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Gamma Radiation as a Potential Phytosanitary Squash Treatment for the Control of *Anastrepha grandis* (Macquart) (Diptera: Tephritidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FBB and AR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ALCHV provided the irradiation equipment and author FLS conducted the quality analyses. All authors read and approved the final manuscript.

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are the primary pests of horticultural crops worldwide, causing both direct and indirect damage to the fruit production, including *Anastrepha grandis* (Macquart, 1846), which exclusively infests Cucurbitaceae fruit. Dose-response tests were used to examine the effects of gamma radiation exposure on 24 – 48 h old eggs and third instar larvae, both *in vitro* and in Atlas squashes (*Cucurbita moschata*). The following physicochemical properties of Atlas squashes

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exposed to gamma radiation were evaluated: titratable total acidity, sugar, pH, external colouration, and texture. It was found that under *in vitro* conditions, a 20.0 Gy dose of gamma radiation was able to prevent larval eclosion, but a 200 Gy dose was necessary to prevent emergence after the exposure of third instar larvae. In Atlas squashes, 200 and 250 Gy doses of gamma radiation were able to prevent the emergence of *A. grandis* adults from eggs and larvae, respectively. The radiation doses estimated by Probit 9 to prevent emergence from squashes infested with eggs and third instar larvae were 257.13 Gy and 270.25 Gy respectively. The use of gamma radiation did not significantly affect the physicochemical properties of Atlas squashes, which suggested that gamma radiation may represent a potentially useful technique for the quarantine control of *A. grandis* in cucurbits.

Keywords: Insecta; fruit flies; Cucurbitaceae; quarantine treatment; post-harvest treatment.

1. INTRODUCTION

Cucurbitaceae species include the watermelon (*Citrullus lanatus* Schrad.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poir, orange pumpkin (*Cucurbita maxima* Duch. ex Lam.), and mogango (*Cucurbita pepo* L.) [1,2]. The total production of *Cucurbita* (including *C. moschata, C. maxima., C. pepo,* and hybrids), watermelon and melon varieties in Brazil reached 516,716; 1321,394; and 873,196 tons, respectively, in 2017 [3]. Only the exportation of Brazilian melon produced in fruit fly-free zones during 2019-2020 season reach more than US$ 100 million.

The presence of exotic insects represents a major problem of international trade in food and agricultural products [4]. Many insect pests, such as fruit flies (Tephritidae), are important quarantine targets in various global regions because they may be absent within the region or the importing country, and many countries have implemented ‘zero tolerance’ policies regarding the importation of any living insects, regardless of whether they are economically significant [5].

Anastrepha grandis (Macquart, 1846), also known as the South American cucurbit fruit fly, attacks several native and introduced Cucurbitaceae species grown in many regions of South America [6], is registered in eleven Brazilian states [7], and is considered to be a significant pest for crops belonging to the genus *Cucurbita* [8,9]. The mean duration of biological cycle (egg-adult) is 49 days (Fig. 1A).

Post-harvest phytosanitary treatments are used to disinfest fruit that are known to host pests and can be used to guarantee that pests are not transported to importing countries that do not have such occurrences [10]. Depending on the plant species and the country of destination, Brazilian cucurbits intended for export must be produced in free areas or under a low *A. grandis* prevalence. Because of this quarantine restriction, only a few Brazilian agricultural regions are allowed to export cucurbit species. An alternative strategy for achieving phytosanitary security is the submission of cucurbit fruits to effective quarantine treatments.

Irradiation is an emerging technology [4] and represents one of the newest phytosanitary measures that has been applied for quarantine purposes [11] to prevent the introduction or spread of regulated pests. Unlike all other commercially applied phytosanitary treatments, the disinfesting effectiveness of phytosanitary irradiation can be measured by the prevention of adult emergence from eggs or larvae within the fruit that are exposed to the irradiation process [12,13].

Irradiation technique has been developed for the disinfestation of many fruits against tephritids, including *Anastrepha* [14], *Bactrocera* [15-19], and *Ceratitis* [15,20,21]. In addition, determining the effects of irradiation on the fruit characteristics plays an important role for promoting the acceptance of this process by fruit growers, packers, and consumers [22].

Due to the need to develop quarantine treatments for cucurbits grown in regions infested with *A. grandis*, in the present study, we attempted to determine the minimum level of gamma irradiation necessary to prevent adult emergence from Atlas squashes infested with *A. grandis* eggs and larvae.

2. MATERIALS AND METHODS

2.1 Test Insects

The bioassays were conducted in the Laboratory of Economic Entomology of the Biological

Institute, located in Campinas, São Paulo State (SP), Brazil. The *Anastrepha grandis* colony used in this study was established in 2002, and the larvae were reared in orange pumpkin (Fig. 1B), according to the methodology described by Raga et al. [23].

2.2 Tested Fruit

In all tests using fruit, Atlas (American Butternut - Sakata®) squashes (*C. moschata*) were used (Fig. 1C), which is one of the Brazilian exportation agenda. The free-insecticide fruit were obtained from a farm located in Campinas (SP). The fruit was individually weighed before infestation, using an analytical balance (Balmak, model EL'C-6/15/30).

2.3 Irradiation Equipment, Immature Stage Tested and Criteria of Efficacy

Radiation experiments were performed at the Radiation Technology Centre of the Nuclear and Energy Research Institute (IPEN/CNEN) in São Paulo (SP). The irradiation equipment (^{60} Co) used in this study was a Gammacell irradiator (Atomic Energy of Canada Limited model 220 - serial number 142), with an initial activity of 1,588,734 Ci. The dose rate of the cobalt-60 irradiation equipment used during these experiments was 773 Gy/h.

We used approximately 10,000 *A. grandis* eggs (Fig.1D) and larvae (Fig. 1E) during the squash disinfestation tests. The disinfestation efficacy was compared among fruit treated with varying doses of gamma radiation based on the prevention of adult emergence as the end-point criterion. Initial *in vitro* irradiation tests provided the basis for the use of this technology to achieve the disinfestation of fruit against *A. grandis.*

2.4 Irradiation (^{60}Co) of *A. grandis in vitro*

The tool for *A. grandis* egg collection consists of red rigid PVC tubing (40 mm height x 15 cm diameter), containing approximately 40 holes in the side measuring 8 mm (in diameter) with a capped base and top. The side of the tube was covered with a layer of Parafilm ® and its interior was filled with distilled water. The *A. grandis* female perforates the parafilm in the area corresponding to the hole and lays the eggs inside the tube (Fig. 1F).

Anastrepha grandis eggs were submitted to the cobalt-60 irradiation process at the following treatment doses: 0.0 (untreated), 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 20.0 Gy. Each treatment consisted of 20 replicates, and each replicate consisted of 10 eggs that were 24 – 48 h- old. After 168 hours of treatment, we counted the number of dead and alive *A. grandis* eggs under a stereomicroscope.

Third instar larvae were submitted to the irradiation process at the following treatment doses: 0.0 (untreated), 10.0, 20.0, 30.0, 40.0, 50.0, 70.0, 100.0, 150.0, 200.0, and 250.0 Gy. Each treatment consisted of 20 replicates, and each replicate consisted of 10 larvae. After exposure, the larvae from each replicate were transferred to Petri dishes (10 cm × 1.5 cm) to become pupae (Fig. 1G) and adults (Fig. 1H). The Petri dishes were kept into Biological Oxygen Demand (BOD) at 25 ± 2 °C for 25–35 days. The efficacy was evaluated based on the number of adults that emerged.

2.5 Tests using Gamma Radiation on Squash Fruit Infested by *A. grandis*

Fruit were exposed to *A. grandis* females of 15 - 20 day-old during 24h. Eight fruit were used for each treatment condition, and each fruit was considered one replicate. After the irradiation treatment, each fruit was stored in a separate plastic container, and elastic bands were used to secure a cover cloth to the container. The fruit were maintained at a temperature of 25 ± 2 °C, at a relative humidity of approximately 70% ± 10%.

The cobalt-60 treatment doses tested against egg-infested fruits were 0.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 70.0, 100.0, 150.0, 200.0, and 250.0 Gy. The evaluation of the treated eggs occurred 45 to 60 days after exposure to each respective gamma radiation dose, by counting the number of emerged adults.

The irradiation doses tested for larvae-infested fruit were the same as described for the *in vitro* irradiation of *A. grandis* larvae. The efficacy was evaluated between 25 and 35 d after the treatments, by counting the number of emerged adults.

2.6 Post-treatment Fruit Quality

Tests to evaluate the colour and texture of the fruit shell and pulp were performed at the Laboratory of Instrumental Analysis of the

Faculty of Food Engineering (FEA) of the State University of Campinas (Unicamp).

The following physicochemical properties of Atlas squashes exposed to gamma radiation treatments were evaluated: titratable total acidity, sugar, pH, external colouration, and texture (adapted from Silva et al. [24]. For the evaluation of all parameters, we used three squashes per irradiation dose, and each squash was considered to be a replicate.

The irradiation doses were chosen based on the previously described *A. grandis* disinfestation tests. The fruit were exposed to 0 (untreated control), 40, 50, 70, 100, and 150 Gy.

2.6.1 Peel and pulp textures

We used the texture analyser, model TA-XT2i (Stable Micro Systems, Godalming, Surrey, England), with a load cell of 25 kg and texture expert software for Windows. The samples were evaluated by performing a drilling test, using a 2-mm diameter cylindrical probe at a constant speed of 1 mm/s. The texture analyzer settings during the pre-test and post-test rates were 1 mm/s and 10 mm/s, respectively. The penetration distance of the probe was 20 mm, and the plateau region was evaluated between 5 and 15 mm of fruit penetration. The textures of three fruit were analysed with five perforations performed in the perimeter of each fruit. The results are expressed in terms of the maximum force measured for the bark perforation and the average force required for penetration into the pulp region (plateau region).

2.6.2 Colouration

The colour index of the squash skin was evaluated using the HunterLab Colorimeter spectrum, with a MiniScan XE Plus. Data acquisition was performed using the Universal version 4.0 software. The evaluation of colour changes was performed using the Hunter - L * co-ordinate system, a* and b*, where L* varied from white (100) to black (0), a* varied from red (+a) to green (−a), and b* varied from yellow (+b) to blue (−b). Four readings per fruit were obtained, in the regions of the bulge and around the neck.

2.6.3 pH and titratable acidity (AT)

A pH meter (DM-20 from Digimed), which was calibrated with a buffer solution at 20°C and 95% sensitivity, was used to perform the titratable acidity and pH tests. For the test, 10 g of crushed fruit was placed into a 250 ml beaker, to which 90 ml of distilled water was added. The beaker containing the solution was placed on a shaker, and the pH meter electrode was placed in the solution to measure the pH of the diluted juice. The sample was then titrated using a standard solution of sodium hydroxide (NaOH) at 0.10 N until a pH of 8.1 was obtained. The volume of NaOH added was recorded for the calculation of titratable acidity (TA):

$$TA\ (\%) = [(V \times N \times M) / (m \times n)] * 100\%$$

Where,

V = volume of NaOH solution (L) added to the sample
N = Normality of NaOH solution (mol/L)
M = 134,1 = molecular weight of malic acid (g/mol)
m = sample mass (g)
n = number of carboxylic groups of the main acid (malic acid = 2)

2.6.4 Evaluation of soluble solids

The soluble solid contents were analysed by performing a direct reading in a digital refractometer (Reichert r2i300 from Ametek), which was calibrated with distilled water at 20°C. For this analysis, a small amount of the squash juice was used, which was obtained by wrapping a piece of squash in cotton and compressing the fruit until one or two drops of juice fell onto the prism of the refractometer. The results obtained were expressed in % of soluble solids.

2.7 Statistical analysis

The radiation experiments were analysed with a completely randomised design using statistical software StatPlus 2009 Professional and Assistat 7.7 [25]. The averages were compared using the Tukey test ($p \leq 0.05$). The *in vitro* and fruit test mortality values were submitted to probit analysis, using the Statplus 2009 Professional program (AnalystSoft), to estimate the 90% and 95% mortality rates; the 99.9968% mortality rate (probit 9) was estimated using PoloPlus version 0.03 [26-28].

The results obtained from the physicochemical fruit quality tests were subjected to comparisons by Tukey's test ($p \leq 0.05$), using the program Assistat 7.7 [25].

3. RESULTS

3.1 *In vitro* bioassays performed on the immature *A. grandis*

Approximately 2,000 *A. grandis* eggs were used during the *in vitro* gamma radiation tests. The mean viability of 24–48-h-old *A. grandis* eggs in the untreated control was 73%. The viability of *A. grandis* eggs treated with 2.0 Gy did not differ significantly different that of the untreated control. Hatchability began to be affected when *A. grandis* eggs were subjected to doses starting at 4.0 Gy. No larval eclosion of *A. grandis* was observed from the eggs treated at 20.0 Gy (Table 1).

The radiosensitivity of *A. grandis* eggs increased with radiation dose (Fig. 2A). However, to achieve Probit 9, a dose of gamma radiation approximately 10 times higher is required in comparison that to kill 50% of eggs. The dose required to induce 99% mortality in *A. grandis* eggs was estimated to be 25.78 Gy (Table 2).

We tested 2,200 *A. grandis* larvae in the *in vitro* radiation assay. Doses of up to 40 Gy did not significantly reduce the emergence of adults (Table 1) compared with the emergence rate of the untreated control. Doses starting at 50 Gy significantly reduced the emergence of *A. grandis* adults, and doses starting at 150 Gy completely prevented the emergence of adults (Table 1). The estimated dose for the non-emergence of adults at the probit 9 level for irradiated *A. grandis* larvae was 222.88 Gy (Table 2).

A significant decrease in immature *A. grandis* survival was observed when third instar larvae were exposed to doses ranging from 50 to 100 Gy, which suggested that this dose range may be related to the tolerance threshold of *A. grandis* larvae to irradiation (Table 1; Fig. 2B).

Table 1. Effects of gamma radiation treatment against *A. grandis,* both *in vitro* and when infesting Atlas squash fruit

Dose (Gy)	Treated stage/substrate							
	Eggs in vitro [a] *(n=10)*		Larvae in vitro[b] *(n=10)*		Eggs in fruit[b]		Larvae in fruit[b]	
0.0	2.70	g	7.75	a	83.50	a	68.37	a
2.0	2.95	fg	-	-	-	-	-	-
4.0	3.65	ef	-	-	-	-	-	-
5.0	-	-	7.80	a	52.25	ab	67.00	a
6.0	4.20	e	-	-	-	-	-	-
8.0	5.50	d	-	-	-	-	-	-
10.0	6.70	c	7.70	a	43.37	bc	62.12	ab
12.0	8.10	b	-	-	-	-	-	-
16.0	9.65	a	-	-	-	-	-	-
20.0	10.00	a	7.70	a	38.87	bcd	52.62	abc
30.0	-	-	7.00	ab	31.87	bcde	47.25	abc
40.0	-	-	7.20	a	26.75	bcde	46.00	abc
50.0	-	-	6.00	b	24.87	bcde	31.50	abcd
70.0	-	-	2.85	c	22.75	bcde	33.50	abcd
100.0	-	-	0.65	d	11.87	cde	19.12	bcd
150.0	-	-	0.00	d	4.37	de	10.75	cd
200.0	-	-	0.00	d	0.00	e	0.62	d
250.0	-	-	0.00	d	0.00	e	0.00	d

[a] *Average number of dead eggs per plot*
[b] *Average number of emerged adults per plot*
Values within columns followed by the same letter are not significantly different ($p < 0.05$) by Tukey´s test

Table 2. Probit analysis showing the lethal doses (LD) for *A. grandis* eggs and third instar larvae treated with gamma radiation, both *in vitro* and when infesting Atlas squash fruits

LD (%)	Probit (Y)	p-level	Dose Gy	Default error
Egg mortality in vitro				
50	5	0.323	5.23	0.967
90	6.2817	0.323	18.12	5.502
95	6.6452	0.323	25.78	10.214
99.9968	9	0.323	53.79	1.035
Non emergence of adults from larvae treated in vitro				
50	5	0.208	34.72	2.783
90	6.2817	0.208	158.08	154.220
95	6.6452	0.208	212.97	78.756
99.9968	9	0.237	222.88	1.187
Non emergence of adults from eggs treated in fruit				
50	5	0.1695	13.81	2.783
90	6.2817	0.1695	130.97	31.609
95	6.6452	0.1695	247.86	79.179
99.9968	9	0.323	257.13	0.059
Non emergence of adults from larvae treated in fruit				
50	5	0.1695	48.98	4.209
90	6.2817	0.1695	177.05	26.044
95	6.6452	0.1695	254.90	45.492
99.9968	9	0.323	270.25	0.092

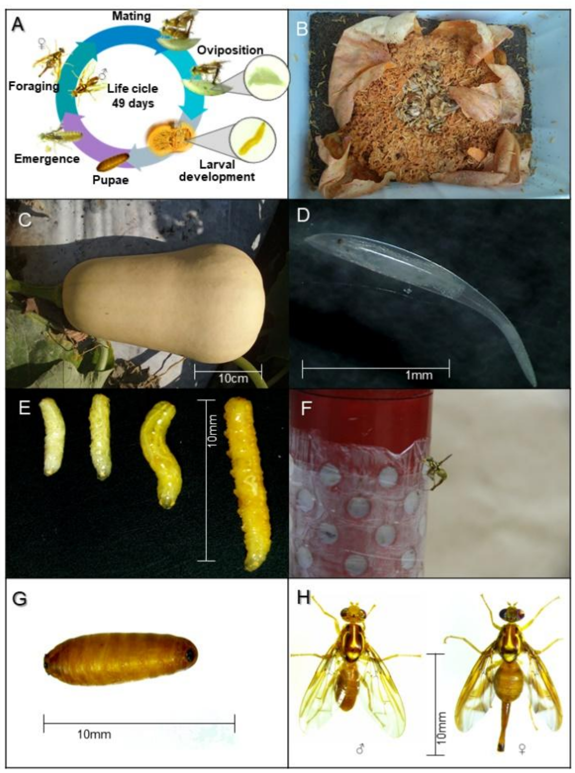

Fig. 1. Life cycle (A) and larval rearing (B) of *A. grandis*; 'Atlas' squash (C); egg (D) and larvae (E) of *A. grandis*; tool for oviposition (F); pupa (G) and adults (H) of *A. Grandis*

Table 3. Average values for pH, titratable acid – AT (%), soluble solids (%), texture, and colouration after Atlas squash fruit were submitted to gamma radiation

Dose	pH	AT	Soluble solids (%)	Texture	Coloration
Control	6.250 a	0.579 a	2.600 a	22.856 abc	34.075 a
40.0 Gy	6.187 a	0.789 a	2.467 a	19.072 c	35.808 a
50.0 Gy	6.210 a	0.909 a	3.033 a	23.727 abc	36.300 a
70.0 Gy	6.363 a	0.831 a	2.667 a	25.195 ab	34.978 a
100.0 Gy	6.443 a	0.627 a	2.800 a	20.450 bc	35.788 a
150.0 Gy	6.287 a	0.889 a	2.600 a	28.194 a	35.496 a

Values within columns followed by the same letter are not significantly different ($p < 0.05$) by Tukey´s test

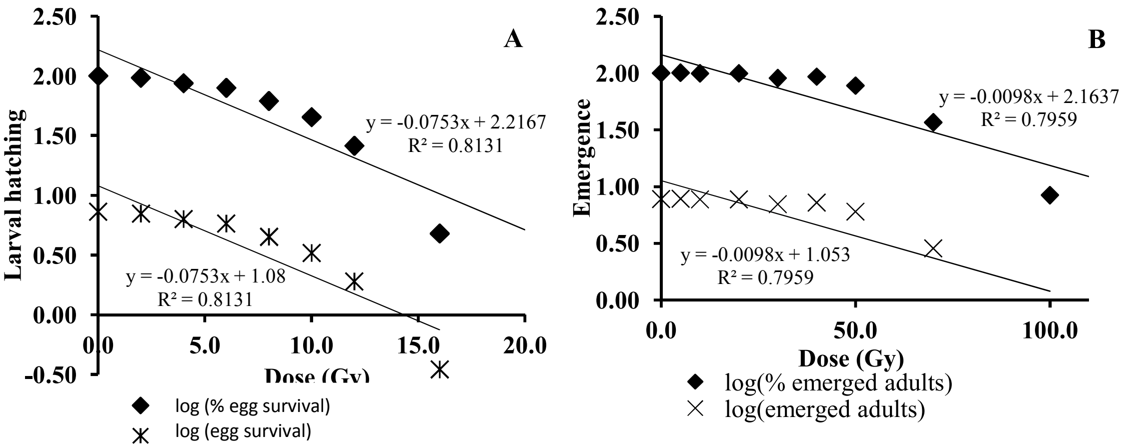

Fig. 2. Linear regression of larval hatching (A) and adult emergence (B) after eggs and larvae of *Anastrepha grandis* were submitted to gamma radiation doses *in vitro*

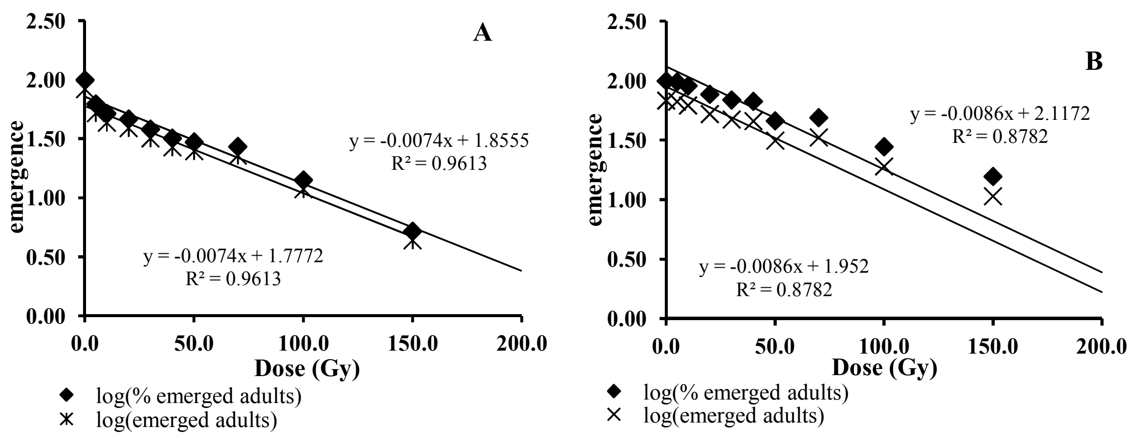

Fig. 3. Regression analyses modelling the relationship between adult emergence of *Anastrepha grandis* and gamma radiation doses after irradiation of eggs (A) and larvae (B) in Atlas squash fruit

3.2 Gamma Irradiation of *A. grandis* in Squash Fruit

The mean weight of the tested fruits was 324.5 g. The dose of gamma radiation required to prevent the adult emergence from squashes infested with *A. grandis* eggs increased to 200 Gy (Table 1), which was 10 times the dose required to prevent larval hatching *in vitro* (20 Gy). Doses ranging from 10 Gy to 70 Gy resulted in statistically similar results, in terms of number of emerged adults. A strong reduction in the number of emerged *A. grandis* adults was observed when fruit infested with eggs were irradiated at doses between 70 and 100 Gy (Fig. 3A). When squashes infested with *A. grandis* eggs were irradiated at 150 Gy, adult emergence was reduced by 99%. To prevent the emergence of adult *A. grandis* from eggs infesting squashes at the 50% and 99.968% levels, gamma radiation doses of 13.81 Gy and 257.13 Gy, respectively, were required (Table 2).

To perform tests evaluating the effect of cobalt-60 irradiation on *A. grandis* larvae infested in squash fruit, a minimum of 3,511 *A. grandis* larvae were treated. Squashes infested with *A. grandis* larvae and treated with irradiation doses between 100 Gy and 250 Gy showed significant reductions in the number of emerged adults (Table 1). A gradual reduction in adult emergence was observed when increasing radiation doses of up to 70.0 Gy were applied to *A. grandis* larvae (Fig. 3B), although this radiation level was not associated with any significant differences in the number of adults compared with the untreated control (Table 1), which indicated that the third instar larva was not strongly affected by gamma radiation when inside the fruit.

A significant effect of radiation on the emergence of adults was observed when fruit were treated at 200 Gy, whereas no *A. grandis* adults emerged at 250 Gy (Table 1). In the dose-response test, 100 Gy provided a 72% reduction in the number of *A. grandis* adults (Fig. 3B). A dose of 270.25 Gy was determined to be necessary to prevent the adult emergence at the 99.9968% level for squashes infested with *A. grandis* third instar larvae (Table 2).

3.3 Post-treatment Fruit Quality

No significant differences were found for the values of pH, titratable acidity (AT), or soluble solids treated squashes compared with the untreated control (Table 3), indicating that irradiation of squashes at levels up to 150 Gy had no significant effects on these quality attributes.

Generally, the values obtained for the texture and colouration of squashes subjected to various doses of gamma radiation (Table 3) were not significantly different from those for the untreated control. The texture displayed a clear non-linear dose-response.

4. DISCUSSION

In the present study, the untreated fruit (control) present 73% *A. grandis* egg viability (Table 1). The estimated values obtained using probit 9 analyses for determining the mortality rate of immature *A. grandis* eggs and larvae demonstrated the great potential value of using gamma radiation as a quarantine treatment for cucurbits.

Insect eggs are expected to be one of the most radiation-sensitive insect stages, particularly in the early stages [29]. The eggs of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), from the beginning of the embryonic period and through 24 h of age, have been shown to be more sensitive to radiation than later stages [20]. Radiation doses between 20 and 25 Gy applied to medfly eggs 4–6-h-old were sufficient to prevent adult emergence [30,31], which likely exhibit a similar gamma radiation tolerance as the *A. grandis* eggs in the present study. Similar to our results, the treatment of *Bactrocera tryoni* (Froggatt) eggs with 20 Gy prevented larvae from hatching [32].

The sensitivity of *A. grandis* eggs to gamma radiation may be associated with their relatively long length and large size (Fig. 1D). Consequently, *A. grandis* eggs may suffer from greater side effects in response to irradiation, caused by the release of free radicals during hydrolysis of water. When infesting squashes, *A. grandis* was found to be more tolerant to gamma radiation than other tephritids infesting softer fruits [18,31,33,34], which is likely because the eggs were situated below the squash shell, approximately one centimetre deep.
Third instar larvae of *A. grandis* were more radiotolerant than the eggs, with estimated probit 9 values of 53.79 Gy required to kill eggs compared with 222.88 Gy required to prevent adults from emerging from treated larvae. To achieve the desired level of reduction (Probit 9) in the number of *A. grandis* adults that emerge from fruit infested with third instar larvae (270.25 Gy), higher doses of radiation were required (Table 2) compared with the estimated doses calculated for larvae *in vitro* (222.88 Gy). Costa & Artur [34] needed 50 and 200 Gy to prevent adult emergence when *C. capitata* eggs and third larvae, respectively, were irradiated in two citrus varieties. The irradiation of peaches and

nectarines infested with 68–72 h medfly eggs required 400 and 450 Gy, respectively, to prevent hatching [35].

In a study attempting to define a generic dose for all species of the genus *Anastrepha,* Hallman [36] suggested that 70 Gy would be sufficient for use as a quarantine treatment. In contrast, based on our dose-response data, a dose of 250 Gy is necessary to prevent the emergence of adults from irradiated squashes. In many cucurbits, the mature larvae of *A. grandis* prefer to feed on the deeper sections of the pulp region, near the seeds. This behaviour may inhibit the efficacy of many quarantine disinfestation methods.

However, for irradiation to be successful as a disinfestation process, the applied dose must be adjusted for each fruit species, in addition to considering the characteristics of each cultivar, the fruit size, and the ripening stage [37]. The doses necessary to prevent the emergence of adults are lower than those necessary to kill the treated immature stage because cause successive and slow physiological effects on the development of the insect. Thus, depending on the applied dose, some irradiated larvae are able to pupate, but they are not able to reach the adult stage [38]. The statistical analysis of the data demonstrated that the survival kinetics were best represented by a first-order reaction (Figs. 1 and 2). For this phenomenon, we assumed that insect survival effects were proportional to increased irradiation exposure.

There are likely sizeable differences in the radiotolerance among genera and between species from the same genus of fruit flies. *Bactrocera tau* (Walker), which is found in Asia and also develops in squashes, was found to be more sensitive to radiation than *A. grandis*, requiring an estimated dose of 70.9 Gy to prevent the emergence of adults at the probit 9 level [18]. However, 46% of the *Bactrocera dorsalis* (Hendel) third instar larvae irradiated at 250 Gy *in vitro,* survived through pupation [39].

Using an X-ray machine as a source of radiation, Bachmann et al. [40] observed that the application of 100 Gy to *A. fraterculus* larvae prevented the emergence of adults. This species belongs to the *fraterculus* group, which present a distinct phylogenetic relationship with *A. grandis* (*grandis* group) [41]. Thus, the estimated Probit 9 level for *A. grandis* appears to be higher than the values obtained for other species of Tephritidae [18,21,42]. The amount of eggs and larvae tested in the present study made it possible to estimate the Probit 9 values, which are parameters for a future tests of the large-scale confirmatory phase [43], when a minimum of 93,613 individuals must be tested [44].

One hypothesis that might explain the large difference in the necessary levels of radiation required to treat eggs *in vitro* (20 Gy) compared with eggs inside of fruit (200 Gy) is that the squashes provide a measure of radioprotection. Foods rich in lipid and protein compounds also offer protection by competing for interactions with free radicals [45].

Although the mortality of larvae *in vitro* was not evaluated in our study, the observed radiotolerance of this stage of *A. grandis* was noticeable. When squashes infested with *A. grandis* larvae were irradiated from 100 to 250 Gy, we obtained similar results to those reported by Follett & Armstrong [46] for the third instar larvae of *Bactrocera cucurbitae* (Coquillett), *B. dorsalis,* and *C. capitata.*

The probit analysis indicated that to achieve the desired levels of reduction in the numbers of *A. grandis* adults that emerged from fruit infested by larvae, higher doses of cobalt-60 radiation were required for all LDs studied (Table 2). When compared with the doses obtained for the *in vitro* tests*,* the doses obtained for fruit were higher, indicating that the fruit are likely acting as radioprotectors during the treatment process. In trials examining guavas infested with *B. dorsalis* at 1, 2, 3, and 7 d of age, Jupeng et al. [42] did not recover any adults from a total of 100,684 irradiated larvae at doses of 97 and 116 Gy, resulting in an efficacy of 99.9970%.

However, a minimum absorbed dose of 250 Gy is used commercially as a quarantine treatment for some fruit against fruit fly species [14]. The lack of adult emergence should be used as the primary criterion for effectiveness [47].

To facilitate comparisons of the sterilisation efficiencies between the tests in which the third instar larvae were irradiated with gamma rays, the probit analysis was performed to estimate the probit curves (Table 2). The Fig. 3 showed an increase in dose and respective reduction in the number of adults, and the most dose-response curves assume a sigmoid shape [48].

The application of irradiation levels up to 750 Gy did not affect the quality and ripening of mangoes cv. 'Totapuri' [49]. Similar results were verified in guavas cv. 'Taiwan', which were evaluated at 1, 3, and 7 days, revealing that the fruit tolerated radiation doses of up to 800 Gy without displaying significant changes in chemical and nutritional content (sucrose, total soluble solids, titratable acid, vitamin C, and soluble solids) [42]. Jo et al. [50] reported that in citrus fruits irradiated with 400Gy gamma-ray, the total soluble solids and titratable acidity remained unaltered.

Gamma radiation represents a promising method for the disinfestation of squashes against *A. grandis* eggs and larvae. In addition, the tests examining the physicochemical qualities of Atlas squashes did not indicate any significant alterations when treated with doses of up to 150 Gy.

The loss of fruit firmness occurs as the result of two factors: excessive water loss and the associated decrease in cellular turgidity pressure, or the enzymatic decomposition of pectin and the cell wall [51,52]. Some species of Cucurbitaceae can reach 96% water content [53]. This high water content may result in increased radioprotection [54], especially when the larvae are deep inside the fruit.

Soluble solids and titratable acid were not significantly affected in citrus varieties irradiated with doses of up to 500 Gy [14]. Cantaloupe melons treated between 50 and 900 Gy were trade-viable according to the physicochemical and nutritional characteristics assessed during the post-harvest period [55]. No substantial differences in physiological parameters were detected in papayas and mangoes that were irradiated with doses of up to 2 kGy [56].

5. CONCLUSIONS

Increasing the dose of gamma radiation resulted in increased *A. grandis* egg and third-instar larva mortality. Third-instar larvae of *A. grandis* are more radioresistant than eggs. The prevention of *A. grandis* adult emergence can be achieved when Atlas squashes infested with *A. grandis* eggs or larvae are irradiated at 200 Gy and 250 Gy, respectively. We estimated a dose of 270.25 Gy to prevent the adult emergence at Probit 9 level for squashes infested with *A. grandis* third instar larvae. Physicochemical parameters of Atlas squashes exposed up to 150 Gy are unaltered. In summary, gamma radiation techniques have the potential for use as quarantine treatments to disinfest Atlas squashes infested with *A. grandis.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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