

## Ionizing Radiation for Quarantine Control of *Opogona sacchari* (Lepidoptera: Tineidae)

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**ABSTRACT** A discriminating irradiation dose of 150 gray (Gy) was used to determine the most tolerant immature stages of *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae). Based on adult emergence, early and late pupae were determined to be the most tolerant stages, and they were significantly more tolerant than eggs, neonate larvae, and larvae that were 1, 2, or 3 wk old. Irradiation treatment of eggs, neonates, 1-wk-old larvae, 2-wk-old larvae, 3-wk-old larvae, early pupae, and late pupae at 150 Gy resulted in a 96, 96, 95, 73, 61, 8, and 9% reduction in adult emergence, respectively. Pupae were treated with irradiation doses between 60 and 400 Gy. Emergence to the adult stage was significantly reduced by irradiation, averaging 90% in experimental controls and 29% in the 400-Gy treatment. Egg production was also reduced by irradiation, although the average age of pupae at the time of irradiation had a larger effect on fecundity. In total, 2,527 pupae treated with 120 Gy eclosed and produced 47,221 eggs and three F<sub>1</sub> larvae. In the 150-Gy treatment, 2,927 adults in total emerged from the 4,626 insects treated as pupae. These adults laid 62,878 eggs, none of which hatched. We conclude that a minimum dose of 150 Gy should be sufficient for sterilization of immature *O. sacchari* infesting export commodities.

**KEY WORDS** *Opogona sacchari*, sterilizing dose, irradiation, quarantine treatment, biosecurity

*Opogona sacchari* (Bojer) (Lepidoptera: Tineidae), is a pest of row crops, ornamental crops (including *Dracaena*, *Cordyline*, *Chamaedorea* palms, *Philodendron*, *Cycas revolute* Thunb.), and tropical fruit trees (including papaya and banana) (Peña et al. 1990). Around the world, this pest is commonly referred to as the “banana moth,” although the Entomological Society of America does not yet recognize a common name for this species. Larvae usually feed internally, burrowing within the stem or roots of host plants. In the United States, *O. sacchari* is recorded only from Florida and Hawaii. It was discovered in Hawaii in 1990 (Davis and Peña 1990). There is evidence that *O. sacchari* is expanding its host range in Hawaii, because it has been recently noted as a twig girdler of coffee and a root/stem feeder on orchids. In potted plants, larvae can even feed on the planting medium itself if it is high in organic matter (R.G.H., unpublished data). *O. sacchari* is a generalist feeder, and infestations typically begin on diseased, damaged or dead tissue, or on other organic material associated with the crop or commodity. Older larvae may burrow directly into healthy plant tissues (R.G.H., unpublished data). This opportunistic feeding pattern is probably responsible for occasional *O. sacchari* infestations on banana and many other horticultural commodities produced in Hawaii.

*O. sacchari* originates in tropical and subtropical Africa, but it was introduced into Brazil and Central America in the 1970s (CAB International 1997). At about this same time, it was detected in a number of countries in the European Union, primarily in glasshouses (CAB International 1997). In the United States, China, and the European Union, *O. sacchari* is regulated as a quarantine pest (CAB International 1997, Ju et al. 2004, USDA-APHIS 2005). All immature stages of *O. sacchari* are found on agricultural commodities, including eggs laid as masses beneath leaf sheaths or in other hidden or recessed areas; larvae that burrow into fruits, vegetables or plant stalks; and pupae, typically formed within the host material used for larval feeding.

Treating infested commodities with irradiation before export could prove a practical quarantine treatment method for this pest. Irradiation is an approved quarantine technology for 15 Hawaiian fruits and vegetables, several of which are hosts for *O. sacchari*. A commercial x-ray irradiation facility located on the island of Hawaii is already treating papaya (*Carica* spp.) and sweet potato (*Ipomoea* spp.) for export (Follett and Armstrong 2004), but banana (*Musa* spp.) also could become an important export crop treated with irradiation (Koger 2006; Federal Register 2006). A generic treatment dose of 150 Gy has been approved for tephritid fruit flies, whereas 400 Gy is approved as a generic dose for all other insects except adults or pupae of Lepidoptera (Follett 2006). Commodities infested with the latter cannot be exported using an

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irradiation protocol unless a specific irradiation treatment has been approved (Federal Register 2006).

A nonhost status protocol for bananas in Hawaii was developed for export to the U.S. mainland (Armstrong 2001). The protocol, which was developed for fruit flies, was seldom used due to problems with surface insects and the associated threat of rejection by quarantine inspectors. New federal quarantine regulations allow banana exports from Hawaii to the U.S. mainland by using irradiation as the quarantine treatment (Federal Register 2006). This banana protocol requires inspection for pupae and adults of *O. sacchari* and irradiation at a dose of 400 Gy (to control tephritid fruit flies and surface insects). Hence, detection of *O. sacchari* in a consignment will prevent export. Irradiation studies to date with *O. sacchari* were insufficient to recommend a treatment dose (Potenza et al. 1999, 2000). The purpose of this test was to determine the most radiotolerant stage of *O. sacchari* and to identify a potential dose that provides quarantine security.

### Materials and Methods

The research was conducted at the facilities of the USDA-ARS U.S. Pacific Basin Agricultural Research Center, Hilo, HI. Insects used in tests were taken from a colony of *O. sacchari* that has been in continuous laboratory culture since 1999, and it was originally collected from infested rambutan bark in Kurtistown, HI. Temperature during rearing and holding of *O. sacchari* averaged  $\approx 24^{\circ}\text{C}$  (range,  $19\text{--}27^{\circ}\text{C}$ ). Photoperiod was not controlled and reflected ambient laboratory conditions.

**Discriminating Dose Test.** Based on preliminary tests, a dose of 150 Gy was selected as a discriminating dose to determine which immature stage of *O. sacchari* was most tolerant to irradiation. Insects used in the discriminating dose test were reared on an artificial bean- and wheat-based diet (modified from Follett and Lower 2000), containing the following proportions of blended ingredients: 468 g of cooked great northern beans, 100 g of wheat mill, 70 g of yeast flakes, 7.0 g of ascorbic acid, 4.4 g of Na benzoate, 2.2 g of sorbic acid, 40 g of agar, 1,400 ml of hot water, and 1.2 g of Terramycin antibiotic (Pfizer, Morris Plains, NJ), to help reduce mold.

Life stages tested were 1-5-d-old eggs; 0-2-d-old (neonate) larvae; 1-, 2-, or 3-wk-old larvae; early pupae ( $\leq 1$  wk since pupation); and late pupae ( $> 1$  wk since pupation). Egg masses used in tests were collected on pieces of folded wax paper placed inside an oviposition chamber holding adult moths provisioned only with water. Egg masses were placed in 30-ml insect diet cups for irradiation treatment. The number of eggs present in each egg mass was counted after irradiation treatment but before hatching. After hatching was complete, the number of neonate larvae in the diet cup was counted. In the experimental controls, this number underestimated potential egg hatch, because neonate larvae often fed on unhatched eggs.

Larvae for tests were transferred by brush from larger ventilated plastic rearing containers (35 by 24 by 12.5 cm high; Rubbermaid, Fairlawn, OH) to ven-

tilated 400-ml plastic containers (11.5 cm in diameter by 4.2 cm in height; Sweetheart Plastics, Wilmington, MA) holding the artificial diet described above. Fifty or 100 larvae were added to each container. After irradiation treatment, container tops were removed, and each container holding larvae was placed within a larger ventilated plastic container (17 cm in diameter by 12.5 cm in height) holding water supplied via a wick extruding from a reservoir. Additional artificial diet was added as required. The insects pupated en masse in the bottom or on the sides of the containers. After all emerged moths had died, the pupal exuviae were counted, and this number was used to calculate percentage of emergence.

Pupae used in tests were taken from large ventilated rearing containers where they had attached themselves to the inside surfaces, or they were gently extracted from the cells of corrugated cardboard pieces that had been added to rearing containers to facilitate collection of pupae. Pupae  $\leq 6$  d old were divided equally into two groups (treatment replicates); the first group was irradiated the next day, whereas the second group was irradiated  $\approx 8$  d later. Pupae were irradiated within 200-ml ventilated plastic containers. Emergence of pupae was determined in the same manner as for larvae. On each date that insects were irradiated, we tested as many different life stages as were available from our colony, until we had completed at least three separate irradiation tests (by using different insect cohorts) for each life stage. For each replication of each life stage, two containers were prepared, one holding insects to be treated, and one holding insects that traveled to the irradiator facility but were not treated (the experimental control).

**Sterilizing Dose Tests.** Based on the finding that pupae were the most radiotolerant stage, we carried out a dose-response study with pupae to determine a putative sterilizing dose, followed by two validation tests using larger numbers of pupae. *O. sacchari* larvae were reared on diced (1-2 cm-thick) sweet potato roots (purple-fleshed Okinawan variety) within ventilated plastic containers. Pupae used in these tests were obtained from pieces of corrugated cardboard that were left for various periods in containers holding *O. sacchari* larvae. Periods of pupal collection (ranging from 6 to 20 d) were recorded, and the mid-point of collection period was used to compute the average age of pupae at the time of treatment. After pupae (within their pupal cases, or cocoons) were extracted, they were held in separate containers, and moths emerging before the irradiation treatment were removed daily, to minimize the chance that eggs would be laid on pupae used in irradiation treatments. As an extra precaution, cocoons were removed from pupae 1-2 d before the irradiation treatment. Pupae of each cohort were randomly divided into five equal groups and randomly assigned to a treatment or to the experimental control. Four experiments were carried out in sequence, including two dose-response tests and two validation tests. The first experiment compared treatments of 0, 100, 250, 325, and 400 Gy (eight replications carried out over time). The second compared 0, 60, 90,

120, and 150 Gy (six replications carried out over time). The third experiment compared 0 and 120 Gy, and the fourth compared 0 and 150 Gy (4 and 11 replications, respectively, carried out over time). In the latter two validation experiments, four of the five groups of pupae in each cohort were assigned to the irradiation treatment, yet each group was handled and set up individually (placed in separate potatoes for irradiation and transferred to separate holding containers). For analysis purposes, the four groups were considered as a single replicate. In all of the sterilizing dose experiments, the pupae in each treatment group were placed within a separate sweet potato root within a hole (2 cm in diameter), which had been bored into each root for this purpose. The hole was plugged with tissue paper before treatment. Dosimeters were placed inside an extra root of similar size and weight.

After treatment, pupae were transferred to ventilated, round, plastic containers (21 cm in diameter by 13 cm in height; Berry Plastics, Evansville, IN) holding folded wax paper (to collect eggs) and provisioned with water wicks. Containers also held a 300-ml plastic cup (9.0 cm in diameter by 4.7 cm in height; Highland Plastics, Mira Loma, CA) filled three-quarters full with artificial diet. The cup was fitted with a fiberglass window-screen cover and turned upside down on the bottom of the container to maximize the chance that neonate larvae would find the diet. A small amount of hot glue added to the screen cover raised it slightly off the bottom of the container, allowing small larvae access to the diet. To eliminate contamination problems encountered in preliminary trials, holding containers for irradiated pupae were prepared for use by soaking previously used containers and lids in bleach solution, followed by freezing of the containers and lids. Containers holding pupae were placed inside sealed paper bags, and held in different rooms to physically separate insects associated with different treatment levels. After all adults had emerged and died within containers (30–32 d after irradiation of pupae), the containers were opened, and data were collected on the number of pupal exuviae, the number of eggs (categorized as hatched or unhatched) on wax paper, and the number of  $F_1$  larvae in the containers. The sex ratio of insects that emerged to the adult stage was not determined. However, our informal observations are consistent with the hypothesis of a 50:50 sex ratio.

**Irradiation Process.** Treatments were carried out at a commercial irradiation facility (Hawaii Pride LLC, Keaau, HI) that uses an electron linear accelerator (5 MeV, model TB-5/15, L-3 Communications Titan Corp., San Diego, CA) at ambient temperatures to create x-rays to sterilize insects pests of quarantine significance in papayas, sweet potatoes, and various types of fresh fruit exported to the mainland U.S. To estimate the actual doses received by insects in each irradiation exposure, we averaged the readings from two dosimeters (Opti-chromic detectors, FWT-70–83M, Far West Technology, Goleta, CA) placed among the insects being treated or placed within the same types of containers or potatoes as those holding insects. To minimize variation, a cardboard box was

used to elevate the samples to the middle of the carrier as it passed by the x-ray source. The dosimeters were read with a FWT-200 reader (Far West Technology) at 600-nm absorbance.

**Analysis.** In the discriminating dose test, data on percentage of emergence of the treated insects in each developmental stage were adjusted for control mortality (Abbott 1925), arcsine (square-root) transformed (Steel and Torrie 1980), and used in one-way analysis of variance (ANOVA) with “stage” as the independent variable (SAS Institute 2001). In two of the four replications involving late pupae, percentage of adult emergence was higher for treated than for control insects (6 and 13% higher); in these cases, corrected mortality for treated insects was adjusted to zero before data analysis.

In the sterilizing dose studies, data associated with the first two experiments (dose–response tests) were analyzed and presented separately from data associated with the experiments 3 and 4 (validation tests at 120 and 150 Gy, respectively). Data were kept separate because the average age of pupae used in the validation tests was greater than the average age of pupae used in the first two experiments. Data were omitted when the difference between actual doses and target doses was >10% of target dose. Regression analyses used actual dose as the independent variable and percentage of adult emergence, number of eggs per adult, or percentage of egg hatch as the dependent variable. Before analysis, percentage data were transformed as described above. In a separate analysis using data pooled from both dose–response and validation tests, the number of eggs per adult was regressed on actual dose and the average age of pupae at the time of treatment. The purpose of this multiple regression analysis was provide a possible explanation for the observation that egg production was greater in the validation tests compared with the dose–response tests. Regression analyses and Tukey’s multiple comparison tests were performed using SAS statistical software (SAS Institute 2001).

## Results

**Discriminating Dose Test.** Although the target dose was 150 Gy, actual irradiation dose levels measured in 14 replicates averaged 148.8 Gy (SEM = 1.2 Gy, range 140.8–158.8). Irradiation of immature stages of *O. sacchari* at this level reduced adult emergence by 8–96%, depending on the stage treated (Table 1). Based on ANOVA, there were significant differences in tolerance among the different stages ( $F = 23.8$ ;  $df = 6, 21$ ;  $P < 0.01$ ). Early and late pupae were significantly more tolerant to irradiation than eggs, neonates, and larvae of any age by using adult emergence as the required criterion ( $P \leq 0.05$ , error  $df = 21$ , Tukey’s honestly significant difference [HSD]). The reduction in adult emergence associated with treatment of early and late pupae was 8 and 9%, respectively; the difference between the two was not significant ( $P > 0.05$ , error  $df = 21$ , Tukey’s HSD). There was no significant difference in tolerance among eggs and larvae of any age; how-

**Table 1.** *O. sacchari* mortality after ionizing radiation treatment at 150 Gy

Insect stage	Age within stage	No. replications	N	% mortality ± SEM
Eggs		4 <sup>a</sup>	1,637	96 <sup>b</sup> ± 3.1a
Larvae	Neonates	4	250	95 ± 3.8a
	1 wk old	4	200	95 ± 4.6a
	2 wk old	4	200	73 ± 5.1a
	3 wk old	3	150	61 ± 18.7a
Pupae	≤1 wk old	5	1,072	8 ± 2.9b
	>1 wk old	4	644	9 ± 9.0b

Means followed by a different letter are statistically different from one another ( $P \leq 0.05$ , Tukey's HSD).

<sup>a</sup> Replications were carried out over time and used similar numbers of control and treated insects.

<sup>b</sup> Percentage of mortality, except for the egg stage, was calculated as  $100 \times [(\text{initial no. insects}) - (\text{no. of insects emerging to adult stage})] / [\text{initial no. insects}]$ . For eggs, percentage of mortality was calculated as  $100 \times [(\text{initial no. eggs}) - (\text{no. of neonates that hatched from eggs})] / [\text{initial no. eggs}]$ . Mortality data were corrected for control mortality according to Abbott (1925).

ever, there was a trend toward increasing tolerance with larval age (Table 1).

**Sterilizing Dose Data, Dose-Response Tests.** The average age of pupae at the time of irradiation treatment over treatment dates was 8.6 d (SEM = 0.7,  $N = 14$ ). Percent adult emergence was significantly affected by irradiation treatments in the range of 60–400 Gy (Table 2) ( $P < 0.01$ ,  $F = 130$ ,  $df = 1, 67$ ;  $R^2 = 0.66$ ). At dose levels of 250 and 325 Gy, adult emergence was reduced 55 and 68%, respectively. Exposure to 400 Gy did not further reduce adult emergence compared with results obtained using 325 Gy (Table 2).

The number of eggs produced per adult moth was also significantly affected by irradiation ( $P < 0.01$ ,  $F = 54$ ,  $df = 1, 65$ ;  $R^2 = 0.45$ ). Egg production in the 250-, 325-, and 400-Gy treatments averaged 1.2–2.3 eggs per adult, <15% of egg production in the untreated control (Table 3).

**Table 2.** Adult emergence of *O. sacchari* after irradiation treatment during the pupal stage

Nominal dose (Gy)	Actual dose (Gy) (SEM)	Dose range	No. replications <sup>a</sup>	N <sup>b</sup>	% adult emergence ± SEM
0	0 ± 0		14	1,365	89.5 ± 1.3
60	61.6 ± 0.8	59–65	6	670	85.8 ± 2.2
90	91.6 ± 0.9	90–95	6	666	80.4 ± 2.4
100	101.6 ± 1.6	98–109	8	695	68.1 ± 4.9
120	120.1 ± 2.6	113–130	6	663	70.6 ± 2.3
150	149.8 ± 2.7	142–156	5	595	69.3 ± 2.7
250	252.1 ± 3.8	238–267	8	689	40.7 ± 7.7
325	325.4 ± 4.3	310–343	8	684	28.2 ± 9.2
400	394.8 ± 4.8	377–411	8	684	28.6 ± 8.6

Regression of percentage of emergence on actual dose was significant at  $P < 0.01$  ( $F = 130$ ;  $df = 1, 67$ ;  $R^2 = 0.66$ ;  $y = 1.24 [\mu\text{inv}\sigma]$   $0.0020x$ , where  $y$  is percentage of emergence, and  $x$  is dose (Gy), by using arcsine (square-root) transformed data.

<sup>a</sup> Replications were carried out over time. Data shown were pooled from 2 separate tests as explained in Materials and Methods.

<sup>b</sup> N is total number of pupae treated. The number of pupae treated in each replicate varied from 45 to 184.

**Table 3.** Number of eggs and larvae produced by *O. sacchari* exposed to irradiation as pupae

Nominal dose (Gy)	No. pupae treated	Total eggs produced	No. eggs per adult ± SEM	% egg hatch ± SEM	Total no. larvae found
0	1,365	15,821	15.7 ± 1.7	86.2 ± 1.2	2,920
60	670	10,789	17.9 ± 2.9	4.8 ± 1.5	234
90	666	8,851	14.9 ± 3.3	0.04 ± 0.03	5
100	695	4,108	8.1 ± 2.5	0.05 ± 0.05	1
120	663	7,866	16.4 ± 2.7	0 ± 0	0
150	595	3,688	8.6 ± 2.2	0 ± 0	0
250	689	2,130	2.3 ± 1.6	0 ± 0	0
325	684	1,134	1.2 ± 1.0	0 ± 0	0
400	684	1,362	1.8 ± 1.3	0 ± 0	0

Regression of number of eggs per adult on actual dose was significant at  $P < 0.01$  ( $F = 54$ ;  $df = 1, 65$ ;  $R^2 = 0.45$ ;  $y = 16.5 - 0.043x$ , where  $y$  is number of eggs per adult and  $x$  is dose (Gy).

Regression of percentage of egg hatch on actual dose was significant at  $P < 0.01$  ( $F = 35$ ;  $df = 1, 46$ ;  $R^2 = 0.43$ ;  $y = 0.70 - 0.0032x$ , where  $y$  is percentage of egg hatch and  $x$  is dose (Gy), by using arcsine (square-root) transformed data.

Percentage of egg hatch was significantly affected by irradiation ( $P < 0.01$ ,  $F = 35$ ,  $df = 1, 46$ ;  $R^2 = 0.43$ ), and the effects were much greater than those observed with egg production (Table 3). In the experimental control, egg hatch averaged 86%. Egg hatch was reduced by 94% at 60 Gy, the lowest level of irradiation tested.

Based on dose-response data, no viable progeny were produced when irradiation levels  $\geq 120$  Gy were used (Table 3). Of the 663 pupae exposed to the 120 Gy level of irradiation, 468 emerged as adults and produced a total of 7,866 eggs. None of the eggs were observed to hatch, and no larvae were recovered.

**Sterilizing Dose Data, Validation Tests.** The average age of pupae at the time of irradiation treatment over treatment dates was 11.6 d (SEM = 0.3,  $N = 15$  dates). Based on the results obtained in dose-response tests, 120 Gy was tested as a putative sterilizing dose in the first validation test that used 1,864 pupae. Adults emerging from pupae treated at 120 Gy laid 39,355 eggs, none of which were observed to hatch (Table 4). However, single larvae were recovered from three of the 16 treatment containers, involving two of the four test dates (replicates) (Table 4). Therefore, the dose level was raised to 150 Gy, and a second validation test was carried out using an additional 4,031 pupae in 11 replicates. The 2,588 adults that emerged produced a total of 59,190 eggs on the wax paper placed within containers. No hatching of these eggs was observed, and no larvae were recovered in any of the holding containers.

## Discussion

At the 150-Gy dose level, a total of 4,626 pupae were irradiated in 16 experimental replicates. From these pupae, 2,927 insects emerged as adults and produced 62,878 eggs. None of the eggs hatched, and no larvae were found in any of the containers holding treated insects. In the experimental control, there were a total

**Table 4.** Number of eggs and larvae produced by *O. sacchari* exposed to irradiation as pupae in validation tests at 120 and 150 Gy

Nominal dose (Gy)	Actual dose (Gy) ± SEM	Dose range	No. pupae treated	No. replicates	% adult emergence ± SEM	Total eggs produced	No. eggs per adult ± SEM	% egg hatch ± SEM	Total no. larvae found
0	0 ± 0		1,485	15	80.8 ± 2.9a	33,824	32.9 ± 2.7a	87.8 ± 1.7a	1,877
120	120.2 ± 0.8	114–126	1,864	4	75.2 ± 1.5a	39,355	27.8 ± 4.7ab	0 ± 0b	3
150	142.7 ± 0.6	135–151	4,031	11	64.2 ± 2.7b	59,190	23.6 ± 2.4b	0 ± 0b	0

Means followed by the same letter(s) are not statistically different from one another ( $P \leq 0.05$ ; Tukey's HSD). Percentage data were arcsine (square-root) transformed before analysis.

of 2,850 pupae divided among 29 replicates. In total, 2,411 of these emerged as adults, and the females produced a total of 49,646 eggs. Eighty-seven percent of these eggs hatched, and 4,480 larvae were recovered from the holding containers. These results, together with our finding that pupae are the immature stage most tolerant to irradiation, indicate that a dose level of 150 Gy should be sufficient as a sterilizing dose for immature *O. sacchari*. The results are important because current quarantine treatment schedules for irradiation in the United States do not allow shipment of a commodity infested with pupae of *O. sacchari*. An irradiation dose of 150 Gy is easily tolerated by most fruit and vegetable commodities during commercial treatment without injury (Morris and Jessup 1994).

No egg hatch was observed in the egg masses laid on wax paper by females exposed to the 120-Gy treatment. However, three larvae were found in treatment containers, indicated that egg hatch had nevertheless occurred. Whereas the majority of eggs found within containers were laid as masses on the folded wax paper pieces placed inside, eggs were occasionally laid singly on other types of surfaces within the container. Occasionally small egg masses were found on exuviae of pupae. Eggs laid in these locations were not routinely examined. A hatched egg could also have escaped detection if a neonate larva completely consumed its shell. In several replicates associated with 120-Gy treatments, it was noted that some of the apparently dead embryos had developed to a stage where head capsules could be seen through the shells of the eggs. Head capsules were never seen through the shells of any of the eggs produced by females exposed to the 150-Gy treatment.

In the validation tests, the average number of eggs laid per adult in the 0-, 120-, and 150-Gy treatments (shown in Table 4) was approximately double the number of eggs laid per adult for the same dose levels, respectively, in the dose-response tests (shown in Table 3). This difference can be explained by differences in the average ages of pupae used. In the two validation tests carried out at 120 and 150 Gy, irradiation tests were carried out less frequently (generally every 2 wk instead of every week). Consequently, the pupae used in the validation tests were collected over a longer period, thereby increasing their average age at the time of irradiation. The pupal stage of *O. sacchari* lasts  $\approx 12.5$  d at 24°C (Davis & Peña 1990). The average age of untreated pupae in the validation tests was 11.6 d, and fecundity averaged 32.9 eggs per adult. The average age of untreated pupae in dose-response tests

was 8.6 d, and fecundity averaged 15.7. Using the complete data set, results of multiple regression analysis showed that egg production per emerged adult was negatively affected by irradiation dose ( $t < 0.01$ ) but positively related to average pupal age at the time of treatment ( $t < 0.01$ ) [ $F(\text{model}) = 49.0$ ;  $df = 2, 93$ ;  $P < 0.01$ ,  $R^2 = 0.51$ ]. Parameter estimates from multiple regression yielded the equation:  $y = 6.3 - 0.06x + 1.72z$ , where  $y$  is eggs per adult,  $x$  is dose (Gy), and  $z$  is average age of pupae (d).

In Brazil, Potenza et al. (2000) compared the radiotolerances of 2- and 7 d-old *O. sacchari* eggs, 2- and 22-d-old larvae, and 2- and 11-d-old pupae. Pupae that were 11 d old were determined to be the most tolerant stage as measured by percentage of adult emergence. A dose of 450 Gy completely prevented adult eclosion, whereas 3% of pupae treated with 400 Gy emerged. For 2-d-old pupae, a dose of 350 Gy but not 300 Gy completely prevented adult emergence. In a separate study, Potenza et al. (1999) treated fifth instars, and they found that 300 Gy completely prevented development to the adult stage, whereas a dose of 240 Gy resulted in a 2% rate of development to adult. Reproductive performance of emerging adults was not reported. Adult sterility is the critical measure in determining the lowest level of irradiation that will provide quarantine security (Follett and Neven 2006, Follett and Griffin 2006). In our study,  $\approx 29\%$  of pupae treated with 400 Gy developed to the adult stage. Using the same dose level, Potenza et al. (2000) found that only 3% of pupae developed to the adult stage. This difference is likely a consequence of age differences within the cohorts of pupae used in our tests. In general, older pupae are more likely than younger pupae to eclose after irradiation treatments. To increase our sample sizes, we used pupae of mixed ages. A proportion of the pupae we used would have been very close to the time of adult emergence (within 1 or 2 d) at the time of irradiation treatments. Similar reasoning can be used to explain why adult emergence in our study was very similar in the 325- and 400-Gy treatments; we hypothesize that both the 325- and 400-Gy doses prevented emergence in nearly all of the pupae tested except those within several days of eclosion.

In this study, 4,626 pupae in total were irradiated at the 150-Gy dose level. The probit 9 standard for the development of quarantine treatments requires that 93,613 insects are treated with no survivors (Couey and Chew 1986). However, the probit 9 standard was initially recommended for tropical fruit heavily infested with fruit flies (Baker 1939). The alternative

approach for determining treatment efficacy is to calculate the probability of a mating pair, gravid female or parthenogenic individual surviving in a shipment (Landolt et al. 1984). Follett and McQuate (2001) have demonstrated that quarantine security (defined as 99% confidence that <1 mating pair survives in a given volume or weight of a commodity shipment) in certain cases can be achieved using sample sizes of test insects that are several orders of magnitude lower than those required to achieve quarantine security as defined by the probit 9 approach. For example, for *Bactrocera dorsalis* (Hendel) and *Cryptophlebia* spp. infesting rambutan, *Nephelium lappaceum* L., in Hawaii, Follett and McQuate calculated that the minimum number of insects required in tests to demonstrate quarantine security (assuming commodity shipment sizes of 2,000 kg) was 639 and 923, respectively. In this example, the required number of test insects was low because rambutan is a poor host for these pests. For *O. sacchari*, data are not available on infestation rates on bananas and other potential host fruits and vegetables after these commodities have been prepared for export (after washing and culling practices). However, each shipment of bananas leaving Hawaii receives a mandatory USDA-APHIS inspection for *O. sacchari* and other external pests before export. These inspection records might be used as a basis for estimating infestation rates of *O. sacchari* on bananas, which could be incorporated in mathematical probability models to assess levels of quarantine security associated with potential irradiation treatments, used alone or in combination with inspection results.

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