Rapport final

No projet : IA113135

Titre : Les lâchers d’insectes stériles comme méthode de lutte biologique contre la drosophile à ailes tachetées : protocole de stérilisation, compétitivité des mâles irradiés et lâchers en conditions semi-naturelles.

Responsable scientifique : Annabelle Firlej

Établissement : IRDA

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Section 1 - Chercheurs impliqués et responsable autorisé de l'établissement (ces personnes doivent également faire parvenir un courriel pour attester qu'ils ont lu et approuvent le rapport.)

Annabelle Firlej, Ph.D., IRDA, chercheure en entomologie fruitière.

Jacques Brodeur, Ph.D., Université de Montréal, professeur et titulaire d'une chaire de recherche en lutte biologique.

Véronique Martel, Ph.D., Ressources Naturelles Canada, chercheure en entomologie forestière.

François Fournier, M.Sc., Cégep Montmorency, professeur et consultant.

Stéphane P. Lemay, ing., P.Eng., agr., Ph.D., IRDA, directeur de la recherche et du développement et responsable autorisé de l' Institut.

Section 2 - Partenaires

Geneviève Lanouette, M.Sc., Université de Montréal, étudiante.

Franz Vanoosthuyse, M.Sc., IRDA, professionnel de recherche.

Michèle Grenier, M.Sc., IRDA, statisticienne.

Marc Vreysen, Ph.D., Agence Internationale de l'Énergie Atomique, chercheur et directeur du laboratoire de contrôle des insectes nuisibles.

Carlos Cáceres,, Ph.D., Agence Internationale de l'Énergie Atomique, chercheur et responsable de la section recherche sur les mouches à fruits.

Section 3 – Fiche de transfert

Les lâchers d'insectes stériles comme méthode de lutte biologique contre la drosophile à ailes tachetées

Annabelle Firlej, Jacques Brodeur, François Fournier et Véronique Martel

No de projet : IA113135 **Durée :** 05/2014 – 12/2017

FAITS SAILLANTS

La drosophile à ailes tachetées est un ravageur exotique particulièrement dommageable pour les cultures de petits fruits puisqu'elle s'attaque aux fruits en murissement. Depuis son invasion sur les continents américain et européen en 2008, ses populations sont contrôlées majoritairement avec des insecticides chimiques. L'objectif de cette étude était d'explorer le potentiel de la technique des insectes stériles comme approche de lutte à la drosophile à ailes tachetées, d'abord en irradiant des pupes à différentes doses pour identifier la dose optimale pour la stérilisation des mâles et, par la suite, en évaluant les capacités reproductives de ces mâles irradiés en laboratoire et en condition semi-naturelle (lâchers d'individus en cage avec deux pots de framboisier). En premier lieu, il a été observé que l'irradiation n'avait pas d'effet significatif sur l'émergence, la malformation et la longévité des adultes à chacune des doses testées. Suite à l'accouplement de mâles irradiés avec des femelles saines, deux équations ont été obtenues permettant d'établir une relation entre la dose d'irradiation et le taux d'éclosion des œufs et la survie des individus jusqu'au stade adulte. Les capacités reproductives des mâles irradiés à la plus haute dose testée (120 Gy) ont ensuite été comparées à celles des mâles non-irradiés. Les mâles irradiés se sont révélés aussi efficaces que les mâles non-irradiés pour accoupler et transmettre du sperme aux femelles en absence de compétition. En conditions de compétition, le succès d'accouplement des mâles irradiés a été de 37,5% et de 62,5% par les mâles non-irradiés. Une expérience sur le ré-accouplement des femelles a conclu que celui-ci était peu fréquent et n'était pas influencé par l'irradiation des mâles. Lors de lâchers en cage en situation de compétition en laboratoire, les quatre doses d'irradiation (80, 90,100, 120 Gy) se sont avérés équivalentes alors que lors de lâchers en condition semi-naturelle, ce sont les doses de 80 et 120 Gy qui ont générés les mâles irradiés les plus compétitifs.

OBJECTIF(S) ET MÉTHODOLOGIE

Les deux principaux objectifs de cette étude étaient de déterminer la dose d'irradiation optimale de stérilisation de *D. suzukii* et d'analyser, en laboratoire et en condition semi-naturelle, la compétitivité des mâles irradiés. Plus précisément, nous avons irradié avec des rayons gammas des pupes de *D. suzukii* à sept doses différentes (30, 50, 70, 80, 90, 100, 120 Gy), puis évalué l'émergence des adultes, leur malformation et leur longévité. Nous avons accouplé les individus irradiés à des individus sains du sexe opposé afin de déterminer la fécondité des femelles (nombre d'œufs pondus), la fertilité des oeufs (taux d'éclosion), ainsi que le taux de survie (d'œuf à adulte). Par la suite, nous avons étudié les capacités d'accouplement des mâles irradiés à 120 Gy (la plus haute dose) par rapport à celles des mâles non-irradiés, ceci afin de déterminer si la dose induisant la meilleur stérilité diminue les capacités des mâles irradiés. Une expérience a testé la quantité de femelles qu'un mâle peut accoupler en 24 h, une deuxième expérience a identifié le mâle gagnant en conditions de compétition (un mâle sain et un mâle irradié pour une seule femelle). Puis une dernière expérience a eu pour but de déterminer si les femelles acceptent un second accouplement, et si ce choix est influencé par l'irradiation de son premier partenaire. Deux dernières expériences en laboratoire et en condition semi-naturelle ont testé la compétitivité des mâles irradiés aux quatre plus hautes doses (80, 90,100,120 Gy).

RÉSULTATS SIGNIFICATIFS POUR L'INDUSTRIE

1) Mise au point d'un protocole de stérilisation pour *D. suzukii*

Le taux d'émergence moyen de 88,1%(Fig. 1), le pourcentage moyen de *D. suzukii* déformés de 4,0% (Fig. 1) et les courbes de survies des mâles et femelles *D. suzukii* (Fig. 2) ne sont pas affectés par les différentes doses d'irradiation testées. Les femelles irradiées à la dose de 50 Gy sont complétement stériles. La fertilité des œufs pondus par des femelles accouplées à des mâles irradiés atteint 4% pour la dose de 120 Gy comparativement à 82,6% pour les œufs issus d'accouplement avec des mâles témoins (Fig. 3).

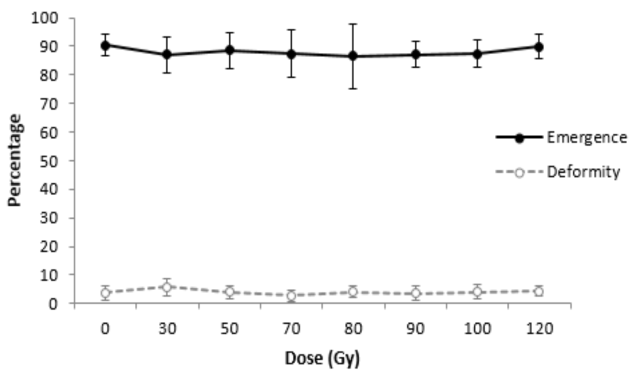

Figure 1: Effet de la dose d'irradiation (Gy) sur le pourcentage d'émergence et d'adultes déformés de *D. suzukii*. Modèle binomial linéaire généralisé (P> 0,05).

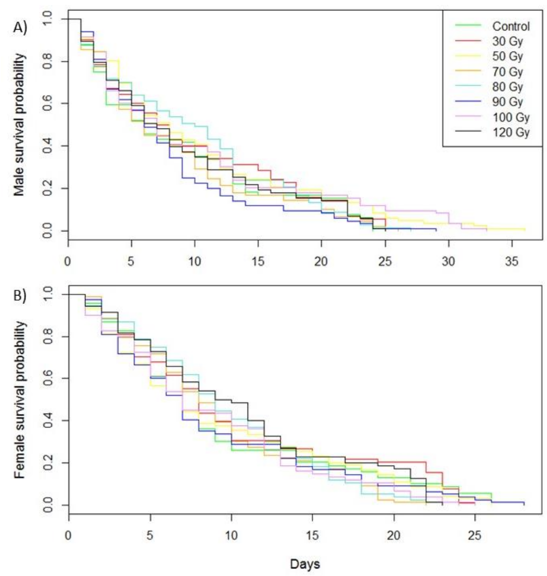

Figure 2: Effet de la dose d'irradiation (Gy) sur la longévité des mâles (A) et femelles (B) de *D. suzukii*. Mantel-Cox log-rank test (P>0,05).

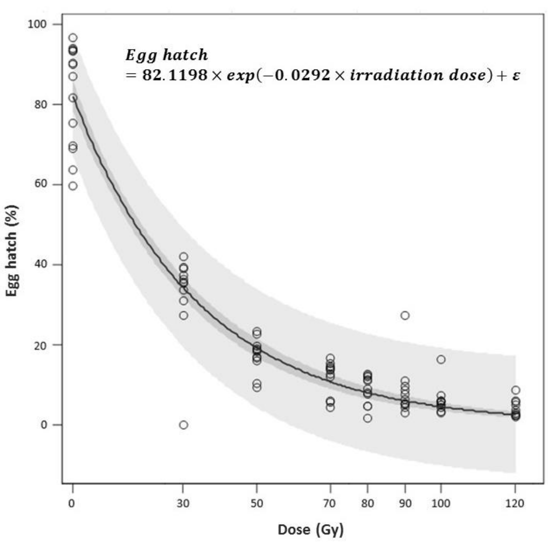

Figure 3 : Effet de la dose d'irradiation (Gy) sur l'éclosion des œufs chez des femelles *D. suzukii* non irradiées et accouplées avec des mâles irradiés à différentes doses (Les zones grisées représentent les limites de confiance de 95% et 99%).

2) **Évaluation de la compétitivité des mâles irradiés en laboratoire et conditions semi-naturelles.**

Les males irradiés à 120 Gy sont capables de copuler et de transférer du sperme aux femelles ; ils peuvent inséminer 6,4 femelles en 24 h comparativement à 6,9 femelles pour un mâle non-irradié (Fig. 4). La compétitivité des mâles irradiés a été évalué à 37,5 % et ne diffère pas des mâles non-irradiés (Fig. 5). Le ré-accouplement des femelles est relativement peu fréquent, soit 18,8 % des cas deux jours après que les femelles aient été accouplées à un mâle irradié à 120 Gy.

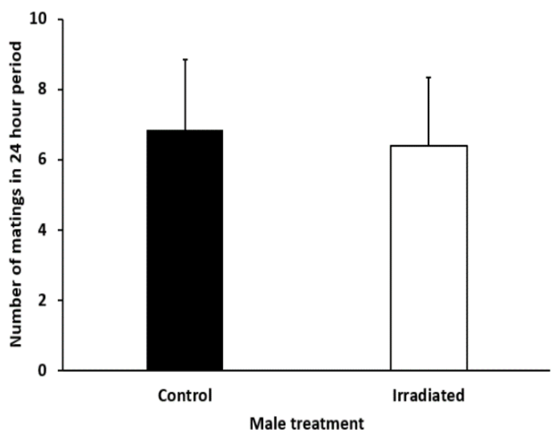

Figure 4 : Nombre de femelles inséminées en 24 h par un mâle irradié à 120 Gy et un mâle non-irradié. Modèle binomial linéaire généralisé (P> 0,05).

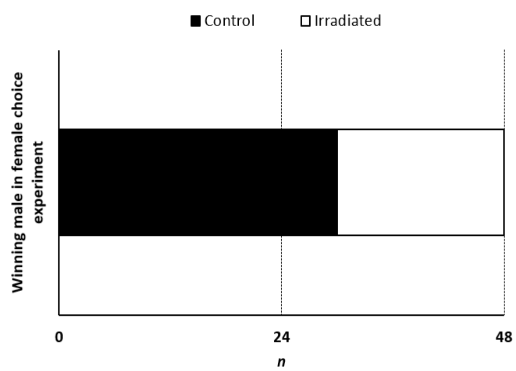

Figure 5 : Fréquence observée du mâle irradié à 120 Gy ou non-irradié (contrôle) ayant accouplé une femelle en situation de compétition. Modèle binomial linéaire généralisé (P> 0,05).

APPLICATIONS POSSIBLES POUR L'INDUSTRIE ET/OU SUIVI À DONNER

L'irradiation de pupes de *D. suzukii* à une dose de 120 Gy permet d'atteindre un niveau de stérilité acceptable sans diminuer significativement les attributs biologiques et compétitifs des mâles irradiés. Afin de finaliser l'évaluation du potentiel de la technique des insectes stériles pour *D. suzukii*, les étapes suivantes seraient d'étudier 1) les capacités d'envol et de dispersion de ces mâles irradiés, 2) les capacités d'accouplement avec des femelles sauvages en nature, 3) les effets de différents ratio de lâcher en condition « producteur », 4) l'optimisation de l'élevage de masse du ravageur et 5) l'analyse des paramètres économiques associés à une telle technique.

POINT DE CONTACT POUR INFORMATION

Nom du responsable du projet : Annabelle Firlej
Téléphone: 450-653-7368 poste 363
Télécopieur : 450-653-1927
Courriel : annabelle.firlej@irda.qc.ca

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Section 4 - Activité de transfert et de diffusion scientifique (joindre en annexe la documentation en appui)

Lanouette, G., J. Brodeur, F. Fournier, V. Martel, M. Vreysen, C. Caceres et A. Firlej. 2016. Utilisation de mâles stériles pour le contrôle de la drosophile à ailes tachetées *Drosophila suzukii* (Diptera : Drosophilidae): établissement de la dose optimale d'irradiation. Congrès de la Société d'entomologie du Québec. Québec, Canada. (Annexe 1)

Lanouette, G., J. Brodeur, F. Fournier, V. Martel, M. Vreysen, C. Caceres et A. Firlej. 2016. Preliminary results of radiation dose responses of *Drosophila suzukii* (Matsumura) for use in the Sterile Insect Technique (SIT). International Congress of Entomology. Orlando, USA. (Annexe 2)

Firlej, A. 2016. Past and ongoing projects on Spotted Wing Drosophila. Canadian Webinar Series, first session : Spotted wing Drosophila (Fruit fly). (Annexe 3)

Lanouette, G. 2017. La technique des insectes stériles comme méthode de lutte intégrée contre la drosophile à ailes tachetées (*Drosophila suzukii*). Séminaire de maîtrise, Université de Montréal. Montréal, Canada. (Annexe 4)

Firlej, A., G. Lanouette, M. Vreysen, C. Caceres-Barrios, F. Fournier, V. Martel et J. Brodeur. 2017. Radiation Dose Responses of *Drosophila suzukii* (Matsumura) for Use in the Sterile Insect Technique (SIT). Third FAO–IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques. Vienne, Autriche. (Annexe 5)

Firlej, A., G. Lanouette, M. Vreysen, C. Caceres-Barrios, F. Fournier, V. Martel et J. Brodeur. 2017. Recent advance in the development of the sterile insect technique for *Drosophila suzukii*. Société d'entomologie du Canada. Winnipeg Manitoba. (Annexe 6)

Lanouette, G., J. Brodeur, F. Fournier, V. Martel et A. Firlej. 2017. Capacité d'accouplement et compétitivité des mâles *Drosophila suzukii* suite à des irradiations. Congrès de la Société d'entomologie du Québec. Longueuil, Canada. (Annexe 7)

Section 5 - Activités de diffusion et de transfert aux utilisateurs (joindre en annexe la documentation en appui)

Lanouette, G. 2016. La technique des lâchers de mâles stériles appliquée à la DAT : les premiers résultats laboratoire. Journées de formation du RAP petits fruits. Drummondville, Canada. (Annexe 8)

Firlej, A. 2016. Potentiel de la technique des lâchers d'insectes stériles pour lutter contre la drosophile à ailes tachetées. Visite des parcelles de recherche à la Plateforme d'innovation en agriculture biologique de Saint-Bruno-de-Montarville dans le cadre de la 2ième Conférence scientifique canadienne en agriculture biologique. Saint-Bruno-de-Montarville, Canada. (Pas de documentation à l'appui car présentation orale sans support)

Section 6 – Grille de transfert des connaissances

1. Résultats Présentez les faits saillants (maximum de 3) des principaux résultats de votre projet.	**2. Utilisateurs** Pour les résultats identifiés, ciblez les utilisateurs qui bénéficieront des connaissances ou des produits provenant de votre recherche.	**3. Message** Concrètement, quel est le message qui devrait être retenu pour chacune des catégories d'utilisateurs identifiées? Présentez un message concret et vulgarisé. Quels sont les gains possibles en productivité, en rendement, en argent, etc.?	**4. Cheminement des connaissances** a) Une fois le projet terminé, outre les publications scientifiques, quelles sont les activités de transfert les mieux adaptées aux utilisateurs ciblés? (conférences, publications écrites, journées thématiques, formation, etc.) b) Selon vous, quelles pourraient être les étapes à privilégier en vue de maximiser l'adoption des résultats par les utilisateurs.
Des doses d'irradiation de 80 à 120 gray induisent des niveaux de stérilité des œufs de 8,5 à 4% des drosophiles à ailes tachetées. La dose la plus élevée de 120 Gy ne diminue pas significativement la compétitivité des mâles stériles	Scientifiques, producteurs et conseillers	La technique des insectes stériles démontre un potentiel de développement pour la drosophile à ailes tachetées et nécessite de réaliser des essais en conditions naturelles. Les gains possibles si la technique voit le jour sera la réduction des dommages dû à la drosophile et la réduction des applications d'insecticides utilisés pour la contrôler.	a) -Scientifiques : présentation dans des congrès internationaux -Producteurs : conférence aux Journées horticoles de St-Rémi; -Conseillers : Conférence à la journée de formation du RAP petits fruits b) Obtenir le financement d'un projet pour la poursuite des expériences terrain

Section 7 - Contribution et participation de l’industrie réalisées

La compagnie Phytodata a été approchée lors de l’élaboration du projet et les résultats avancés leur ont été présentés en mars 2017. Ce transfert a ainsi mené à la rédaction d’un nouveau projet déposé au programme Prime-Vert Volet 4 en novembre 2017 afin de passer à l’étape d’essais sur le terrain avec ce partenaire.

Section 8 - Rapport scientifique et/ou technique (format libre réalisé selon les normes propres au domaine d'étude)

Introduction

La drosophile à ailes tachetées, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), un nouveau ravageur apparu au Québec en 2010, impose de nouveaux défis à l'industrie des petits fruits. Cette mouche à fruits attaque tous les petits fruits cultivés à peau molle, et au contraire de *D. melanogaster* qui utilise les fruits en décomposition, *D. suzukii* se reproduit dans les fruits en mûrissement. Ses capacités de reproduction impressionnantes, sa rapidité de développement et ses stades larvaires inatteignables dans le fruit rendent la lutte à ce ravageur difficile. Les dommages aux récoltes pour certaines cultures non protégées peuvent avoisiner les 100 %; c'est pourquoi la lutte chimique reste actuellement la seule méthode de lutte pour les producteurs. Les lâchers d'insectes stériles s'avèrent une technique de lutte biologique utilisée dans plusieurs pays pour contrer divers diptères ravageurs des cultures (mouches à fruits et mouches légumières) ou des diptères vecteurs de maladies humaines (insectes hématophages). Les lâchers de *D. suzukii* stériles constituent une technique prometteuse à évaluer selon Cini et al. (2012) et font partie des priorités de recherche identifiées par l'Association des producteurs de fraises et framboises du Québec (APFFQ). Les lâchers de mâles stériles pour *D. suzukii* est donc une technique dont le potentiel doit être évalué afin de trouver des solutions de lutte pour ce ravageur. Le présent projet a évalué différents objectifs de recherche: 1) Mettre au point un protocole de stérilisation pour *D. suzukii* et 2) évaluer la compétitivité des mâles *D. suzukii* irradiés en laboratoire et conditions semi-naturelles.

Volet 1 : Mettre au point un protocole de stérilisation pour *D. suzukii*

Suite aux expériences réalisées pour répondre aux objectifs spécifiques de ce volet (1-Déterminer la dose d'irradiation pour la stérilisation de *D. suzukii* et 2-étudier la stérilité induite par les mâles irradiés sur la progéniture F1), un article scientifique a été publié en septembre 2017 dans la revue Plos One et illustre tous les résultats scientifiques originaux obtenus (voir ci-dessous).

RESEARCH ARTICLE

The sterile insect technique for the management of the spotted wing drosophila, *Drosophila suzukii*: Establishing the optimum irradiation dose

Geneviève Lanouette[1]*, Jacques Brodeur[2], François Fournier[3], Véronique Martel[4], Marc Vreysen[5], Carlos Cáceres[5], Annabelle Firlej[1]

1 Institut de recherche et de développement en agroenvironnement, Saint-Bruno-de-Montarville, Québec, Canada, **2** Institut de recherche en biologie végétale, Université de Montréal, Montréal, Québec, Canada, **3** Collège Montmorency, Laval, Québec, Canada, **4** Centre de foresterie des Laurentides, Ressources naturelles Canada, Québec, Québec, Canada, **5** Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria

* genevieve.lanouette@umontreal.ca

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Abstract

The spotted wing drosophila *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), a pest of berries stone fruits, invaded North America and Europe in 2008. Current control methods rely mainly on insecticides. The sterile insect technique (SIT) has potential as an additional control tactic for the integrated management of *D. suzukii.* As a step towards the development of the SIT, this study aimed at finding the optimum irradiation dose to sterilize *D. suzukii* under controlled laboratory conditions. Four-day-old *D. suzukii* pupae were irradiated 12 to 24 hours prior to adult emergence in a ^{60}Co Gamma Cell 220 and in a ^{137}Cs Gamma Cell 3000 with doses of 30, 50, 70, 80, 90, 100 or 120 Gy. Emergence rate (88.1%), percent of deformed flies (4.0%) and survival curves were not affected by the tested irradiation doses. However, some reproductive parameters of the flies were affected by irradiation. Females irradiated with a dose of 50 Gy or more had almost no fecundity. When non-irradiated females were mated with irradiated males, egg hatch decreased exponentially with irradiation dose from 82.6% for the untreated control males to 4.0% for males irradiated with 120 Gy. Mortality of F1 individuals from the irradiated treatment also occurred during larval and pupal stages, with an egg to adult survival of 0.2%. However, descendants produced by the irradiated generation were fertile. These results are an encouraging first experimental step towards the development of the SIT for the management of *D. suzukii* populations.

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive pest of berries and stone fruits. It is a native from Southeast Asia and was first reported in North America (California) and Europe (Spain) in 2008 [1, 2]. By 2010, the fly was present in nine states in the USA and four Canadian provinces [1] and in 2013 had reached South America [3]. Unlike

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Competing interests: The authors have declared that no competing interests exist.

other *Drosophila* species that thrive on decaying fruits, *D. suzukii* has a serrated ovipositor that allows females to lay eggs in ripening and marketable fruits [4]. Not only will the developing larvae damage the fruit, but the puncture made by the ovipositor is an entry point for pathogenic fungi and bacteria [5]. Initially a pest of cherries in Asia, *D. suzukii* has expanded its host range to more than 15 commercial plant species including raspberries, blueberries, strawberries and grapes within invaded regions where severe damaged are observed [4]. Between 2009 and 2014, California alone experienced revenue losses of $36.4 million and $3.4 million in conventional and organic production, respectively [6]. To protect crops from *D. suzukii* infestation, growers mostly have been using chemical insecticides [7–10], requiring an average of four to six applications per growing season [6, 11]. Insecticide use leads to environmental pollution, public health issues, pest resistance and mortality of natural enemies [12]. Alternative methods are urgently needed to reduce our dependence on insecticides for the sustainable management of *D. suzukii*.

Alternative methods to insecticides, effective or under evaluation, include mass-trapping of adult flies using attractants [13, 14], protecting crops with exclusion nets [15], cultivating crops inside closed tunnels [16], spraying crops with natural repellents [17, 18], harvesting more frequently [4] and postharvest refrigeration, fumigation or irradiation [19–21]. Other avenues include the use of native and/or exotic parasitoids [22–24], generalist predators [25] or entomopathogenic fungi [26]. One alternative approach that remains unexplored is the sterile insect technique (SIT).

The SIT consists of mass-rearing the insect pest in specialized facilities, exposing pupae or adults to ionizing radiation to induce reproductive sterility and releasing them in the target area. The released sterile males will mate with virgin wild females and, as a result, the females will lay unfertile eggs. A sustained release of sterile males in a target area aims at reducing the pest population over time below an acceptable economic threshold, or even reaching local eradication in certain ecological settings [27]. The sterilization of males and females is achieved by ionizing radiation, mainly using gamma or X rays which have high levels of energy and penetration power [28, 29]. The irradiation triggers lethal mutations in the sperm and, following insemination of the oocyte, the death of the developing embryo [30].

The SIT has been used effectively against many crop and livestock pests and disease vectors. Following the successful eradication of the New World screwworm *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae) from the USA, Mexico, Central America and Panama [31], the technique has been increasingly used worldwide for the management of several Tephritidae fruit flies, tsetse flies and Lepidoptera [32]. Its success depends on the knowledge of the pest biology, the competitiveness of the irradiated males and the sustained and area-wide release of sterile insects [33, 34].

A crucial and initial step in the development of an SIT program is to determine the optimal irradiation dose for the targeted pest species. The optimal dose should sterilize individuals without impairing critical traits of their biology such as their ability to mate. Males exposed to high irradiation doses are potentially less likely to compete and mate with wild females than non-irradiated males. A dose inducing 100% sterility is rarely used as it usually incurs excessive somatic damage to the insects [30, 35]. For example, Toledo et al. [36] found that an irradiation dose of 40 Gy induced an average of 99.5% sterility in male *Anastrepha obliqua* Macquart (Diptera: Tephritidae) and that these irradiated males were twice as effective in increasing the amount of sterile eggs in the population than fully sterile males irradiated with 80 Gy. For an optimal use of the SIT, irradiation should cause high sterility without affecting emergence rate, rate of deformed males, adult longevity, and the fecundity of healthy females mated with irradiated males.

The biological quality of irradiated males is assessed using various parameters, e.g. percentage adult emergence, percentage deformed insects, flight ability, longevity with or without access to food, fecundity of inseminated females (number of eggs produced), fertility (percentage of hatching), presence or absence of sperm transfer, male mating competitiveness, and overall capacity to reduce pest populations under semi-field or field conditions [37–41].

This study is part of a large research program that aims to determine the feasibility of using the SIT as an additional control tactic for the integrated pest management of *D. suzukii* populations in fruit crops. The present objective is to quantify the effects of different gamma irradiation doses applied to *D. suzukii* pupae on several biological attributes of irradiated individuals and their descendant (emergence, deformed males, longevity, fecundity, fertility of parent and descendant flies).

Material and methods

Insect colonies and rearing method

A *D. suzukii* colony was established from individuals collected in a vineyard near San Michele all Adige, Trentino, Italy. The colony had been in culture for one year before being sent to the Insect Pest Control Laboratory (IPCL) of the Joint Food and Agriculture Organization of the United Nation (FAO)/International Atomic Energy Agency (IAEA) Division of the Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria. A first set of laboratory experiments (fertility and fecundity tests) were carried out at the IPCL and the colony was thereafter sent to the Institut de Recherche et de Développement en Agroenvironnement (IRDA), St-Bruno-de-Montarville, Canada, where a second set of experiments was carried out (fecundity, emergence, deformity, longevity, F1's survival and F1's fertility tests). The colony was kept at 23 ± 1˚C, 50 ± 10% HR, and under a 16:8 L:D photoperiod. Two types of larval diet were used: a carrot powder diet developed for the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) [42] and a fresh banana diet developed for *D. suzukii* [22]. The carrot diet was used to produce large and uniformly sized pupae to be irradiated and for fecundity tests. The banana diet was used for the fertility, F1 survival and F1 fertility experiments because it provides higher egg to pupa survival (survival of 22.1% in previous unpublished test using carrot diet showed in S7 Table vs. survival of 72.1% in banana diet in present study using banana diet). When used as oviposition site, the carrot diet was spread inside a 1L rectangular container, covered with thin slices of fresh banana and put inside a rearing cage for 2 to 3 days to attract adult females. Thereafter, the diet was removed from the cage, covered with a mesh and placed under rearing conditions for larval development. Pupal extraction was done on days 6, 7 and 8 after oviposition using soft clips. Pupae were briefly washed in water and deposited on a wet makeup cotton pad. Following emergence, adults had access to water and a diet composed of white sugar and brewer's yeast (3:1). Adults were reared in 29 X 29 X 29 cm plexiglass cages ventilated with muslin netting.

Irradiation

Four day-old *D. suzukii* pupae (12 to 24 h prior to adult emergence) were irradiated in a ^{60}Co Gamma Cell 220 (MDS Nordion, Canada) for experiments conducted at the IPCL and in a ^{137}Cs Gamma Cell 3000 (Best Theratronics, Canada) at the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) for experiments in Canada. Gamma rays emitted by ^{60}Co or ^{137}Cs are similar (C. Cáceres, personal communication), but the two radiation sources differed in their dose rate, which is known to have no effect on emergence, longevity and flight ability of tephritid fruit flies such as *Dacus cucumis* French [43] and *Bactrocera tryoni* Froggatt [44]. Experiments conducted in Austria and Canada can therefore be merged. Pupae for irradiation and control pupae were transported in a thermal bag to the irradiation center and were thus exposed to the same temperature and humidity conditions. The irradiation doses used in all experiments were 30, 50, 70, 80, 90, 100 or 120 Gy, whereas control pupae were not irradiated.

Adult emergence and deformed flies

A first objective was to correlate irradiation dose applied to pupae with percentage of adult emergence and deformed flies. We first developed a method to allow sexing of the flies and to assure that all individuals were virgin before being transferred to rearing cages following irradiation. A few hours following irradiation, *D. suzukii* pupae were placed individually in the wells of an ELISA plate identified with irradiation dose and date and covered with Parafilm®. On the following day, plates were observed every few hours from morning to late afternoon for adult emergence. From visual observation, without using anesthesia, each fly was identified through the plastic plate as male or female, and as being healthy or deformed. Sex was assessed through the presence/absence of an ovipositor at the end of the abdomen. Deformed flies included individuals that partially emerged from the pupal case, and individuals whose wings did not fully deploy following emergence. Healthy flies were removed from the plate with an insect aspirator shortly after emergence and used in experiments. The plates were observed for an additional day for late emergence, and the remaining unemerged pupae were classified as dead individuals. A total of 8,958 pupae were examined (~3000 pupae from the control and ~800 pupae for each experimental doses), allocated in 11 replicates (irradiation dates) at CRCHUM. To test for an irradiation dose effect on adult emergence and deformity, two binomial generalized linear models were used in R 3.3.2 [45].

Longevity

We tested the effect of irradiation on the average lifespan of virgin adults. Upon emergence and sexing, *D. suzukii* adults were placed in groups of ten flies of the same sex in a 15 X 15 X 15 cm plastic cage with access to water and adult diet (white sugar and brewer's yeast). Mortality was recorded each day at 9 AM until all flies had died. Two cages of ten virgin females and two cages of ten virgin males were set up for each dose and the experiment was repeated four times (n = eight cages, for a total of 80 individuals, per sex and per dose). Longevity per dose was analyzed using Kaplan-Meir survival analyses and, for each sex, survival curves were compared using Mantel-Cox log-rank tests using R 3.3.2 [45].

Fecundity

The effect of irradiation dose on fecundity was assessed for (1) ten non-irradiated females mated with ten irradiated males, (2) ten irradiated females mated with ten non-irradiated males and (3) ten non-irradiated virgin females. After emergence and sexing, *D. suzukii* adults were assigned to one of the three treatments. Flies were placed in cages similar to the ones used for longevity test with access to water, adult diet, and an egg-laying site consisting of a Petri dish (4 cm diameter) filled with carrot diet covered with slices of fresh banana. The egg-laying site was renewed three times a week. Following the pre-oviposition period, oviposition was monitored for seven days. Following oviposition, egg-laying sites were immediately observed under a stereomicroscope. *Drosophila suzukii* eggs have two visible white filaments near the anterior end coming out above the surface of the diet and allowing the embryo to breathe, which enable precise egg counting [46]. For analysis, the numbers of eggs per cage were pooled for the seven-day period.

For each male-female combination, two cages containing ten couples were set up per treatment. The experiment was replicated four times (n = eight cages per combination per dose for mated flies and n = 13 cages for virgin females). This experiment was conducted at both the IPCL and the IRDA, but virgin females were tested only at the IPCL. Data were analyzed separately for irradiated males and females. For irradiated males, a mixed model was used to examine the effect of irradiation dose on fecundity (total number of eggs laid in seven days per cage containing ten couples), adding the irradiation date as a random variable to consider possible differences between irradiation sites. For irradiated females, a mixed model with Poisson distribution and Dual Quasi-Newton test for over dispersion of data were used to examine the effect of irradiation dose on fecundity. Analysis was done using R 3.3.2 [45].

Fertility

The effect of irradiation dose on male fertility was examined by assessing egg hatch when non-irradiated females were mated with irradiated males. After emergence and sexing, ten irradiated adult males were place together with ten non-irradiated females in a cage with access to water, adult diet, and a banana slice. Flies were left together for five days for sexual maturation and mating. Thereafter, with both male and female flies present in the cages, the banana slice was changed twice a day for three consecutive days to sample the eggs. Each day, one of the banana slices was checked under a stereomicroscope (the first one was from 8 AM to 4 PM, the second and third were from 4 PM to 8 AM), and all eggs were carefully taken out with forceps and put on a black filter paper placed on a wet sponge. Eggs were incubated at rearing conditions for 48 h, after which, egg hatch was scored under a stereomicroscope. An average of 150 ± 62 eggs was harvested per cage over three days. Percent hatch was calculated for each cage and used as a measure of male fertility. Two cages containing ten couples were set up per treatment, except for the first replicate when three cages were used per treatment. The experiment was replicated five times (n = eleven cages per treatment). The data were analyzed using a non-linear method (NLIN) describing the decreasing exponential function using SAS 9.4 [47].

F1 survival

Survival of F1 larvae, i.e. descendants of parents of which the male had been exposed to irradiation, was assessed. In contrast to the previous fertility experiment, we used the banana diet because it provided higher survival of *D. suzukii* larvae. Following adult emergence and sexing, ten irradiated males were placed together with ten non-irradiated females in a cage with access to water, adult diet and 25 ml of banana diet poured into a 4 oz Solo cup without cover as oviposition site. The oviposition site was changed three times a week for two weeks. The eggs on the oviposition site were taken out of the cage and counted under a stereomicroscope. A muslin-covered top was put on the Solo cup that was kept under rearing conditions for eight days, and humidified when necessary. Then, the patch was re-examined for counting the number of pupae formed on top of the diet. The pupae were carefully taken out with forceps and put on a wet makeup cotton pad in a new clean Solo cup of the same size with muslin-covered top. The cotton pad was re-humidified when necessary until adult emergence. Adults were thereafter sexed and counted. The number of eggs, pupae and adults of each sex produced in two weeks were determined for each cage. Two cages containing ten couples were set up per treatment. The experiment was repeated four times (n = eight cages per treatment) and the data were analyzed using a non-linear method (NLIN) describing the decreasing exponential function using SAS 9.4 [47].

F1 fertility

We next assessed the fertility of F1 descendants (produced in the previous experiment), i.e. descendant flies from crossings of partially sterile irradiated males with non-irradiated females

(F0). Inherited sterility has been observed in several insect species, particularly in Lepidoptera [48]. Only descendants produced from pairs where the males had been irradiated at doses of 70 Gy and higher were tested. Upon emergence, each descendant was put individually in a 1 oz Solo cup with a muslin-covered top, 5 ml of banana diet and two non-irradiated adults of the opposite sex. Those two non-irradiated adults were replaced if they died before the end of the test. The diet was changed three times during a ten-day period. The diet was humidified and kept for eight days to determine the total number of pupae produced by each descendant. If the descendant was a male, the number of pupae produced was divided by two since he had been coupled with two females. Since the F0 couples with males irradiated at doses of 70 Gy or more produced few descendants, the number of individuals for this experiment was low, i.e. from two to eleven descendants per dose. Only average and standard deviation of the number of descendants produced per female by each F1 individuals were calculated using R 3.3.2 [45].

Results

Adult emergence and deformed flies

For each dose, gamma ray irradiation of four-day-old *D. suzukii* pupae did not have a significant effect on adult emergence (Binomial generalized linear model; $F = 0.2031$, d.f. = 7, $P = 0.663$) and percentage deformed adults (Binomial generalized linear model; $F = 0.3031$, d.f. = 7, $P = 0.580$). For each treatment, percentage emergence was high ($88.1 \pm 6.5\%$), and percentage deformed flies was low ($4.0 \pm 2.4\%$) (Fig 1).

Longevity

For both males and females, irradiation did not have a significant effect on longevity (Mantel-Cox log-rank; $X^2 = 13.5$, d.f. = 7, $P = 0.062$ for males; $X^2 = 5.2$, d.f. = 7, $P = 0.635$ for females) (Fig 2). Males survived from 1 to 36 days while females survived from 1 to 28 days.

Fecundity

On average, 320.4 ± 160.0 eggs were laid per week in control cages, while 275.8 ± 140.9 eggs were laid in cages containing ten irradiated males at any dose and ten non-irradiated females. The number of eggs sampled in one week did not differ significantly with irradiation dose given to the males (control consisted of non-irradiated males and non-irradiated females)

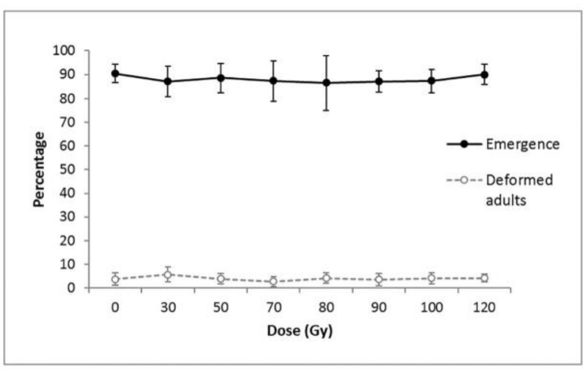

Fig 1. Effect of irradiation dose on percent *D. suzukii* adult emergence and percentage deformed adults. Significances were measured with binomial generalized linear models (P> 0.05).

https://doi.org/10.1371/journal.pone.0180821.g001

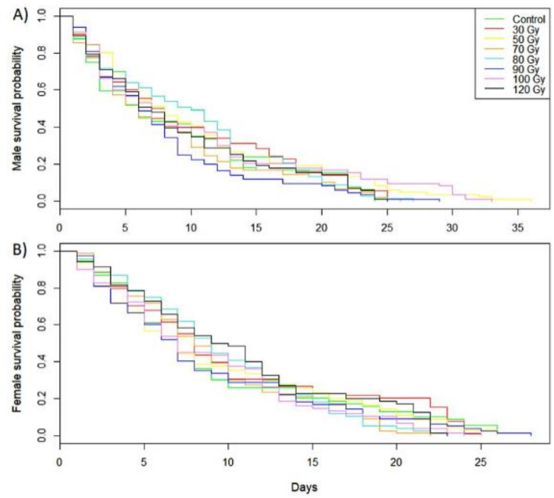

Fig 2. Effect of irradiation dose on longevity of *D. suzukii* (A) males and (B) females when flies had access to food and water. Significance were measured with Mantel-Cox log-rank test (P>0.05).

https://doi.org/10.1371/journal.pone.0180821.g002

(Linear mixed model, F = 0.2290, d.f. = 7, P = 0.634). In cages containing ten non-irradiated males and ten irradiated females, all irradiation doses drastically reduced the fecundity of *D. suzukii* females (Poisson generalized linear model, F = 53.52, d.f. = 7, P<0.0001). Females irradiated with 30 Gy laid 6.3 ± 2.0 eggs and less than one egg per cage was found for all other doses, except with 80 Gy where females had a fecundity of 3.6 ± 1.3 eggs per cage. No difference was found in fecundity of females irradiated with doses of 50 Gy and more (Multiple t-tests, P>0.05). Virgin females also laid sterile eggs, with an average of 34 ± 40 eggs per week per ten females (Fig 3).

Fertility

The hatchability of eggs laid by non-irradiated *D. suzukii* females mated with irradiated males decreased exponentially with irradiation dose (Regression, pseudo-R^2 = 0.93); i.e. from 82.6% in the untreated control cages to 4.0% in cages with males irradiated with 120 Gy (Fig 4).

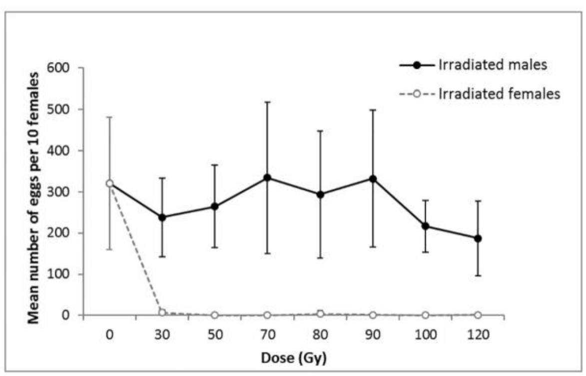

Fig 3. Effect of irradiation dose on *D. suzukii* fecundity (number of eggs oviposited in one week per ten couples) when either males or females were irradiated. Significance was measured with a Linear mixed model for irradiated males (P>0.05) and a Poisson generalized linear model for irradiated females (P<0.0001).

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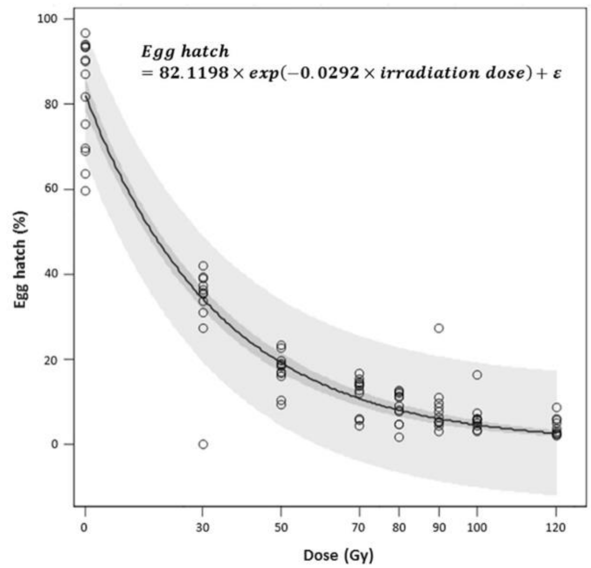

Fig 4. Effect of irradiation dose on egg hatch when non-irradiated *D. suzukii* females were mated with irradiated males. Dark and pale shaded areas represents 95% confidence limits and 95% prediction limits, respectively.

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F1 survival

Survival from egg to adult of the F1 generation decreased with irradiation dose following an exponential regression curve (pseudo-R^2 = 0.86), i.e. from 59.2% in the control cages to 0.2% in cages with males irradiated with 120 Gy (Fig 5). The difference between the egg hatch curve and the survival from egg to adult results from natural mortality at the larval and pupal stages.

F1 fertility

Too few data were obtained on the fertility of the descendants to be statistically analyzed. Nevertheless, results presented in Table 1 suggest that individuals from the F1 generation that reached the adult stage were fertile, regardless of the irradiation dose of the F0, except for the only F1 female obtained from the 120 Gy irradiated F0.

Discussion

The objective of the present study was to assess the effects of gamma irradiation on several biological attributes of *D. suzukii*. The selection of the optimal irradiation dose for use in an SIT program is a crucial step that requires careful evaluation under laboratory conditions. The optimal dose should balance high levels of insect sterility with minimal impact on their overall biological quality [35]. A small residual fertility can be accepted in situations where the competitiveness of irradiated males is preserved [29, 36]. The optimal dose is thus a trade-off between complete sterility and alterations induced in the somatic cells by irradiation.

Gamma irradiation did not cause apparent morphological damage to males and females *D. suzukii*, even at the highest dose tested (120 Gy). Provided that these results are confirmed by further evaluation of the competitiveness of irradiated males under laboratory and field conditions, this suggests that released irradiated males would be as successful as wild males to find

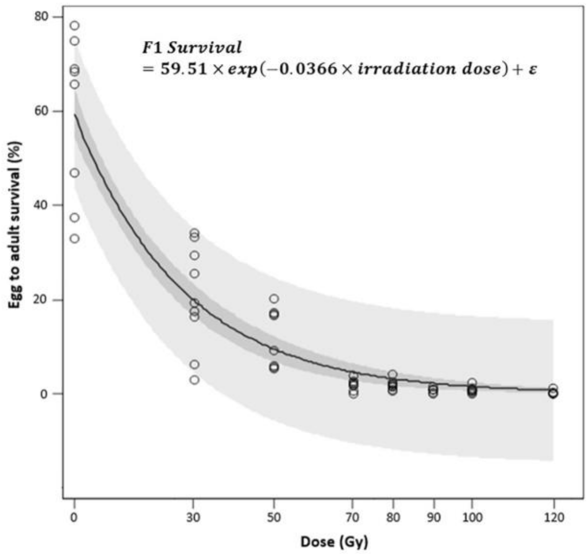

Fig 5. Effect of irradiation dose on survival to adult stage of eggs laid by non-irradiated *D. suzukii* females when mated with irradiated males. Dark and pale shaded areas represents 95% confidence limits and 95% prediction limits, respectively.

https://doi.org/10.1371/journal.pone.0180821.g005

and mate with wild females. In addition, sterile insect production facilities will not suffer losses from non-emerging pupae or deformed adults. Such a pattern has been observed in other insect species: irradiation up to 100 Gy did not impair *Anopheles arabiensis* Patton (Diptera: Culicidae) emergence and longevity [39]; irradiation with 170 Gy had no effect on emergence and longevity, with or without access to food, of the American serpentine leafminer *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) [40]. On the other hand, Amoako-Atta et al. [38] showed that irradiation of the almond moth *Cadra cautella* Walker (Lepidoptera: Pyralidae) with 100 to 300 Gy as late stage pupae had no effect on adult emergence but percentage deformed adults increased from 2 to 10%, while irradiation of younger pupae induced high mortality and a high percentage of deformed adults.

Drosophila suzukii males and females cannot be differentiated at the pupal stage. Since both sexes will have to be released in the field, it is crucial to ensure complete female sterility following irradiation. Females irradiated with doses of 50 Gy and higher were sterile, indicating that they are more sensitive to gamma irradiation than males. Irradiation with 50 Gy did not fully sterilized males; when mated with a non-irradiated female, egg hatching and survival from egg

Table 1. Effect of gamma irradiation dose applied to *D. suzukii* males on the fertility of males and females of the F1 generation.

Irradiation dose (Gy)	Descendants produced by females F1 (pupae ± SD)	*n*	Descendants produced by males F1 (pupae ± SD)	*n*
70	34.5 ± 28.5	4	46.5 ± 46.3	3
80	22.8 ± 21.0	5	46.0 ± 32.5	6
90	5.3 ± 5.51	3	9.7 ± 9.9	4
100	21.7 ± 28.2	3	64.9 ± 65.9	4
120	0	1	57.0	1

n = number of F1 descendants.

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to adult were reduced to 17.3% and 12.2%, respectively. This higher radio-sensitivity of females facilitates the development of an SIT program as it allows the selection of the optimal dose for males. Difference in radio-sensitivity between sexes is a common finding in many insects like the beet leafhooper *Circulifer tenellus* Baker (Hemiptera: Cicadellidae) [49], the almond moth [50], the Indian mealmoth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) [51] and the South American fruit fly *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) [37].

As observed in other insects [27], fecundity of non-irradiated *D. suzukii* females was similar for those mating with non-irradiated or irradiated males for all doses tested. This implies that wild females mated with irradiated males would still damage the fruits when laying sterile eggs, and these oviposition scars would be an origin for bacterial and fungal infections [5]. However, this drawback will gradually wane along with the reduction of wild female *D. suzukii* populations resulting from the release program. Released irradiated females do not oviposit eggs, however it remains unclear if they still pierce the fruits, for feeding. If they do, the development of a genetic sexing strain that produces only males, like the one for the Mediterranean fruit fly [52], could be an option not only to avoid producing sterile females, but also to reduce production cost and enhance efficiency of sterile males.

The relationship between *D. suzukii* male fertility (expressed as egg hatch) and irradiation dose is similar to what has been observed in other insects, i.e. a rapid decrease of fertility with increasing dose rates [53], with the lowest fertility (4.0%) obtained with a dose of 120 Gy. Our study provides the first dose-response curves for *D. suzukii*. When examining the use of irradiation for quarantine purposes on fresh fruits infested with *D. suzukii*, Follett et al. [20] concluded that a dose of 80 Gy applied to late-stage pupae completely prevented reproduction of emerging adults. In our study, we tested the sterility of *D. suzukii* males and females separately, by mating them with non-irradiated individuals of the opposite sex instead of mating irradiated males with irradiated females.

Although very few individuals were available to explore aspects of the fertility of the F1 generation, our results suggest that offspring of partially sterile males and non-irradiated females are fertile. Therefore, selecting an optimal irradiation dose for *D. suzukii* must be based on the dose-response of the parental F0 generation, and not on inherited sterility since it was not observed in this species. Most cases of inherited sterility have been observed in lepidopteran species [48], but partial inherited sterility has also been shown in the large milkweed bug *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae) [54]. The pattern of sterilization of *D. suzukii* following irradiation is therefore similar to patterns observed in other fruit flies belonging to the *Tephritidae* family.

This study is the first to consider the SIT as a control technique for *D. suzukii*. Irradiated sterile males could be released in the environment to reduce *D. suzukii* populations in targeted areas. They are expected to mate with wild females and prevent them from producing descendants. Further research is required, for instance to examine competitiveness of irradiated males [29]. Experiments have been undertaken to compare the mating behavior of irradiated and non-irradiated males in the laboratory. The irradiation dose of 120 Gy seems promising as it sterilizes *D. suzukii* pupea without reducing their emergence and longevity or increasing the rate of deformed flies.

Supporting information

S1 Table. Supporting data of the adult emergence and deformity experiment.
(XLSX)

S2 Table. Supporting data of the longevity experiment.
(XLSX)

S3 Table. Supporting data of the fecundity experiment.
(XLSX)

S4 Table. Supporting data of the fertility experiment.
(XLSX)

S5 Table. Supporting data of the F1 survival experiment.
(XLSX)

S6 Table. Supporting data of the F1 fertility experiment.
(XLSX)

S7 Table. Supporting data from an unpublished previous test of non-irradiated D. suzukii survival on carrot diet.
(XLSX)

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Author Contributions

Conceptualization: Geneviève Lanouette, Jacques Brodeur, François Fournier, Véronique Martel, Marc Vreysen, Carlos Cáceres, Annabelle Firlej.

Data curation: Geneviève Lanouette, Jacques Brodeur, Annabelle Firlej.

Formal analysis: Geneviève Lanouette.

Funding acquisition: Annabelle Firlej.

Investigation: Geneviève Lanouette, Annabelle Firlej.

Methodology: Geneviève Lanouette, Jacques Brodeur, François Fournier, Véronique Martel, Marc Vreysen, Carlos Cáceres, Annabelle Firlej.

Project administration: Annabelle Firlej.

Resources: Jacques Brodeur, François Fournier, Marc Vreysen, Carlos Cáceres, Annabelle Firlej.

Supervision: Jacques Brodeur, Annabelle Firlej.

Validation: Geneviève Lanouette, Jacques Brodeur, Annabelle Firlej.

Visualization: Geneviève Lanouette.

Writing – original draft: Geneviève Lanouette.

Writing – review & editing: Jacques Brodeur, François Fournier, Véronique Martel, Marc Vreysen, Carlos Cáceres, Annabelle Firlej.

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Volet 2 : Évaluer la compétitivité des mâles *D. suzukii* irradiés en laboratoire et en condition semi-naturelle.

Suite aux expériences réalisées pour répondre à trois des quatre objectifs spécifiques du volet 2 (1- Établir le degré d'accouplement multiple des mâles irradiés et des femelles non-irradiées, 2- étudier la préséance des spermatozoïdes lors de deux accouplements successifs et 3- étudier la compétitivité des mâles pour l'accouplement en laboratoire), un deuxième article scientifique est actuellement en voie d'être soumis au journal *Entomologia Experimentalis and Applicata* et illustre tous les résultats scientifiques originaux obtenus (voir ci-dessous).

TITRE: Mating capacity and competitiveness of *Drosophila suzukii* males after irradiation treatment and study of female re-mating

G. Lanouette[1], J. Brodeur[2], F. Fournier[3], V. Martel[4], and A. Firlej[1]
[1]Institut de recherche et de développement en agroenvironnement, Saint-Bruno-de-Montarville (Québec), Canada J3V 0G7
[2]Institut de recherche en biologie végétale, Université de Montréal, Montréal (Québec), Canada H1X 2B2
[3]Collège Montmorency, Laval (Québec), Canada H7N 5H9
[4]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre , PO Box 10380, Stn. Sainte-Foy, Québec (Québec), Canada G1V 4C7

Abstract

The sterile insect technique is a new approach for the integrated management of the *Drosophila suzukii*, a new invasive pest in North America. We evaluated under laboratory conditions the mating capacities of irradiated *D. suzukii* males in absence of competition and their competitiveness as their mating success in situations with one female, one irradiated male and one control male. Finally, we explored female's remating, and if it was affected by males' treatment. Four-day-old pupae were irradiated with the sterilizing dose of 120 Gy. We observed that irradiated males are fully capable to mate and transfer sperm to females, copulating with 6.4 ± 1.9 females in 24 h period, vs. 6.9 ± 2.0 females for control males without significant difference. Irradiated males competitiveness was evaluated at 37.5% but not significantly different from competitiveness of control males. Female remating can be considered infrequent and non-significantly influenced by male treatment; as we observed 7.4% of the females first mated with control males and 18.8% of the females first mated with irradiated males to remate when given the opportunity two days and four days after the first mating. Latency and mating duration were not significantly influenced by male treatment, but by presence of male competition. We suggest a dose of 120 Gy be revised and considered to be lowered by further research into SIT as a control method for *D. suzukii*.
Keywords: Spotted wing drosophila, sterile insect technique, sterilization, latency, mating duration

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae) has rapidly become a major pest of berries and stone fruits since it invaded both Europe and America in 2008 (Walsh et al., 2011). Unlike other fruit flies, it has the ability to laid eggs in healthy, ripening fruits, causing revenue losses of $US 39.8 million to California raspberry production between 2009 and 2014 and significantly increasing production costs (Farnsworth et al., 2016). The industry relies mainly on chemical management of *D. suzukii*, thereby increasing the risks and consequences of the pest developing insecticide resistance. Potential alternatives to insecticides mainly include biological control with parasitoids (Chabert et al., 2012; Daane et al., 2016; Guerrieri et al., 2016; Knoll et al., 2017) and predators and microorganisms (Cuthbertson et al. 2014, Cuthbertson et Audsley 2016), physical control like exclusion nets (Cormier et al., 2015), and the sterile insect technique (SIT) (Lanouette et al., 2017).

The SIT consists of mass-rearing the pest species, exposing individuals to radiation to induce sterility, and releasing overwhelming numbers of sterile insects in the environment. Sterile males are then expected to seek and mate with wild females, preventing them to produce viable progeny. Releases of sterile males over a number of generations allow the reduction of the pest population under an acceptable threshold (Klassen, 2005). Several pest species, including Tephritid fruit flies, have been successfully controlled with SIT worldwide (Klassen, 2005). A research initiative has recently been launched to examine the feasibility of using SIT to control *D. suzukii* populations in berry fruit production. As a first step, Lanouette et al. (2017) reported that a suitable level of sterilization of four-day-old pupae can be achieved using gamma irradiation at a dose of 120 Gy. Furthermore, irradiation did not induce morphological deformations in males. The success of a SIT depends greatly on the capacity of producing sterile males that can find and mate with wild females in the field (Pérez-Staples et al., 2013). We will therefore study the mating capacity of the sterile males without competition, as it would be if releases reach high levels of sterile to wild males, but also their capacity under competitive conditions, as if wild males were still present in the fields. Released sterile males should display the same courtship behavior and be as competitive as wild males in order to mate with wild females.

Sexual behavior varies greatly among *Drosophila* species (Markow & O'Grady, 2008). For *D. suzukii*, Revadi et al. (2015) showed under laboratory conditions that mating can occur all over the photoperiod, but the highest copulation rate was observed in the morning, within the first 30 minutes following the onset of the photoperiod. *Drosophila suzukii* females do not respond to a male sex pheromone (*cis*-vaccenyl acetate) as observed in many species from the *melanogaster* group (Dekker et al., 2015), but they produced a cuticular hydrocarbon (CHC) used by males for recognition (Revadi et al., 2015). *Drosophila suzukii* males' courtship behavior includes substrate borne vibrations produced by abdominal quivering while males remain motionless or as "toots" while males are chasing females (Mazzoni et al., 2013). Furthermore, *D. suzukii* males have black spots on their wings that can be visually displayed by wing extension during courtship; wing vibration is also a part of courtship (Fuyama, 1979; Mazzoni et al., 2013; Revadi et al., 2015).

Competitiveness of sterile males is a prerequisite of a SIT program, but is unlikely that irradiated males would be as competitive as wild, control conspecifics (Lance & McInnis, 2005). While other factors can have a deteriorating effect on male quality in SIT programs, such as continuous laboratory rearing for years, high population density, artificial diets and handling conditions during mass-rearing, the irradiation process can also directly induce competitiveness loss. In addition to reproductive cells, somatic cells are also irradiated and can undergo mutations (Robinson, 2002). This is why irradiation is generally performed at late-pupal or adult stage, when almost only reproductive cells are multiplying and subjected to malformations (Lance & McInnis, 2005).

Another aspect to consider in a SIT program is the propensity of female for re-mating. This causes a problem when females primary mated to irradiated males are more inclined to remate or when combine with a lower mating success for irradiated males in competitive conditions, giving control males a higher probabilities of inseminating wild females with fertile sperm (Barclay, 2005). For example, the receptivity of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) females is less reduced following a mate with an irradiated, sterile male than with a wild male (Kraaijeveld & Chapman, 2004). For *D. suzukii*, re-mating behavior was observed by Revadi et al. (2015) but not investigated further. According to Markow (2002), *Drosophila* species displaying a sexual dimorphism, as for *D. suzukii* males having black spots on their wings, tend to have infrequent female re-mating. *Drosophila* males invest in external morphological characters when sexual selection occurs before mating (infrequent female re-mating), or in ejaculate features, such as sperm gigantism, when sexual selection occurs after mating (overlapping ejaculates, frequent female re-mating) (Markow, 2002). The same analysis also showed that *Drosophila* species from the group *Melanogaster*, the one that *D. suzukii* belongs to (Hauser, 2011), all share the evolutionary feature of infrequent female re-mating.

No previous research has studied the behavior of irradiated *D. suzukii* males, a critical aspect to consider for SIT. The objectives of this study were to (i) determine the mating capacity of irradiated males, (ii) perform competition experiments to compare mating success of irradiated males vs. control males, and (iii) explore female re-mating behavior following a first mating with irradiated or control males.

Material and methods

All experiments took place at the Institut de Recherche et de Développement en Agroenvironnement (IRDA), St-Bruno-de-Montarville, Québec, Canada using an Italian strain of *D. suzukii* collected from wine grapes in 2012 in Trentino region and reared at IRDA since February 2016. Rearing procedures and methods of egg laying and extraction of pupae from the artificial diets are described in Lanouette et al. (submitted). Tests were performed under controlled conditions at 23 ± 1ºC, 50 ± 10% HR, and a photoperiod of 16:8 L:D.

Irradiation

Four-day-old *D. suzukii* pupae (12 to 24 h before emergence) were irradiated at the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) in a Gammacell 3000 (Best Theratronics, Canada) with ^{137}Caesium source. Pupae for irradiation and control pupae were brought in a thermal bag to the irradiation center and were thus exposed to the same temperature and humidity conditions. All tests included the same numbers of males irradiated with dose of 120 Gy and control males (non-irradiated). Pupae from a given rearing cohort were assigned randomly to each treatment.For all experiments, three-day-old adult males and five-day-old adult females were used to ensure sexual maturity and high levels of mating (G. Lanouette, unpublished data). Pupae were isolated after irradiation and individuals sexed on the day of emergence. They were separated in 1L box according to their sex, treatment and day of emergence. Adults were provided with sucrose, yeast (3:1), a piece of banana diet (Chabert et al., 2012) and water.

Mating capacity

We first compared the mating capacity of irradiated males and control males in the absence of competition, when males were alone with females. Each male was placed in a Petri dish (ø = 100mm) with ten females and a small piece of banana diet for 24 h. The male was next removed from the Petri dish and females were kept alive at 8 ± 2 ºC for zero to three days until dissection to detect stored sperm and assess the number of females inseminated by each male. The spermatheca and seminal receptacle were taken out of the female abdomen by pulling on the ovipositor. The organs were put on a slide with a drop of 1% saline solution, covered with a coverslip. A light pressure was then applied with the thumb to break the spermatheca and release the spermatozoa. Immediate observation of all structures was performed using a microscope at 400 magnification to score the presence/absence and location of spermatozoa in three different storage organs: spermatheca, spermathecal tubes, seminal receptacle.

This experiment was conducted using five irradiated males and five control males per cohort, and repeated four times (total of 20 males per treatment). The number of mated females per male and the presence/absence of spermatozoa in each storage organ per mated female were compared using binomial general linear models according to male treatment.

Competitiveness

The competitiveness of irradiated vs. control males was next assessed by scoring mating success in Petri dish (ø = 47 mm) containing three virgin flies: one irradiated male, one control male and one female. The behavior of the flies was recorded using a camera (Dino-Lite Digital microscope Model AM4012NZT with 720 x 576 pixels resolution and 25 pictures/second) placed above the experimental arena. Filming started when flies were introduced in the Petri dish and ended following a first mating, for a maximum of three hours in case of no mating. Males were introduced first in the arena, alternating the sequence between irradiated and control individuals; the female was always introduced last. All flies were introduced into the 10 arenas replicates within a 20 minutes period. In the laboratory, *D. suzukii* mate mostly in the first half-hour after lights opening (Revadi et al., 2015), tested flies were therefore introduced in the arena in darkness conditions, at the end of the scotophase, using a red light. Low level of activity was observed in darkness, but in a few cases mating occurred and flies were then discarded. A small piece of banana diet was also put inside the arena prior to the flies since *D. suzukii* use substrate-borne vibrations during courtship (Mazzoni et al., 2013). A total of 60 arenas were set-up and 48 mating events were recorded.

Videos were watched with video player Kinovea (open source) using the slide by slide tool, if necessary. Sequences were analyzed with CowLog (Hänninen, 2009) to quantify the frequency and duration of the following behaviors: immobile (include grooming), walking and courting (any behaviors aimed at attracting a mate) (Revadi et al., 2015). Number of rejections (decamping, kicking, spinning or abdominal depression) by the female following a mating attempt by a male was also scored (Revadi et al., 2015). Latency period (i.e., time between opening of the light and beginning of mating) and mating duration were calculated and finally, the male successful in mating the virgin female was consider as the “winner” of the competitiveness experiment.
Recording the competitive experiment allows to compare time budget of the males, as we know irradiation can cause passivity and loss of reproductive investment in males (Lux et al., 2002). Percentage of time allocated to different behaviors was calculated for both males and compared with a non-parametric Kruskal-Wallis test. Numbers of rejections for each type of males was compared using a Poisson general linear model according to male treatment. Proportion of females mating with irradiated vs. control males was compared with using Exact test of Goodness of fit.

Female re-mating

We examined the propensity of *D. suzukii* females to remate depending on the treatment of their first mate (irradiated vs. control). Twenty irradiated males and 20 control males were put individually in Petri dishes (ø = 47 mm) with a small piece of banana diet. As a first step, each of them was provided with one virgin female until mating, for a maximum of two hours. Flies were introduced in the experimental arena following the method described above. The observer noted the beginning and ending of mating, if it occurred, and calculated the latency period and mating duration. After two hours, males and virgin females were discarded. Mated females were kept individually in a small cup (ø = 30 mm) with banana diet to allow oviposition. As a second step, two days after the first mating, each female was provided with a new male, once again for two hours at the opening of the lights. The same observations were done. If no mating occurred during this second encounter, the female was provided with a third male from the same treatment as the second male four days after the first mating using the same conditions. In contrary if mating occurred, the female was not presented again to a new male. The following four scenarios were tested:

1- First mate with an irradiated male, then access to an irradiated male
2- First mate with an irradiated male, then access to a control male
3- First mate with a control male, then access to a control male
4-First mate with a control male, then access to an irradiated male

The experiment was repeated on four different days, for a total 80 females first presented to a control male and 80 females first presented to an irradiated male. Damaged flies and those that were not active during the test were not included in the analysis (total of six flies eliminated).
Mating success of irradiated and control males with virgin females was compared using a binomial linear model. The same model was used to determine if female re-mating was influenced by the following factors: treatment of the first mate (irradiated or control), treatment of the second male, first mate duration, time since the first mate (two or four days).

Latency and mating duration were compared between male treatments (irradiated or control) and between mating conditions (non-competitive with results from the female re-mating experience or competitive with results from the competitiveness experiences; both tests were performed one week apart) by an ANOVA using the data from the mating capacity experiment and re-mating experiment (from the first mating). Latency indicates the efficacy of the males to find, courtship and be accepted by the females, knowing that in a SIT context, males will have to find the females over a much larger arena. Mating duration is positively correlated to the males' ejaculate investment and to its sperm being increasingly used in a polygamy context (Bretman et al., 2009). All data analysis were performed using R 3.3.2 (R Development Core Team, 2016).

Results

Mating capacity

There was no significant difference in the mating capacity of irradiated and control males (Binomial linear model: Z=-0.951, d.f.=1, P=0.342). During the 24 h period, irradiated and control males (at 120 Gy) mated an average of 6.9 ± 2.0 females and 6.4 ± 1.9 females, respectively.

Irradiation had no effect on sperm storage in female reproductive organs. Sperm (presence/absence) was observed in similar proportions in the spermatheca (Binomial linear models: Z=-0.899, d.f.=1, P=0.369), spermathecal tubes (Z=-0.950, d.f.=1, P=0.342) and seminal receptacle (Z=-0.842, d.f.=1, P=0.400) following mating with irradiated or control males (Fig. 1).

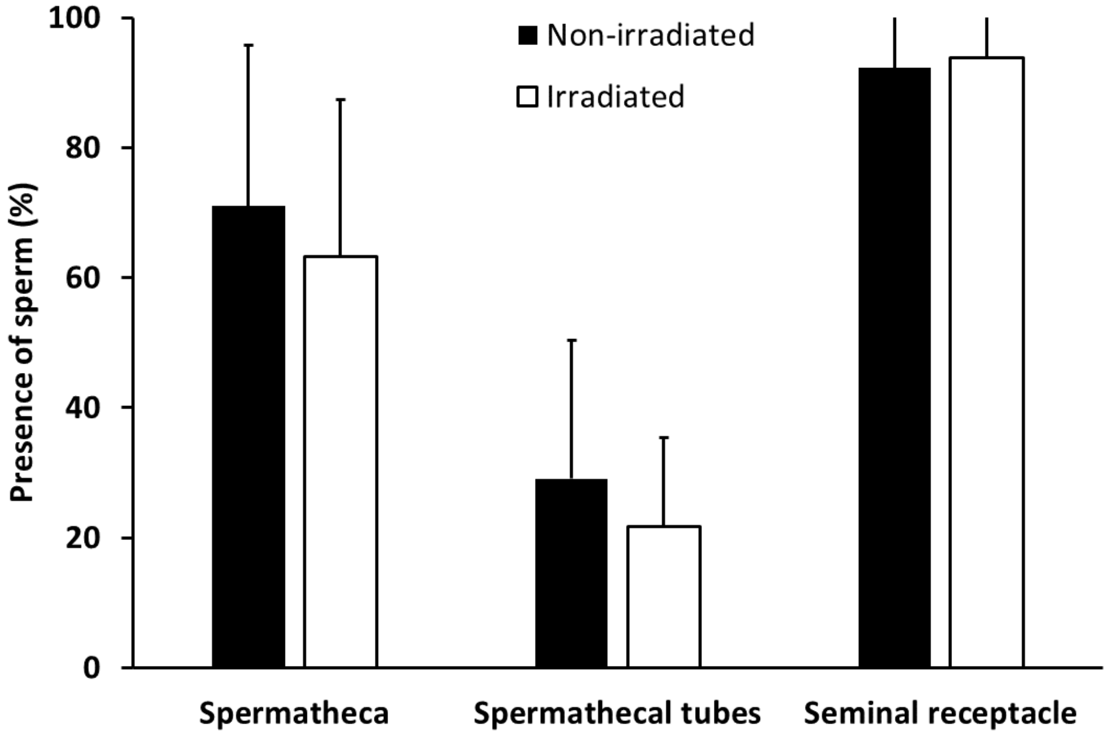

Figure 1: Effect of gamma irradiation at the pupal stage with dose of 120 Gy of males *Drosophila suzukii* on sperm storage in females' reproductive organs. Statistical significance was tested with a binomial linear model.

Competitiveness

Irradiated *D. suzukii* males were as successful (37.5%) as control males (62.5%) in mating with females (Exact test of goodness to fit: 95% confidence interval= 0.240 - 0.527, P=0.111), although there is trend in favor of control males.

Irradiation had no effect on the amount of time spent by males resting, walking without interacting with females or courting the female (Kruskal-Wallis: X^2=0.475, d.f.=1, P=0.491; X^2=0.409, d.f.=1, P=0.5223; X^2=2.895, d.f.=1, P=0.089; respectively) (Fig. 2). Number of rejections made by females before accepting a mate did not vary between irradiated (1.4 ± 2.7) and control (1.4 ± 2.2) males (Poisson linear model: Z=0.475; d.f.=1, P=0.635).

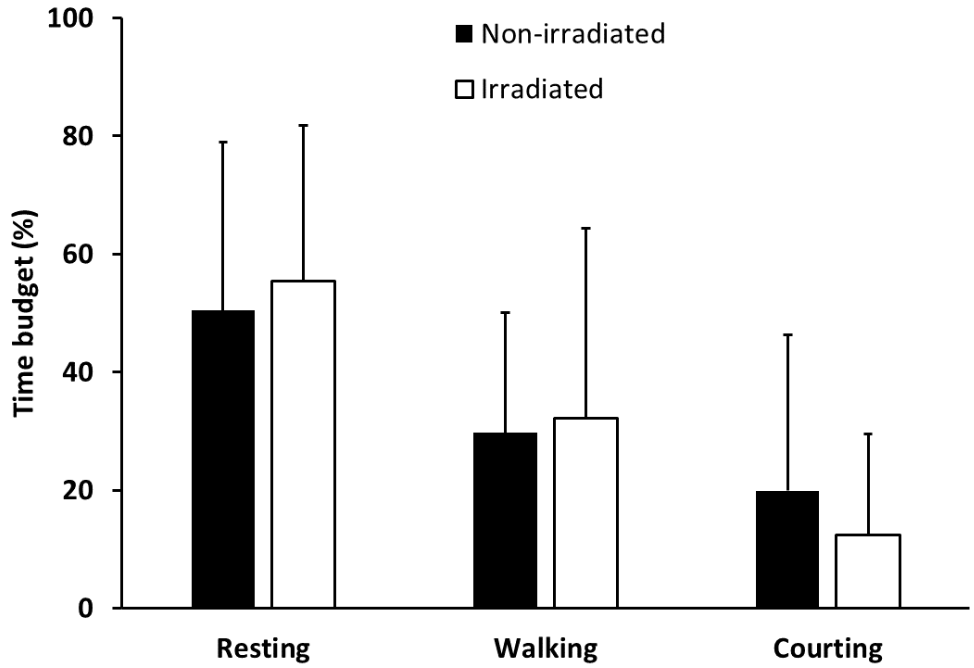

Figure 2: Effect of gamma irradiation at the pupal stage with dose of 120 Gy of males *Drosophila suzukii* time budget when irradiated males were in competition with non-irradiated males. Significance was tested with Kruskal-Wallis tests.

Female re-mating

During the first mating period (2 h), irradiated males (59.8%; 49 matings out of 82) were more successful in mating with virgin females than control males (40.8%; 29 matings out of 71) (Z=0.789, d.f.=1, P=0.017). Remating occurred uncommonly after two (8.1%; 6 females out of 74) and four (7.7%; 5 females out of 65) days, either for females first mated with irradiated (18.8%) or control (7.4%) males. None of the tested variables had a significant impact on remating: treatment of the first mate (Z=1.212, d.f.=1, P=0.223; Binomial linear model), treatment of the second mate (Z=-0.666, d.f.=1, P=0.505), duration of the first mating (Z=0.969, d.f.=139, P=0.333), interval between mates (Z=-0.066, d.f.=1, P=0.947) (Fig. 3).

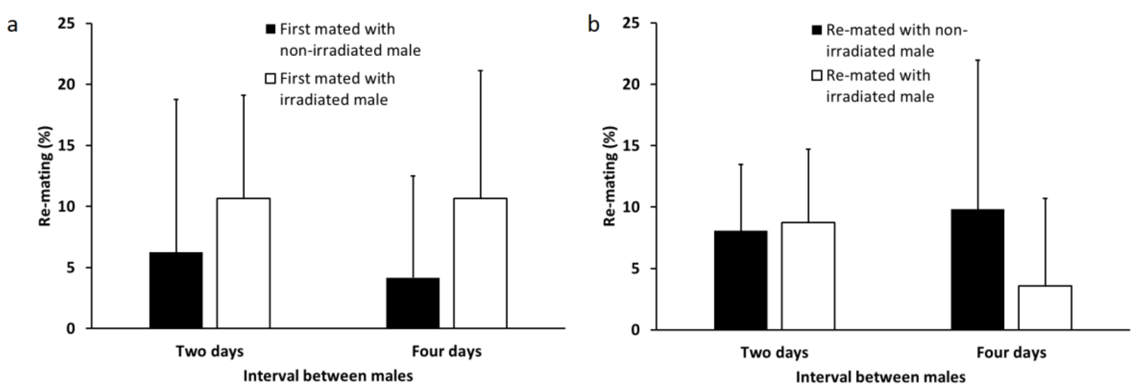

Figure 3: Effect of gamma irradiation at the pupal stage with dose of 120 Gy on *Drosophila suzukii* female re-mating percentage a) if her first mate has been irradiated b) if the second male presented to her has been irradiated. Significance was tested with binomial linear models.

Latency before mating was shorter when males were in competitive conditions compared to when alone (ANOVA: F=27.042, d.f.=1, P<0.0001), but latency was not affected by irradiation (F=0.208, d.f.=1, P=0.448) (Fig. 4). Similarly, mating duration was shortened when males were in competition (ANOVA, F=7.306, d.f.=1, P=0.008), but it was not affected by male irradiation (F=0.379, d.f.=1, P=0.539) (Fig. 4).

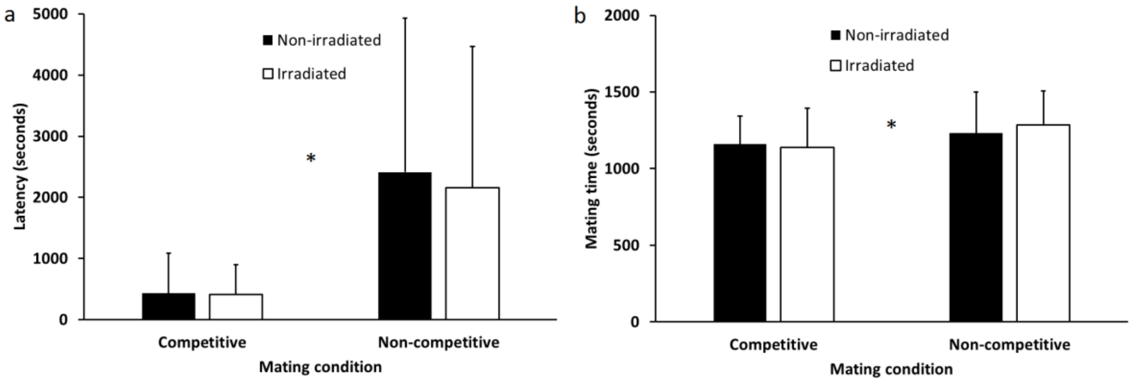

Figure 4: Effect of gamma irradiation at the pupal stage with dose of 120 Gy of *Drosophila suzukii* males on mating properties a) Latency b) Mating duration. Significance was measured with ANOVA, asterisks indicate P<0.05.

Discussion

The objective of the study was to compare mating capacities of males irradiated at 120 Gy and control *D. suzukii* males in non-competitive and competitive conditions. We also examined if male irradiation had any effect on propensity of females to remate. We observed no difference in the number of mates within a 24 h period between irradiated and control males. However, during the re-mating experiment, irradiated males mated more females than control males did during the two hours period.

When males were competing for a female, the performance of irradiated males did not significantly differ from control males. This result is interesting in the context of a SIT program, as reduction of mating capacity should be avoided in irradiated males. Similar results have been observed in other species irradiated in SIT context: for the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), the low dose of irradiation (35 Gy) did not affect the male's competitiveness, while the high doses (70 and 140 Gy) reduced its ability to mate a virgin female in non-competitive conditions (Lux et al., 2002). For the West Indian fruit fly *Anastrepha obliqua* Macquart (Diptera: Tephritidae): the competitiveness of the irradiated males, estimated as proportion of eggs laid by females in competitive conditions that did not hatch, was reduced with the augmentation of the irradiation dose (40, 60 and 80 Gy were tested) (Toledo et al., 2004). In contrast, Allinghi et al. (2007) found no difference in the competitiveness of the South American fruit fly *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) males irradiated at three different doses (40, 70 and 100 Gy) and control males in field cages. Although competitiveness was not reduced in irradiated males for the dose tested, reduced competitiveness of irradiated males needs to be monitored during the following steps of adaptation of the SIT to the pest to confirm the efficacy of the program, especially if the dose varies. Lower competitiveness of irradiated males could (i) be compensated for by using high ratios of irradiated to wild males in SIT releases, (ii) be avoided by reducing the irradiation dose (Parker & Mehta, 2007) or (iii) be compensated by increasing irradiated male size when mass-produced. Size has been associated with higher irradiated males competitiveness in fruit fly (Artiaga-López et al., 2004) and with greater lifetime mating success in the congeneric *D. melanogaster* (Partridge & Farquhar, 1983). Competitiveness of males *D. melanogaster* has also been showed to improve with age from two to eight-day-old (Long et al., 1980), and we tested the competitiveness of males *D. suzukii* with two-day-old individuals.

We observed infrequent female re-mating when given the opportunity two and four days after the first mating. The occurrence of remating was not influenced by the treatment of the first partner (7.4% of females mated to control males and 18.8% of females mated to irradiated males remated), by treatment of the second partner, or by the length of the first mating. Female re-mating has been studied in *D. melanogaster.* Using repetitive 30 minutes contact periods, Manning (1962) did not observe re-mating for a 48h period, while Fuerst et al. (1973) observed re-mating during a continuous 24 h contact period. They concluded that multiple mating is influenced by the length of the contact period and by female age. In our experiment, we used repetitive 2 h contact periods, which could explained the low re-mating. Female re-mating could be further studied using longer contact period or expanding the interval between males presentation to learn if females will accept at a higher rate a second mating later. Additional study could also check sperm usage by females mated to both irradiated and control males by observing their egg hatch.

The latency and the duration of mating vary between control and irradiated males of different species. Latency was longer for irradiated *C. capitata*, shorter for irradiated *B. tryoni* and similar for irradiated *A. fraterculus*, while mating duration was similar for irradiated *C. capitata* and *B. tryoni* and shorter for irradiated *A. fraterculus* (Allinghi et al., 2007; Lux et al., 2002; Radhakrishnan et al., 2009). For *D. suzukii*, the irradiation treatment did not affect the mating latency and duration. This suggests that males irradiated at dose of 120 Gy have a normal mating behavior, which is important when used in an SIT program. Interestingly, we observed that mating occurring under competitive conditions occurred more rapidly and were shorter for both control and irradiated males. Having two males in the experimental arena likely increases the probability of encounter with the female. During mating, interference by the second male may result in shorter mating duration.

Overall, we observed that an irradiation dose of 120 Gy applied to four-day old pupae does not affect male mating performance in non-competitive or competitive conditions. Irradiated males are fully able to mate with similar number of females as control males when in absence of competition, have similar latency and mating duration as control males. Furthermore, the experiments took place in limited space (Petri dishes) where interactions between males and females are «forced». The copulation rates obtained this way can be higher then what will be observed in the field. Experiments would gain to be repeated in semi-field conditions with larger cages that better represent the conditions of a SIT program.

This is the first study of irradiation effects on *D. suzukii* behavior and represents an important step in the development of an SIT program against the pest. Other aspects still need to be studied to allow a better judgement of the optimal irradiation dose. We suggest the investigation be continued by obtaining the Fried competitiveness index (Fried, 1971) of males irradiated at various doses. This index represent the proportion of non-fecund eggs being laid in a population where females, control males and irradiated males cohabit. It can be obtained from semi-field cages and allow seeing if a lower irradiation dose would be more efficient, but should also be continuously obtained from wild flies during a SIT program to verify its performance (Vreysen, 2005).

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Le dernier objectif du volet 2 était d'étudier **la compétitivité des mâles suite aux lâchers en condition semi-naturelle.** Avant de procéder aux tests terrain qui nécessitent beaucoup de temps de préparation et ressources, des tests similaires à ceux planifiés en condition semi-naturelle ont été réalisé en laboratoire.

Compétitivité des mâles suite à des lâchers en condition de laboratoire
Matériel et méthodes
Des lots de 100 pupes de quatre jours ont été stérilisés avec un Gamacell 3000 aux doses de 0 (témoin), 80, 90, 100 et 120 Gy. Les adultes émergeants ont été placés en cage de mousseline selon les traitements suivants pour chaque dose afin de mesurer la compétitivité des mâles irradiés : 10 mâles irradiés + 10 mâles non-irradiés + 10 femelles non-irradiées (identifié comme Dose-10-10 sur le graphique), (2) 20 mâles irradiés + 10 femelles non-irradiées (identifié comme Dose-20-0 sur le graphique). Pour chaque traitement, 5 répétitions d'irradiation ont été effectuées. Les témoins comprenaient 10 femelles et 20 mâles non-irradiés. Après 5 jours en cage, des sites de ponte composés de diète à base de poudre de carotte et rondelle de banane ont été ajoutées. Les pontes de 3 jours consécutifs ont été récoltés et les œufs ont été conservés sur un papier filtre noir humide pour vérifier leurs éclosion après 48h.

Résultats
Les analyses statistiques démontrent que pour les doses 80 à 120 Gy, les traitements avec 50% de mâles irradiés et 50% de mâles non irradiés ont des pourcentages d'éclosion d'œufs autour de 38% à 49%, ce qui est significativement différent des taux d'éclosion d'œufs pour les témoins (61%) (ANOVA : F=31,75; df=8, 285;.P<0,0001) (Fig. 1). Les traitements avec 100% de mâles irradiés ont des pourcentage d'éclosion d'œuf très bas (2,8 à 7,9%) et significativement différents du témoin et des traitements en situation de compétition. Suite à ces expériences en laboratoire nous avons obtenus les résultats suffisants pour passer au test de compétitivité en milieu semi-naturel. Cependant, la souche de *D. suzukii* utilisée pour les expériences ne pouvant être relâchée à l'extérieur (réglementation de l'ACIA), nous avons donc réalisé des tests pour utiliser notre souche québécoise.

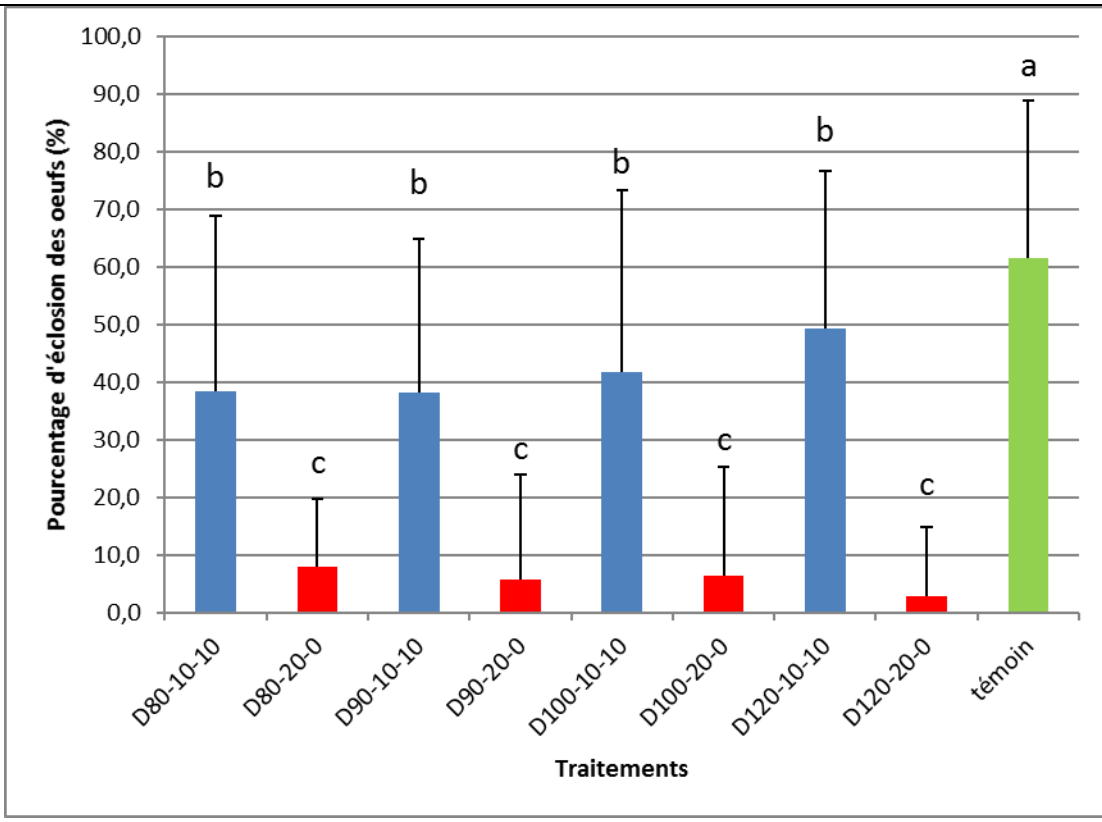

Figure 1: Pourcentage d'éclosion des œufs issus de mâles irradiés en situation de compétition ou non avec des mâles non-irradiés (Des lettres différentes indiquent une différence significative à α=0,05 avec un test de Tukey-Kramer).

Comparaison de la stérilité des souches québécoise et viennoise

Matériel et méthodes

Des lots de 100 pupes de quatre jours de la souche *D. suzukii* québécoise ont été stérilisés avec un Gamacell 3000 aux doses de 0 (témoin), 80, 90, 100 et 120 Gy. Dix mâles émergeants pour chaque dose ont été mis en couple dans des cages avec eau et nourriture, selon les combinaisons suivantes : (1) 10 mâles irradiés + 10 femelles non-irradiées et (2) 10 mâles non-irradiés + 10 femelles non-irradiées. Après 5 jours en cage, des sites de ponte composés de diète à base de poudre de carotte et rondelle de banane ont été ajoutées. Les pontes de 3 jours consécutifs ont été récoltées et les œufs ont été conservés sur un papier filtre noir humide pour vérifier leurs éclosion après 48h.

Résultats

La souche québécoise soumise à quatre doses d'irradiation montre les mêmes niveaux de stérilité que la souche viennoise (Fig. 2). Suite à cette expérience nous avions les résultats suffisants pour passer au test de compétitivité avec la souche québécoise de *D. suzukii*.

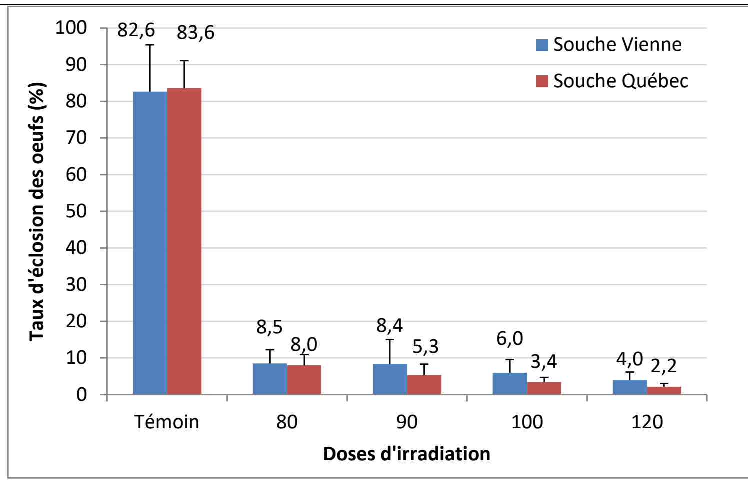

Figure 2 : Comparaison du pourcentage d'éclosion des œufs issus de femelles accouplées à des mâles irradiés de souche québécoise et viennoise.

Compétitivité des mâles suite à des lâchers en condition semi-naturelle

Matériel et méthodes

L'expérience a été réalisée pendant huit semaines sous des grand tunnels avec des framboisiers d'automne de variété Polana et Polka (Photo 1). Pour réaliser les lâchers de *D. suzukii* en condition semi-naturelle, 18 cages en bois d'un volume de 3 m³ (1,2m x 1,2m x 2,15) ont été utilisées pour l'expérience avec à l'intérieur deux pots de framboisier (Photo 2). Les cages étaient rendues hermétiques à l'aide filet Proteknet 80gr/m^2. Elles étaient réparties sur trois rangs et l'emplacement de chaque traitement a été effectué par tirage au sort (Fig. 3). Des lots de 100 pupes de quatre jours ont été stérilisés avec un Gamacell 3000 aux doses de 0 (témoin), 80, 90, 100 et 120 Gy. Les adultes émergeants ont été placés dans les cages de 18 cages selon les traitements suivants pour chaque dose afin de mesurer la compétitivité des mâles irradiés : 10 mâles irradiés + 10 mâles non-irradiés + 10 femelles non-irradiées (identifié comme Dose-10-10 sur le graphique), (2) 20 mâles irradiés + 10 femelles non-irradiées (identifié comme Dose-20-0 sur le graphique). Pour chaque traitement, quatre répétitions d'irradiation ont été effectuées. Les témoins comprenaient 10 femelles et 20 mâles non-irradiés. Quatre récoltes de fruits ont été effectuées trois, six, huit et dix jours suivants les lâchers de *D. suzukii* dans les 18 cages. La totalité des fruits mûrs de chaque cage étaient récoltés puis disposés individuellement dans une solo cup® de 37 ml (1 ¼ oz). Les contenants avec les fruits étaient ensuite entreposés au laboratoire à une température de 23 °C et 50% HR pendant deux semaines. Les *D. suzukii* mâles et femelles produit pour chaque fruit récolté ont été notés afin de calculer un nombre moyen de *D. suzukii* produit par fruit, ceci afin de tenir compte du nombre de fruits disponibles qui était variable selon la semaine de réalisation des tests.

Photo 1 : Framboisiers d'automne utilisés pour les tests en condition semi-naturelle

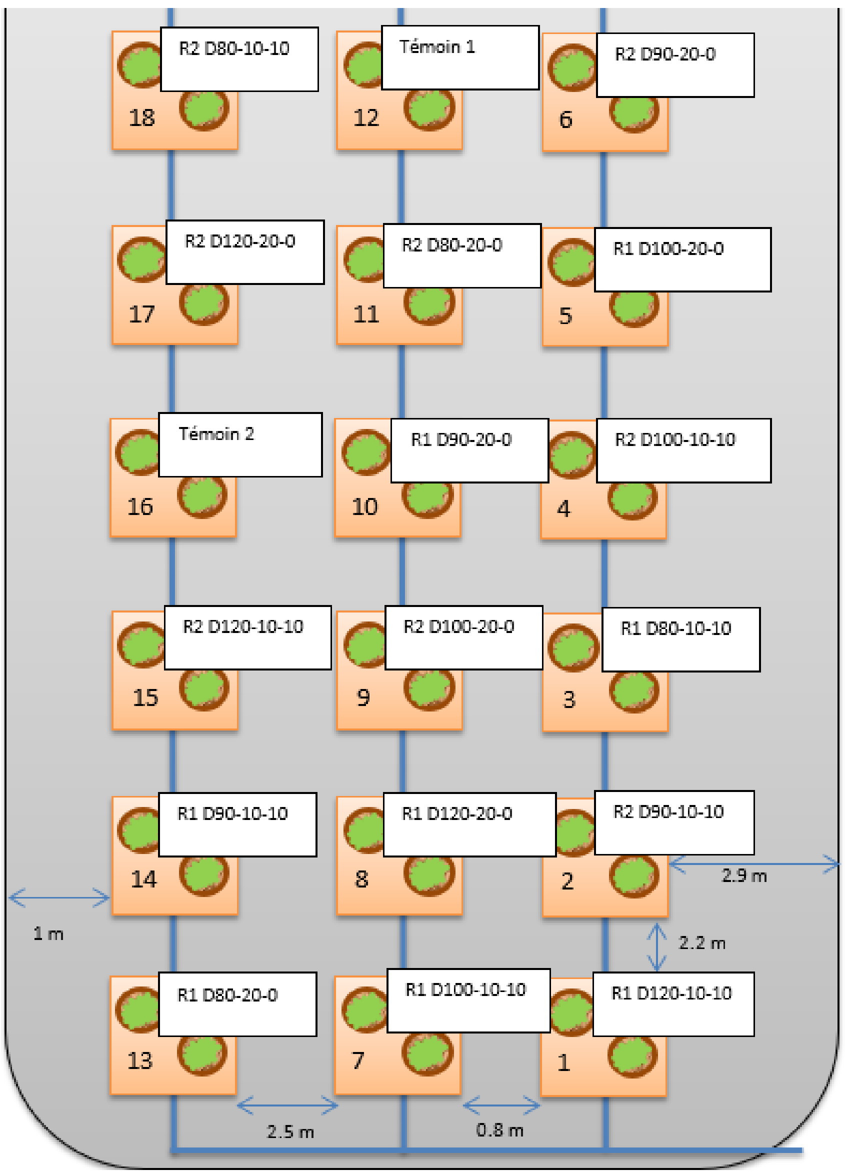

Figure 3: Dispositif expérimental utilisé pour étudier la compétitivité en condition semi-naturelle avec 18 cages contenant deux pots de framboisier d'automne

Photo 2 : Cage contenant deux framboisiers d'automne utilisés comme répétition pour les différents lâchers.

Résultats

Les analyses statistiques démontrent que pour les doses 80 à 120 Gy, les traitements avec 50% de mâles irradiés et 50% de mâles non irradiés sont différents significativement du témoin (ANOVA : F=140,64; df=8, 12544;.P<0,0001) (Fig. 4). Les doses de 80 Gy et 120 Gy produisent significativement moins de *D. suzukii* que les traitements aux doses de 90 et 100 Gy. Les traitements avec 100% de mâles irradiés produisent très peu d'individus par fruits et sont significativement différents du témoin et des traitements en situation de compétition.

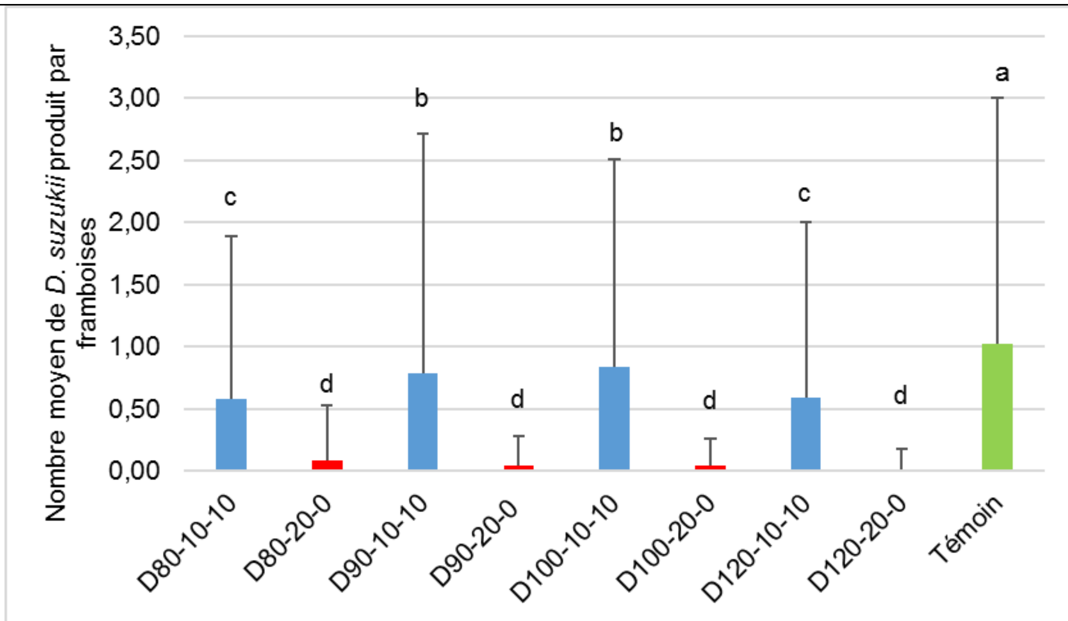

Figure 4: Nombre moyen de *D. suzukii* produit par framboise en situation où des femelles sont en présences de mâles irradiés et/ou mâles non-irradiés (Des lettres différentes indiquent une différence significative à α=0,05 avec un test de Tukey-Kramer).

CONCLUSION GÉNÉRALE

Les différentes expériences ont montré que les doses de 80 à 120 Gy induisent une stérilité des œufs significative chez les mâles de *D. suzukii* sans diminution de leurs attributs biologiques. Lors des expériences en laboratoire, les mâles irradiés à la plus haute dose de 120 Gy démontrent des capacités reproductives et une compétitivité similaire aux mâles non irradiés.

Les expériences en situation de compétition en laboratoire et en condition semi-naturelle montrent des résultats légèrement différents entre eux mais identifient que la dose de 120 Gy produit des individus mâles compétitifs.