Phytosanitary irradiation of the mealybugs, *Dysmicoccus neobrevipes*, *Planococcus lilacinus,* and *Planococcus minor* (Hemiptera: Pseudococcidae), infesting dragon fruit in Viet Nam

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Abstract

Dysmicoccus neobrevipes Beardsley, *Planococcus lilacinus* (Cockerell), and *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) are invasive species that infest agricultural and ornamental plants. They infest dragon fruit (*Hylocereus undatus*: Caryophyllales: Cactaceae) in Viet Nam and are rated with a medium invasive risk potential. Before this research was conducted, 400 Gy was the minimum radiation dose required to disinfest dragon fruit of all pest species. Irradiation studies were conducted to determine the most radiotolerant stage of each of the above mentioned species, as well as which of these 3 species is the most radiotolerant. All stages of the life cycle were exposed to gamma radiation at doses ranging from 100 to 400 Gy and the mortality rate of each stage, the development of nymphs, and the reproduction of female adults were determined. The mature adult female was the most radiotolerant stage in all 3 of these mealybug species of which *D. neobrevipes* was the most radiotolerant species. Radiation doses to completely prevent the reproduction of adult females of *D. neobrevipes*, *P. minor* and *P. lilacinus* were found to be 200, 150 and 100 Gy, respectively. In large scale confirmatory testing involving 31,750 female adults of the most radiotolerant species, *D. neobrevipes*, the dose range of 206–231 Gy prevented 99.99% F_1 egg hatch. The few F_1 generation 1st instars surviving were very weak and died before reaching the 2nd instar. The dose of 231 Gy was recently approved by the International Plant Protection Convention for the phytosanitary treatment of hosts of these 3 mealybug species. Whereas irradiation of dragon fruit with 400 Gy moderately reduced its vitamin C content and the lightness of the peel color, these reductions were diminished somewhat by irradiation with 200 Gy. Irradiation with 231 Gy is projected to reduce the cost of phytosanitary treatment of dragon fruit and to ameliorate the moderate loss in quality at the previously adopted dose rate.

Key Words: gamma radiation; tolerant stage; radiotolerance; confirmatory test

Resumen

Dysmicoccus neobrevipes Beardsley, *Planococcus lilacinus* (Cockerell) y *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) son especies invasoras que infestan las plantas agrícolas y ornamentales. Ellos infestan la pitahaya (*Hylocereus undatus*: Caryophyllales: Cactaceae) en Vietnam y se han valorado como un riesgo potencial invasivo mediano. Antes de que se realizó esta investigación, 400 Gy fue la dosis mínima de radiación necesaria para desinfectar la fruta del dragón de todas las especies de plagas. Se realizaron estudios de irradiación para determinar el estadio más radiotolerante de cada una de las especies antes mencionadas y la más radiotolerante de estas 3 especies. Todas los estadios del ciclo de vida fueron expuestos a la radiación gamma en dosis que van de 100 a 400 Gy y se determinaron las tasas de mortalidad de cada estadio, el desarrollo de las ninfas, y la reproducción de las hembras adultas. La hembra adulta madura fue el estadio más radiotolerante de las 3 especies de cochinillas de la cual *D. neobrevipes* fue la especie más radiotolerante. Se encontró que la dosis de radiación efectiva para extinguir por completo la reproducción de las hembras adultas de *D. neobrevipes*, *P. minor y P. lilacinus* fue 200, 150 y 100 Gy, respectivamente. En las pruebas de confirmación a gran escala con las especies más radiotolerante, *D. neobrevipes,* que incluyó 31,750 hembras adultas, el rango de dosis medida (206 a 231 Gy) previno la reproducción de esta especie en una 99.99%. Los pocos estadios de la 1ª generación F_1 producidos por las hembras irradiadas fueron muy débiles y murieron antes de llegar al segundo instar. La dosis de 231 Gy fue aprobada recientemente por la Convención Internacional de Protección Fitosanitaria para el tratamiento fitosanitario de las plantas hospedantes de estas 3 especies de cochinillas. Mientras que la irradiación de la fruta del dragón con 400 Gy moderadamente reduce su contenido de vitamina C y la claridad del color de la piel, estas reducciones disminuyeron un tanto por irradiación a 200 Gy. La irradiación con 231 Gy se proyecta para reducir sustancialmente el costo de tratamiento fitosanitario de la fruta del dragón y para aminorar la pérdida moderada en la calidad de la tasa de dosis aprobada previamente.

Palabras Clave: radiación gamma; estadio tolerante; radiotolerancia; prueba de confirmación

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The mealybugs *Dysmicoccus neobrevipes* Beardsley, *Planococcus lilacinus* (Cockerell), and *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) are pests of dragon fruit (*Hylocereus undatus* (Haworth) Britton & Rose; Caryophyllales: Cactaceae) in Viet Nam (APHIS 2008b). These pests are known for feeding on young growth that causes severe stunting and distortion of leaves, thickening of stems, and a bunchy-top appearance of shoots; in severe cases the leaves may fall prematurely. Furthermore, honeydew deposited on leaves and fruits by mealybugs serves as a medium for the growth of black sooty mold, which interferes with photosynthesis and reduces the crop's market value (Jahn 1993).

The gray pineapple mealybug, *D. neobrevipes,* attacks a wide range of plants, including agricultural, horticultural, and forest species and is present throughout Central American, northern South America, the Caribbean, Indochina, the Philippines, and parts of Oceania (Ben-Dov et al. 2015).

The cacao mealybug, *P. lilacinus*, is found in Bangladesh, Cambodia, India, Laos, Myanmar, Taiwan, Viet Nam, Yemen, Indonesia, Java, Malaysia, the Philippines, and Papua New Guinea (CABI 2006). The host range of *P. lilacinus* is extremely broad, comprising species in at least 35 plant families (Ben-Dov et al. 2015). The main dispersal stage of mealybugs is the first-instar crawler, which may be transported locally by wind or on animals. Throughout its geographic range *P. lilacinus* is a pest of cacao, causing severe damage to young trees by killing the tips of branches (CABI 2006). *Planococcus lilacinus* is also considered a major pest of tamarind (*Tamarindus indica* L.; Fabales: Fabaceae) in India (Hill 1983) and causes damage to a wide range of other economically important crops, such as coffee, custard apple, coconut, citrus, grape, guava, and mango (CABI 2006).

Planococcus minor—with the common name passion vine mealybug—is extremely polyphagous, being recorded on host species in at least 65 plant families. This species attacks several economically important crops, including grape, pear, various cucurbits, and various solanaceous species (Ben-Dov et al. 2015). Dispersal over long distances is accomplished through the movement of infested plant materials in commerce (CABI 2006; Sugimoto 1994).

Utilization of phytosanitary irradiation (PI) as a treatment to disinfest agricultural commodities in trade has expanded in recent years. Since 2008 and 2011, respectively, dragon fruit and rambutan (*Nephelium lappaceum* L.; Sapindales; Sapindaceae) have been exported from Viet Nam to the United States, and this was made possible by PI. Because they are rated with medium risk potential in a pathway-initiated risk assessment, the aforementioned mealybugs must be disinfested at the minimum dose of 400 Gy before the host fruit can be exported (APHIS 2008a, b). A generic dose of 400 Gy was adopted by the Animal and Plant Health Inspection Service (APHIS), US Department of Agriculture, to disinfest commodities of all insect pest species except lepidopteran pupae and adults (Hallman 2012). The goal of a quarantine treatment is to prevent the establishment of invasive species, and the required response to PI can be acute mortality, the prevention of adult emergence when eggs and larvae are irradiated, prevention of oviposition, prevention of hatching of F_1 generation eggs, or the development subsequent to the F_1 generation after P generation pupae or adults have been irradiated (Heather & Hallman 2008). However, irradiation at the doses typically used in PI does not cause high levels of acute mortality, so acute mortality generally is not an objective of research to establish radiation doses suitable for PI.

If the efficiency of disinfestation can be maintained while the dose is lowered, the cost of treatment will be reduced and the capacity of the treatment facility may be increased because of a shorter treatment time. Follett (2009) reported that in Hawaii a reduction in the dose from 400 Gy to 150 Gy for sweet potato resulted in a 60% reduction in the cost of treatment. Additionally, quality of commodities, especially fresh fruits, treated at low doses can be retained better.

Development of generic doses for groups of common plant pests would be beneficial. Efforts are being made to provide generic treatments for more groups of quarantine pests and reduce the 400 Gy dose accepted by APHIS for all insect species except pupae and adult Lepidoptera (Hallman 2012). In 2009, the Joint Division of Nuclear Techniques in Food and Agriculture of the United Nations Food and Agriculture Organization and the International Atomic Energy Agency, initiated a 5-yr Cooperative Research Project with the objective of developing additional generic PI treatments (IAEA 2009). In part, these studies were conducted to determine the most radiotolerant stage in the life cycle of each species and the most radiotolerant species among the aforementioned mealybug species infesting Vietnamese dragon fruit through measures of efficacy such as mortality and the extinction of developmental and/or reproductive abilities of survivors of the treatment. A large scale confirmation test with the most radiation resistant species was carried out to verify that an effective dose could be lower than the currently required dose of 400 Gy for these pests.

Materials and Methods

INSECT REARING

Dysmicoccus neobrevipes, *P. lilacinus,* and *P. minor* were collected from fields of dragon fruit (*Hylocereus* sp.; Caryophyllales: Cactaceae) in Binh Thuan province, Viet Nam and reared in the laboratory at 28 ± 2 °C and 70 ± 5% RH for *D. neobrevipes* and *P. lilacinus,* and at 29 ± 2 °C and 75 ± 5% RH for *P. minor*. Because the shelf life of dragon fruit is relatively short (4–5 d) pumpkin (*Cucurbita moschata* Duchesne ex Poir.; Cucurbitales: Cucurbitaceae) fruits were used as hosts for *D. neobrevipes* and *P. lilacinus* and sprouting potato (*Solanum tuberosum* L.; Solanales: Solanaceae) tubers were used as the host for *P. minor*. Twenty female adults of each species were reared on the surface of a pumpkin fruit or sprouting potato tuber with 3 replicates to determine the developmental stages and life cycles of the 3 mealybug species. Observations of their reproduction and the development of nymphs were conducted daily until all had died. Based on the time of molting, the developmental stages were determined. Even-aged individuals of each stage were collected for irradiation experiments. The removal of individual insects was done by using a soft paint brush to avoid injury. To conduct a large scale confirmatory test, female adults of the most radiotolerant species, *D. neobrevipes,* were reared on pumpkin fruits to obtain > 30,000 individuals. The measure of efficacy to be achieved at the end of this test was the prevention of the development in the F_1 generation of 2nd instar nymphs.

IRRADIATION TREATMENT

Irradiation was conducted in a gamma Co-60 irradiator (SVST-Co-60/B, Hungary) with the dose rate of 8.3 Gy/min at the Research and Development Center for Radiation Technology, Ho Chi Minh City, Viet Nam. Cardboard boxes (30 cm × 22 cm × 13 cm) containing samples were placed on the stainless steel table with different positions facing toward the source racks in the irradiation chamber. Host surfaces with insects were positioned to face the Co-60 source. The absorbed dose was measured by Fricke dosimeters attached to surfaces of the host (pumpkin fruits or potato tubers) (ASTM 2004).

EXPERIMENTAL DESIGN

To investigate the effect of irradiation on different developmental stages of each species 1st, 2nd, and 3rd instars (2–4 days old) and adults (3–6 days old) were irradiated at doses between 100–400 Gy. Also the experiment included a non-irradiated control. In each of 3 replicates, 100 individuals of each life stage were irradiated with each dose and held for observation until all that had not reached the adult stage had died. The non-irradiated control was moved to a nearby facility to have the same conditions as the irradiated samples but to prevent accidental contamination of the treated insects by the non-irradiated controls. The adult female was the focus for all tests, because it is essential that a phytosanitary treatment actually renders all female pests incapable of reproduction, for in that case—even if the males are fully fertile—no progeny can be produced. Mortality was determined by absence of movement when an individual was probed with a needle. After irradiation the irradiated samples and the control (non-irradiated) were returned to the laboratory and held under standard rearing conditions for observation on the mortality rate, the survival rate, and adult female reproduction. They were held until all individuals had died or reached the adult stage.

To assess the effect of irradiation on reproduction of adult mealybugs, 30 females (3–6 d old) were kept on the surfaces of the hosts and irradiated at the each target dose with 3 replicates. The observations of fertility and survivability of young nymphs produced from irradiated females were recorded every 2 days until they had died.

QUALITY ANALYSES OF IRRADIATED DRAGON FRUIT

After harvest, dragon fruit were cleaned, dried, and packed in perforated polyethylene bags and placed inside cardboard boxes. Dragon fruits were irradiated at target doses of 200, 400, 600, and 800 Gy with 3 replicates for each treatment. Then the irradiated fruits were stored at 5 ± 1 °C and 90 ± 5% RH for 3 wk to determine firmness, vitamin C content, total soluble solids (TSS), titratable acidity (TA), and the color of the fruit peel at defined time intervals. Non-irradiated fruits were handled in the same manner as the irradiated fruits. Flesh firmness (N) was measured by a penetrometer (FT327, Effegi, Italy) with a force gauge having a 6-mm diam flathead probe. Measurements were taken at 3 locations on the surface of fruit by the peeling back a flap of the exocarp. Vitamin C content (mg/100g) was determined by titration against 2,6-dichlorophenol indophenol (AOAC 967.21). TSS was measured with a portable refractometer (TIRBX32, Trans Instruments Pte Ltd., Singapore). Fruit acidity (citric acid equivalents by titration) of juice expressed from composite fruit samples was determined by following a titrimetric method (AOAC 942.15). Using the scale of the Commission Internationale de l'Eclairage, i.e., CIELAB scale, peel color measurements of lightness (L*), red (a*), and yellow (b*) were taken at 3 positions around the equator using a chroma meter (Model CR 200, Konica Minolta, Japan).

DATA ANALYSIS

Data were analyzed using SAS (2002). Mortality data were arcsine transformed before analysis. Means were separated at the 5% level of probability by the *t*- test. Survival percentages of treated nymphs to female adults and reproduction data were subjected to ANOVA and regression analyses. For quality assessment of irradiated dragon fruit, analysis of variance was carried out using the software Statgraphic Centurion, version XVI (2005). The mean values were considered significantly different when the P value was < 0.05.

Results

LIFE CYCLE OF THE MEALYBUG SPECIES

The developmental stages and life cycle of each mealybug species are presented in Table 1. Young nymphs pass through 2 molts with 3 instars to become adults. Life cycles were 30.5 ± 0.3 and 31.6 ± 0.60 d for *D. neobrevipes* and *P. lilacinus,* respectively. Development of *P. minor* proceeded through 5 stages, i.e., egg, 3 larval instars, and the adult, with a total developmental time of 36.07 ± 0.58 d.

DOSIMETRY

During the research, the dose uniformity ratio (DUR) was consistently < 1.2 for all applied doses. Repeated readings were taken at each position with differences less than 0.5%. The ranges of absorbed dose measurements are shown in Table 2.

MORTALITY RATES OF IRRADIATED MEALYBUG DEVELOPMENTAL STAGES

The effect of radiation at 0, 100, 200, 300, and 400 Gy on the mortality rates of *D. neobrevipes, P. minor,* and *P. lilacinus* by 17 d after treatment are presented in Figs. 1, 2, and 3, respectively. The results show that mortality of all stages and the radiation dose had significant interactions (F = 4.66; P = 0.0001 for *D. neobrevipes*, F = 13.94; P < 0.0001 for *P. minor* and F = 5.5; P < 0.0001 for *P. lilacinus*). Radiotolerance was directly related to stage of development. The highest dose (400 Gy) in these experiments was insufficient to cause high levels of acute mortality of the 3 mealybug species even during a period of > 2 wk—the time required for shipping commodities by sea to various destinations.

EFFECT OF IRRADIATION ON DEVELOPMENT OF NYMPHS TO ADULTS

Data on the percentages of nymphs of *D. neobrevipes, P. minor,* and *P. lilacinus* that developed to the adult stage after irradiation are presented in Table 3. The results indicate that the survival rate of nymphs until the adult stage decreased with increasing radiation doses. All doses affected the survival of nymphs, but 1st and 2nd instars were much more sensitive to radiation than 3rd instars. Among these 3 mealybug species, *D. neobrevipes* proved to be more radiotolerant than the 2 other species. Indeed only in the case of *D. neobrevipes* did some 2nd and 3rd instars when treated with 300 or 400 Gy survive and develop to the adult stage.

Table 1. Life cycle parameters of the 3 mealybug species that infest dragon fruit in Viet Nam. The observations were conducted on cultures reared in a laboratory under constant conditions.

	Developmental duration (Mean ± SE) (days)		
Developmental stage	*D. neobrevipes*	*P. lilacinus*	*P. minor*
Eggs	—	—	3.27 ± 0.69
1st instars	6.6 ± 0.2	6.30 ± 0.30	6.03 ± 0.76
2nd instars	7.9 ± 0.3	9.20 ± 0.36	7.07 ± 0.78
3rd instars	6.3 ± 0.2	9.55 ± 0.32	7.80 ± 0.71
Total nymphal stages	20.9 ± 0.3	25.05 ± 0.20	20.90 ± 1.03
Pre-oviposition adult ♀	9.6 ± 0.2	6.60 ± 0.20	11.90 ± 0.66
Total life cycle	30.5 ± 0.3	31.65 ± 0.60	36.07 ± 0.58

Table 2. Dosimetry* of target doses explored as possible phytosanitary treatments of the 3 mealybug species that infest dragon fruit in Viet Nam, i.e., *Dysmicoccus neobrevipes*, *Planococcus lilacinus*, and *Planococcus minor.*

Target dose (Gy)	Range of measured dose (Gy)	Measured dose (Mean ±SE) (Gy)	DUR
100	103–119	113.7 ± 9.2	1.15
150	151–159	153.7 ± 4.6	1.05
200	196–218	210.3 ± 12.4	1.11
250	246–257	252.0 ± 5.6	1.03
300	296–326	312.0 ± 15.1	1.10
400	376–423	405.0 ± 25.4	1.12
600	590–649	620.3 ± 7.6	1.10
800	792–840	816.0 ± 15.2	1.06

*Irradiation was conducted in a gamma Co-60 irradiator with the dose rate of 8.3 Gy/min. Cardboard boxes (30 cm × 22 cm × 13 cm) containing samples were placed on a stainless steel table, and host surfaces with insects were positioned to face the Co-60 source. The absorbed dose was measured by Fricke dosimeters attached to surfaces of the host plant part (pumpkin fruits or potato tubers).

REPRODUCTION OF IRRADIATED ADULT MEALYBUGS

The fate of the progeny of adult females that developed either from γ-irradiated nymphs or that were the F_1 progeny of irradiated adult females are recorded in Table 4. Efficacy was measured by the prevention of hatching of F_1 generation eggs, development of 1st instars to 2nd instars, and reproduction of adults. The reproductive ability of the mealybugs decreased with increasing radiation dose, but the severity of the impairment depended on the species and the developmental stage being irradiated. First and 2nd instars of *P. lilacinus* and *P. minor* were killed when irradiated with 100 Gy. The reproduction of irradiated 3rd instars and of adult females of *P. lilacinus*—as measured by failure of their eggs to hatch—was prevented at 100 Gy. In the cases of *P. minor* adult females and 3rd instars irradiated with 100 Gy, the adults and the 3rd instars—that survived to become adults—laid fertile eggs. Also all stages of *D. neobrevipes* irradiated with 100 Gy produced some normal nymphs. Thus the data clearly prove that *D. neobrevipes* was the most radiotolerant of the 3 species.

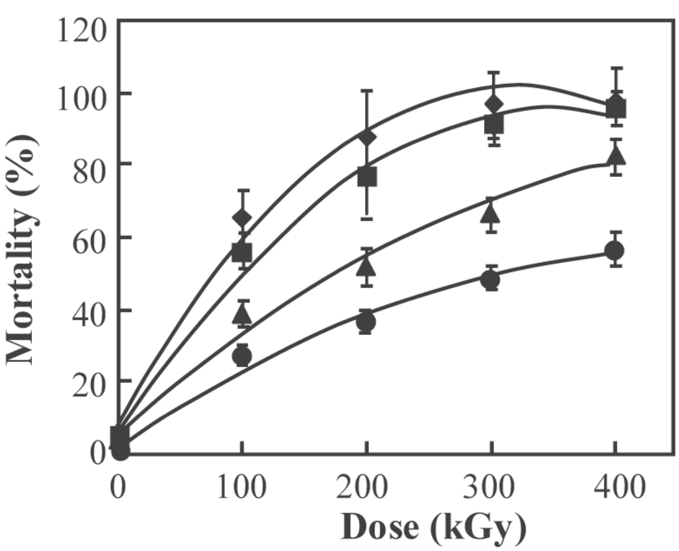

Fig. 1. Trends in percent mortality of each of the life stages of *Dysmicoccus neobrevipes* with increasing doses of irradiation at 17 d after irradiation.

♦ **1st instars;** ■ **2nd instars;** ▲ **3rd instars;** ● **Adults**

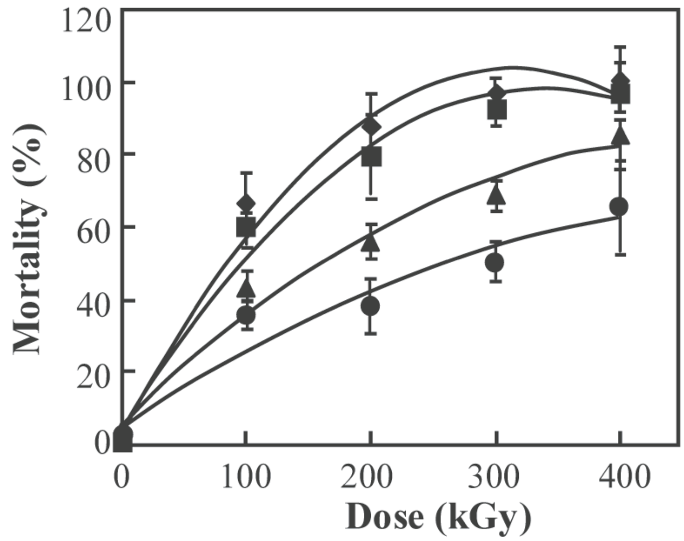

Fig. 2. Trends in percent mortality of each of the life stages of *Planococcus minor* with increasing doses of irradiation at 17 d after irradiation.

♦ **1st instars;** ■ **2nd instars;** ▲ **3rd instars;** ● **Adults**

Unlike the viviparous species, *D. neobrevipes* and *P. lilacinus*, *P. minor* lays eggs. Thus, it was necessary to investigate the effects of the irradiation of *P. minor* eggs on hatch rate, development of nymphs and adults from irradiated eggs, and fecundity or fertility of the resulting adults. Table 5 shows that *P. minor* eggs treated with ≥ 150 Gy could emerge and develop to adults, but they were infertile, i.e., not eggs hatched.

The effect of radiation on *P. minor* immatures was assessed in the dose range 100–250 Gy (Table 6). No 1st and 2nd instars survived to the adult stage at 100 Gy. Survival of 3rd instars decreased with increasing dose, and none survived at 250 Gy. At 150 and 200 Gy, 15 and 4%, respectively, of the 3rd instars molted into adult females, which were completely sterile, i.e., no eggs hatched.

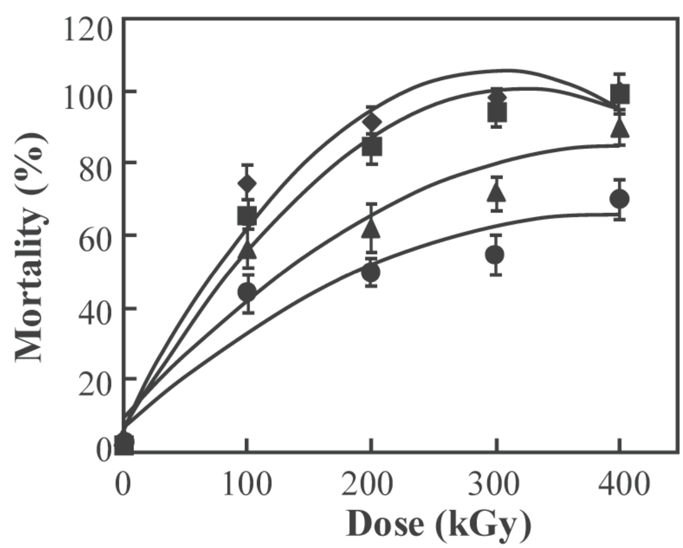

Fig. 3. Trends in percent mortality of each of the life stages of *Planococcus lilacinus* with increasing doses of irradiation at 17 d after irradiation.

♦ **1st instars;** ■ **2nd instars;** ▲ **3rd instars;** ● **Adults**

Table 3. Percentages of individuals of each of the 3 mealybug species that developed to the adult stage after having been irradiated either as 1st, 2nd or 3rd instars at doses ranging from 0 to 400 Gy. One hundred individuals of each life stage were irradiated with each dose and observed until all that failed to reach the adult stage had died.

		Percent adult development ± SE at dose (Gy)				
Species	Instar irradiated	0	100	200	300	400
D. neobrevipes	1st	94.67 ± 4.93a	15.33 ± 5.13b	1.33 ± 0.58c	0c	0c
	2nd	95.67 ± 1.10a	24.00 ± 3.61b	3.00 ± 1.00c	2.33 ± 0.58c	1.33 ± 0.58c
	3rd	96.33 ± 1.53a	27.67 ± 7.55b	9.67 ± 4.73c	7.67 ± 1.15c	6.00 ± 2.00c
P. minor	1st	96.67 ± 0.58a	0b	0b	0b	0b
	2nd	97.00 ± 1.00a	0b	0b	0b	0b
	3rd	97.00 ± 1.73a	20.53 ± 1.53b	5.67 ± 2.52c	0d	0d
P. lilacinus	1st	93.67 ± 1.15a	0b	0b	0b	0b
	2nd	95.00 ± 1.00a	0b	0b	0b	0b
	3rd	95.33 ± 0.58a	11.33 ± 6.03b	3.00 ± 1.10c	0d	0d

Note: Means within a row followed by the same letters are not significantly different ($P < 0.05$, t-test).

CONFIRMATORY TESTING OF ADULT *DYSMICOCCUS NEOBREVIPES*

In comparison of reproduction among the 3 mealybug species after irradiation, *D. neobrevipes* was the most radiotolerant. Large scale confirmatory tests of adult *D. neobrevipes* were performed to verify the minimum dose for preventing F_1 generation 2nd instars. Thus, 31,750 of 3–4 d-old adult females were irradiated at measured doses of 206–231 Gy. All female adults laid ovisacs from which some F_1 generation crawlers emerged that were very weak and that died without transforming into 2nd instars. The level of efficacy was 99.99% at the confidence level of 95% for the dose of 231 Gy applied to adult females; a control of non-irradiated adult females exhibited normal fertility.

QUALITY ASSESSMENT OF IRRADIATED DRAGON FRUIT

Effects of radiation at 0 (non-irradiated control), 200, 400, 600, and 800 Gy followed by 17 d of storage are presented in the Table 7. Firmness values did not differ significantly between treatments in the range, 0–600 Gy. TA (titratable acidity) values did not differ significantly for 0, 200 and 400 Gy and TA at 400 Gy was not significantly less than at 600 Gy, and the latter value was not significantly less than at 800 Gy, but TA at 800 Gy was significantly greater than at 400 Gy or smaller doses. Vitamin C content was significantly greater in the untreated control than in all other treatments, and the content at 200 Gy was significantly greater than at 400 Gy; which was significantly greater than at both higher doses. Vitamin C contents at 200 and 400 Gy were 72 and 65%, respectively as great as in the control. The increasing values of L* suggest that the shade of the fruit became progressively darker as the dose increased. However, the fruit was not significantly darker in the 200 Gy treatment than in the control, but it was slightly but significantly darker in the 400 Gy treatment than in the control. Fruit in the 400 Gy treatment was significantly lighter than in the 600 Gy treatment, which was significantly lighter than in the 800 Gy treatment. However, irradiation did not have a significant effect on the total soluble solids (TSS), nor on a* (redness) and b* (yellowness) color parameters of dragon fruit. At 600 and 800 Gy bract wilting and browning and peel injury was observed. This irradiation scald appeared as diffuse darkening of the peel surfaces, which was easily recognized by the unaided eye.

Discussion

Ionizing radiation is a promising phytosanitary treatment that is increasing in use worldwide. Many tropical fruits, when subjected to phytosanitary irradiation, are good candidates for export to lucrative markets. Research has already been done to establish the feasibility of irradiation by determining the minimum dose needed for these fruits

Table 4. Status or fate of the progeny of adult females that developed either from γ-irradiated nymphs or that were the F_1 progeny of irradiated adult females.

		Radiation dose (Gy)			
Mealybug	Irradiated stage	0	100	200	300-400
D. neobrevipes	1st instar	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	1st instars killed
	2nd instar	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀
	3rd instar	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀
	Adult ♀	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀
Pl. minor	Egg	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀
	1st instar	Fertile ♀♀	1st instars killed	1st instars killed	1st instars killed
	2nd instar	Fertile ♀♀	2nd instars killed	2nd instars killed	2nd instars killed
	3rd instar	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	3rd instars killed
	Adult ♀	Fertile ♀♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀
Pl. lilacinus	1st instar	Fertile ♀♀	1st instars killed	1st instars killed	1st instars killed
	2nd instar	Fertile ♀♀	2nd instars killed	2nd instars killed	2nd instars killed
	3rd instar	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀	3rd instars killed
	Adult ♀	Fertile ♀♀	Sterile ♀♀	Sterile ♀♀	Sterile ♀♀

Table 5. Effects of various doses of γ-irradiation of *Planococcus minor* eggs on the efficacy parameters: hatch rate, percent development of nymphs and adults from irradiated eggs and percent of these adults that laid eggs (*n* = 15 ovisacs/replicate, 3 replicates).

Dose (Gy) applied to eggs	Hatch rate (%)	Eggs that survived to adults (%)	Adult reproduction rate (%)	Number of oviposited eggs per female	Percent hatch of F_1 eggs
0	94.30 ± 2.73a	88.17 ± 3.20a	95.21 ± 6.77a	146.0 ± 10.61a	74.14 ± 0.10a
100	3.23 ± 2.76b	87.50 ± 17.68a	56.30 ± 8.84b	53.00 ± 7.78b	37.23 ± 7.30b
150	0.30 ± 2.76b	50.00 ± 10.71b	50.00 ± 20.71c	21.00 ± 8.99bc	0c
200	0b	0c	0c	0c	0c
250	0b	0c	0c	0c	0c

Means within a column followed by the same letters are not significantly different (*P* < 0.05, t- test).

and the tolerance of the fruit to radiation (Bustos-Griffin et al. 2012). Ionizing radiation does not cause significant acute mortality at the doses used for PI, but renders pests incapable of completing development and/or reproducing (Hallman 2012).

All of the results showed that diminished survival of irradiated insects was directly related to the impairment of development, as was previously observed in studies on the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Jacobsen & Hara 2003), on *Planococcus minor* (Ravuiwasa et al. 2009), and on the solenopsis mealybug, *Phenacoccus solenopsis* (Huang et al. 2014). These trends are also consistent with the conclusion of a general study on insect radio-tolerance (Hallman et al. 2010).

Dysmicoccus neobrevipes was found to be the most radio-tolerant of the 3 species as measured by prevention of the development of the F_1 generation progeny of irradiated adults to reach the 2nd instar. 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 would be higher than most fresh commodities could 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 (Hallman et al. 2013).

In our study the predicted dose causing 100% acute mortality for the most radio-tolerant species *D. neobrevipes* was ~700 Gy. If this dose were prescribed it might harm the quality of dragon fruit. Even at the currently minimum required dose of 400 Gy dragon fruit might be exposed to damagingly high doses given that the DUR may be in the range of 1.5–2.0 fold in commercial irradiation. Fruit firmness, vitamin C content, acidity, and peel color of treated dragon fruit could be changed significantly due to irradiation at 600–800 Gy followed by 17 d of storage. In a previous research Wall & Khan (2008) indicated that minimum softening occurred in outer flesh layers of dragon fruit treated at 400 or 600 Gy after 12 d storage at 10 ºC. During storage, softening of fruit tissue or a decrease in color intensity following irradiation at 1,000 Gy or less has been observed in other non-climacteric fruits such as strawberry, blueberry, cherry, orange, and rambutan (Yu et al. 1995; Boylston et. 2002; Drake & Neven 1997).

Ravuiwasa et al. (2009) report that 150 Gy prevented the hatch of F_1 generation eggs of irradiated adult *P. minor*. Jacobsen & Hara (2003) found that the minimum dose needed to ensure quarantine security against *M. hirsutus* is 100–250 Gy. In our study F_1 generation egg hatch of *P. minor* was prevented at 150 Gy. At the same dose, some 3rd instars could survive to adults, but they could not reproduce, and only few of the irradiated female adults that developed from irradiated nymphs were able to oviposit. The result was similar to studies on *P. minor* conducted by Ravuiwasa et al. (2009) who reported that eggs did not hatch at ≥ 150 Gy. Huang et al. (2014) also reported that 150 Gy could be an effective sterilizing dose for both *P. solenopsis* nymphs and adults. Results of the effects of radiation on *D. neobrevipes* were reported by Doan et al. (2012). At 200 and 250 Gy adults laid cottony sacs with eggs instead producing 1st instars. Some nymphs emerged from these egg sacs but did not develop to the 2nd instar. In summary, doses of ~200 Gy prevented the reproduction of all 3 mealybug species. Therefore, it was concluded that a dose < 400 Gy could be used to control these aforementioned mealybugs infesting dragon fruit.

Confirmatory testing requires that large numbers (up to 30,000 or possibly more) of the most radio-tolerant stage be irradiated at a dose that achieves the endpoint without failure. Based on the results obtained in our preliminary test, the target dose of 200 Gy was used as an effective quarantine dose for *D. neobrevipes*—which we had determined to be the most radio-tolerant species—in large-scale confirmatory testing. An estimated 31,750 individuals of female adults were irradiated with doses ranging from 206–231 Gy. The results showed that 100% of the F_1 generation 1st instars died before reaching the 2nd instar. The level of efficacy achieved was 99.99% at the 95% confidence level.

Normally the maximum dose recorded in large-scale confirmatory testing becomes the minimum dose for commercial application (Hallman et al. 2010, 2013); hence, 231 Gy would be recommended for

Table 7. Quality assessment of dragon fruit γ-irradiated with various doses followed by 17 d in storage at 5 °C and 90 ± 5% RH.

Dose (Gy)	Firmness (N)	TSS (%)	TA (%)	Vitamin C (mg/100g)	L*	a*	b*
0 (control)	11.32a	12.33a	0.09a	6.36a	44.12a	25.52a	15.03a
200	11.08a	12.03a	0.10a	4.57b	42.09ab	26.41a	11.56ab
400	10.94a	12.00a	0.10ab	4.13c	40.49b	29.84a	10.90ab
600	10.51a	12.00a	0.11bc	3.45d	36.80c	28.35a	10.00ab
800	9.64b	11.97a	0.12c	3.39d	33.96d	27.87a	8.28b
P	0.031	0.8904	0.0032	0.0001	0.0001	0.8827	0.2221
F	4.15	0.27	8.29	87.19	25.24	0.28	1.72

Within a column, means with different letters indicate significantly different values at *P* < 0.05.

Abbreviations: TSS, total soluble solids and TA, titratable acidity. Also lightness (L*) is a measure of the lightness of the color of the fruit's exocarp; L* values range from black = 0 to white = 100. The parameter, a*, pertains to its redness (red ± green), and the smaller the number, the redder is the fruit. The parameter, b*, pertains to the fruit's yellowness (yellow ± blue), and the larger the number, the more yellow is the fruit; for comparison grapefruit has a value of about 25 (McGuire 1992).

Table 6. Percentage of *Planococcus minor* nymphs that survived to the adult stage after the γ-irradiation of 1st, 2nd or 3rd instars (2–4-d-old) with doses between 100–400 Gy.

Target dose (Gy)	Percent survival to adult stage (mean ± SE) of irradiated instar		
	1st instars	2nd instars	3rd instars
0	96.67 ± 0.58a	97.00 ± 1.00a	97.00 ± 1.73a
100	0b	0b	18.00 ± 1.36b
150	0b	0b	14.67 ± 5.51c
200	0b	0b	3.67 ± 2.16c
250	0b	0b	0d

Means within a column followed by the same letters are not significantly different ($P < 0.05$, t- test).

quarantine security of mealybugs infesting dragon fruit. A proposal of a treatment dose at 231 Gy against these mealybugs was submitted to the International Plant Protection Convention by the Plant Protection Department, Ministry of Agriculture and Rural Development, Viet Nam and was approved (IPPC 2015). Lowering the dose for dragon fruit from 400 Gy to 231 Gy would minimize any deleterious effects on fruit quality. This conclusion is in accordance with Follett & Weinert (2012) that lowering of the dose for the commodity of interest, the cost of treatment will be reduced, any quality problems will be minimized, and the capacity of the treatment facility may be increased due to a shorter treatment time. It was also reported by Hallman (2012) that the 400 Gy dose is probably excessive for almost all insect pest species other than lepidopteran pupae and adults and a reduction in the dose should reduce the risk of damage to fresh commodities as well as reduce cost and time of treatment.

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