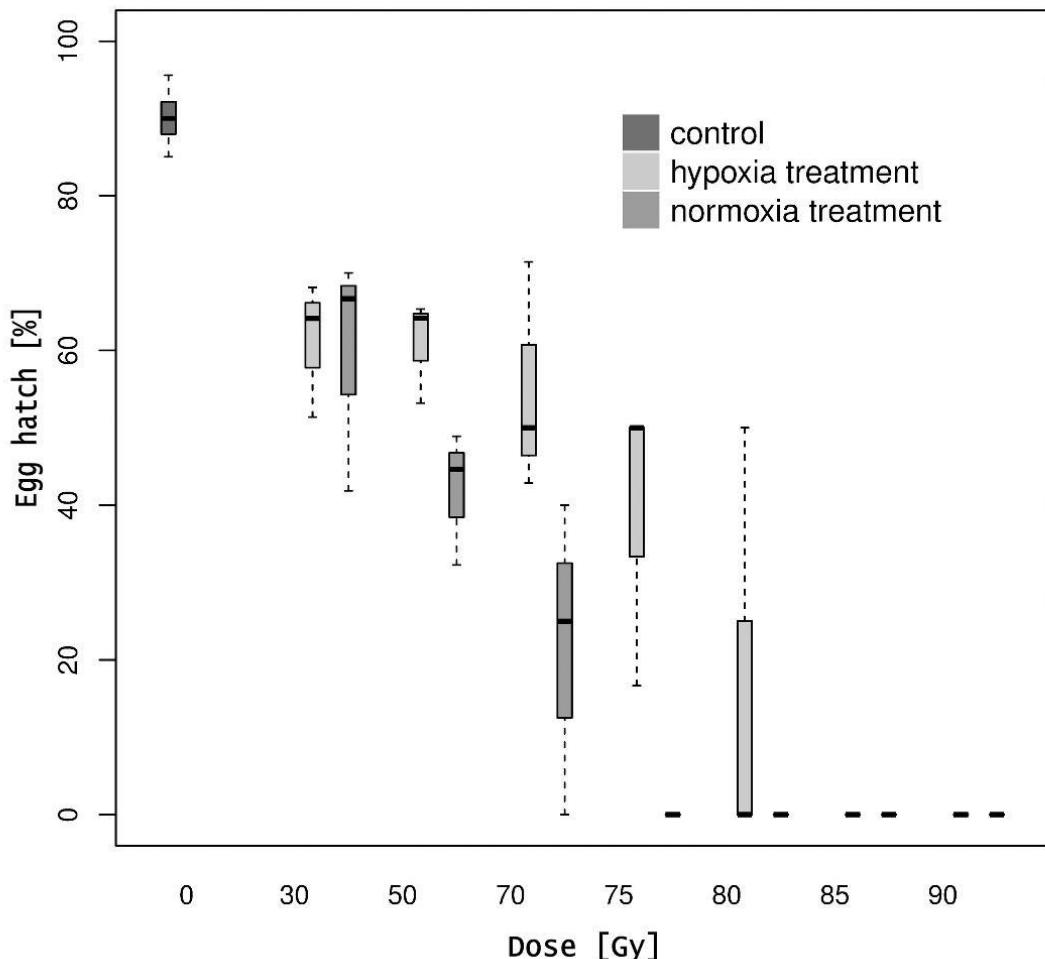


Fig 3. Percentage of egg hatch of irradiated females and non-irradiated males at different irradiation doses under hypoxia and normoxia atmosphere treatments. The effect of different irradiation doses on *D. suzukii* egg hatch under hypoxia and normoxia atmospheres in a cross of irradiated females with non-irradiated males. Bold lines represent medians and dashed lines represent upper and lower whiskers.

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and irradiation exposure (hypoxia: z -value = -0.220, P = 0.974; normoxia: z -value = 1.045, P = 0.548). Flight ability rate was 88.8% and 91.6% at 170 Gy, and 91.6% and 88.4% at 220 Gy under hypoxia and normoxia, respectively (Fig 5 and S3 Table).

Discussion

In this study, a wide range of gamma radiation doses was tested on *D. suzukii* pupae to determine the reproductive sterility dose for the potential application of SIT on this pest. Due to the radiological protection given by hypoxia atmosphere during irradiation treatment in several

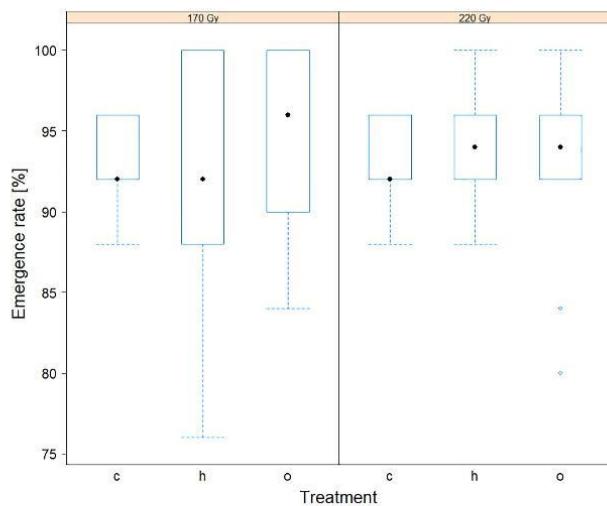


Fig 4. The effect of irradiation dose on the emergence rate. The effect of irradiation dose on the emergence rate of non-irradiated ("c") and irradiated pupae at 170 and 220 Gy under hypoxia ("h") and normoxia ("o") conditions. Full dots represent medians, dashed lines represent upper and lower whiskers and circles represent outliers.

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insect species [26,33], doses were assessed under normoxia and hypoxia atmosphere treatments for *D. suzukii* males and females.

In both atmosphere conditions, sterility increased with the increase of the irradiation doses as already described for other insects [36,45]. Full male sterility was achieved when pupae were exposed to 190 and 210 Gy in normoxia and hypoxia, respectively. This confirmed that *D. suzukii* pupae are more radiological resistant under both atmosphere conditions than tephritid species which can be sterilized between a dose range of 70–120 Gy [46–51] and *D. melanogaster* that requires 160 Gy [52].

As currently, no sexing system is available for *D. suzukii*, both sexes will be released in the field in future SIT applications. In irradiated females and non-irradiated males crosses, the fecundity and fertility and consequently the reproductive index decreased as the doses increased. A 75 Gy dose was sufficient to obtain complete female sterility under normoxia, confirming previous studies [18,20]. However, a higher dose of 85 Gy was required to obtain the same full female sterility under hypoxia treatment. In both atmospheres, females required much lower doses than males to achieve the same sterility confirming that females are more radiologically sensitive compared to males [45, 32, 33].

We determined a suitable range of radiation doses that will allow choosing the dose and the sterility level to optimize the efficiency of SIT. Our results suggest that a dose of 220 Gy induces 99.8% sterility (based on egg hatch data) in males and it would be recommended in an eradication strategy while a lower dose of 170 Gy (97% sterility based on egg hatch data) would be recommended for suppression programmes. In any of those two-choice scenarios for SIT application, irradiated females will be fully sterile.

The assessment of hypoxia conditions as mentioned above is of crucial importance for the implementation of SIT against *D. suzukii* since low oxygen levels reduce the negative effects on cells caused by free radicals generated from ionizing radiation [53]. These highly reactive molecules can cause irreversible intracellular alterations [31]. Therefore, in AW-IPM programs

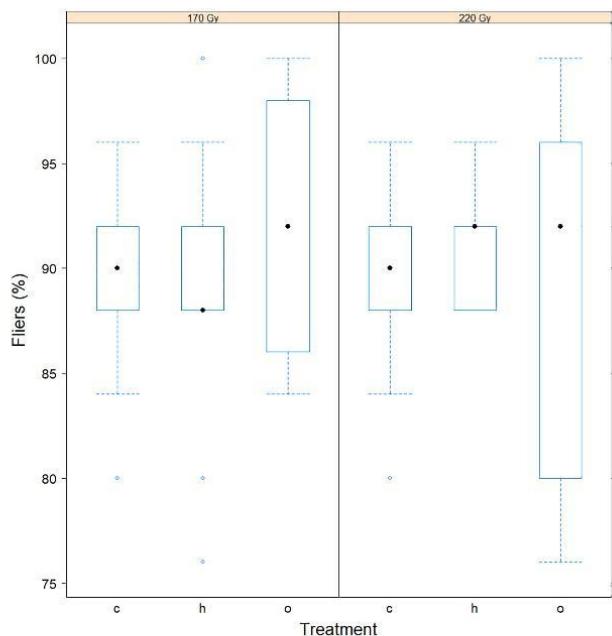


Fig 5. The effect of irradiation dose on the flight ability. The effect of irradiation dose on the flight ability rate of non-irradiated ("c") and irradiated pupae at 170 and 220 Gy under hypoxia ("h") and normoxia ("o") conditions. Full dots represent medians, dashed lines represent upper and lower whiskers and circles represent outliers.

<https://doi.org/10.1371/journal.pone.0226582.g005>

that include releases of sterile adults, pupae have to be kept under hypoxia or anoxia conditions during the irradiation, facilitating also transportation to the final release center since hypoxia will also prevent emergence during shipment [54]. In our study, *D. suzukii* pupae were treated under hypoxia prior and during irradiation and were compared with pupae irradiated under normoxia. For the sterilization of *D. suzukii* males, higher doses of approximately 15 Gy were required to obtain the same level of sterility in individuals irradiated under hypoxia than under normoxia. This phenomenon has been previously reported for tephritid fruit flies [23,24,33,55], tsetse flies [56], and lepidoptera species such as *Cydia pomonella* [57] and *Trichoplusia ni* [58].

To determine the effect of irradiation doses under different atmospheric conditions on the quality of *D. suzukii*, emergence rate and flight ability were assessed. Our results suggest that the examined quality parameters were not influenced by exposure to radiation at either a dose of 170 Gy or 220 Gy. Despite the protective effects of the low-oxygen atmosphere, no differences were detected in the emergence rate or flight ability. Additional parameters should be tested to complement our understanding of the effect of both atmosphere and irradiation dose on the biology of *D. suzukii* males. These additional parameters must include mating studies under semi-field conditions to assess the competitiveness of irradiated males when in competition with fertile males for mating with wild females. Nevertheless, the development of a protocol to irradiate *D. suzukii* pupae under hypoxia is an important step toward the development of the SIT package for *D. suzukii* and its implementation in confined areas such as greenhouses.

Supporting information

S1 Fig. Linear regression of the transformed fertility of the irradiated females and non-irradiated males. Linear regressions (bold lines) of the dose response fertility values (full dots) under hypoxia ("h") and normoxia ("n") atmosphere in a cross of irradiated females with non-irradiated males. The y-axis represents the irradiation doses (Gy); the x-axis represents the normal equivalent deviate transformation (N.E.D.) of the corrected fertility. Blue and red shaded areas represent the 95% confidence level interval for predictions from the linear model for the normoxia and hypoxia atmosphere, respectively. (TIF)

S2 Fig. Fecundity of irradiated females and non-irradiated males at different irradiation doses under hypoxia and normoxia atmosphere treatments. The effect of different irradiation doses on *D. suzukii* fecundity under hypoxia and normoxia atmosphere conditions in a cross of irradiated females with non-irradiated males. Bold lines represent medians, dashed lines represent upper and lower whiskers. (TIF)

S1 Table. Data of non-irradiated females and irradiated males experiment. Fertility, pupae recovery, adult emergence and sex ratio in crosses between irradiated males under hypoxia ("h") and normoxia ("n") conditions and non-irradiated females. The averaged percentage +/- SD of all replicates at different irradiation doses is presented. The sex ratio is presented in proportion. (PDF)

S2 Table. Data of irradiated females and non-irradiated males experiment. Fecundity, fertility, pupae recovery, adult emergence and sex ratio in crosses between irradiated females under hypoxia ("h") and normoxia ("n") conditions and non-irradiated males. The averaged percentage +/- SD of all replicates at different irradiation doses is presented. The sex ratio is presented in proportion. (PDF)

S3 Table. Data on emergence rate and flight ability experiment. Emergence and flight ability tests. The averaged percentage +/- SD of all replicates at the irradiation doses of 170 and 220 Gy is presented. (PDF)

S4 Table. Raw-data of non-irradiated females and irradiated males experiment. The number of eggs laid, egg hatch, pupae recovery, adult emergence and male adults in crosses between irradiated males under hypoxia ("h") and normoxia ("n") conditions and non-irradiated females. The mean +/- SD of all replicates at different irradiation doses is presented. (PDF)

S5 Table. Raw-data of irradiated females and non-irradiated males experiment. The number of eggs laid, egg hatch, pupae recovery, adult emergence and male adults in crosses between irradiated females under hypoxia ("h") and normoxia ("n") conditions and non-irradiated males. The mean +/- SD of all replicates at different irradiation doses is presented. (PDF)

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2.2 Mass-rearing of *Drosophila suzukii* for SIT application: Evaluation of two oviposition systems

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Article

Mass-Rearing of *Drosophila suzukii* for Sterile Insect Technique Application: Evaluation of Two Oviposition Systems

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Abstract: *Drosophila suzukii* (Diptera: Drosophilidae) is an invasive pest of a wide range of commercial soft-skinned fruits. To date, most management tactics are based on spraying of conventional and/or organic insecticides, baited traps, and netting exclusion. Interest has been expressed in using the sterile insect technique (SIT) as part of area-wide integrated pest management (AW-IPM) programs to control *D. suzukii* infestations. Mass-rearing protocols are one of the prerequisites for successful implementation of the SIT. To establish mass-rearing methods for this species, two different egg-collection systems were developed and compared with respect to the number of eggs produced, egg viability, pupa and adult recovery, adult emergence rate, and flight ability. Female flies kept in cages equipped with a wax panel produced significantly more eggs with higher viability and adult emergence rate, as compared to the netted oviposition system. The wax panel system was also more practical and less laborious regarding the collection of eggs. Furthermore, the wax panel oviposition system can be adapted to any size or design of an adult cage. In conclusion, this system bears great promise as an effective system for the mass production of *D. suzukii* for SIT.

Keywords: wax panel system; netted system; spotted wing drosophila; pest management; female oviposition behavior

1. Introduction

The successful implementation of the sterile insect technique (SIT) as part of an area-wide integrated pest management (AW-IPM) approach against a target pest species requires the development of technologies such as the mass-rearing of the target species, the optimal irradiation doses to sterilize the males and the females, adequate quality control protocols, and packing, shipping, holding, and release procedures [1–4]. Efficient mass-rearing methods are required to allow for continuous and stable production of sufficient numbers of insects at an economically viable cost [5].

Adapting wild insects to artificial laboratory rearing conditions is the first step in development of a mass-rearing system. The adaptation process should avoid adverse effects like modification of the natural behavior of the insect, resulting in poor field performance; inadequate sexual competitiveness; or incompatibility with the wild target insect population [6]. Mass-rearing of fruit flies comprises several

steps, starting with the adaptation of the females' oviposition behavior under artificial conditions to maximize production and ensure optimal quality of the eggs [7,8]. Female oviposition behavior plays an essential role in the development of suitable oviposition devices to achieve the required production goal [9,10]. When a large number of an insect species is reared, natural hosts are no longer practical as an egg-collection system. Consequently, a functional and cost-efficient method must be developed for females to oviposit in artificial substrates or devices, allowing the best possible egg recovery [11]. Under artificial conditions, the adaptation of a female's oviposition behavior may occur after several generations due to the low heritability or genetic complexity involved in oviposition behavioral traits [12,13]. The adaptation of the female's oviposition from the natural host to an artificial diet and from an artificial diet to a non-diet device results in bottleneck events that may reduce the colony genetic variability in addition to selecting for a specific rearing behavior [14].

To date, 13 species of Tephritidae are mass-reared worldwide for pest-control purposes [15], and a practical artificial oviposition method has been developed based on the biology and ethology of each species [16].

Artificial egg-collection systems for large-scale rearing of insects are highly variable, e.g., parasitoid species lay eggs on the natural host egg, larva, or pupae [17,18] and some species of mass-reared mosquitoes and Lepidoptera lay eggs on moist or dry paper substrates where eggs are either manually removed by a brush [19–21] or placed directly onto the larval diet [22]. Perforated bottle or domes simulating fruits are used as an oviposition device for some species of fruit flies (*Ragoletis cerasi*, *Bactrocera* spp., *Dacus* sp.) [23–25], whereas a broad range of either flat membranes, panels, or nets with different composition, size, color, and thickness are used as a collection method ("egg-dropping") for the Mediterranean fruit fly *Ceratitis capitata* and *Anastrepha* spp. [26,27].

The "egg-dropping" technique is the most common method applied in fruit fly mass-rearing facilities worldwide [9,28]. More than two billion eggs of *C. capitata* are collected weekly with this system (Cáceres C., personal communication). Females oviposit eggs through the netting and the eggs are collected in a metallic or PVC container filled with water. The *C. capitata* colony is a strain that has been adapted to laboratory conditions for decades, and females can therefore lay eggs through this artificial system in the same quantity as the natural host without any external stimulants. Therefore, the amount of eggs produced and collected can be easily quantified, which is important for the estimation of colony production and management.

Spotted wing drosophila (SWD) *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is a destructive insect pest of fruit crops. Female *D. suzukii* possess a saw-shaped ovipositor that allows them to pierce the skin of ripening fruit, contrary to other Drosophilidae flies that prefer laying eggs in decaying fruits [29–31]. *D. suzukii* females oviposit eggs and larvae develop on a large number of different soft skinned hosts [32,33], making many fruit crops in Europe and America susceptible to infestation with high economic losses [34–36]. These losses are mitigated by applying insecticides [37], performing field sanitation [38], mass-trapping [39,40], and introducing mechanical impediments such as nets to physically protect the fruit [41,42].

As a result of the invasion of *D. suzukii* into the European and American continents, government agencies and growers have been looking for alternative control methods that are more efficient than the existing methods and more benign to the environment [36,43]. Therefore, different research groups have been focusing on the development of an SIT package for the control of *D. suzukii*, making use of the experience and knowledge that has been accumulated over the years with Tephritidae species.

The main objective of this study was to develop an efficient, practical, and economically viable oviposition system that would allow production of eggs under artificial conditions for large-scale *D. suzukii* production for SIT application. In this study, a comparison was made of two oviposition systems with respect to the total egg production, egg viability, egg to pupa developmental time, pupa and adult recovery, and the quality of the produced adults as assessed by adult emergence and flight ability.

2. Materials and Methods

2.1. *Drosophila suzukii* Colony

The *Drosophila suzukii* used in this study came from a colony that was established from flies collected in San Michele All'Adige, Trento (Italy) in 2014, and reared at the Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria. Adult flies were maintained in aluminum-framed cubic cages ($45 \times 45 \times 45$ cm) that were loaded with approximately 30,000 pupae per cage. Pupae volume was measured in a graduated cylinder (1 mL = 220 pupae). Emerging adult flies had unlimited access to water and a diet consisting of a blend of sugar and enzymatic hydrolyzed yeast at a 3:1 ratio. Subsequently, insects were transferred to larval diet consisting of 25.7% wheat bran, 6.4% brewer yeast, 11.9% sugar, 0.4% sodium benzoate, 0.4% nipagin, and 55.1% water. They were kept at a temperature of 22 ± 5 °C, 65 ± 5% RH, and a 14:10 h L:D photoperiod.

2.2. Oviposition Systems

The “netted oviposition system” was developed and has been used as a rearing method for *D. suzukii* at the IPCL since 2017. Thereafter, “the wax panel system” was developed as a more cost-effective oviposition system (Figure 1). Two colonies were maintained with both systems for ten generations, and after this period of adaptation, a comparison was made in terms of production efficiency and quality.

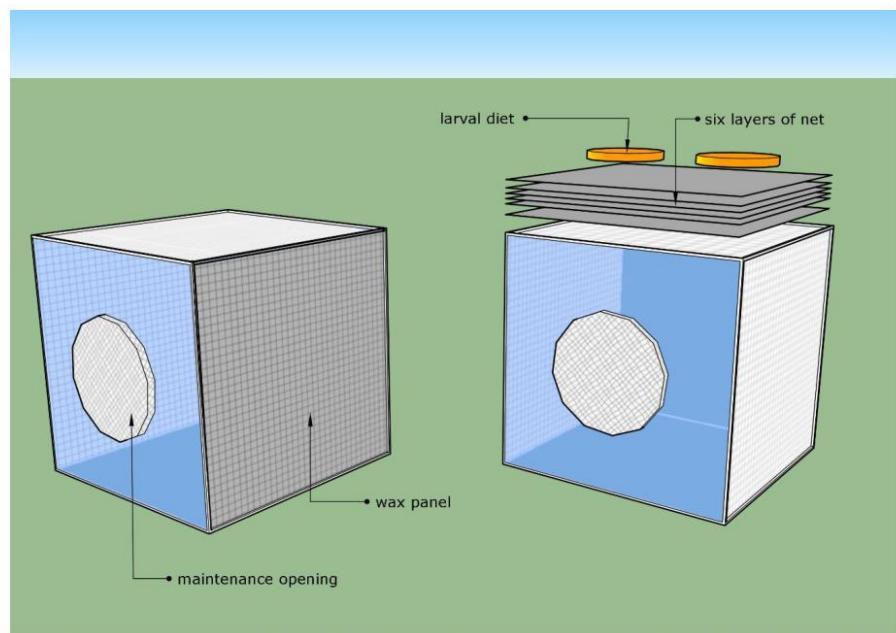


Figure 1. Design of rearing cages. (Left) Wax oviposition system; (right) netted oviposition system.

2.2.1. Net System

The net system consisted of a cubic aluminum frame and three transparent PVC plates, i.e., one each for the floor, the back, and the front. The front panel had a round hole (200 mm diameter) for introducing and removing water, adult diet, and pupae. Two sides of the cage were covered with mesh

netting (mesh hole size: 0.22×0.22 mm) to allow for air exchange. The top of the cage consisted of one fixed internal mesh net (hole size: 1.0×1.5 mm), and six removable layers of synthetic black nylon net (Art. Velo, Sirio Tendaggi S.r.l., Magnago, Italy) (hole size: 0.22×0.22 mm) were placed on the top of the cage and used as an oviposition substrate. Petri dishes filled with the larval diet were used as a stimulant and placed on the top of the sixth layer of the synthetic black nylon, allowing females to lay eggs through the first five layers without having direct contact with the diet. This system allowed the collection of eggs that were trapped among the first five layers of net, avoiding contact with the artificial larval diet which only served as an oviposition attractant. Every day, the six netted layers were removed from the top of the cage and the eggs were collected by washing the first five layers with tap water. The sixth, top layer was changed daily. After the daily egg collection, the quantity of eggs produced was assessed volumetrically using graduated cylinders.

2.2.2. Wax Panel

The wax panel system was similar to the design of the netted system. However, in this system, one of the lateral mesh facets of the cage was used as an oviposition site. This layer comprised an internal net with a mesh hole size of 1.0×1.5 mm, and an external layer of a finer black net with a mesh hole size of 0.22×0.22 mm. Both nets were fully immersed in a hot liquid solution containing 3% beeswax, 20% liquid paraffin, 49% solid paraffin (52–54), and 28% glycerin, and left at room temperature (22 ± 5 °C) to solidify. As a result, the two layers of nets were stuck together to form a single sealed waterproof layer. The internal net with the larger mesh hole size served as a resting and support area, while the external one served as an oviposition surface. The waxed panel was dabbed with guava juice daily to stimulate female oviposition. Females oviposited by inserting the ovipositor directly through the waxed panel. Due to the filament of *D. suzukii* eggs, the eggs stuck to the panel. The oviposited eggs were washed off the wax panel daily with water and collected in the containers placed along the bottom of cage.

2.3. Evaluation of the Artificial Oviposition Systems

To assess the two oviposition systems, 33 mL of pupae (approximately 7000 pupae) were placed in each cage ($30 \times 30 \times 40$ cm) on moist filter paper to prevent desiccation and to facilitate emergence. Emerged adults had unlimited access to water and a blend of sugar and yeast hydrolysate enzymatic (MP Biomedicals, Eschwege, Germany) (3:1) as adult diet [44]. All experiments were carried out under the same environmental conditions as the rearing colony. The same number of pupae was used for each experiment and they were replicated five times. Replicates were implemented at different times and thus five consecutive generations were evaluated.

2.4. Egg Production and Larvae Rearing

In both oviposition systems, eggs were filtered after collection, and the total volume of eggs (in mL) was recorded daily. Eggs were then incubated on moist black filter paper and maintained in Petri dishes. After 24 h, eggs were washed with water from the Petri dishes and placed on the artificial larval diet.

Additionally, samples of 100 eggs from both the netted and wax panel systems were sampled daily, incubated on a moist black cloth, and placed in Petri dishes filled with the rearing diet. The following parameters were assessed:

- Egg hatch: closed eggs were counted 48 hours after the collection day;
- Egg to pupa time: 4–5 days after egg collection, Petri dishes were checked for pupae (cryptocephalic pupae) to assess the duration of larval development;
- Pupa recovery: about 11 ± 1 days after the egg-collection day, pupae were removed from the diet, counted, placed on moist paper, and left in sealed boxes without water and food for adult emergence;

- Adult production: once dead, the emerged adults were removed from the boxes and then counted and sexed under stereoscope.

2.5. Adult Emergence and Flight Ability

The total egg production for each cage (with the exception of the samples mentioned above) was separately transferred daily to the larval diet. To allow larval development, about 0.3 mL of eggs was transferred to 80 ± 5 g of larval diet. Approximately 11 ± 1 days after the egg transfer, pupae had completed their development and were removed from the diet. These were counted and a sample was taken for evaluation of the quality control parameters.

For each oviposition system, 10 pupae were sampled daily (860 pupae in total for each oviposition system) and placed in a Petri dish to assess adult emergence. The experiment was replicated five times, and each replicate represented a different generation. The percentage of adult emergence was recorded to estimate the number of adults available in the next generation.

For each oviposition system, 10 pupae were sampled daily (860 pupae in total for each oviposition system) to assess the ability of adults to fly (hereafter: “flight ability” or “flyers”). The experiment was replicated five times, with each replicate representing a different generation. Flight ability tests were carried out to assess the quality of emerged adults. A black PVC cylinder (10 cm height and 8.4 cm internal diameter) was used for the test, as described in the FAO/IAEA/USDA quality control manual [2]. Pupae were placed inside a paper ring in a Petri dish and covered with a black PVC cylinder coated internally with talcum powder to prevent emerged adults crawling out of the cylinder. Flies outside the tubes were removed every hour to avoid re-entry into the cylinders. No food or water was provided during the experiments.

Adult emergence and flyers were calculated as described in the FAO/IAEA/USDA quality control manual [2].

2.6. Data Analysis

The data were analyzed in R, version 3.4.4 [45] using the package lme4 for all models [46]. Linear regression followed by Kruskal–Wallis test was used to test the effect of the generations (replicates) on the egg production for each egg-collection system. Statistical tests on the egg production and egg to pupa time were performed using the Mann–Whitney–Wilcoxon test (hereafter: “W”). The egg hatch was analyzed using a linear mixed model where the two egg-collection systems was included as a fixed effect and the replicates were included as a random effect. The proportions of pupa recovery and adult production were determined as the number of pupae and adults, respectively, per total number of egg hatch. The pupae recovery and adult production proportions were analyzed using a linear mixed model where the two egg-collection systems and the day of collection (number of days since the given replica started) were included as fixed effects and the replicates were included as random effect. To analyze the quality control parameters (adult emergence and flight ability), we used a linear mixed model where the two egg-collection systems were included as a fixed effect and the replicates were included as a random effect. In all data, the mean \pm standard error is reported. The statistical analyses (Supplementary Materials, S1) and raw data (Supplementary Materials, S2, S3), are available online.

3. Results

3.1. Production Parameters

Initially, the number of eggs per volume unit was assessed to enable volumetric measurements of the eggs. On average, 2303.8 ± 269.6 eggs were counted in a volume of 0.1 mL over seven replicates.

The replicates (generations) had no effect on the egg production for either egg-collection system (Kruskal–Wallis: $\chi^2 = 6.0276$, $df = 9$, $p = 0.7372$). The daily egg production was not significantly different for the two oviposition systems ($W: 3739.5$, $p = 0.0521$, $N = 182$), with an average of 0.48 ± 0.24 mL

of eggs collected daily with the wax oviposition system and 0.43 ± 0.21 mL with the netted system (Figure 2).

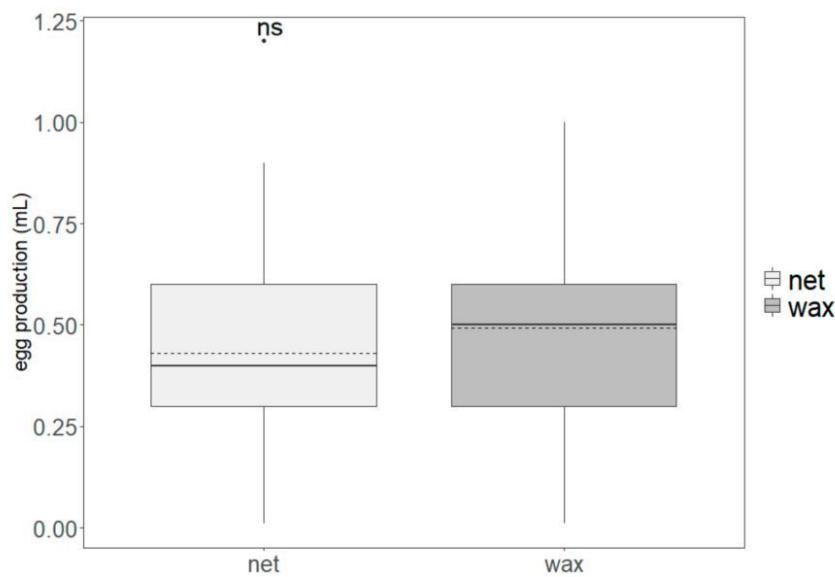


Figure 2. Volume of eggs of *Drosophila suzukii* produced in cages with netted and wax panel oviposition systems. Boxes indicate the interquartile range, bold lines indicate medians, dashed lines indicate means, whiskers indicate minimum and maximum values, and the dot indicates an outlier. Non-significant differences between treatment groups are indicated with “ns” ($p > 0.05$).

The egg hatch was significantly higher for the wax system compared to the netted system ($t\text{-value} = 5.30$, $df = 182$, $p < 0.0001$). The average of the daily percentage of egg hatch (\pm S.E.) was 42.7 ± 22.6 and 57.3 ± 16.9 for the net and wax cages, respectively (Figure 3).

For both egg-collection systems, the time from egg to pupation was similar ($W = 2883.5$, $p = 0.1538$, $N = 161$) with an average of 7.4 ± 0.8 and 7.6 ± 0.8 days from the egg-collection day to the first pupa for the net and wax system, respectively.

Pupae recovery and adult production proportions were significantly higher for the wax panel system compared to the netted system, i.e., a mean of 63.5 ± 17.1 and 56.5 ± 21.7 of pupae for the wax panel and netted system, respectively ($t\text{-value} = 2.583$, $df = 182$, $p = 0.0106$) and 56.9 ± 16.3 adults for the wax panel, compared with 50.4 ± 21.6 adults for the netted system ($t\text{-value} = 2.435$, $df = 182$, $p = 0.0158$) (Figure 3).

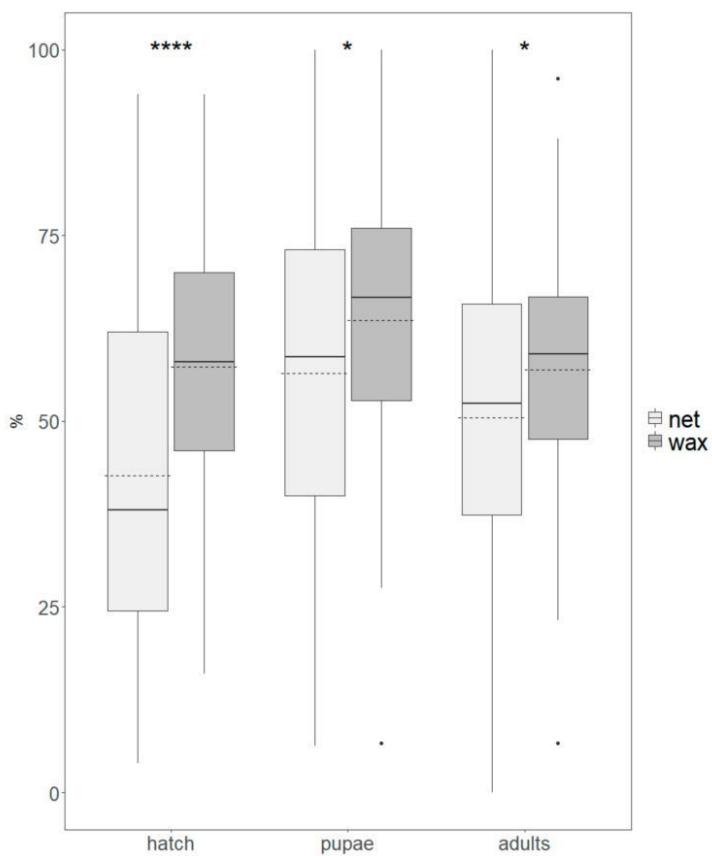


Figure 3. Percentages of egg hatch, pupa recovery, and adult production of *Drosophila suzukii* from netted (light grey) and wax (dark grey) cages. Boxes indicate the interquartile range, bold lines indicate medians, dashed lines indicate means, whiskers indicate minimum and maximum values, and dots indicate outliers. Significant differences between treatment groups are indicated with asterisks (* $p < 0.05$; *** $p < 0.0001$).

3.2. Quality Assessment

The average percentage of adults emerged from pupae produced in the netted oviposition system was $74.9\% \pm 21.2\%$, compared with an average of $85.5\% \pm 12.5\%$ adults emerged in the wax panel system. The average number of adults that were able to fly was higher in the wax system compared with the netted system ($77.6\% \pm 16.5\%$ and $66.5\% \pm 24.1\%$, respectively).

Both the adult emergence and flyer data showed that there were significant differences between the netted and wax system ($t\text{-value} = 4.101$, $df = 163$, $p = 0.0001$, and $t\text{-value} = 3.700$, $df = 163$, $p = 0.0003$, respectively) (Figure 4).

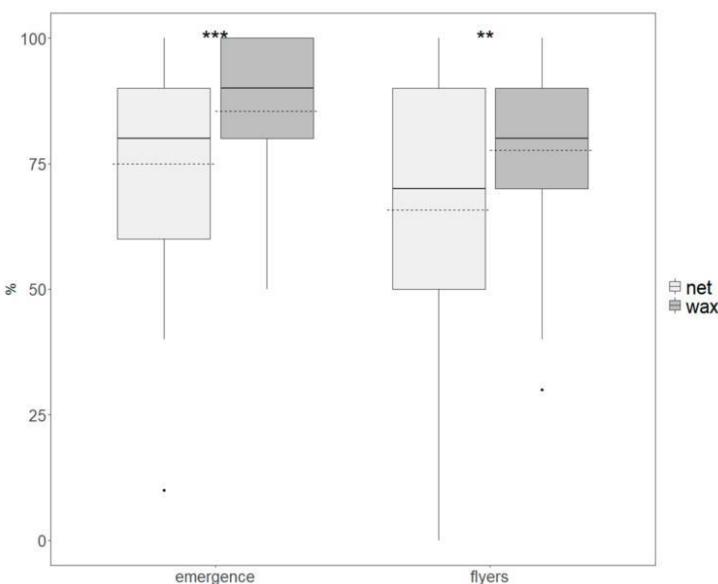


Figure 4. Adult emergence (left) and flyers (right) of *Drosophila suzukii* produced by the netted (light grey) and wax panel (dark grey) oviposition systems. Boxes indicate the interquartile range, bold lines indicate medians, dashed lines indicate means, whiskers indicate minimum and maximum values, and dots indicate outliers. Significant differences between treatment groups are indicated with asterisks (** $p < 0.001$; *** $p < 0.0001$).

4. Discussion

An efficient egg-collection system is a prerequisite for the development of a cost-effective and practical mass-rearing system for insects. In tephritids that oviposit eggs in fresh fruits, several methods have been assessed and developed for artificial mass production. Those methods range from perforated plastic egg bottles that contain fruit juice as attractants for *Bactrocera* species to mesh plastic nets for *Ceratitis* species and silicon panels for *Anastrepha* species [25,47]. All these systems enable the designing of a rearing cage prototype that enables easy handling and cleaning [48,49].

The SIT can be integrated into the IPM practices for the control of *D. suzukii*, but for the mass-rearing process, it is necessary to develop a cost-effective artificial egg-collection system. We have developed two potential systems that may be used as routine protocol for the artificial egg production of *D. suzukii*. In this study, we compared netted and wax panel systems. The rearing in both systems was artificial and eggs were collected without the need for further separation from any natural or semi-natural host substrate. Consequently, all eggs oviposited were quantified, allowing an accurate estimate of total colony production.

The wax panel system and as the netted system were based on the same principle as the widely used “egg-dropping” technique [10]. This method has proven to be a practical and productive oviposition system for the mass-rearing of various tephritid species [25,27], having been routinely used for more than 30 years in mass-rearing facilities [50].

The wax panel system was more efficient than the netted system with respect to egg hatch and number of pupae and adults produced. Previous studies have shown that the number of eggs oviposited by females can be influenced by the artificial rearing method, reducing the quantity and quality of all development stages [51]. The results revealed that the netted method caused damage to a

relatively high percentage of eggs during oviposition or during the egg-collection process. The risk of egg damage is important in *D. suzukii*, of which the eggs have two respiratory appendages that can easily be harmed.

In this study, the quality of the flies produced with the two methods was assessed. In tephritid fruit flies as well as in other insect species, mass-rearing conditions often compromise the quality of adults, e.g., damaged wings or shortened lifespan, reducing the performance of males after release in the field [52]. Our results showed that flies produced using the wax panel system were of better quality with regards to adult emergence and flight ability compared with the netted system. Therefore, production efficiency and quality of the *D. suzukii* was higher in the wax panel system.

Following an initial stressful period due to host restriction or deprivation, egg-laying behavior may be altered, resulting in females that can oviposit in unusual oviposition sites. Hence, after several generations and due to selection pressure, females can adapt their egg-laying pattern to artificial rearing conditions [53]. Therefore, considering the relatively short adaptation time of the colony to the two rearing systems, there is potential for future improvement of the *D. suzukii* mass-rearing system. Large quantities of high-quality sterile males are crucial to ensure optimal implementation of the SIT as a pest control tactic for *D. suzukii*. In spite of the fact that specific data were not collected in this study, our experience suggests that the use of a wax panel as an artificial oviposition system could facilitate the daily practices of colony maintenance and thus become more cost-effective for the mass-rearing of *D. suzukii*.

This study presents an important improvement in the development of the mass-rearing cage for *D. suzukii* regarding oviposition. An ultimate rearing cage design to optimize egg female production would require additional investigations on optimal cage size, adult density, quality, and quantity of adult food. It will be necessary to undertake more research and to assess the impact of different larval diets, establishing a pupae separation system, and refining the environmental parameters required for maintaining the colony.

5. Conclusions

This was the first study to develop and compare two oviposition systems for the rearing of *D. suzukii*. It was also the first to evaluate an oviposition system in large-scale rearing cages for this pest. The two oviposition systems were tested and the wax panel was more efficient as well as more practical compared with the netted system. Further studies will be crucial for the development of the whole SIT technology package targeted at *D. suzukii*.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2075-4450/10/12/448/s1>, S1: Statistical analyses, S2: Dataset all parameters, S3: Dataset quality control parameters.

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3. Conclusion

In the last decade, the imminent need for a solution to its invasiveness has made SWD a perfect species for pest management studies (Atallah, 2014). The ongoing progress on alternative biological methods to fight this pest has brought promising results, however all of the areas impacted by SWD are mostly subjected to chemical-dependent control strategies (Sial et al., 2019).

The objectives of this thesis focused on the SIT part of the project by developing species-specific protocols for the irradiation and mass-rearing of SWD. In particular, a dose-response sterility curve for sterilization through irradiation of SWD was established (Sassù et al., 2019a) and a practical oviposition method was identified for the mass-rearing of this pest (Sassù et al., 2019b).

A wide range of gamma radiation doses was tested on *D. suzukii* pupae and the differences in radiological sensitivity of the treated pupae assessed under two atmosphere conditions. As previously reported in other insect species, irradiation under hypoxia atmosphere required higher doses to obtain the same sterility in SWD than under normoxia (Yamada, 2019). As a result, potential target doses that allowed for the full and partial sterility of the males were identified and these would be recommended for an eradication and suppression programme, respectively. Consequently, the emergence rate and flight ability tests were used as quality control measurements to evaluate the effect of the selected doses on SWD adults under both atmosphere conditions. Results did not show negative effects on the studied parameters, concluding that sterility does not compromise the ability of SWD flies to emerge or fly. There were also no differences in the emergence rate or flight ability despite the atmosphere

conditions. Therefore, sterile males can be employed for SIT purposes, and the hypoxia atmosphere applied so as to facilitate SIT procedures such as transportation and shipment to the final release centre (Sassù et al., 2019b). As a second objective, easy-to-use egg-collection systems were developed and adapted to specifically designed cages for the mass-rearing of SWD. The rearing in both systems was completely artificial and all the eggs collected could be quantified, allowing an accurate estimate of total colony production. Consequently, one of the two methods was selected based on the comparison of the total colony production and quality, creating the first available practical egg-collection system so far for the mass rearing of SWD (Sassù et al., 2019a). The establishment of an optimal irradiation sterility curve as well as an oviposition system of SWD represent indispensable starting points for the successive development of SIT protocols. However, the negative effects on the insect's fitness caused by the irradiation exposure and/or mass-rearing processes required further assessments. High-quality sterile males are indeed essential to guarantee successful matings and the efficacy of the programme (Parker, 2007). Therefore, additional investigations to determine the mating competitiveness of SWD sterile males are strongly recommended. A recent study conducted under laboratory conditions has demonstrated that sterility did not influence the mating likelihood of sterile males compared to fertile males (Krüger et al., 2019). Likewise, preliminary laboratory tests assessing the percentage of male mating success when pupae are treated under hypoxia prior and during irradiation confirmed similar results (Sassù et al, unpublished data), bringing promising expectations for male performance once in the field. The damage that may be caused to the irradiated pupae and/or adults by the handling and releases procedures also need to be characterized whereas the effect of the prolonged hypoxia treatments due to the shipment is already under revision. (Sassù & Enriquez, unpublished data). Until the

assessment of male quality is evaluated in various settings, the IIT can be considered as a credible alternative in assisting SIT in the population suppression of SWD. The combined action of irradiation and CI can significantly reduce radiation dose and its subsequent effect on male quality (Nikolouli, 2020). The effort required for the advancement and future implementation of SIT on SWD is still considerable. However, the novel prospective offered by the gene editing technology CRISPR/Cas9 (Kyrou et al., 2018) and genomics of SWD opens a range of possibilities of genetic modifications that might foster this process (Buchman et al., 2018; Kalajdzic & Schetelig, 2017).

In conclusion, this study improves our knowledge of SIT and encourages its use to control SWD in an environment-friendly way.

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<https://doi.org/10.1038/s41586-019-1407-9>

5. Appendix

5.1 Curriculum vitae

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Research experience

July 2016 – October 2019

Ph.D. student in Insect pest control laboratory, FAO/IAEA, Vienna.
Supervisor: Ph.D. C. Cáceres

October 2015 – February 2016

Internship in the chemical ecology group, Foundation Edmund Mach, San Michele All'Adige.
Supervisor: Univ. Prof. G. Anfora

May 2015 – September 2015

Internship in the Department of Biology and Biotechnology "Charles Darwin", University Sapienza, Rome.
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Publications

Tait, G., Vezzulli, S., Sassù, F., Antonini, G., Biondi, A., Baser N, Sollai G, Cini A, Tonina L, Ometto L, & Anfora, G. (2017). Genetic variability in Italian populations of *Drosophila suzukii*. BMC Genetics, 18(1), 87.

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