Journal of Economic Entomology, XX(XX), 2023, 1–8
https://doi.org/10.1093/jee/toad170
Research

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Commodity Treatment and Quarantine Entomology

Phytosanitary irradiation treatment of the aerial root mealybug, *Pseudococcus baliteus* (Hemiptera: Pseudococcidae)

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Subject Editor: Dong H. Cha

Received on 25 May 2023; revised on 31 July 2023; accepted on 16 August 2023

The aerial root mealybug, *Pseudococcus baliteus* Lit (Hemiptera: Pseudococcidae), is an important invasive and quarantine pest that poses a potential threat to fruits, vegetables, and ornamental plants. As a result, phytosanitary treatments are necessary to ensure the commodities of international trade are free from these pests. To determine the minimum absorbed dose required for phytosanitary irradiation (PI) application, irradiation dose-response and large-scale confirmatory tests were conducted. Eggs that were 2, 4, and 6 days old and late gravid females (containing 0-day-old eggs) of *P. baliteus* were X-ray irradiated with doses of 10, 20, 40, 60, 80, 100, and 120 Gray (Gy). The efficacy of preventing egg-hatching (mortality) was compared using two-way ANOVA, 95% confidence interval overlapping and lethal dose ratio test in probit analysis. The radiotolerance sequence of mealybugs egg was found to be 0 < 2 ≈ 4 < 6-day-old eggs, and their estimated $LD_{99.9968}$ values with 95% confidence interval were 132.0 (118.9–149.5), 137.6 (125.2–153.7), 145.5 (134.5–159.1), and 157.4 (144.6–173.6) Gy, respectively. Subsequently, target doses of 135 and 145 Gy were used in the confirmatory gamma radiation treatments. No F_1 generation neonates developed from a total of 47,316 late females irradiated at the measured dose of 107.7–182.5 Gy, resulting in the treatment efficiency of 99.9937% at the 95% confidence level. Therefore, the highest dose of 183 Gy measured in the confirmatory tests is recommended as the minimum absorbed dose in PI treatment of *P. baliteus* for establishing national and international standards.

Key words: invasive species, radiation biology, quarantine

Introduction

In 1984, the aerial root mealybug, *Pseudococcus baliteus* Lit (Hemiptera: Pseudococcidae), was first identified on the aerial roots of *Ficus elastica* Roxb. ex Hornem in the Philippines, and subsequently named after its host plant. This pest attacks a range of agricultural, horticultural, and forest plant species, including litchi, durian, guava, orange, longan, rambutan, wax-apple, mangrove, fragrant conehead, and olive (Lit and Calilung 1994a, b, Williams 2004). Its strong reproductive ability, with an average of 550 eggs laid per female, results in significant damage to host plans, leading to reduced yield, delayed ripening, leaf shedding, yellowing, and loss of commodity value in serious cases. (Jiao et al. 2011, Zhao et al. 2021a).

Currently, *P. baliteus* is distributed in various countries, including Indonesia, Myanmar, India, Cambodia, Singapore, Philippines, Thailand, and Vietnam, and China (He et al. 2011, Cai et al. 2023). It is listed as a quarantine pest for the importation of durian from Thailand and mangosteen from Indonesia, and it is also a quarantine pest in Japan, the United States, and other countries (Zhao et al. 2021a). Aerial root mealybugs are small, usually live in concealed portions of their hosts, and transported with commodities in international trade. Commodities originated from quarantine area and/

or infested with *P. baliteus* should be subjected to phytosanitary treatments.

Postharvest disinfestation treatments include fumigation, cold, heat, ionizing radiation, modified atmospheres, pesticide, and their combinations (Heather and Hallman 2008). Methyl bromide (MB) fumigation is currently a common measure to disinfest regulated pests in commodities internationally, but MB can deplete the atmospheric ozone layer, and it is therefore restricted in use excepted for quarantine and pre-shipment uses (IPPC 2008).

Cold treatment is another widely used disinfestation measure for quarantine pests, accomplished by holding commodities at low temperatures. For instance, treating fresh grapes for 12 days at 1 °C, 14 days at both 2 and 3 °C can eliminate the Queensland fruit fly *Bactrocera tryoni* Froggatt (Heather and Hallman 2008, De Lima et al. 2011). However, phytosanitary cold treatment requires a long treatment time, and it may damage some tropical fruits, e.g., mango and papaya. In such cases, hot-water immersion and vapor heat treatment are often utilized to disinfest fruit fly from these tropical fruits (Heather and Hallman 2008, Ndlela et al. 2017, Hsu et al. 2018).

In comparison with other postharvest disinfestation treatments, irradiation of produce has further advantages. It is highly penetrative in commodities; therefore, it offers complete control of insect pests within fruit and prevents insect pests form subsequent development and reproduction; there is no residues, and there are no negative effects that could endanger consumers (Hallman et al. 2010). Phytosanitary irradiation (PI) treatment measures have been developed and used in international trade since the establishment of the International Standard on Phytosanitary Measure (ISPM) (ISPM 18: *Guidelines/Requirements for the use of irradiation as a phytosanitary measure*) in 2003 and revised in 2023, and the international shipment trials between Australia and New Zealand in 2004 (IPPC 2003, 2023, Hallman et al. 2016). Moreover, PI is an alternative to MB fumigation in disinfesting mealybugs of fruits to overcome quarantine barriers in trade (Hallman 2012, IPPC 2023). Determination of the minimum absorbed radiation dose for providing effective quarantine security and biosecurity against specific pests and for specific traded products is critical (IPPC 2015, Hallman et al. 2016).

Several studies have been performed to determine the minimum absorbed doses, establish treatment schedules, and develop a generic dose for PI treatment of the family Pseudococcidae (Hofmeyr et al. 2016a, Zhan et al. 2016, Ma et al. 2019, 2022). A minimum dose of 231 Gy is adopted by the International Plant Protection Convention (IPPC) to prevent the reproduction of adult females of *Dysmicoccus neobrevipes* Beardsley, *Planococcus lilacinus* Cock, and *Pl. minor* Maskell (IPPC 2015).

There are no standards available for the PI treatment of *P. baliteus*, the aerial root mealybug. Here, in order to determine the minimum absorbed dose for PI treatment of *P. baliteus*, we conduct dose-response and large-scale confirmatory tests based on the research protocol specified in ISPM 18 (IPPC 2003, 2007).

Materials and Methods

Insect Rearing

The colony of *P. baliteus* used in this research was intercepted at Pingxiang Port, Guangxi Autonomous Region, China, from mangosteens imported from Thailand. The insects were reared in the Laboratory of Phytosanitary Treatment and Equipment, Chinese Academy of Inspection and Quarantine, Beijing, China on insecticide-free pumpkins, *Cucurbita maxima* Duch. (Violales: Cucurbitaceae). The infested pumpkins were incubated in plastic boxes (13.5 × 12.5 × 9.5 cm) with a meshed ventilation window in the laboratory at 26 ± 2 °C and 60–80% RH, under a photoperiod of L:D = 14:10 h (Zhao et al. 2021a).

X-ray Irradiation

Preparation of adult females.

The adult stage is the most radio-tolerant life stage, and so adults were used as the target stage for PI treatment (IPPC 2007, Hallman et al. 2010), whereas egg-hatching rate or emergence of second-instar nymphs were used for efficacy-evaluation (Hofmeyr et al. 2016a, Zhan et al. 2016, Ma et al. 2019, 2022). Eggs of *P. baliteus* are found in the ovisac attached to the body of adult females. Thus, all the eggs in the body and ovisac were irradiated with gravid females. Before irradiation treatment, 10 gravid adult females (40–45 days old) reared on pumpkin fruits were transferred to a 9-cm polystyrene Petri dish with moist filter paper on the bottom on each (as a treatment batch), were subjected to irradiation at the target dose of 10, 20, 40, 60, 80, 100, and 120 Gy.

Preparation of eggs.

To collect eggs, 20 gravid adult females were transferred from pumpkin using a fine brush to a 9 cm Petri dish containing a layer of moist filter paper to maintain humidity. The Petri dish was sealed with a plastic film punched with pinholes for ventilation and placed in the rearing room, the rearing conditions are consistent with the laboratory environments. Adult females were removed 24 h later and the eggs were counted under a stereomicroscope (SteREO Discovery V12, ZEISS, Germany) and transferred to a new Petri dish with moist filter paper on the bottom (120 eggs/dish). Petri dishes were then placed back in the rearing room and the eggs allowed to develop for 2, 4, or 6 days before treatment. Eggs hatched from 7 to 11 days after laying.

X-ray irradiation treatment.

An RS-2000 Pro X-ray irradiator (Rad Source Technologies, Inc., USA) was used for the dose-response test of eggs and adult females. Operating parameters were 220 keV and 17.6 mA. There was a reflector (width of 43 cm, depth of 38 cm, height of 43 cm) which was placed at the exposure platform (35 cm from the X-ray source) to provide even dose distribution about 90–95% dose uniformity detected before irradiation. A Petri dish containing eggs or adult females was placed in the center of the chamber to be irradiated, and a dosimeter (model 2086, RadCal Corp., CA) with a 10 × 6-6 Ion Beam Chamber was set beside the dishes (Ma et al. 2019, Zhao et al. 2021b).

Eggs and adult females were subjected to irradiation treatments of 0 (as control), 10, 20, 40, 60, 80, 100, and 120 Gy. Each treatment was replicated 3 times. The cumulative irradiation dose was recorded from the dosimeter. Petri dishes were rotated 180° horizontally when half of the target dose were reached to get a better dose uniformity. The monitored dose rate was about 9.0 Gy/min.

Bioassays.

After irradiation treatment, the 2, 4, and 6-day-old eggs were transferred back to the rearing room and allowed to hatch, which occurred 7–11 days after laying. In order to simplify counting the hatchlings, the irradiated eggs were placed in the center of a filter paper, and the outer perimeter of the paper was treated with sticky insect glue to ensnare the recently crawlers emerged from the eggs became adhered to the sticky paper and were counted. Control eggs (120 eggs/dish) and adults (10 gravid adult females as a batch) were treated as above but without irradiation treatment, all of control groups have 3 replicates.

Large-scale Confirmatory Trials With Gamma Radiation

Cobalt-60 radiation facility and treatment.

To validate the estimated minimum dose for probit-9 prevention of reproduction of *P. baliteus* adults at 95% confidence level (CL), all the large-scale confirmatory tests were conducted with a Cobalt-60 radiation source from the National Institute of Metrology Research Irradiator, Beijing, China. The Fricke system (ISO/ASTM 51026: 2013) was used to monitor the irradiation dose. A total of 307 pumpkin fruits weighing between 350 and 500 g with a diameter of 9.5–11 cm were placed in square plastic boxes (side length 10.0–12.5 cm, height 16.0 cm) containing aerial root mealybugs were placed 100 cm from the radiation source. Each plastic box contained 1 pumpkin. In order to achieve a test population of at least 30,000 adult females for large-scale confirmatory trials, the experiment was conducted 3 times. In each experiment, the number of testing insects exceeded 10,000. Dosages of 135 and 145 Gy were respectively used for the first and 2 other tests; 31 pumpkin fruits remained as untreated controls. During treatments, the boxes were rotated 180° horizontally at mid-exposure to get a more uniform dosage. Fifteen Fricke dosimeters were used in each irradiation to measure dose variations.

Post-treatment rearing and bioassays.

After dose-response irradiation, each replicate treatment of *P. baliteus* adult females was transferred to new pumpkins (10 adults per pumpkin) and then placed in the rearing room for continuous rearing; eggs laid by treated females were collected every 3 days and placed in a Petri dish with filter paper coated with insect glue so that the emerging crawlers (first-instar nymphs) could be counted. Egg mortality (nonhatching) was checked under a microscope 2 wk later. For the irradiated eggs, the Petri dishes were placed in a controlled temperature and humidity incubator to continue the development of eggs for an additional 8–12 days, after which they were examined under a microscope to record mortality.

The aerial root mealybugs and pumpkin fruits in the large-scale confirmatory tests were returned to the laboratory and kept separately from the control group to avoid cross-infestation, both the irradiated and controlled mealybugs are kept in the same conditions. After treatment, the adult females on all pumpkins were counted and then transferred to a new, larger plastic box (4 irradiated and 2 new pumpkins), if any nymphs hatch after large-scale confirmatory tests, they can crawl onto the new pumpkins for feeding; eggs laid by females were checked within 1 wk and then transferred to a petri dish with moist filter paper in the bottom, after which the eggs were collected every 4 days until the female died; egg-hatching for all the treated eggs and controls were checked 2 wk later under a microscope. The number of eggs in the large-scale confirmatory tests in this research was estimated by multiplying the number of adult females by 550.

Data Analysis

To determine the effects of radiation dose on different ages of eggs, and to compare radiotolerance among 0-, 2-, 4-, and 6-day-old eggs, mortality data in the X-ray irradiation dose-response tests were corrected by using Abbot's formula (Abbot 1925), and then subjected to two-way ANOVA. Means (±SD for all mortality) were compared by Tukey's multiple comparison tests using DPS software (DPS 2010). The dose-response data were also subjected to probit analysis (probit and logit model) by using the PoloPlus 2.0 program to estimate the minimum absorbed dose leading to 99% or 99.9968% (probit-9) mortality at 95% CL, where any dose causing <100% mortality and the lowest dose causing 100% mortality were used (LeOra Software 2002, Nicholas and Follett 2018). To compare the significance of tolerance among developing ages, pairwise comparison tests were also performed by calculating the 95% confidence intervals (CI) of the lethal dose ratios (LDR) at LT_{90} and LT_{99}; if the 95% CI excludes 1, the LDs values are significantly different (LeOra Software 2002, Wheeler et al. 2006). In addition, the overlapping tests on the 95% CI of LD_{90} and LD_{99} were performed.

For the confirmatory tests, the irradiation treatment efficacy was calculated by the equation 1 when there was no survivor emergence:

$$1 - Pu = (1 - C)^{1/n} \tag{1}$$

where *Pu* is the acceptable level of survivorship (normally 0.01% or 0.0032%), *C* is the CL, and *n* is the number of treated females (Couey and Chew 1986, Nicholas and Follett 2018). Normally, the 95% CL is used for calculating of mortality proportion (1 – *Pu*), which is based on the number of treated *P. baliteus* late adult females.

Results

Two-Way ANOVA on Dose Mortality of X-ray Irradiation

When *P. baliteus* eggs (including those within the female body) were exposed to X-ray irradiation, the corrected mortality increased with the increasing dose within an age. The minimum doses causing 100% mortality for 0-, 2-, 4-, and 6-day-old eggs were 100, 100, 100, and 120 Gy, respectively. Mortality decreased with the increasing age within a dose (Fig. 1). The reason for a few 0-day-old eggs hatching at 100 Gy may be due to the uneven distribution of absorbed dose and the large number of eggs (9,471–13,528) used in each dose compared to individuals of other ages. The 6-day-old eggs, which were the most developed eggs used, were more tolerant to radiation than eggs of younger ages.

To investigate the significant difference of egg mortality, the dose-mortality data were subjected to two-way ANOVA (Table 1), which showed that the effects on mortality were highly significant for both age ($F = 40.39$; df = 3,83; $P \le 0.0001$) and dose ($F = 588.9$; df = 6,83; $P \le 0.0001$), and age × dose interaction effects ($F = 93.29$; df = 18,56; $P \le 0.0001$). For the effect of radiation dose, mortality was significantly different at 10, 20, 40, 60, and 80 Gy. For comparison of the mean mortality in ages, the 6-day-old eggs with the minimum mortality (62.4 ± 35.1%) were the most tolerant life age, followed by the 4 and 2-day-old eggs; while the 0-day-old eggs showed the maximum mortality (78.1 ± 26.1%) was the most sensitive age. Consequently, the sequence for tolerance to X-ray irradiation is of 0 < 2 ≈ 4 < 6-day-old eggs.

Probit Analysis on Dose-mortality of X-ray Irradiation

The results of the probity analysis (using probit and logit model) on the dose-mortality data are listed in Table 2, including the estimated minimum absorbed dose that leading to LD_{90}, $LD_{99,}$ and $LD_{99.9968}$ (probit-9) prevention of egg-hatching at 95% CL, heterogeneity, slope, intercept, and comparison of the lethal dose ratio (LDR). The estimated LDs values increased with the increasing age, but the 95% CI overlapped between adjacent ages, especially for the 2- and

4-day-old eggs that had similar LD_{90} and LD_{99} values either, which means that the difference in radiotolerance is not significant. The only significant difference took place between 0- and 6-day-old eggs for the fiducial test (Table 2). However, the pairwise comparison of LDR (Tables 2 and 3) by using the probit and logit model revealed there were significant differences in LD_{90} and LD_{99} for different ages: the 0-day-old eggs had the minimum LD values, followed by significant increases with the 2 and 4-day-old eggs, then the 6-day-old eggs had the maximum LD values, which means the older eggs were more tolerant. Furthermore, the results of LDR (Table 3) showed that the 95% CI of the LDR at LD_{90} and LD_{99} include 1, so the differences in radiotolerance were insignificant in 2- and 4-day-old eggs. Thus, the sequence of radiotolerance is suggested as follows: 0 < 2 ≈ 4 < 6-day-old eggs. Therefore, the radiotolerance sequence is the same as the result derived from two-way ANOVA (Table 1). In addition, probity-9 mortality is an extrapolated value, so the pairwise comparison of LDR at $LD_{99.9968}$ have not been conducted (LeOra Software 2002).

The estimated probit-9 value is ordinarily used as the target dose for conducting the large-scale confirmatory tests, through there are big differences among analyzing models, for instance, the value estimated by logit model is 39.9, 39.3, 39.6, and 36.0% higher than that of probit model (Table 2). Since all the eggs died after 120 Gy irradiation (Table 1), and late females may contain eggs attached to their bodies, therefore, the estimating $LD_{99.9968}$ value for females (132.0 Gy, 95% CI: 118.9–149.5 Gy) and the mean probit-9 values for 2 and 4-day-old eggs derived from probit model (137.6, 145.5 Gy) may be selected as the target dose for conducting confirmatory tests; a dose of 135 Gy and 145 Gy were hence used as the target dose in the following confirmatory tests.

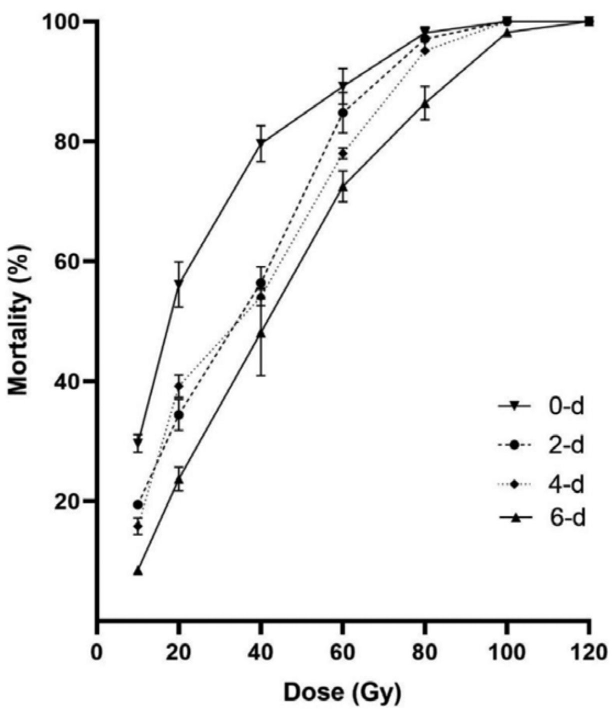

Fig. 1. Corrected mortality of 0-, 2-, 4-, and 6-day-old eggs of *Pseudococcus baliteus* irradiated at doses of 10, 20, 40, 60, 80, 100, and 120 Gy.

Large-scale Confirmatory Trials

To validate the estimated dose for 99.9968% mortality (at the 95% CL), large-scale confirmatory tests were conducted using a cobalt-60 radiation source with 3 repeats, the dose rate measured were 1.9–2.1 Gy/min. This is because X-ray and gamma rays have the same biological effects with an equal absorbed dose, and a commercial X-ray irradiator was not available (Ma et al. 2022). The results (Table 4) showed that the absorbed dose ranged from 107.7 Gy (first test, target dose: 135 Gy) to 182.5 Gy (third test, target dose: 145 Gy) measured by Fricke dosimeter, and the dose uniformity ratio (DUR, the ratio of the maximum dose divided by the minimum dose) was of 1.35, 1.28, and 1.49, respectively. No F_1 generation crawler emerged from an estimated 47,316 irradiated late females reared on pumpkin fruits, and there were no dead females found in the radiation treatment to the oviposition period, whereas the rate of egg-hatching in untreated controls was 98.1, 98.2, and 98.2% respectively for the first–third confirmatory tests, indicating that the F_1 generation developed normally in the controls (Table 4). Thus, efficacy for gamma irradiation treatment calculated by Equation 1 is 99.9937% at 95% CL, whereas, the number of late females used for calculation was not adjusted since no dead adult was founded in the treatments and controls, and the lack of egg hatch in the control group was very low which indicate that any adjustments made would have a minimal impact on the calculated treatment efficacy (Follett and Neven 2006, NAPPO 2011).

Discussion

The relative radiotolerance for *P. baliteus* 2-, 4-, and 6-day-old eggs, and late females which contained immature eggs in the body, were compared in the dose-response tests and then validated in the confirmatory tests. Prevention of egg-hatching (mortality) was used as the efficacy-evaluation criterion. The significant difference in mortality response of different ages was determined through two-way ANOVA on mortality (Table 1), LDR test (Tables 2 and 3), and overlapping test on fiducial intervals of LD_{90} and LD_{99} (Table 2). As a result, tolerance to radiation increased significantly with the increasing age except for the 2 and 4-day-old eggs that had similar

Table 1. Result of two-way ANOVA on the corrected mortality (mean ± SD, %) of *Pseudococcus baliteus* eggs irradiated with X-ray at different dose

Age	Corrected mortality (%) of eggs irradiated at dose[a]							Means of ages[b]
	10 Gy	20 Gy	40 Gy	60 Gy	80 Gy	100 Gy	120 Gy	
0 day	28.1 ± 1.5aD	55.6 ± 6.2aC	76.2 ± 0.6aB	89.0 ± 5.0aA	97.9 ± 1.4aA	99.8 ± 0.2aA	100.0 ± 0.0aA	78.1 ± 26.1a
2 day	19.4 ± 0.2abE	34.4 ± 4.3bD	56.4 ± 4.6bC	84.8 ± 5.9abB	97.1 ± 3.5aA	100.0 ± 0.0aA	100.0 ± 0.0aA	70.3 ± 32.1b
4 day	15.7 ± 2.4bcE	39.2 ± 3.4bE	54.1 ± 2.6bC	78.0 ± 1.5bcB	95.1 ± 1.2abA	100.0 ± 0.0aA	100.0 ± 0.0aA	68.9 ± 31.6b
6 day	8.5 ± 1.5cF	23.7 ± 3.1cE	48.1 ± 12.6bD	72.5 ± 4.7cC	86.4 ± 4.9bB	98.2 ± 0.8aA	100.0 ± 0.0aA	62.4 ± 35.1c

[a]Mean (±SD, %)s of *P. baliteus* eggs followed with different upper-case letters (for each age within a column) and lower-case letter (for dose within a row) are significant difference ($P < 0.05$; Tukey test).
[b]Mean mortality (±SD, %) of ages followed by different letters in the same row are significant different ($P < 0.05$, Tukey test).

Table 2. Comparison different model analysis on the dose-mortality for different aged eggs of *Pseudococcus baliteus* irradiated at 225 keV X-rays

		Analyzed by probit model with nontransformation of dose					Analyzed by logit model with nontransformation of dose				
			Estimating LD and 95% CI (Gy)[a]					Estimating LD and 95% CI (Gy)[a]			
Age (days)	No. eggs	Slope ± SE	LD_{90}	LD_{99}	$LD_{99.9968}$	Hetero-geneity[b]	Slope ± SE	LD_{90}	LD_{99}	$LD_{99.9968}$	Hetero-Geneity[b]
0	72431	0.036 ± 0.000	55.9 (51.6–61.2)c	85.1 (77.7–94.9)c	132.0 (118.9–149.5)	80.44	0.064 ± 0.001	54.2 (48.6–60.0)c	91.7 (82.7–103.8)c	181.9 (161.1–210.0)	78.95
2	2911	0.038 ± 0.002	66.5 (61.9–2.2)b	93.8 (86.5–103.3)b	137.6 (125.2–153.7)	2.34	0.065 ± 0.003	66.5 (61.3–73.2)b	103.3 (93.7–116.2)b	191.7 (170.5–220.8)	2.59
4	3088	0.036 ± 0.001	70.6 (66.3–75.6)b	99.4 (92.6–107.6)b	145.5 (134.5–159.1)	2.07	0.062 ± 0.002	70.5 (65.4–76.9)b	109.5 (100.3–121.3)b	203.1 (183.3–228.9)	2.55
6	2764	0.035 ± 0.001	79.9 (74.9–86.0)a	109.7 (101.9–119.4)a	157.4 (144.6–173.6)	2.76	0.061 ± 0.002	79.2 (73.6–86.3)a	118.8 (108.8–132.0)a	214.1 (192.5–242.9)	3.06

[a]LT_{90}, LT_{99}, and $LT_{99.9968}$ are the lethal dose in the X-ray irradiation that resulted in 90, 99, and 99.9968% mortality at the 95% CL of *P. baliteus* egg and was calculated with probit or logit model (PoloPlus 2.0, LeOra Software 2002). Within each column, values followed with different letters are significantly different ($P < 0.05$, lethal dose ratio test).
[b]χ^2 divided by the degree of freedom (df).

tolerances. Our results are generally in agreement with the radiation treatment of the mealybug *P. comstocki* Kuwana (Dohino and Masaki 1995), *Dysmicoccus lepelleyi* Betrem (Ma et al. 2019), and two-spotted spider mites *Tetranychus urticae* Koch (Dohino and Tanabe 1993), where radiotolerance increased with the age of eggs, with the exception of 0-day-old eggs (immature eggs in the body of late females) of *P. baliteus* that were significantly more sensitive to radiation than 2-, 4-, and 6-day-old eggs. While a radiation dose of 100 Gy caused 100% mortality in 2-day-old eggs, a few 0-day-old eggs remained viable and hatched (Tables 1 and 2). This result also suggests that the LDR test is more sensitive than fiducial intervals overlapping comparison, and it is very useful for relative tolerance comparison in phytosanitary research, examples being cold treatment of 6 *Bactrocera* species (Myers et al. 2016), irradiation treatment of *Pl. lilacinus* and *Bactrocera dorsalis* Hendel under normal and low-oxygen atmosphere (Zhan et al. 2020, Ma et al. 2022), and modified atmosphere in combination with irradiation treatment of *Trogoderma granarium* Everts (Zhao et al. 2021b).

For PI treatment research and standard establishment, it is required to determine the most radio-tolerant stage in insect pests, and then validate the minimum absorbed dose to provide quarantine security for the most tolerant stage in the shipped commodity according to ISPM 18 and 28 (IPPC 2003, 2007, 2023). An extensive review of the PI literature (Hallman et al. 2010) revealed that radiotolerance in insects increases with the developmental stage and increasing age when a common measure of efficacy is used. The most developed stage is invariably the most radio-tolerant. This general conclusion has subsequently been validated across a range of target insects, for instance in the study of mealybugs and scale insects, *D. neobrevipes* and *D. lepelleyi* Betrem (Doan et al. 2012, Ma et al. 2019), *P. jackbeardsleyi* (Shao et al. 2013, Hofmeyr et al. 2016a, Zhan et al. 2016), *Aspidiotus destructor* Signoret and *Pseudaulacaspis pentagona* Targioni-Tozzetti (Follett 2006a, b), *Aonidiella aurantia* Maskell (Khan et al. 2016), *Paracoccus marginatus* Williams and Granara de Willink (Seth et al. 2016a), *Planococcus citri* Risso and *Pl. ficus* Signoret (Hofmeyr et al. 2016b), *Phenacoccus solenopsis* Tinsley (Seth et al. 2016b), *Exallomochlus hispidus* Morrison (Kuswadi et al. 2016, Indarwatmi et al. 2017), and *Pl. lilacinus* Cock (Ma et al. 2022). PI researchers can only work with the most developed stage that could be present in/on the shipped commodities and preventing the emergence of F_1 generation crawlers is generally used as the efficacy-evaluation criterion (Hallman et al. 2010, Hallman 2011, Ma et al. 2022). For PI studies on oviparous mealybugs, preventing egg-hatching of the F_1 generation could be used as the efficacy criterion, thereby using eggs to replace females in the dose-response tests to determine the relative tolerance and estimate the probit-9 mortality value (Ma et al. 2019). With regard to the deuterotokous ovoviviparous reproduction type mealybugs such as *D. neobrevipes*, *E. hispidus*, and *Pl. lilacinus*, a suitable efficacy criterion is prevention of the emergence of second-instar nymphs since the first-instar nymphs are always attached to the body of the gravid females (Doan et al. 2012, Indarwatmi et al. 2017, Ma et al. 2022).

To develop a technical schedule for PI treatment of the aerial root mealybug, the adult females present on the fruit commodity should be treated in a dose-response test to estimate the minimum absorbed dose for preventing F_1-generation egg-hatching, followed by validating the dose in confirmatory tests (IPPC 2003, 2007, 2023). An important aim and reason for conducting the dose-response testing is to predict the treatment intensity for large-scale confirmatory tests. Probit and logit models are among the most widely used generalized linear models in the case of binary

Table 3. Pairwise comparison of LDR for the different aged eggs of *Pseudococcus baliteus* treated at 225 keV X-rays

		95% CI of LDR for LD (Probit model)		95% CI of LDR for LD (Logit model)	
Reference age (days)	Pairwise age (days)	LD_{90}	LD_{99}	LD_{90}	LD_{99}
0	2	3.1×10^7 to 6.6×10^{13}	3.4×10^3 to 7.0×10^{13}	3.9×10^{11} to 2.4×10^8	6.2×10^3 to 6.1×10^{15}
	4	4.2×10^{11} to ∞	2.0×10^9 to ∞	3.6×10^{12} to ∞	8.2×10^{10} to ∞
	6	∞ to ∞	∞ to ∞	∞ to ∞	∞ to ∞
2	4	0 to 2.1	0 to 29.4	0 to 2.0	0 to 17.1
	6	0–0	0–0	0–0	0–0
4	6	0–0	0–0	0–0	0–0

Table 4. Results of the irradiation confirmatory tests of *Pseudococcus baliteus* using gamma rays

						F_1 generation eggs	
Irradiation date	Exposure time (min)	Absorbed dose (Gy)	DUR	No. pumpkins	No. females	No.	Mortality(%)
20 Dec2020	67.5	107.7–145.1	1.35	105	14,919	7.5×10^6	100.00
(control)	—	0	—	10	1,302	7.3×10^5	1.89
24 Mar 2021	72.5	133.0–170.0	1.28	98	18,262	10×10^6	100.00
(control)	—	0	—	10	1,266	7.0×10^5	1.83
31 Oct 2022	72.5	122.8–182.5	1.49	104	14,135	7.8×10^6	100.00
(control)	—	0	—	11	1,055	5.8×10^5	1.75

dependent variables; data are typically fitted to a probit model (LeOra Software 2002, Follett and Neven 2006). The probit model is commonly used to analyze the dose-mortality data in fumigation and temperature treatments (IPPC 2018, 2019a, Fang et al. 2019). With regards to the irradiation treatment, mortality is replaced by using prevention of development or reproduction, for example, preventing the emergence of F_1 generation second instars of *D. neobrevipes and Pl. lilacinus*, and preventing adult emergence from third instar larvae of *Zeugodacus tau* (Doan et al. 2012, Zhan et al. 2015, IPPC 2022, Ma et al. 2022). In this research, the dose led to probit-9 mortality of eggs estimated by the probit model (Table 2) and validated in the confirmatory tests (Table 4). The estimated dose for the LD_{90} and LD_{99} was nearly equal, but the $LD_{99.9968}$ value derived from logit model was 36-40% larger than that from the probit model (Table 2), suggesting that the probit model is more extensively used than logit model. When logarithmic transformation of dose was used in the regression, the estimated $LD_{99.9968}$ value in Table 2 will be increased by 3.4- to 5.3-fold and 10.3- to 24.3-fold, respectively, in the analysis of probit and logit model with a large CI. Therefore, the traditional logarithmic transformation of radiation dose is not suitable for dose-response data analysis in this investigation. To evaluate the significance of *P. baliteus* tolerance to irradiation treatment among different ages, pairwise comparison tests were conducted. This involved calculating the 95% confidence intervals (CIs) of the lethal dose ratios at LD_{90} and LD_{99}. Both 2-way ANOVA analysis and lethal dose ratios were found suitable for analyzing the effects of age and dose on egg mortality. Consistent results were obtained for the radiation tolerance of eggs at different ages using both methods, with the sequence of $0 < 2 \approx 4 < 6$-day-old eggs.

During the large-scale testing at the target dose of 135 and 145 Gy (considering the large DUR), an estimated 47,316 late females were irradiated at monitored doses of 107.7–182.5 Gy, and resulted in mortality of all F_1 generation eggs (Table 4); indicating that the treatment efficacy calculated by Equation 1 is not less than 99.9937% at 95% CL. A total number of 33,181 late females were irradiated in the first 2 confirmatory tests (Table 4), resulting in the efficacy of 99.9910% at 95% CL (Couey and Chew 1986). These two-efficacy levels satisfy the requirements in some countries of not less than 99.99% (95% CL), for example in Australia, New Zealand, and Japan (Follett and Hennessey 2007). Therefore, the largest radiation dose of 183 Gy (nominally 182.5 Gy) or 170 Gy in the confirmatory tests, which is also larger than the estimated upper limit for the late females (149.5 Gy), 4-day-old eggs (159.1 Gy), and 6-day-old eggs (173.6 Gy) in Table 2, should be the minimum absorbed doses required for commercial uses (Hallman et al. 2010, Zhan et al. 2015; IPPC 2022, 2023). This result also supports the recommendation regarding 250 Gy as a generic dose for PI treatment of mealybugs in the family Pseudococcidae (Hallman 2011, 2012). Therefore, minimum absorbed doses of 183 or 170 Gy, can provide adequate quarantine security for the PI treatment of the aerial root mealybug infesting produce, and is recommended to relevant organizations including IPPC to develop technical standards.

Additionally, the IPPC Technical Panel on Phytosanitary Treatments (TPPT) evaluated the listed topic 2017-012: Irradiation treatment for all stages of the family Pseudococcidae (generic) in 2019; however, the TPPT suggested that further research is necessary to be conducted on more species with economic importance globally to identify the most resistance species in this family (IPPC 2019b). This research will contribute 1 more species to the list of those required for the further investigation.

Acknowledgments

We would like to thank Dr. Yong Zhong (the Technical Centre of Nanning Customs) for mealybug sampling, Dr. Hui-Ping Li (the Technical Centre of Taiyuan Customs) for insect identification, and the anonymous reviewers for their valuable suggestions and modifications to the draft of this manuscript. This study was supported by the Chinese National Key R&D Program (2021YFF0601901), and the International Atomic Energy Agency CRP Research Contract No. 25002.

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