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Phytosanitary irradiation as an effective treatment for *Drosophila suzukii*

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Drosophila suzukii is a pest native to Southeast Asia that causes significant economic losses to soft fruit crops. Phytosanitary irradiation is a promising treatment for _D. suzukii_ hosts; yet an internationally recognized irradiation protocol is lacking. To fulfil specific requirements for proposing an irradiation treatment for _D. suzukii_, naturally infested blueberries and cherries containing a total of 37,489 late pupae were irradiated with a maximum absorbed dose of 80 Gy. Infested hosts containing a total of 9578 late pupae were considered unirradiated controls. Prevention of egg laying by females that emerged from treated pupae was considered the treatment endpoint. The fecundity and egg viability of females that emerged from treated pupae mated with their siblings were evaluated using blueberries. While females from unirradiated pupae laid a total of 43,142 eggs, no egg was laid by females that emerged from irradiated pupae. In addition, 1-day-old adults were irradiated with nominal doses of 20 and 72 Gy to evaluate whether egg laying could be prevented in flies emerging before the irradiation treatment. Females irradiated with 72 Gy laid eggs that did not hatch. Our findings suggest the minimum absorbed dose of 80 Gy as a phytosanitary irradiation treatment against _D. suzukii and_ may support its inclusion as a treatment option in the annex of the International Standard for Phytosanitary Measures 28 (ISPM 28).

Keywords Postharvest treatment, Radiation tolerance, Phytosanitation, Ionizing radiation, Spotted wing drosophila

Phytosanitary treatments are applied to disinfest fresh agricultural commodities and prevent insect pest invasions worldwide[1,2]. Ionizing radiation provided by gamma rays (^{60}Co or ^{137}Cs), X-rays (up to 7.5 MeV), and electrons (up to 10 MeV) are effectively used as phytosanitary treatments for many insect pests[3]. Phytosanitary irradiation has increased consistently over the years, partially due to its advantages over other treatments[4]. Irradiation treatments do not leave harmful residues in the commodity[5], can be applied in the country of destination[6], are well tolerated for most fruits and vegetables[7], and simultaneously cover many insect taxa through generic doses[8,9].

The large number of treatment schedules available for a wide range of insect pests reflects the steady growth of phytosanitary irradiation. Irradiation treatments comprise more than 50% of all phytosanitary treatments adopted by the Commission on Phytosanitary Measures (CPM), the governing body of the International Plant Protection Convention (IPPC) in the International Standard of Phytosanitary Measures 28 (ISPM 28). Adopted treatments in ISPM 28 are based on robust data evidence meticulously evaluated and recommended by the Technical Panel on Phytosanitary Treatment (TPPT) of IPPC. Extensive research data reviewed by the TPPT have resulted in the adoption of harmonized irradiation treatments in ISPM 28 for numerous species of beetles[10–13] moths[14–17], mealybugs[18,19], and tephritid fruit flies[20–24].

Although phytosanitary irradiation has been recommended and used for several insect pests, there remains a need to validate treatments for some species, such as *Drosophila suzukii*, the spotted wing drosophila (SWD), *that* is a serious threat to soft-skinned and small fruit crops[25]. Originating from Eastern and South-eastern Asia and spreading to Africa, Asia, Europe, North America, and South America, *D. suzukii* infests more than 140 hosts[26]. The only countries where *D. suzukii* is still absent are Australia and New Zealand. To prevent the invasion and

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establishment of *D. suzukii*, Australia and New Zealand have imposed quarantine restrictions on the domestic and international trade of several hosts, including cherries, peaches, plums, strawberries, and grapes[26,27]. These restrictions are justified as the North Island of New Zealand, Tasmania, and a narrow range in the southeast and southwest of Australia have been identified as regions of great environmental suitability to *D. suzukii*[28].

Currently, the phytosanitary treatment options for *D. suzukii* include cold treatment[29] and methyl bromide[30]. Due to the global reduction in MB use and the potential for chemical residues in fresh fruit[31,32], phytosanitary irradiation constitutes a promising alternative for host commodities of *D. suzukii*. In 2017, an irradiation treatment for *D. suzukii* was submitted to the TPPT for evaluation[33]. The treatment submitted was based on large-scale confirmatory tests with an estimated number of more than 33,000 late pupae of *D. suzukii* irradiated with 80 Gy, considering prevention of F1 adults as the treatment endpoint[34]. In 2019, after multiple rounds of revision, the TPPT recognized that more research was needed to support such irradiation treatment for *D. suzukii*[33]. According to the TPPT, further studies should provide supporting data with a large number of *D. suzukii* individuals tested, enabling the calculation of the efficacy level, and should carefully evaluate the absence of eggs post-irradiation[34].

The validation of an irradiation treatment for *D. suzukii* will expand the range of phytosanitary treatment options used to mitigate the risk of further spread and establishment in areas free of this pest. Determining a phytosanitary irradiation dose and its efficacy against *D. suzukii* is fundamental for supporting harmonized treatments and promoting the safe and sustainable trade of fresh commodities. Hence, addressing the TPPT's recommendations is essential to validate a phytosanitary irradiation treatment against *D. suzukii*. In this paper we present research to validate the already proposed phytosanitary irradiation treatment for *D. suzukii*[34] through large-scale confirmatory tests with the late pupal stage. To this end, we also evaluated the extent to which radiation treatments impair the reproduction and development of *D. suzukii* irradiated as late pupae or newly emerged adults.

Results

Confirmatory test and treatment efficacy

A total of 37,489 late pupae were irradiated in cherries (14,122) and blueberries (23,367) at absorbed doses ranging from 60.0 to 79.9 Gy across 285 replicates (Table 1). No egg was laid in fruit exposed to 20,603 females emerging from irradiated pupae, while 5795 non-irradiated females laid 43,142 eggs (Table 1). Based on the range of absorbed doses and the absence of eggs observed in fruit exposed to irradiated females, we confirm that the minimum absorbed dose of 80 Gy can be used as a phytosanitary treatment for *D. suzukii*. The total number of insects treated (37,489) was adjusted using Abbot's correction[52] based on control mortality and calculated as 36,887 with the resulting efficacy of 99.9919% at the 95% confidence level.

Irradiation of newly emerged flies

We found that 1-day-old females irradiated with a nominal dose of 72 Gy laid fewer eggs as compared with the moderate numbers laid by 20 Gy-irradiated and high numbers by non-irradiated females (Table 2, $\chi^2 = 1957$, $df = 2$, $p < 0.0001$). However, none of the eggs oviposited by 1-day-old females irradiated with 72 Gy hatched (Table 2). Eggs laid by 1-day-old females irradiated with 20 Gy yielded F1 flies with viability similar to non-irradiated flies (Table 2).

Dosimetry

The dosimetry system used in the experiments indicated an estimated level of uncertainty of 2.5%. The insects used in our experiments were irradiated with dose rates ranging from 40.7 to 43.9 Gy/min. The absorbed doses measured in irradiated fruit infested by *D. suzukii* were obtained from routine dosimetry protocols using nominal doses of 70 and 72 Gy (Table 1), resulting in average minimum and maximum absorbed doses of 65.0 Gy and 74.6 Gy. The maximum absorbed dose measured over the 285 irradiation treatments was 79.9 Gy, indicating that 80 Gy should be the minimum absorbed dose recommended as a phytosanitary irradiation treatment for *D. suzukii*. For the experiment assessing irradiation treatment efficacy for newly emerged adults, the nominal doses used were 20 Gy and 72 Gy (Table 2), resulting in average minimum absorbed doses of 20.9 Gy, 74.7 Gy, and maximum absorbed doses of 24.2 Gy, 82.3 Gy, respectively.

		Absorbed dose (mean ± SE) [min, max]				No. emerged F0 adults			No. emerged F1 adults	
Host fruit	Nominal dose	Bottom (Gy)	Top (Gy)	Replicate	No. pupae treated	Males	Females	F0 fecundity (no. eggs)	Males	Females
Cherry	0 Gy	0	0	17	3641	1236	2223	19,832	3227	4565
	70 Gy	65.0 ± 0.3 [61.5, 69.6]	67.7 ± 0.3 [62.7, 71.2]	60	5194	1983	2595	0	0	0
	72 Gy	70.9 ± 0.4 [64.5, 77.0]	73.3 ± 0.4 [65.1, 78.8]	60	8928	3678	4663	0	0	0
Blueberry	0 Gy	0	0	20	5907	2195	3572	23,310	5921	7425
	70 Gy	65.0 ± 0.6 [61.4, 68.4]	67.7 ± 0.5 [65.6, 70.1]	10	1922	671	816	0	0	0
	72 Gy	70.7 ± 0.2 [60.0, 78.5]	74.6 ± 0.2 [68.3, 79.9]	155	21,445	787	12,529	0	0	0

Table 1. Large-scale confirmatory tests with *Drosophila suzukii* late pupal stage irradiated in cherries and blueberries.

Host fruit	Nominal dose	Replicates	Absorbed dose (mean ± SE) [min, max]		No. 1d-old adults treated	Total no. eggs laid	No. eggs/five fruit (mean ± SE)[a]	No. emerged F1 adults (mean ± SE)	
			Bottom (Gy)	Top (Gy)				Males	Females
Blueberry	0 Gy	4	0	0	200	5921	1480 ± 462 A	308	643
	20 Gy	8	20.9 ± 0.3 [19.1, 22.6]	22.8 ± 0.3 [21.3, 24.2]	400	1419	177 ± 27 B	107	90
	72 Gy	8	70.7 ± 1.7 [66.1, 81.0]	74.7 ± 1.2 [71.2, 82.3]	400	314	39 ± 23 C	0	0

Table 2. Irradiation of newly emerged adults (1 day old), their fecundity, and egg viability. [a]Different letters indicate statistically significant differences (estimated marginal means contrasts, $p < 0.05$).

Discussion

Confirmatory tests aim to evaluate a sufficiently large number of insects to provide a high level of confidence of the efficacy of the treatment[35]. Our confirmatory tests showed that an irradiation treatment of 80 Gy provided a high level of effectiveness in impairing the development of *D. suzukii* as the 20,603 females emerged from the 37,489 irradiated pupae laid no eggs. The purpose of phytosanitary treatments with high-level efficacy is to provide quarantine security through the disinfestation of internationally traded commodities[36,37]. The dose of 80 Gy used in this study prevented emerged females from laying eggs. Our results corroborate a previous study where a nominal dose of 80 Gy was applied as a phytosanitary irradiation treatment against *D. suzukii* to prevent F1 adult production[34]. Additionally, our findings indicate that 80 Gy can be safely used to treat commodities infested by *D. suzukii*, aiming to prevent oviposition, an earlier endpoint than prevention of F1 adults.

Establishing a target dose for *D. suzukii* with a high level of efficacy is a crucial step toward using phytosanitary irradiation as an alternative to methyl bromide treatment for commercial application. *Drosophila suzukii* is a regulated pest for Australia and New Zealand, and both countries require phytosanitary treatment of host commodities using methyl bromide and SO_2/CO_2 fumigation as a condition of entry[30,34,38,39]. However, concerns regarding the use of methyl bromide and other fumigants as phytosanitary treatments have been raised since their use is harmful to the environment and may leave residues on the fruit[40]. This is where phytosanitary irradiation offers a sound alternative. The Food Standards Australia New Zealand (FSANZ) has approved the use of phytosanitary irradiation to treat commodities associated with regulated pests at absorbed doses ranging between 150 and 1000 Gy for commercial trade in Australia and New Zealand[41]. Among the fresh commodities that could be treated using phytosanitary irradiation are cherries and berries, some of the most preferable hosts of *D. suzukii* (CABI, 2024). Cherries and blueberries tolerate irradiation treatments up to 400 Gy, without showing radiation-induced damage that could compromise fruit quality[42,43].

The prevention of egg laying observed in our study for females that emerged from irradiated late pupae suggests that the absorbed doses ranging from 60 to 79.9 Gy resulted in severe radiation-induced ovarian damage. Previous studies investigated the effects of radiation exposure on female *D. suzukii* and demonstrated that 50[44] to 75[45,46] Gy effectively induced female sterility. A similar outcome has been observed for the tephritid fruit fly *Anastrepha fraterculus*[47] and for lepidopteran *Spodoptera litura*[48], a lepidoptera pest, wherein radiation-induced damage also resulted in female sterility. Radiation-induced sterility in insect females is attributed to the heightened susceptibility of germinative cell structures to radiation damage, which subsequently leads to ovarian impairment[49].

We observed that newly emerged female *D. suzukii* irradiated with 20 and 72 Gy could lay eggs, but these did not hatch in the latter treatment. Studies evaluating the sterility of *S. litura* females irradiated with 100, 130, 150, and 200 Gy indicated a decrease in the number of eggs laid with increasing radiation doses[50]. The same was observed for *Aedes aegypti* mosquitoes irradiated with 40, 50, 60, 70, and 80 Gy[51]. In both studies, egg hatch was reduced in females irradiated with low radiation doses as compared with the untreated control, but when irradiated with high radiation doses, none of the eggs hatched. Therefore, our results on the irradiation of newly emerged adults are important to confirm the effectiveness of the minimum absorbed dose of 80 Gy in preventing development in extreme cases where flies could emerge inside the packages containing fresh commodities before the treatment.

In conjunction with prior research[34], our results provide robust evidence supporting a minimum absorbed dose of 80 Gy for phytosanitary irradiation against *D. suzukii.* Our study addressed the research gaps on the efficacy level and treatment endpoint identified by the TPPT[33] and provided additional data supporting an irradiation treatment for *D. suzukii* in ISPM 28.

Materials and methods

Rearing conditions

The *D. suzukii* colony used in the study was established in 2014 from flies collected at San Michele All 'Adige, Trento (Italy). The *D. suzukii* colony has been maintained at the Insect Pest Control Laboratory of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria since then. Immature stages were reared on an artificial diet based on 25.7% wheat bran, 6.4% brewer's yeast, 11.9% sugar, 0.4% sodium benzoate, 0.4% nipagin, and 55.1% water[52]. The diet was placed in Petri dishes and offered to sexually mature *D. suzukii* females for 24 h. After infestation, multiple Petri dishes containing the artificial diet with newly laid eggs were individually placed into plastic containers (38 × 37 × 21 cm) to allow larval development. After 12 days, *D. suzukii* reached the late pupal stage, and the Petri dishes with the artificial diet were washed in fresh water to collect the pupae (FAO/IAEA, 2022). The washed pupae were individually screened before being placed inside the rearing cages (30 × 30 × 30 cm) to prevent contamination with *Drosophila melanogaster*. Adults were reared in emergence cages containing water and a dry artificial diet (1 hydrolysed yeast: 3 sucrose). Insects were reared under laboratory conditions at 23 ± 1 °C, 65 ± 5% RH, and 12L:12D photoperiod.

Fruit infestation

Blueberry (*Vaccinium* spp.) and cherry (*Prunus* spp.) were chosen to assess the efficacy of 80 Gy as the minimum absorbed radiation for a phytosanitary irradiation treatment as they are of the preferred hosts of *D. suzukii* (CABI, 2022). Blueberries and cherries from Spain, Italy, Morocco, and Peru were obtained from a local market. The fruit was washed, rinsed, soaked for 15 min in an antifungal solution (4% sodium benzoate, 1% sodium hypochlorite), re-rinsed, dried, and kept in the fridge. Before infestation, fruits were weighed. Fruits were infested by exposing them to ~ 4000 sexually mature *D. suzukii* adults in rearing cages (30 × 30 × 30 cm) for a period ranging from 4 to 24 h. Infested blueberries and cherries were placed into separate plastic containers. Infested fruit were elevated using a piece of voile fabric held with an elastic band to prevent them from touching the juice leaking out during *D. suzukii* development. Containers with fruit were individually labelled and closed with a lid covered by voile fabric. Fruit was incubated at 25 ºC and 90% HR (Forma Reach-In Incubator, Model 3951, Thermo Fischer Scientific Inc, Waltham, Massachusetts, USA) until the insects reached the late pupal stage (10 days for cherries and 11 days for blueberries). The late pupal stage was used in the experiments because it is the most tolerant *D. suzukii* stage associated with the commodity[33].

Confirmatory test

Large-scale confirmatory tests were carried out using the late pupal stage of *D. suzukii* reared in blueberries and cherries. We evaluated if 80 Gy could be used as a phytosanitary irradiation dose for *D. suzukii*. The first step was to establish criteria to measure the success of the irradiation treatment and its impact. Prevention of oviposition was used as the treatment endpoint to evaluate the efficacy of the treatment. Blueberries and cherries infested with late pupae of *D. suzukii* were placed into a square plastic container (6.4 × 8.0 cm) made of polyethylene terephthalate glycol (PETG) and irradiated in a Co-60 self-contained irradiator (Model 812, Foss Therapy Services Inc., California, USA) located at the IPCL. Prior to treatment, the containers with infested fruit were placed inside the irradiation chamber on a 10 cm high support of stalked Petri dishes to optimize the dose distribution across the sample. Each irradiation container and control with 100 g of infested fruit were considered as a replicate. The dose rate during the confirmatory test ranged from 40.7 to 43.9 Gy/min. A random sample of 10% of the infested fruit was set apart, left unirradiated as control, and otherwise handled similarly to treated fruit.

Irradiation of newly emerged flies

An experiment was performed using 1-day-old adults to evaluate if the absorbed dose of 80 Gy (nominal dose of 72 Gy) is also effective against newly emerged flies, simulating a situation of emergence inside fruit packaging before treatment. Flies were separated into groups of 25 couples and placed inside conical tubes (12.1 × 1.6 × 1.7 cm) (Eppendorf AG, Hamburg, Germany) for irradiation and control. To prevent the flies from moving inside the tube and guarantee a better radiation dose distribution, we limited their movement using a cotton pad to keep all the flies in the bottom of the conical tube. After that, flies were irradiated with nominal doses of 20 Gy and 72 Gy. The sub-optimal dose of 20 Gy was chosen to evaluate moderate radiation effects compared with non-irradiated and 72 Gy. The post-radiation routine was followed by keeping the flies inside the emergency cages containing water and food. After five days, blueberries were exposed to sexually mature females mated with siblings from the same treatment, and the number of eggs laid and their viability from egg to adult were evaluated. Eight replicates for each irradiation treatment and four for non-irradiated treatment were performed. To compare the number of eggs oviposited across different doses, we used a Poisson Generalized Linear Mixed Model using the g*lmer* function, considering block and replicate as random factors. For post hoc tests, the *emmeans* function was used. These analyses were performed in R (version 4.4.0; R Core Team, 2024) and RStudio (version 2022.07.2 + 576; RStudio Team, 2022).

Dosimetry

Dose mapping and calibration

Dose mapping was performed to detect the maximum and minimum doses inside the irradiation chamber of our self-contained irradiator. After checking the dose distribution in the irradiation chamber, we selected the radiation source and the turntable position that best met our irradiation target dose. Furthermore, we used the Gafchromic™ HD-V2 film dosimetry system to measure the absorbed doses delivered to infested fruit. The calibration of our dosimetry system followed the Standard Operating Procedures for Gafchromic™ Film Dosimetry System for Gamma Radiation v1.0 of the FAO/IAEA[53].

Routine dosimetry

The absorbed dose of each replicate exposed to irradiation was assessed using three pieces of Gafchromic™ HD-V2 films. The films were cut (1 × 1 cm) and individually packed in paper envelopes (2.5 × 2.5 cm) (FWT-80, Far West Technologies, Goleta, CA, USA) and then sealed in plastic bags (3 × 3 cm) to avoid them to get wet by fruit juice leak. One plastic bag containing three packed films was placed on the top and bottom of the infested fruit to allow the measurement of the absorbed dose. The absorbed dose for all replicates was verified using the

Gafchromic™ dosimetry system. A portable densitometer (DoseReader 4®, RadGen, Budapest, Hungary) was used to read the Gafchromic™ films 24 h after the radiation exposure.

Post-radiation routine

After the irradiation treatment, control and treated fruit were carefully placed in a Petri dish inside a plastic cage (20 × 14 × 20 cm) with water and dry artificial diet. The cage with the fruit was placed inside a black voile bag (100 × 60 cm) to avoid contamination by errant *Drosophila* flies. Five days after adult emergence, non-infested blueberries were placed inside the cages as oviposition resources for sexually mature females for 24 h every day for two weeks. After fruit infestation, the number of eggs laid by the untreated control and treated insects (if eggs were found), were counted, and recorded. If eggs were found in the fruit exposed to treated insects, egg viability (hatching) was checked daily for three consecutive days since the prevention of egg hatch determined the treatment efficacy. If eggs were not found in the fruit exposed to treated insects, the fruit were discarded. Infested fruit from cages with adults emerging from control or irradiated pupae were placed inside the incubator chamber to allow development of the immature stages. Fruit was checked daily for three consecutive days to score egg viability (hatching). After immature development, fruit with pupae were placed inside cages and held for two weeks to monitor for adult emergence. The total number of adults and pupae in the control and treatment groups was recorded.

Data availability

The datasets used in the current study are available from the corresponding author on reasonable request.

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Author contributions

I.V.G., V.S.D., and S.W.M. conceived the experiments; I.V.G. and V.S.D. performed the dosimetry; I.V.G., A.C.F.P.S., J.S.A., L.G.C., and J.L.A. carried out the confirmatory tests; I.V.G. and L.G.C. conducted experiments on irradiation of newly emerged flies; I.V.G. and V.S.D. analysed the results and drafted the early version of the manuscript; V.S.D., C.C., M.J.B.V., S.W.M., and C.S. supervised the project; I.V.G., V.S.D., C.C., M.J.B.V., S.W.M., and C.S. edited the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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