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Gamma and X-ray irradiation as a phytosanitary treatment against various stages of *Planococcus lilacinus* (Hemiptera: Pseudococcidae)

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ABSTRACT

The cacao mealybug, *Planococcus lilacinus* Cock, is an important quarantine pest. Infested commodity should be subject to appropriate phytosanitary treatment, while irradiation is recommended for the cacao mealybug. Radio-tolerance comparison tests were conducted on the crawler, nymphs, and adult females of *P. lilacinus* at the X-ray radiation doses of 40, 80, and 120 Gy, respectively. The results showed that irradiation had a strong effect on preventing of development and reproduction; the adult female stage was identified as the most tolerant. During the following dose–response tests, among young and late females X-ray-irradiation (20–100 Gy), the late females were most tolerant when preventing F_1 generation 2nd instars emergence was used as the evaluation criterion. Minimum absorbed dose and its 95 % fiducial limits to provide probit 9 efficacy at 95 % confidence level (100 % mortality/inhibition in an estimated population of 93,616 individuals) were 131.5 Gy (122.5, 142.6 Gy) and 144.4 Gy (132.7, 159.4 Gy), estimating from the probit analysis on dose-mortality data of 1–30 and 1–10-day-old neonates laid by late females, respectively. In the large-scale confirmatory tests, a total of estimating 97,384 late females of *P. lilacinus* rearing on the pumpkins fruits were irradiated with gamma-ray at the target dose of 135 or 145 Gy (measured doses 126.1–163.0 Gy), which resulted in no F_1 generation 2nd nymphs developing during a 6-week post-treatment period. The treatment efficacy calculated is 99.9969 % at the 95 % confidence level. Therefore, a minimum absorbed dose of 163.0 Gy is recommended for phytosanitary treatment of *P. lilacinus* in infested commodity.

Introduction

The cacao mealybug, *Planococcus lilacinus* Cock (Hemiptera: Pseudococcidae), is considered a destructive mealybug and a serious quarantine pest for China and many other countries (Chen et al., 2016). It is polyphagous and attacks more than 36 plant families; including high-value crops for export, such as citrus (*Citrus* spp.), potato (*Solanum tuberosum* L.), coffee (*Coffea arabica* L.), lychee (*Litchi chinensis* Sonn.), mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), and other tropical and sub-tropical fruit trees and shade trees (Cox, 1989; Ben-Dov, 1994; USDA, 2014). The mealybug mainly attacks the fruits, leaves, and twigs of the host, but it has also been reported to attack roots (coffee and tamarind) and peduncles (palms). The feeding activity of *P. lilacinus* causes reduced yield, retard or delay ripening, chlorosis, and leaf loss like other mealybugs; the infestation promotes the growth of sooty mold fungus, honeydew, and sooty mold, and also causes defects to plants and fruits and makes fruit unattractive (USDA, 2014; Chen et al., 2016).

The mealybug is native to southern Asia, but it has been distributed in at least 31 countries or territories worldwide, including India, Indonesia, Malaysia, Philippines, Vietnam, Thailand, Guam, Guyana, Haiti, and Madagascar. The mealybug is often intercepted from fruits imported from Southeast Asia at the port of entry; it is regarded as one of the most commonly intercepted quarantine mealybugs in China, the

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USA, and other countries (USDA, 2014; Chen et al., 2016). Cacao mealybug has the potential to cause significant economic losses in new geographical areas with no natural enemies if it is introduced through plant and plant products. Therefore, phytosanitary treatment should be applied to the infested commodities in the international trade to prevent their introduction and spread to new areas.

Several phytosanitary treatment measures have been developed for the disinfestation of quarantine pests (such as mealybugs, fruit flies, and other insects), including fumigation, temperature (cold and heat), irradiation, modified atmosphere treatment, and their combinations (Follett & Neven 2006; Dohino et al., 2017; Osoulia & Atapourb, 2018; Kwon et al., 2021). Cold treatment and Methyl bromide (MB) fumigation were commonly used to disinfest postharvest pests on fruits. However, the use of MB has been sharply decreased because it depletes the ozone layer, and the development of alternative measures is encouraged (USEPA, 2022).

Low dose ionizing radiation is a potential alternative to MB fumigation for mealybugs on many agricultural commodities because it penetrates packed commodities and typically does no harm to the quality of fresh commodities (Heather & Hallman, 2008; Follett and Weinert, 2009). Phytosanitary irradiation (PI) treatment has been used worldwide to disinfest agricultural commodities of quarantine pests since the establishment of ISPM 18 developed by the International Plant Protection Convention (IPPC) in 2003 and the following the international trade trials between Australia and New Zealand in 2004 (IPPC, 2003; Hallman, 2011; Hallman et al., 2016).

Several kinds of mealybugs have been investigated to establish treatment schedules (Hofmeyr et al., 2016a); for example, a minimum absorbed dose of 150 Gy is needed to inhibit egg hatching from irradiated adult females of *P. minor* (Ravuiwasa et al., 2009). In 2015, the IPPC secretariat recommended adopting an international standard PT 19 annex to ISPM 28; a minimum absorbed dose of 231 Gy is required to prevent the reproduction of *Dysmicoccus neobrevipes* Beardsley, *P. lilacinus*, and *P. minor* Maskell (IPPC, 2015). Yet, it is reported that these two kinds of *Planococcus* mealybugs are less tolerant to radiation than *D. neobrevipes* (Doan et al., 2012; Doan et al., 2016). The minimum absorbed dose for PI treatment of *P. lilacinus* is then needed to be developed to lower the radiation dose.

The objective of the current study was to conduct the dose–response and confirmatory tests based on the research protocol developed in ISPM 18 to determine the minimum absorbed dose for PI treatment of *P. lilacinus* (IPPC, 2003). Based on our findings, we would propose a PI treatment schedule and standard to relevant organization.

Materials and methods

Insects rearing

The colony of *P. lilacinus* used in this study was first intercepted from mangosteens fruits imported from Thailand at Guangzhou port, Guangzhou, China, in 2017; the specimen was identified by Professor Fan Liang (the Technical Centre of Guangzhou Custom, Guangzhou, China). Newly intercepted mealybugs were used to replace the colony-one or two times a year. Insecticide-free pumpkins (*Cucurbita maxima* Duch.) fruits, and potato tubers were used for rearing the mealybugs in the plastic boxes (22.5 × 15.0 × 7.5 cm) with three 2.0-cm diameter holes covered with mesh windows on the lid.

The boxes were maintained in the rearing room in the Key Laboratory of Phytosanitary Treatment, Chinese Academy of Inspection and Quarantine, Beijing, China. The rearing condition was controlled at 25 ± 2°C, 60–80 % RH, under a photoperiod of L:D = 14:10 h. To establish as many individuals as possible within a tightly limited developmental stage, for 2 days, every 10–15 females in the ovipositional period were transferred to a new pumpkin fruit for laying eggs/neonates. Under the laboratory conditions, the eggs hatch within 1 h; and the time for the development of 1st (neonate), 2nd and 3rd instars nymphs, pre-ovipositional (young female), and oviposition period (late female) are 1–9 days, 10–18 days, 19–26 days, 27–32 days, and ≥ 33 days, respectively.

X-ray irradiation treatment

To determine the most tolerance stage(s) and estimate its target dose for conducting the following large-scale confirmatory tests, the radio-tolerance comparison and dose–response tests should be conducted according to the requirement of ISPM 18 (IPPC, 2003). The foodstuff can be irradiated with any kind of radiation source of gamma ray, X-ray, or e-beam which have the same biological effects if equal radiation dose was absorbed; however, the e-beam has lower and decreasing penetration (Hallman, 2011; Hallman et al., 2016), the X-ray and gamma irradiator was accordingly used in the treatment.

X-ray irradiator

A RS-2000 Pro X-ray irradiator (Rad Source Technologies, Inc., Atlanta, Georgia, USA) were performed all the X-ray irradiations with the operating parameters was of 220 KV and 17.6 mA; the irradiator was equipped with a reflector placed in the bottom of the exposure chamber (width, 17″; depth, 15″; height, 17″) where is 40 cm away from the X-ray source to get a good dose uniformity ratio (Gueorguiev, 2002; Zhan et al. 2020).

Irradiation treatment

To achieve more even irradiation, the sample including the potato tubers and Petri dish positions were placed in the middle of the exposure chamber and altered after half of the radiation time. A Dosimeter (model 2086, RadCal Corp., CA, USA) with a 10 × 6–6 Ion Chamber was placed near the irradiated sample to measure radiation and record the cumulative radiation dose (Zhan et al., 2020; Zhao et al., 2021). When the target cumulative dose was reached, the irradiator was stopped, and the samples were taken out. The measured dose rate at the irradiation time was 5.1–5.3 Gy/min.

Irradiation treatment of crawler, nymphs, and adult females

To compare the radio-tolerance of *P. lilacinus*, each of ~200 crawlers (5–15 days old), middle-aged nymphs (16–25 days old), and adult females (26–35 days old) grown on potato tubers were placed in Petri dish and irradiated with X-rays irradiator at the radiation dose of 40, 80, and 120 Gy, respectively. The percentage mortality of adult females and development of the F_1 generation 1st or 2nd instar nymphs were checked under a stereomicroscope (SteREO Discovery. V12, ZEISS, Germany). The results showed that the tolerance to radiation grows with developing time and stages. As Hallman et al. (2010) summarized, the adult female stage is the most tolerant. The irradiated tubers and mealybugs were transferred to the new plastic box (10.5 × 9.5 × 13.5 cm, with one 2.0-cm diameter hole covered with mesh windows on the lid) for further development in the rearing room.

Irradiation treatment of young and late females

The adult females of *P. lilacinus* were divided into young and late females by the developmental time and subject to be irradiated under the X-ray irradiator. Every 40 young and late female individuals were placed into a Petri dish (with filter paper attached to the bottom), were subjected to X-rays irradiation at the radiation doses of 0 (control), 20, 40, 60, 80, and 100 Gy; each of the doses was replicated three times. All adult females were checked to ensure the absence of eggs or neonates attached to the abdomen before the irradiation.

After irradiation, the treated adult females and controls of *P. lilacinus* together with their pumpkin fruits were transferred on to the fresh bean pod in new plastic box (22.5 × 15.0 × 7.5 cm) for reproduction in the rearing room. All the females in the dose–response tests were evaluated for fecundity (neonates), progeny survival, and the number of F_1 generation neonate and 2nd instars nymphs every 10 days; the percent mortality for neonates was calculated as equation (1).

$$Mortality\ (\%) = (no.\ neonates - no.\ 2nd\ instars) \div no.\ neonates \times 100 \quad (1)$$

Large-scale confirmatory tests

Large-scale confirmatory irradiation tests were conducted to validate the estimated dose (through the probit analysis) for 99.9968 % prevention of reproduction against *P. lilacinus* at the 95 % confidence level (probit 9 prevention), a minimum of 93,616 most tolerant individuals (late females) should be treated according to ISPM 18 (Couey & Chew, 1986; IPPC, 2003).

Gamma irradiation

The large-scale X-ray irradiator is not available, but the gamma irradiators are widespread used for commercial treatment, then, all the large-scale confirmatory irradiation treatments were conducted at the National Institute of Metrology Research Irradiator, Beijing, China, with a Cobalt-60 radiation source, where the routine dosimetry was performed using the Fricke system (ASTM E1026-13, 2013). Ten percent of boxes (12.5 × 10.0 × 16.0 cm, with one 2.0-cm diameter hole covered with mesh windows on the lid) remained untreated as controls, and a further 160 or 120 boxes, all containing ~400 late females on pumpkin, were treated. The dose rate monitored was 1.9–2.1 Gy/min at 100 cm from the gamma source, and the dose uniformity ratio (maximum/minimum) ranged from 1.15 to 1.23 during the treatments.

Post-treatment rearing

After gamma irradiation, mealybugs were brought back to the laboratory for their development; new pumpkin fruits were used for rearing (in the 22.5 × 15.0 × 7.5 cm plastic boxes) the F_1 generation instar nymphs produced by treated females and controls. All F_1 generation 2nd instar nymphs were counted and recorded during a 6-week observation as all females had died.

Statistical analyses

Data on the numbers of F_1 neonates laid within 1–10, 11–20, and 21–30-days post-treatment were used to calculate the fecundity of females. For each test, mortality data (neonate calculating by Eq. (1)) on nymphs and adult females were corrected for mortality in control by using Abbott's formula (Abbott, 1925). Differences in mortality and the number of neonates laid by per female were subjected to two-way or three-way analysis of variance (ANOVA), and means separations were done using a Tukey's test (DPS, 2010). Probit and logit models by using PoloPlus 2.0 program (LeOra Software, 2007) were conducted to analyze the mortality data (failed to 2nd nymphs from F_1 neonates laid by irradiated adult females) to estimate the minimum absorbed dose (using non-transformed exposure dose) for phytosanitary control purpose, in which any mortality data between 0 and 100 %, and the lowest dose causing 100 % mortality were used in the analysis (Follett 2004; Zhao et al., 2021). To compare the significance of radio-tolerance, the 95 % confidence limits (CIs) of the lethal dose ratios at LD_{99} and $LD_{99.9968}$ were calculated and subjected to pair-wise comparison tests. The values of LD are significantly different when the 95 % CIs excludes 1 (Wheeler et al., 2006; Zhao et al., 2021).

For the large-scale confirmatory tests, the mortality/prevention proportion (1- *Pu*) for a defined confidence level was calculated using equation (2) when no F_1 generation emerged from the treated mealybugs.

$$1 - Pu = (1 - C)^{1/n} \quad (2)$$

where *Pu* is the maximum allowable infestation proportion, *C* is the confidence level, and *n* is the number of test insects (Couey & Chew, 1986). Furthermore, the number (*n*) treated in confirmatory tests should be adjusted based on control survivorship (Follett & Neven, 2006; NAPPO, 2011).

Results

Radio-tolerance among crawlers, nymphs, and adult females

For the X-ray irradiation treatment of the crawlers, nymphs, and adult females of *P. lilacinus*, results derived from two-way ANOVA showed that the effects (preventing development to ovipositional females) were highly significant for the main factors of stages ($F_{2,8}$ = 377.6; $p \le 0.0001$) and radiation dose ($F_{2,8}$ = 22.3; $p = 0.0068$). The mortality increased significantly with increasing radiation doses but decreased with developing stages; the least mortality (mean ± SD, same as below) for adult females (17.0 ± 5.9 %) means it is the most tolerant stage, followed by nymphs (51.4 ± 10.1 %), while the crawlers were the least radio-tolerant stage with the largest mortality of 87.8 ± 10.2 % (Fig. 1). Furthermore, the reproduction was stopped at 80, 120, and 120 Gy doses for the irradiated crawlers, nymphs, and adults, respectively. Therefore, the adults, which show more resistance to development and reproduction than nymphs and crawlers, were the most radio-tolerant stage.

Radio-tolerance between young and late females

Number of F_1 neonates

Under the laboratory conditions, an adult female of *P. lilacinus* was capable of reproducing > 300 egg/neonates. After irradiation, it was observed that many eggs/neonates died under the abdomen of the adult female. The numbers of F_1 neonates laid by young and late females are shown in Table 1. Adults produced F_1 instars at all doses, however, the mean number observed in the control was 2- to 8-times higher than that in the irradiation treatments.

The results from two-way ANOVA showed that the radiation effect on the number of F_1 neonates was highly significant at stage ($F_{1,35}$ = 65.4; $p \le 0.0001$), radiation dose ($F_{5,35}$ = 214.9; $p \le 0.0001$), and interaction effects ($F_{5,24}$ = 13.4; $p \le 0.0001$). The mean number of neonates from late females (121.3 ± 42.6) was significantly larger than that of young females (95.9 ± 64.9); but the number was decreased with increasing doses for young ($F_{5,17}$ = 384.7; $p \le 0.0001$) and late females ($F_{5,17}$ = 91.8; $p \le 0.0001$), and it was significantly lower than the control group (Table 1).

Mortality of F_1 neonate

In the dose–response tests, the mortality of the cacao mealybug F_1 neonate laid by irradiated young and late females increased with

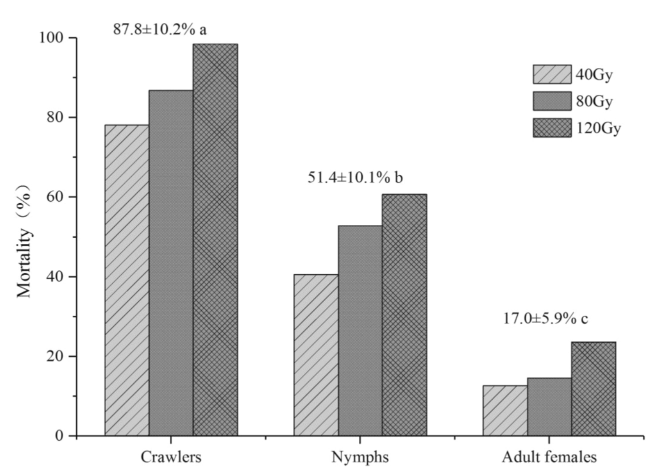

Fig. 1. Corrected mortality (to adults in ovipositional stage) of *Planococcus lilacinus* after X-ray irradiated at the dose of 40, 80, and 120 Gy, respectively.

Table 1
Number of neonates (mean ± SD) laid by young and late females of *Planococcus lilacinus* after irradiation at the dose of 0 (control), 20, 40, 60, 80, and 100 Gy.

Female	No. of neonates per female (mean ± SD) irradiated at the dose of:						Means (±SD) of stage
	0 Gy	20 Gy	40 Gy	60 Gy	80 Gy	100 Gy	
Young	329.6 ± 11.2a	86.0 ± 5.0b	74.6 ± 0.8b	62.9 ± 4.8bc	49.4 ± 2.6c	44.8 ± 2.6c	95.9 ± 64.9B
Late	283.0 ± 19.7a	127.2 ± 1.4b	110.6 ± 0.9bc	94.6 ± 2.4bcd	80.0 ± 1.0cd	71.7 ± 2.8d	121.3 ± 42.6A

*Within each row, means followed with different lower-case letters, and within the column of the mean number of stages are significantly difference ($P < 0.05$; Tukey test).

increasing duration (from 1–10 to 21–30 days old) and radiation dose (from 20 to 100 Gy); the minimum doses leading to 100 % mortality of F_1 neonate was 100 Gy and > 100 Gy, respectively (Table 2). The results derived from three-way ANOVA showed that the differences in corrected mortality were significant for all the main factors (ages, durations, and radiation doses) and their interactions ($p \leq 0.0001$). Thus, the late female is more tolerant to radiation than the young female.

For the irradiation of late females of *P. lilacinus*, mortality of neonate was significantly affected by duration ($F_{2,44} = 2868.5$; $p \leq 0.0001$), radiation dose ($F_{4,44} = 882.9$; $p \leq 0.0001$), and their interaction ($F_{8,30} = 777.9$; $p \leq 0.0001$); for the young females, the mortality was increased significantly with increasing duration ($F_{2,44} = 68.9$; $p \leq 0.0001$) and radiation dose ($F_{4,44} = 271.7$; $p \leq 0.0001$), and the interaction was also significant ($F_{8,30} = 25.0$; $p \leq 0.0001$). Therefore, the least mortality was detected for the 1–10-day-old neonates laid by late (69.2 ± 32.3 %) and young females (95.8 ± 5.9 %); these were the most radio-tolerance neonates. This result showed that the radio-tolerance for eggs in the body of adult females increased with developmental time.

Probit analysis

The dose-mortality data from the neonates laid by irradiated young and late females of *P. lilacinus* were subjected to Probit analysis using the Probit and logit models, in which the radiation dose was not transformed. The derived parameters, including slope, intercept, heterogeneity (chi-square divided by degrees of freedom), and estimated minimum absorbed dose led to 99 % (LD_{99}) and 99.9968 % ($LD_{99.9968}$) prevention of F_1 2nd nymph emergence at 95 % confidence level, are presented in Table 3.

When data based on the neonates laid within 10 days were used for probit analysis, the estimated LD_{99} and $LD_{99.9968}$ values for late females (93.3 Gy, 131.8 Gy) were significantly larger than that for young females (60.9 Gy, 114.5 Gy) from the lethal dose ratio test, and the 95 % confidence interval did not overlap (Table 2). The neonates laid within 10 days were much more radio-tolerant than other neonates. Therefore, the late female containing late-aged eggs in the abdomen was significantly more tolerant than young female of *P. lilacinus*.

When all the data from neonates laid within 30 days were used for analysis, there were no changes in radio-tolerance showing that late females were more tolerant than young females; however, although the lethal dose for young females decreased dramatically, the $LD_{99.9968}$ for late females increased. A possible reason for this difference is that the regression lines become more flattened when all the data are used for analysis (Fig. 2).

Similar results were obtained for the analysis with the logit model, but much higher $LD_{99.9968}$ values were estimated; for example, a minimum absorbed dose of 195.5 (182.4, 211.1) Gy was needed for probit-9 treatment of late female. The estimated dose from the probit model was used for conducting the following validation tests.

Late females—which are more resistant to radiation in fecundly and developed to 2nd instars (Tables 1 and 2)—should be used for large-scale confirmatory tests. However, the estimated dose for $LD_{99.9968}$ based on all the neonates was larger than that based on 1–10-day-old neonates (Table 3, Fig. 2); then, the radiation dose in the range of 132 Gy (the lower confidence limits (132.7) and the $LD_{99.9968}$ value (131.5) estimated from 1 to 10-day-old neonates) and 160 Gy (the upper confidence limits of 159.4 Gy) can be used as the target dose (Table 3) in the confirmatory tests.

Confirmatory tests

The large-scale confirmatory tests were conducted twice to validate the estimated dose for 99.9968 % (at the 95 % confidence level) prevention of F_1 generation 2nd instar nymphal emergence from late females of *P. lilacinus* on pumpkins, where doses of 145 Gy and 135 Gy were selected as the target dose. No F_1 generation 2nd instar nymph emerged from an estimated 97,384 late females reared on pumpkin fruits, whereas most neonates in the control normally developed (Table 4). The absorbed doses measured by the Fricke dosimeter ranged from 132.3 to 163.0 Gy for the first replicate and 126.1 to 145.1 Gy for the second replicate, indicating that the dose uniformity (DUR) was 1.23 and 1.15, respectively (Table 4). In the control condition, no dead females were found from radiation treatment to the oviposition period; therefore, the treatment efficacy can be calculated based on the number of late females used in the treatment without adjusting to the control

Table 2
Corrected mortality (mean ± SD) of neonate produced by young and late females of *Planococcus lilacinus* irradiated at the dose of 20, 40, 60, 80, and 100 Gy.

Duration of neonate	Corrected mortality (mean ± SD, %) and No. of neonate treated at radiation dose of: *						
	20 Gy	40 Gy	60 Gy	80 Gy	100 Gy	means	control
Young females							
1–10-d	85.0 ± 2.1cC	95.0 ± 1.4bB	99.2 ± 0.3aA	99.7 ± 0.1aA	100.0 ± 0.0aA	95.8 ± 5.9c	6.37–8.27
(n)	(715–972)	(652–890)	(418–824)	(363–578)	(333–511)		(1536–1712)
11–20-d	90.2 ± 1.5bB	99.2 ± 0.3aA	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	97.8 ± 4.0b	4.14–5.39
(n)	(1138–1349)	(962–1120)	(867–1004)	(627–741)	(591–672)		4242–4788
21–30-d	95.2 ± 0.2aB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	99.0 ± 2.0a	4.24–5.00
(n)	(430–559)	(366–437)	(312–351)	(277–311)	(245–275)		3409–4154
Late females							
1–10-d	17.9 ± 1.7bA	48.8 ± 2.9bB	85.8 ± 2.3bC	95.1 ± 1.5bD	98.5 ± 0.5aE	69.2 ± 32.3b	4.83–5.26
(n)	(2316–2454)	(1884–2070)	(1620–1716)	(1392–1524)	(1300–1542)		(3972–5649)
11–20-d	97.0 ± 0.7aB	98.9 ± 0.1aAB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	99.2 ± 1.3a	5.69–7.13
(n)	(1137–1236)	(1119–1159)	(917–1099)	(776–851)	(612–658)		(2953–3241)
21–30-d	96.8 ± 2.1aB	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	99.4 ± 1.5a	2.49–3.41
(n)	(268–307)	(143–226)	(145–168)	(99–116)	(97–104)		(442–703)

[*] Within each column, means followed with different lower-case letters within a stage are significantly difference ($P < 0.05$; Tukey test); means followed by different upper-case letters in the same row are significantly different ($P < 0.05$, Tukey test).

Table 3
Probit and logit analysis on prevention of F_1 2nd instar nymphs emergence when young and late females of *Planococcus lilacinus* were irradiated.

Model	Adult female	No. of neonate	Slope ± SE	Intercept ± SE	Estimated doses (95 %CI) (Gy)*		Hetero-geneity
					LD_{99}	$LD_{99.9968}$	
Using the data from the neonates laid within 10 days							
probit	young	14,497	0.032 ± 0.002	0.408 ± 0.057	60.9 (57.0, 65.7)b	114.0 (104.5, 126.1)b	1.14
	late	41,194	0.044 ± 0.001	−1.747 ± 0.026	93.2 (87.9, 99.7)a	131.5 (122.5, 142.6)a	18.25
logit	young	14,497	0.069 ± 0.004	0.339 ± 0.115	61.8 (57.2, 66.6)b	145.4 (131.0, 164.4)b	1.29
	late	41,194	0.078 ± 0.001	−3.092 ± 0.048	93.2 (87.9, 99.7)a	172.7 (162.8, 184.3)a	8.10
Using the data from the neonates laid within 30 days:							
probit	young	58,260	0.037 ± 0.001	0.517 ± 0.042	48.6 (44.9, 53.7)b	93.6 (83.6, 107.7)b	2.15
	late	69,035	0.033 ± 0.000	−0.730 ± 0.018	93.3 (87.0, 101.3)a	144.4 (132.7, 159.4)a	11.45
logit	young	58,260	0.087 ± 0.004	0.396 ± 0.094	48.1 (45.1, 51.9)b	114.2 (103.9, 127.5)b	1.92
	late	69,035	0.060 ± 0.001	−1.433 ± 0.031	100.0 (94.5, 106.4)a	195.5 (182.4, 211.1)a	10.70

*Within each analyzing model, means followed with different letters within a column are significantly difference ($P < 0.05$; lethal dose ratios test);
**during the lethal dose ratio testing, all the 95% confidence level for LD_{99} and $LD_{99.9968}$ comparison between young and late females were from $\infty$to∞.

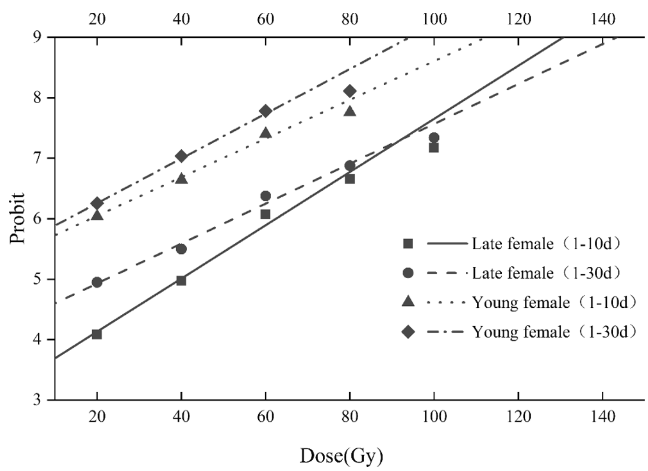

Fig. 2. The mortality and estimated probit curves derived from probit model of the dose-mortality data when young and late females of *Planococcus lilacinus* were irradiated at 20 to 100 Gy with the increment of 20 Gy.

mortality (Follett & Neven, 2006; NAPPO, 2011), which is 99.9969 % at the 95 % confidence level according to Eq. (2).

Discussion

The tolerance to radiation for crawlers, late instar nymphs, and adult (young and late) females of the cacao mealybug were tested by comparing the ability to develop into adults (Fig. 1), producing F_1 generation neonates (Table 1), and their overall development (Tables 2 and 3). The late female stage was identified as the most tolerant to radiation. This result is consistent with the findings of Hallman et al. (2010), who found that radio-tolerance develops with developmental stage and time when a common criterion is used, and the most developed stages are most tolerant.

Several researchers have subsequently confirmed this fundamental principle by irradiating kinds of mealybugs and scale insects, for example, *D. neobrevipes and D. lepelleyi* Betrem (Doan et al., 2012; Ma et al., 2019), *Pseudococcus jackbeardsleyi* Gimpel & Miller (Shao et al., 2013; Hofmeyr et al., 2016a; Zhan et al., 2016), *Aonidiella aurantii* Maskell (Khan et al., 2016a,b), *Maconellicoccus hirsutus* Green (Jacobsen & Hara, 2003), *Paracoccus marginatus* Williams and Granara de Willink (Seth et al., 2016a), *P. citri* Risso and *P. ficus* Signoret (Hofmeyr et al., 2016a), *Phenacoccus solenopsis* Tinsley (Seth et al., 2016b), *Aspidiotus destructor* Tinsley (Khan et al., 2016a), and *Exallomochlus hispidus* Morrison (Kuswadi et al., 2016). In addition, Doan et al. (2016) found that a minimum dose of 100 Gy was effective in preventing the emergence of 2nd nymphs from the 3–6-day-old adults of cacao mealybug, which have the similar ages to the young females in this study. However, they are all less tolerant than the late females (Tables 1–3; Figs. 1 and 2). The radiation tolerance increases as the development of cacao mealybugs progress through its life stages. Therefore, the most advanced developmental stage that can be present on/in the shipped commodity is the stage that should be tested in PI studies. Consequently, it is not necessary to assess the radiation tolerance of earlier developmental stages of the life cycle (Hallman, 2011).

A phytosanitary treatment should be effective against the most tolerant stage that may be present in international shipping commodities (IPPC, 2003, 2007). To develop the technical schedule for phytosanitary treatment of *P. lilacinus*, the late females that may present on the fruits and seedlings should accordingly be treated in the dose–response test to estimate the minimum absorbed dose for providing quarantine security (normally probit 9 mortality at the 95 % confidence level) (Tables 2 and 3), and the estimated dose then validated in confirmatory tests. For this reason, two confirmatory tests were conducted, and none 2nd nymphs were developed from the irradiated late females (Table 4).

The largest radiation dose of 163.0 Gy in the confirmatory tests should be the minimum dose used for phytosanitary treatment (IPPC, 2003; Kuswadi et al., 2016). This measure could prevent the reproduction of at least 99.9969 % (at 95 % confidence level) of adult females of *P. lilacinus*. In addition, a minimum radiation dose of 150 Gy is sufficient to prevent the reproduction of *P. minor* (Ravuiwasa et al., 2009), *P. citri* and *P. ficus* (Hofmeyr et al., 2016b). Furthermore, the ISPM 28 annex PT 19 defines a minimum absorbed dose of 231 Gy to control of *P. lilacinus* (IPPC, 2015). Therefore, the lower radiation dose of 68 Gy (comparing

Table 4
Large-scale confirmatory tests irradiating late females of *Planococcus lilacinus* rearing on pumpkin fruits.

Date of radiation	Target dose (Gy)	Dose monitored (Gy)		no. pumpkin fruits	no. late females	no. F_1 generation	
		Min.	Max.			1st neonates	2nd nymphs
Jun. 2019	145	132.2	163.0	160	66,959	+++	0
(control)	0	0	0	16	6,966	+++	+++
Oct. 2020	135	126.1	145.1	110	30,425	+++	0
(control)	0	0	0	12	3,278	+++	+++

+++: The pumpkin surface was covered with neonates or nymphs, so that the number were not counted accurately.

the minimum absorbed dose of 231 Gy to 163 Gy derived in this investigation) is a significant reduction, and could lead to saving energy, reducing treatment costs, and lessening the moderate loss in fruit quality (especially for radio-sensitive varieties such as lemon and lime) (Kader, 1986; Hallman et al., 2016), thereby facilitating international trade.

Ionizing radiation does not cause significant acute mortality at the doses used for PI treatment but renders pests incapable of completing development and/or reproducing (Hallman, 2012; Ma et al., 2019; Kwon et al., 2021). PI is a unique method among phytosanitary treatments in that acute mortality is not the usual measure of efficacy because acute mortality is not required to prevent the establishment of a pest, and the doses needed to achieve 100 % acute mortality are higher than most fresh commodities can tolerate. PI is effective in completely arresting insect development and preventing reproduction at doses that do not significantly alter the quality of most fresh commodities (Follett & Neven, 2006; Hallman & Blackburn, 2016; Hallman et al., 2016). Acute mortality was not used as the criterion for evaluating the efficacy of irradiation treatment. Conversely, the mortality of F_1 generation eggs (prevent egg-hatching) or neonates (prevent developing to 2nd instars) instead of mealybug adults was used to estimate the minimum absorbed dose to prevent adult reproduction. For PI treatment of mealybugs that oviposits eggs, the suitable criteria are preventing egg hatch, for instance, the passion vine mealybug *P. minor* (Ravuiwasa et al., 2009), *Pa. marginatus* (Seth et al., 2016a,b), and *D. lepelleyi* (Ma et al., 2019). However, preventing the emergence of 2nd instar nymphs should be more convenient for mealybugs that laid neonate, for example, the grey pineapple mealybug *D. neobrevipes* (Doan et al., 2012; Doan et al., 2016).

We chose to investigate the radiation effect on prevention of the emergence of 2nd instar nymphs than neonate (1st instar nymphs) from irradiated females even if the mealybug laid eggs, because it is difficult to find eggs or neonates which lurked in the abdomen of the females. In addition, very high radiation doses are needed to prevent molting of eggs that are ready to be hatched (Shao et al., 2013; Zhan et al., 2016). For instance, the minimum dose to prevent egg-hatch of *P. minor* is different from 7 to 14-day-old old eggs and un-oviposited eggs (in the abdomen of females); they are > 250 Gy and 150 Gy, respectively (Ravuiwasa et al., 2009).

Probit analysis using Probit model has been widely used for analyzing dose-mortality data to estimate the minimum dose for providing quarantine security or comparing tolerance to fumigation and temperature treatments (Liu et al., 2015; IPPC, 2018, 2019; Wang et al., 2020); nevertheless, prevention of development or reproduction for irradiation treatment instead of mortality is used for efficacy evaluation, for example, prevention of *D. lepelleyi* egg-hatching (Ma et al., 2019) and development of *Ps. jackbeardsleyi* neonate (Shao et al., 2013; Hofmeyr et al., 2016a; Zhan et al., 2016). However, the estimation values of LD_{99} are close but $LD_{99.9968}$ from logit model is larger than that from probit model (Tables 3), indicating that probit model is more extensively used than logit model.

In this study, the tolerance to radiation for *P. lilacinus* egg (development within the female body) increased with the developing times for both young and late females (Table 2). The neonates laid within 10 days, which is more tolerant than those between 11 and 30 days, could present the most radio-tolerance egg stages and should be used for probit analysis. Moreover, the estimated LD_{99} or $LD_{99.9968}$ based on 1–10-day-old neonates should be larger than that estimated based on all the neonates developed within 30 days; nevertheless, the estimated $LD_{99.9968}$ from all the 30-day neonates was larger than that of 1–10 days old neonates when late females were irradiated (Table 2, Fig. 2). The reasons for this exception may be: (i) the probit-9 value is an extrapolated value; (ii) the estimated dose-probit line is flattened as the initial mortality was higher than 50 % (Fig. 2); and (iii) the slop decreased from 0.044 for 1–30-day-old neonates to 0.033 for 1–10-day-old neonates, resulting in a crossover of estimated dose-probit lines when the radiation dose is about 95 Gy (Fig. 2). It is suggested that a lower dose, for example, 15 Gy or 10 Gy, can be used for conducting the dose–response test even if dose intervals are different from each other (Shao et al., 2013; Wang et al., 2016). Anyhow, the recommended dose (163 Gy) for PI treatment is also larger than the estimated upper limit (159.4 Gy) for the late females (Table 3). Therefore, a minimum absorbed dose of 163 Gy would provide adequate quarantine security for the phytosanitary treatment of the cacao mealybug infesting commodities.

Author contribution

G-P Z, CM, HL and BL designed and supervised the research. CM, Q-Y Zhao, Z-J, S and G-P Z conducted experiments. J-P Z provided insects and helped with experimental design. CM, G-P Z and Q-Y Z analyzed the data. G-P Z and CM wrote the paper. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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