Minimum gamma irradiation dose for phytosanitary treatment of *Exallomochlus hispidus* (Hemiptera: Pseudococcidae)

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Abstract

Exallomochlus hispidus (Morris) (Hemiptera: Pseudococcidae), is a polyphagous pest, attacking a wide range of plants, including mangosteen (*Garcinia mangostana* L.; Malpighiales: Clusiaceae) in Indonesia. The pest's present distribution is restricted to South East Asian countries, and it has, thus, become a quarantine pest hindering the export of fruits, vegetables and ornamental plants from the region. A series of efficacy tests were conducted to determine the dose of gamma radiation required as a phytosanitary treatment against this pest. In each efficacy test approximately 50 mealybugs were inoculated on a squash (*Cucurbita moschata* Duchesne ex Poir.; Cucurbitales: Cucurbitaceae) fruit and gamma irradiated at various doses in 4 replicates. The mortality and progeny production responses were observed. Tests to determine the most radio-tolerant stage were conducted at doses of 100, 200, 300 and 400 Gy on 1st, 2nd and 3rd instar nymphs and pre-oviposition adults. These tests showed that irradiation inhibited the development of the insects besides inducing mortality and sterility. The 400 Gy generic dose used for arthropods caused only 84.6% mortality. Based on the mortality response, adults and 3rd instar nymphs were found to be the most tolerant stages. Efficacy tests were subsequently conducted on adults at doses of 50, 75, 100 and 125 Gy. These increasing doses did not significantly increase mortality but significantly increased sterility. No treated adult produced progeny except in 1 replicate at a dose of 50 Gy. To determine the minimum dose that would be required to use irradiation as a phytosanitary treatment, other tests on adults were conducted at doses of 40, 60, 80, 100 and 120 Gy and the percent sterility of individual adults was observed and recorded. The results and the probit analysis of the sterility data showed that ED_{50}, ED_{99} and $ED_{99.99683}$ (probit 9) the sterility doses were 85.5, 99.4 and 110.7 Gy, respectively. It is concluded that when sterility is the measure of treatment efficacy, the minimum dose for phytosanitary treatment against *E. hispidus* should be 111 Gy. This is lower than the doses of 200 to 250 Gy necessary to prevent the development of the gray pineapple mealybug, *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae).

Key Words: effective dose, export, mangosteen, fruits, vegetable

Resumen

La cochinilla harinosa de cacoa, *Exallomochlus hispidus* (Morris) (Hemiptera: Pseudococcidae), es una plaga polífagas, que ataca a una amplia gama de plantas, incluyendo mangostán (*Garcinia mangostana* L.; Malpighiales:Clusiaceae) en Indonesia. La distribución actual de esta cochinilla se limita a los países del sudeste asiático, y por lo tanto, se ha convertido en una plaga cuarentenaria que dificulta la exportación de frutas, verduras y plantas ornamentales de la región. Se realizó una serie de pruebas de eficacia para determinar la dosis de radiación gamma requerida como un tratamiento fitosanitario contra esta plaga. En cada prueba la eficacia, se inocularon aproximadamente 50 cochinillas sobre una fruta de calabaza (*Cucurbita moschata* Duchesne ex Poir.; Cucurbitales: Cucurbitaceae) que fueron sometidas a una irradiación gamma a distintas dosis en 4 repeticiones. Se observaron la mortalidad y la respuesta de producción de progenies. Se realizaron exámenes para determinar el estadio más radio-tolerante en dosis de 100, 200, 300 y 400 Gy sobre las ninfas del 1ª, 2ª y 3ª estadio y adultos en pre-oviposición. Estas pruebas mostraron que la irradiación inhibe el desarrollo de los insectos además de inducir la mortalidad y la esterilidad. La dosis genérica 400 Gy utilizada para los artrópodos resultó en una mortalidad de solamente 84.6%. Basado de la respuesta de la mortalidad, se encontraron que los adultos y ninfas de 3ª estadio son los más tolerantes. Posteriormente, se realizaron pruebas de eficacia sobre los adultos a dosis de 50, 75, 100 y 125 Gy. Estas dosis crecientes no incrementaron significativamente la mortalidad, pero incrementó significativamente la esterilidad. No hubo adultos tratados que produjeran progenie excepto en una replicación a una dosis de 50 Gy. Para determinar la dosis mínima que se requiere para utilizar la irradiación como tratamiento fitosanitario, se llevaron a cabo otras pruebas en adultos en dosis de 40, 60, 80, 100 y 120 Gy y la esterilidad se observó en adultos individuales. Los resultados y el análisis de probit aplicado a los datos de esterilidad mostraron que las dosis de esterilidad de SD50, SD99 y SD99.99683 (probit 9) fueron 85.523, 99.385 y 110.729 Gy, respectivamente. Se concluye que cuando la esterilidad es el objetivo del tratamiento, la dosis mínima para el tratamiento fitosanitario contra *E. hispidus* (probit 9) debe ser 111 Gy. Esto es más baja que la dosis de 200 a 250 Gy necesarias para prevenir el desarrollo de la cochinilla gris de la piña, *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae).

Palabras Clave: dosis efectiva, exportación, mangostán, frutas, verduras, hortalizas

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In Indonesia mangosteen (*Garcinia mangostana* L.; Malpighiales: Clusiaceae) is attacked by a number of mealybug (Hemiptera: Pseudococcidae) species including *Exallomochlus hispidus* (Morris), *Pseudococcus cryptus* Hempel, *Dysmicoccus neobrevipes* Beardsley, and *Rastrococcus spinosus* (Hutagaol 2009; Nasution 2012). *Exallomochlus hispidus*, also known as the cacao mealybug, is a polyphagous insect which, besides cacao (*Theobroma cacao*) and mangosteen, also attacks many plant genera, including various plant species of economic importance in genera such as *Annona*, *Durio*, *Garcinia*, *Lansium*, *Licula*, and *Nephelium* (Williams 2004).

Exallomochlus hispidus is sexually dimorphic. The females go through an incomplete metamorphosis, morphologically resembling nymphs, and are wingless though unlike many female scale insects, they often retain legs and can move. Females actively feed on host plants, use waxy layers for egg protection and produce neonates directly. Males do complete metamorphosis but are short lived, winged and smaller. They do not feed at all as adults and only live to fertilize the females.

Exallomochlus hispidus secretes a powdery wax layer on their bodies to protect themselves from predators. While feeding, sucking plant sap on stems, leaves or fruits, the cacao mealybug excretes honeydew. Severe infestations may induce leaf drop but economic losses mostly result from indirect effects of moderate infestations. Deposits of powdery wax and black sooty mold that grows on honeydew droplets on fruits and leaves reduce fruit quality and photosynthesis. Like other mealybugs, the cacao mealybug tends to be a serious pest only in the presence of ants, because the ants protect them from predators and parasites.

Exallomochlus hispidus was intercepted on longan (*Dinocarpus longan* Lour.; Sapindales: Sapindaceae); mangosteen (*G. mangostana*); langsat (*Lancium domesticum* Corrêa; Sapindales: Meliaceae); *Murraya* spp. (Sapindales: Rutaceae) and rambutan (*Nephelium lappaceum* L.; Sapindales: Sapindaceae) from South Asian countries (Williams & Watson 1988). At present, the distribution of this mealybug is restricted to South Asian countries, but it is a potential invasive species for the US and Caribbean Basin (Evans & Dooley 2013). It has become a quarantine pest hindering export of fruits, vegetables and ornamental plants from the Asian region. As an example, phytosanitary treatment is required for mangosteen that Indonesia exports in large quantity to Australia and New Zealand.

Materials and Methods

MEALYBUG REARING

In the field, mangosteen is infested by various mealybug species. To obtain *E. hispidus* colonies, samples of mealybugs were collected from the field, identified (Williams 2004) and *E. hispidus* was isolated and reared in the laboratory on squash (*Cucurbita moschata* Duchesne ex Poir.; Cucurbitales: Cucurbitaceae) fruit as a host at 28 ± 2 °C, RH 80 ± 5% and 12:12 h L:D photoperiod. Squash fruit was used because it has a long shelf-life, is available year round and the cacao mealybug thrives on it.

Laboratory rearing of the *E. hispidus* was conducted to supply adequate numbers of *E. hispidus* of every stage for the experiments. The mealybug's life cycle on squash lasts 27 d with the developmental stages of 1st, 2nd and 3rd instar nymphs and the pre-birthing adult lasting 6, 7, 4, and 10 d, respectively (Fig. 1). To obtain the required number of mealybugs of a uniform life stage, 3 gravid adults were inoculated on squash and when young nymphs were observed on the squash fruit after 1 to 3 d, the adults were removed, and transferred to another squash for another bout of production. On each squash fruit, the required number of mealybugs (about 50) of the stage of interest was selected from the progeny. Using a paintbrush, the selected insects were positioned at the center of the squash in order to obtain a uniform dose distribution during irradiation (Fig. 2).

IRRADIATION

The irradiation treatments were performed at the Center for Isotope and Radiation Application (CIRA) in Jakarta, Indonesia with a Gamma Chamber 4000-A. The source of Cobalt 60 delivered 401 Gy/h. The samples were placed at the center of the irradiator cavity (Ø 15 × 20 cm) (Fig. 2) to get a more uniform dose distribution and then irradiated for the variously appropriate periods of time depending on the required dose. Unlike the 2nd and 3rd instar nymphs and adults, which were irradiated on squash, the 1st instar nymphs (crawlers)—due to their mobility—were irradiated in a plastic jar (Ø 1.0 × 3.0 cm) (Fig. 3).

EXPERIMENTS ON EFFICACY OF GAMMA IRRADIATION

Effect of Irradiation on Nymphal Development. To study the effects of gamma irradiation on the development of *E. hispidus*, the 1st, 2nd and 3rd instar nymphs were irradiated with 100, 200, 300 and 400 Gy. Approximately 50 insects with 4 replicates were used in the experiments. At the end of each observational period (26–44 d), the number of individuals in each of the nymphal instars and the adult stage that were successive to the irradiated instar and the production of progeny from the adults were recorded.

Radio-Tolerance of Various Life Stages. To determine the most radio-tolerant life stage, efficacy experiments were carried out on all life stages except eggs. Approximately 50 mealybugs per each of the 1st, 2nd and 3rd instar nymphs and pre-birthing adults were irradiated with 100, 200, 300, and 400 Gy in 4 replicates. The number of surviving insects and the life stages were observed daily within 26–44 d to record the mortality and the successive development of nymphs toward and to the adult stage. Progeny produced by surviving adults were also recorded. The percent mortality responses to the doses were analyzed using Microsoft Excel 2010 to obtain linear regression so that the most radio-tolerant life stage could be determined.

Minimum Dose of Gamma Irradiation for Phytosanitary Treatment. After the most resistant life stages were identified as the 3rd instar nymph and the adult, 2 efficacy experiments on the adult stage were conducted to determine the minimum dose to be considered as a phytosanitary treatment against the cacao mealybug. In these experiments, the adult stage was used rather than the 3rd instar nymph because it was easier to obtain adults of uniform than even-aged 3rd instars. Each squash fruit was infested with approximately 50 two-d-old pre-birthing adults, and then irradiated. Two efficacy tests were conducted. In the first test, the effects of doses of 50, 75, 100, 125 Gy were evaluated with 4 replicates. Mortality was observed daily for 26 d and the number of progeny produced in each replicate (squash fruit) was also recorded.

In order to determine the minimum dose for phytosanitary treatment using the probit 9 concept with incapacity to reproduce (complete sterility) as the target effect, another efficacy test was carried out. In this experiment, doses of 60, 80, 100 and 120 Gy were used and the sterility of each treated individual adult female was separately observed. For this purpose, each treated adult was individually transferred post-irradiation to a separate squash fruit. Mortality and either partial or full sterility of individual adults were observed and recorded daily for 35 d. Any adult that produced progeny that reached the 3rd

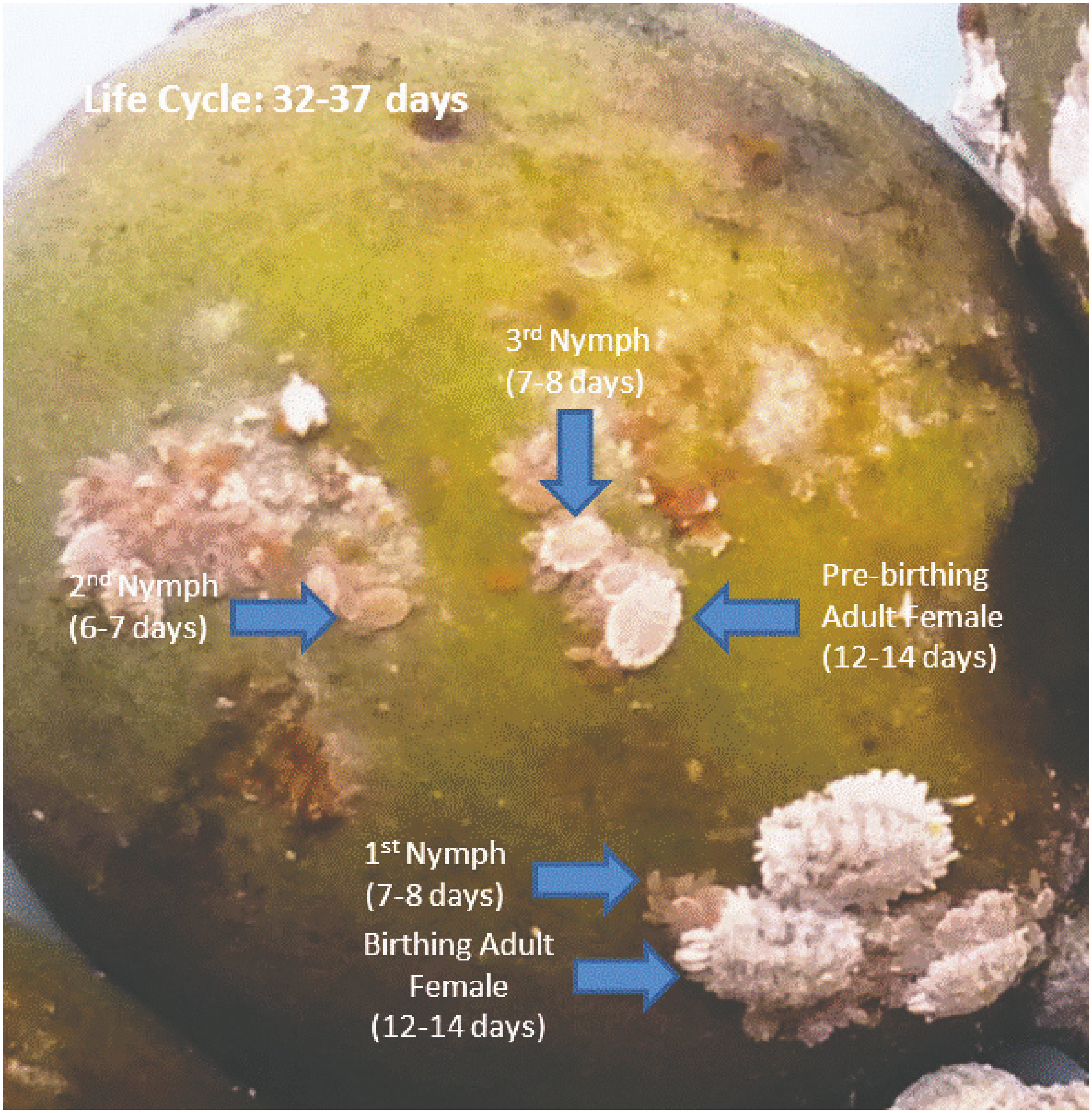

Fig. 1. Life stages of *Exallomochlus hispidus* mealybugs when reared on squash. The 1st, 2nd and 3rd instar nymphs and pre-birthing adults were 6, 7, 4, and 10 d old, respectively, and the total life cycle was completed in 32–37 d.

instar within the 35-d observation period was considered as fertile, but otherwise as sterile. The statistical program PoloPlus ver.1.0 was used for probit analysis of the sterility data.

DATA ANALYSIS

Experimental data were analyzed using SAS (SAS Institute 2002). Means were separated using Student's test at the 5% level of probability. PoloPlus ver. 1.0 probit analysis was used for sterile insect data to determine the ED_{50}, ED_{99} and $ED_{99.9968}$ values, where ED = Effective Dose.

Results

EFFECTS OF IRRADIATION ON NYMPHS

Table 1 shows the number of the next life stages of the insects when *E. hispidus* 1st, 2nd and 3rd instar nymphs and adults were irradiated with various doses of gamma radiation ranging from 100 to 400 Gy. The main effect of irradiation on the mealybugs was mortality. We observed that the earlier the life stage, the more sensitive was the

Fig. 2. Positioning of an *Exallomochlus hispidus* mealybug-infested squash fruit in the cavity of the γ-irradiator.

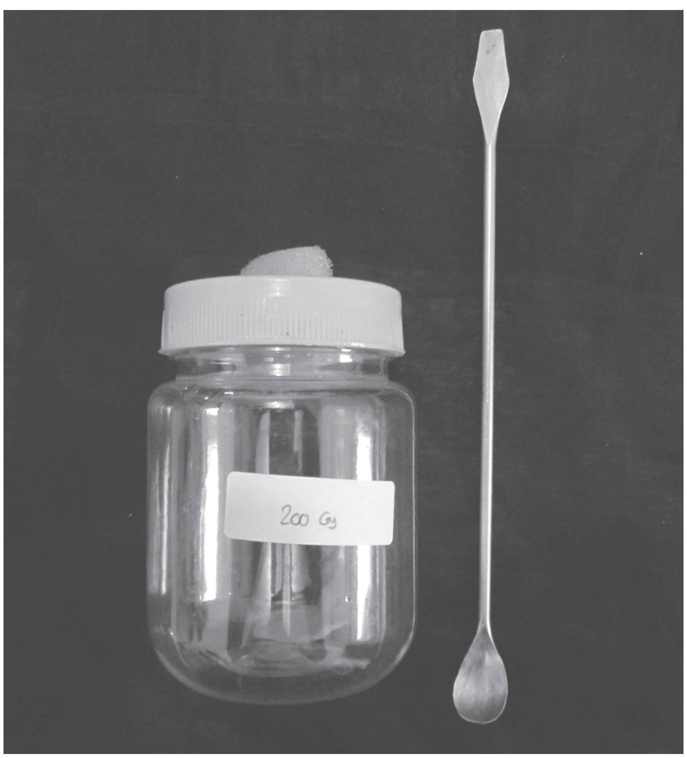

Fig. 3. *Exallomochlus hispidus* crawlers (1st instar nymphs) were irradiated in plastic jars.

cacao mealybug to gamma irradiation. At all tested doses, the number of surviving mealybugs from treated 1st instar nymphs was less than those from treated 2nd instars and from treated 3rd instars. Percentages of survival of 1st instar nymphs irradiated at 100, 200, 300 and 400 Gy were 26.8, 6.3, 4.7 and 6.9, respectively; while, correspondingly, the percentages of survival of treated 2nd instars were 59.3, 32.2, 32.4 and 13.9, respectively; and, correspondingly, the percentages of survival of treated 3rd instars were 96.6, 89.6, 82.5 and 64.1, respectively.

This experiment also showed that besides inducing mortality, irradiation also hinders development of *E. hispidus* instars. While in the control all mealybugs reached the adult stage, in the irradiated nymphal populations a portion of the survivors remained in the nymphal stage and did not reach adult stage. This occurred to the greatest extent when irradiation was applied to 2nd instar nymphs. When the 2nd instar nymphs were treated with doses of 100, 200, 300 and 400 Gy, then—at the end of observation period—8.4, 15.0, 14.6 and 7.3% of the mealybugs remained in the 2nd instar, and 13.1, 13.3, 7.5 and 6.7%, respectively, did not develop beyond the 3rd instar; and only 37.8, 3.9, 10.3 and 0.0%, respectively, reached the adult stage.

MOST RADIO-TOLERANT STAGE OF *E. HISPIDUS*

Table 2 shows the percentage of mortality when 1st, 2nd and 3rd instar nymphs and adults were irradiated at doses ranging from 100 to 400 Gy. In this range, irradiation had a significant effect on mortality at all stages. In all life stages, there was a significant difference among mortalities of mealybugs treated with different doses ($P = 0.05$). The greater the dose the greater was the mortality. The mortalities of 1st instar nymphs irradiated with 100, 200, 300 and 400 Gy were 73.9, 93.7, 95.3 and 93.1%, respectively; 40.6, 67.8, 67.6 and 86.0%, respectively, for 2nd instars; 3.4, 10.4, 17.5 and 35.9% for 3rd instars; and 24.5, 27.0, 33.0 and 46.0%, respectively, for adults.

Figure 4 shows the linear regression of the relationship between dose of irradiation and mortality of the various developmental stages of *E. hispidus.* Table 2 also shows that 3rd instar nymphs and adults were most tolerant to irradiation. The regression equations for the mortality vs. irradiation dose for the 1st, 2nd and 3rd instar nymphs and the adult were:

$y = 0.1864x + 32.982$; $y = 0.1756x + 16.239$; $y = 0.08656x + 12$; and $y = 0.0742x + 0.2224$, respectively, where y is percent mortality and x is the dose of irradiation.

MINIMUM DOSE TO STERILIZE *E. HISPIDUS* ADULTS

Table 3 presents the survival and progeny production of adults, 12 days after pre-oviposition adults had been irradiated at 50, 75, 100 and 125 Gy. At these doses, irradiation did not significantly decrease survival. Thus, the survival rates of adults irradiated with doses of 0 (control), 50, 75, 100 and 125 Gy were 98.0, 86.6, 96.5, 92.2, and 92.5%, respectively. With respect to reproduction, there were about 50 adults/squash fruit and 4 infested fruits/dose. At 12 days after treatment offspring were produced by non-irradiated adults (control), but not by irradiated adults—with the exception that some offspring were produced in 1 of 4 replicates by adults irradiated with 50 Gy. This indicated that more than 50 Gy are required to reliably induce complete sterility of adults.

Table 4 shows the percentages of completely sterile adult females 21 days after 50 pre-oviposition adults per replicate were irradiated at 60, 80, 100, and 120 Gy in a total of 4 replicates. In this experiment complete or partial sterility of each irradiated female was observed individually, each adult being infested on 1 squash fruit in order to isolate the female. The data show that the effect of irradiation on sterility was significant. After irradiation at the various doses, there were significant differences in the percentages of sterile adults. The greater

Table 1. Effect of γ-irradiation with 0–400 Gy of each of the nymphal instars and adults of *Exallomochlus hispidus* on the number of individuals observed in the irradiated and each subsequent instar or adult stage and whether any progeny were produced. The data were recorded at the end of each observational period (26–44 d).

		Percent of individuals[a] in each life stage at the end of each observational period					
		Nymph 1	Nymph 2	Nymph 3	Adult		
Treated as	Doses (Gy)	Percent ± SE (%)	Percent ± SE (%)	Percent ± SE (%)	Percent ± SE (%)	Total percent survival	Progeny[b]
Nymph 1	0	0	0	0	94.52 ± 5.67	94.52 ± 1.22	+
	100	0	0.27 ± 0.26	3.89 ± 1.61	22.66 ± 11.11	26.83 ± 12.54	—
	200	0	0	0.97 ± 0.56	5.29 ± 3.95	6.26 ± 4.20	—
	300	0	0	0	4.73 ± 2.11	4.73 ± 2.11	—
	400	0	0	0.20 ± 0.20	6.70 ± 2.39	6.90 ± 2.41	—
Nymph 2	0	not applicable	0	0	88.30 ± 2.52	88.30 ± 2.52	+
	100	not applicable	8.45 ± 2.78	13.10 ± 3.88	37.80 ± 8.41	59.35 ± 2.85	—
	200	not applicable	14.98 ± 10.49	13.34 ± 7.74	3.92 ± 3.19	32.24 ± 8.39	—
	300	not applicable	14.58 ± 3.97	7.48 ± 2.67	10.31 ± 5.96	32.37 ± 1.53	—
	400	not applicable	7.29 ± 5.50	6.69 ± 3.92	0	13.98 ± 4.96	—
Nymph 3	0	not applicable	not applicable	0	94.15 ± 1.69	94.15 ± 1.69	+
	100	not applicable	not applicable	0	96.57 ± 1.18	96.57 ± 1.18	—
	200	not applicable	not applicable	0	89.59 ± 2.54	89.59 ± 2.54	—
	300	not applicable	not applicable	0	82.50 ± 1.70	82.50 ± 1.70	—
	400	not applicable	not applicable	0	64.08 ± 4.77	64.08 ± 4.77	—
Adult	0	not applicable	not applicable	not applicable	90.00 ± 2.16	90.00 ± 2.16	+
	100	not applicable	not applicable	not applicable	75.50 ± 3.94	75.50 ± 3.94	—
	200	not applicable	not applicable	not applicable	73.00 ± 11.73	73.00 ± 11.73	—
	300	not applicable	not applicable	not applicable	67.00 ± 7.93	67.00 ± 7.93	—
	400	not applicable	not applicable	not applicable	54.00 ± 6.21	54.00 ± 6.21	—

[a]Percent of individuals based on the number of irradiated individuals.
[b](+) Produced progeny, (—) did not produce progeny.

the dose, the greater the percentage of completely sterile adults produced. The pre-oviposition adult females were irradiated at 0, 60, 80, 100, and 120 Gy, the corresponding percentages of completely sterile females were 12.0, 15.3, 26.7, 99.3 and 100. Probit 9 analysis of the data was carried out, and the results are shown in Fig. 5.

Figure 5 shows the correlation between gamma irradiation of pre-oviposition *E. hispidus* adult females and their sterility response. Probit analysis of these data was made using the PoloPlus 1.0 program. Probit analysis showed that the mean doses needed to sterilize 50% (ED_{50}), 99% (ED_{99}) and 99.99683% ($ED_{99.99683}$) of the population of *E. hispidus* were 85.5, 99.4 and 110.7 Gy, respectively.

Discussion

EFFECTS OF IRRADIATION ON DEVELOPMENT OF NYMPHS

Phytosanitary irradiation differs from other phytosanitary treatments because PI does not necessarily result in 100 % acute mortality (Hallman 2011). This experiment confirmed that irradiation could prevent biological development and reproduction of a quarantine pest by causing chronic mortality, hindering development of immature life stages and inducing sterility of the adults. The highest dose used in this experiment, 400 Gy, caused only 46% mortality of *E. hispidus* adult females.

However, the dose required to induce sterility was much lower than the dose necessary to induce mortality. It was demonstrated that the adults that had developed from nymphs irradiated at 100 Gy or more did not produce any progeny. Therefore experiments to determine the minimum dose that induces 100 % sterility in mated *E. hispidus* adult females were done.

Irradiation delayed the development of nymphs into adults, and the latter eventually died without producing progeny.

MOST RADIO-TOLERANT LIFE STAGE

The most tolerant life stage had to be determined in order to establish the minimum dose required as a phytosanitary treatment (Hall-

Table 2. Mortality (%) of *Exallomochlus hispidus* after the various development stages of the insect were γ-irradiated within a range of 100–400 Gy. Means in column followed by the same letter are not significantly different at ά ≤ 0.05 (Duncan's test). (+) produced progeny; (—) did not produce progeny.

	Doses (Gy)									
	0		100		200		300		400	
Development stages	Mortality	Progeny	Mortality	Progeny	Mortality	Progeny	Mortality	Progeny	Mortality	Progeny
Nymph 1	5.48b	+	73.17a	—	93.74a	—	95.27a	—	93.10a	—
Nymph 2	11.69a	+	40.65b	—	67.76a	—	67.63b	—	86.02a	—
Nymph 3	5.85b	+	3.43c	—	10.41b	—	17.50d	—	35.92b	—
Adults	10.00ab	+	24.50bc	—	27.00b	—	33.00c	—	46.00b	—

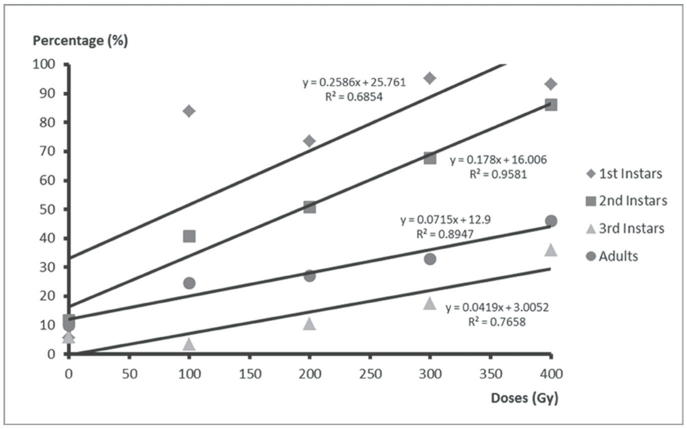

Fig. 4. Linear regression of the relationship between irradiation dose and mortality of each of the 3 instars and of the adult stage of *Exallomochlus hispidus*.

man et al. 2010). All life stages—1st, 2nd and 3rd instar nymphs and immature or pre-birthing mated adult females—were examined after irradiation with doses in the range of 0–400 Gy. Eggs were not included because *E. hispidus* is viviparous.

Results of the efficacy tests showed that the older the life stage the more tolerant it is to radiation. The 3rd instar nymph stage and the adult stage were found to be the most tolerant stages. Mortality rates of 3rd instar nymph and adults were not significantly different.

The experiments also indicated that irradiation of all life stages at 50 Gy or more rendered all adults mostly sterile. These experiments indicated that the gamma irradiation dose needed for total sterility was greater than 50 Gy. Efficacy tests to determine the minimum dose to sterilize the cacao mealybug were then conducted at 50, 75, 100, 125 Gy on mated *E. hispidus* adult pre-birthing females.

MINIMUM DOSE TO STERILIZE ADULT CACAO MEALYBUGS

The minimum dose required to sterilize adults of *E. hispidus* could become the specified dose of a phytosanitary treatment against this pest, because this dose will also sterilize all other life stages. Two efficacy tests were conducted to determine the minimum sterilizing dose, the first with doses of 50, 75, 100, and 125 Gy and the second with doses of 60, 80, 100, and 120 Gy.

Table 3 presents the mortality response using doses of 0, 50, 75, 100 and 125 Gy. It shows that in this dose range, irradiation did not significantly increase mortality. The average survival rates were 98.0, 86.6, 96.5, 92.2, and 92.5%, respectively. Total sterility appeared to be induced by doses greater than 50 and specifically 100 Gy, because no progeny was produced by adults in all 4 replicates irradiated with 100 Gy, but progeny were produced in 1 of 4 replicates irradiated with 50 Gy.

The set of efficacy tests with 60, 80, 100 and 120 Gy was carried out to obtain more accurate sterility data that could be subjected to probit analysis. The objective was to determine the minimum dose causing 99.99683% sterility (Probit 9). Table 4 shows the data obtained by observation of either complete or less than complete sterility of individual adult females that were irradiated as mated pre-oviposition females. Gamma irradiation of pre-birthing adults at 0, 60, 80, 100, and 120 Gy led to complete sterilization of 12.0, 15.3, 26.7, 99.3 and 100.0% of the adult females, respectively. Based on probit analysis using the PoloPlus version 1.0 program, the doses that induced 50% (ED_{50}) and 99% (ED_{99}) sterility were found to be 85.5 and 99.4 Gy, respectively. The probit 9 dose was 110.7 Gy or ~111 Gy. We conclude that the minimum dose to totally sterilize 99.99683% of *E. hispidus* pre-oviposition females at the 95% confidence level is 111 Gy; thus not more than 32 adults are expected to be fertile out of each 1,000,000 adult females irradiated

Table 3. Percent survival and production of progeny of *Exallomochlus hispidus* 12 d after pre-birthing adults were gamma irradiated within a range of 0–125 Gy. Each treatment involved 1 squash fruit infested with approximately 50 pre-birthing adults each about 2 d old; and each treatment was replicated 4 times.

Dose (Gy)	Survivors (%)	Progeny
0	98.0 a	Produced
50	86.0 a	Produced*
75	96.5 a	None
100	92.2 a	None
125	92.5 a	None

Means in column followed by the same letter are not significantly different at $\alpha \leq 0.05$ (Duncan's test)

*Progeny were observed in one replicate (on 1 squash fruit).

Table 4. Percentages of fully sterile *Exallomochlus hispidus* adult females at 21 days after irradiation with 0–120 Gy. Each treatment involved 1 squash fruit infested with approximately 50 pre-birthing adults each about 2 d old; each treatment was replicated 4 times.

Dose (Gy)	Percentage of fully sterile adults
0	12.00 a
60	15.33 a
80	26.67 b
100	99.33 c
120	100.00 c

Means in column followed by the same letter are not significantly different at $\alpha \leq 0.05$ (Duncan's test)

No.	Sterile doses	Stages	Predicted complete sterile doses (Gy)
1	SD50	Adult	85.523
2	SD99	Adult	99.385
3	SDP9	Adult	110.729

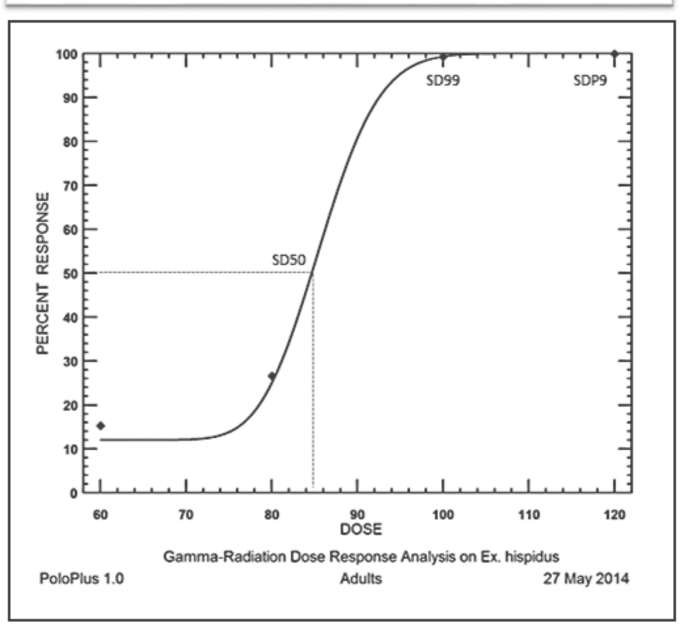

Fig. 5. Correlation between gamma irradiation and the sterility response of *Exallomochlus hispidus* pre-birthing adults to show the probit 9 (99.9968%) estimate based on PoloPlus 1.0.

with 111 Gy. This is lower than the doses required to sterilize several other species of mealybugs. For example, the probit 9 dose was estimated to be between 200 and 250 Gy for the gray pineapple mealybug, *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae) (Doan et al. 2012).

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