Radiation Physics and Chemistry 81 (2012) 97–100

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Effects of gamma irradiation on different stages of mealybug *Dysmicoccus neobrevipes* (Hemiptera: Pseudococcidae)

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ARTICLE INFO

Article history:
Received 13 July 2011
Accepted 26 September 2011
Available online 1 October 2011

Keywords:
Phytosanitary irradiation
Mealybug
Tolerant stage
Sterility

ABSTRACT

Utilization of phytosanitary irradiation as a potential treatment to disinfest agricultural commodities in trade has expanded rapidly in the recent years. Cobalt-60 gamma ray target doses of 100, 150, 200 and 250 Gy were used to irradiate immatures and adults of *Dysmicoccus neobrevipes* (Beardsley) (Hemiptera: Pseudococcidae) infesting dragon fruits to find the most tolerant stage and the most optimal dose range for quarantine treatment. In general, irradiation affected significantly all life stages of *D. neobrevipes* mortality and adult reproduction. The pattern of tolerance to irradiation in *D. neobrevipes* was 1st instars < 2nd instars < 3rd instars < adults, in which the adult is the most tolerant stage. Based on obtained results after 21 days of irradiation, predicted doses for 100% mortality of each different development stage in the above mentioned pattern were 224.6, 241.3, 330.9 and 581.5 Gy, respectively. No survived female adult produced offspring at 200 and 250 Gy. Dose range between 200 and 250 Gy could be efficient to prevent the reproduction of this mealybug.

1. Introduction

Dysmicoccus neobrevipes (Beardsley), *Planococcus lilacinus* (Cockerell) and *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) are pests found on red dragon fruits (*Hylocereus spp.*) in Vietnam (APHIS, 2008b). The gray pineapple mealybug *Dysmicoccus neobrevipes* was described from specimens collected in Hawaii and determined that this is one of the most economically important mealybug pests in Hawaii (Beardsley, 1959). This pest attacks a wide range of plants, including agricultural, horticultural and forest species (Ben-Dov et al., 2006). *D. neobrevipes* goes through three nymphal stages before becoming an adult. The life span (first instar to death as an adult) varies from 59 to 117 days. This species does not lay eggs. Instead they are ovoviviparous, meaning the eggs hatch within the female. Thus, births live young (nymphs). First instars, called "crawlers" are the primary dispersal stage in all mealybug species. Female nymphs molt three times before reaching adult maturity (Kessing and Mau, 1992). Feeding on young growth 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. Also, honeydew deposited on leaves and fruit by mealybugs serves as a medium for growth of black sooty molds, which interfere with photosynthesis and reduce the crop's market value (Jahn, 1993). Since 2008, Vietnam dragon fruit has been exported to the United States using the generic radiation dose of 400 Gy because that dose is currently required if mealybugs are (APHIS, 2008b and Hallman et al., 2010). The generic radiation doses can be lowered for specific pests and commodities if this is practical. If lowering of the dose for the commodity of interest, cost of treatment will be reduced and the capacity of the treatment facility may be increased owing to shorter treatment time. For example, Follett (2009) reported that a reduction in dose for Hawaiian sweet potato from 400 to 150 Gy resulted in a 60% reduction in cost of treatment.

Research data of quarantine irradiation on mealybugs are limited. Dohino and Masaki (1995) found that 200 Gy did not completely prevent hatch of eggs of *Pseudococcus comstocki* (Kuwana) laid by irradiated adults. Some of the hatchlings developed to the adult stage, but did not reproduce. Studies on the radiation tolerance of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) found that at 100 Gy 1.2% of eggs laid by irradiated adults hatched, while non hatched at the next highest does, 250 Gy (Jacobsen and Hara, 2003). Another study showed that while 100 Gy to adult *Planococcus minor* resulted in 49% egg hatch, none hatched at > 150 Gy (Ravuiwasa et al., 2009).

In order to reduce the treatment dose of 400 Gy and to provide more information on a generic dose, the effect of radiation on

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doi:10.1016/j.radphyschem.2011.09.014

different stages and the most tolerant stage of above mentioned mealybugs infesting on dragon fruits is needed to be researched. In this work, we study effects of radiation on different stages and reproduction of adult *D. neobrevipes* to determine the optimum dose lower than of 400 Gy for quarantine treatment.

2. Materials and methods

2.1. Insect rearing

Dysmicoccus neobrevipes was collected from the field on dragon fruit in Binh Thuan province (Vietnam) and reared in the laboratory on the surface of pumpkin (*Cucurbita moschata*) at 28 ± 2 °C, RH $70 \pm 5\%$. The pumpkin was used as a host for *D. neobrevipes* because the shelf life of dragon fruit is rather short (approximately 4–5 days). Development stages and the life cycle of *D. neobrevipes* were determined. Under laboratory conditions, the total life cycle of this mealybug is 30.5 ± 0.3 days. Development stages are identified with four stages including nymphs (1st, 2nd and 3rd instars) and adults. In which, approximate times for the total nymph stage and preoviposition female adult are 20.9 ± 0.3 and 9.6 ± 0.2 days, respectively.

Based on this observation, even-age individuals of each stage were collected for irradiation experiments as shown in Fig. 1. The removal of individual insects was done using soft paint brushes to avoid their injury.

2.2. Irradiation treatment

Irradiation treatment was conducted on 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, Vietnam. The dose uniformity ratio (DUR) during research was ~1.1. Cardboard boxes with the size of $30 \times 22 \times 13$ cm^3 containing samples were placed in irradiation positions. The pumpkin surfaces with mealybugs on them were placed facing the Co-60 source. The absorbed dose was measured using Fricke dosimeters attached on surfaces of host (ASTM E 1026-04, 2004).

2.3. Experimental design

To investigate the effect of irradiation to different development stages of species *D. neobrevipes*, nymphs with 1st, 2nd and 3rd instars (2–3 days old for each) and adults (3–4 days old) were irradiated at the range target dose of 0 (as a control), 100, 150, 200 and 250 Gy with triple replications. 100 individuals for each dose and for each determined life stage were tested and monitored after treatment. The non-irradiated control was moved to nearby the facility to have the same condition with the irradiated samples. Because *D. neobrevipes* reproduces asexually, female adult is the focus for all tests. Mortality was determined by the absence of movement when probed with a needle. After irradiation, the irradiated samples and control were returned to the lab and held under standard rearing condition immediately. After treatment, the insects were held during 1–2 month for observation on mortality rate, survival rate of nymphs and female adult reproduction. In addition, irradiated insects were observed until they died at different dosages.

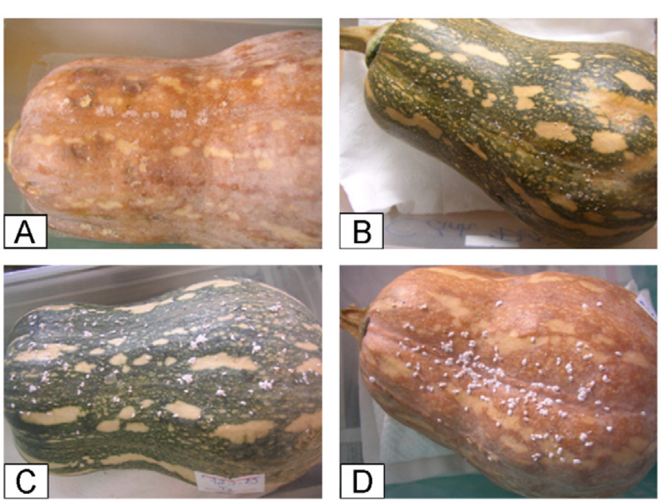

Fig. 1. Different development stages of *D. neobrevipes* mealybug on pumpkin surfaces (A: 1st instars; B: 2nd instars; C: 3rd instars and D: adults).

2.4. Data analysis

Experimental data were analyzed using SAS (SAS Institute, 2002). Mortality data were arcsine transformed before analysis. Means were separated at the 5% level of probability by the Student's test. Percentage of treated nymphs that developed to female adults and reproduction data were subjected to linear regression.

3. Results and discussion

3.1. Effect of gamma irradiation on mortality of nymphs and adults

The mortality of *D. neobrevipes* was determined by day 21 after irradiation treatment at each stage of life cycle. The obtained results in Table 1 indicated that mortality of all stages and irradiation dose had significant interaction (F=4.86, P=0.0002) after treatment. The natural mortality in non-irradiated treatment fluctuated from 0.67 to 5.0% depending on their development stages. It could be concluded that all of treatment doses affected on the survival ability at different development stages of *D. neobrevipes*. The mortality rate increases with the increasing dose. However, the results showed that irradiation doses from 100 to 250 Gy did not cause 100% mortality in all irradiated life stages by 21 days. Immature stages are more sensitive to irradiation than the adult one. The pattern of tolerance to irradiation in *D. neobrevipes* mealybug was 1st instar < 2nd instar < 3rd instar < adult. Linear regression on the data shown in Table 1 was used to predict a radiation dose needed to cause 100% mortality of *D. neobrevipes* in all stages of their life. The adults were determined the most radio-tolerance so the required highest radiation dose was calculated up to 581.5 Gy, whereas the first instars were highly susceptible to irradiation with predicted lower dose of 224.2 Gy. Tolerance of irradiation by *Maconellicoccus hirsutus* (Green) (the pink hibiscus mealybug) tended to increase as maturity increased, and adults were the most radiotolerant stage of *M. hirsutus* (Jacobsen and Hara, 2003). According to Hallman et al. (2010), studies have generally found insect radio-tolerance increases with the age and maturity.

3.2. Effect of gamma irradiation on development of nymphs to adults

After treatment, percentage of nymphs survived to adults of *D. neobrevipes* showed in Table 2. The survival rate of nymphs decreased with the increasing absorbed dose. There was a significant difference in survival from the dose of 0–250 Gy at stages of 1st, 2nd and 3rd instars ($P < 0.05$). Linear regression on the data shown in Table 2 was used to predict a radiation dose needed to cause 100% mortality of *D. neobrevipes* by 35 days after

Table 1
Mortality (%) of *D. neobrevipes* mealybug at different development stages after 21 days of irradiation.

Development stage	Dose (Gy)					Slope ± SE	Predicted dose for 100% mortality (Gy)
	0	100	150	200	250		
1st instars	5.00a	69.33a	85.33a	90.67a	96.33a	0.36 ± 0.16	224.2
2nd instars	4.33a	64.00a	75.67b	84.67a	93.67a	0.35 ± 0.13	241.3
3rd instars	2.33b	42.67b	49.67c	62.67b	74.33b	0.28 ± 0.06	330.9
Adults	0.67c	29.67c	35.00d	39.33c	41.00c	0.16 ± 0.07	581.5

Means within in each column followed by the same letter are not significantly different ($P < 0.05$).

Table 2
Percentage of nymphs survived to adults of *D. neobrevipes* after irradiation (after 35 days in observation).

Development stage	Dose (Gy)					Slope ± SE	Predicted dose for 100% mortality (Gy)
	0	100	150	200	250		
1st instars	95.33a	11.33b	6.00c	1.33d	0	0.38 ± 0.24	201.4
2nd instars	95.00a	21.67b	12.00c	3.33d	2.67d	0.37 ± 0.20	213.5
3rd instars	95.67a	27.33b	18.33c	8.33d	8.00d	0.35 ± 0.18	230.3

Mean within each row followed by the same letter are not significantly different ($P < 0.05$).

Table 3
Reproduction of female adult *D. neobrevipes* after irradiation (offsprings/female).

Development stage	Dose (Gy)					Slope ± SE	Predicted dose for completed sterility (Gy)
	0	100	150	200	250		
1st instars	283.67a	138.67b[a]	127.33b[a]	0	0	1.19 ± 0.34	232.5
2nd instars	207.67a	94.00b[a]	76.33c[a]	0	0	0.87 ± 0.23	227.2
3rd instars	217.00a	95.33b[a]	62.33c[a]	0	0	0.91 ± 0.23	222.6
Adult	179.67a	21.67b[a]	12.33c[a]	0	0	0.70 ± 0.45	201.1

Mean within each row followed by the same letter are not significantly different ($P < 0.05$).

[a] Offsprings/survived female adult.

treatment. Accordingly, required dose was also increased as age increased. The surviving insects were very weak and stunted in growth at 250 Gy in our observations. These results are in general agreement with data of mealybug *Planococcus minor* (with the same family – Pseudococcidae) reported by Ravuiwasa et al. (2009). Hallman (2011) stated that the objective of irradiation is not acute mortality but prevention of development or reproduction, as most fresh commodities do not tolerate the 100% acute mortality required doses.

3.3. Effect of gamma irradiation on reproduction of female adult

Table 3 showed the reproduction of female adults that survived from radiation exposure. Surviving females in four stages were delayed in their reproduction and almost all eggs failed to hatch. In non-irradiated control, the average number of eggs oviposited was 220.0 ± 37.1. The number of offspring was decreased by the increasing dose. At the doses of 200 Gy and 250 Gy, no offspring was recorded during observation time in all life stages. In other words, sterilizing dose for *D. neobrevipes* could be 200 Gy as described in Fig. 2. Dohino et al. (1997) reported that the dose up to 200 Gy using electron beams in this case was also needed to achieve complete sterility in female *Pseudococcus comstocki* (Kuwana).

Based on experimental results and regression analysis, it can be concluded that none of mature mealybug *D. neobrevipes* was able to reproduce at doses between 200 and 250 Gy. Female adults developed from 100 Gy irradiated nymphs can reproduce but the time of preoviposition lasted at least 7–10 days compared with non-irradiated one.

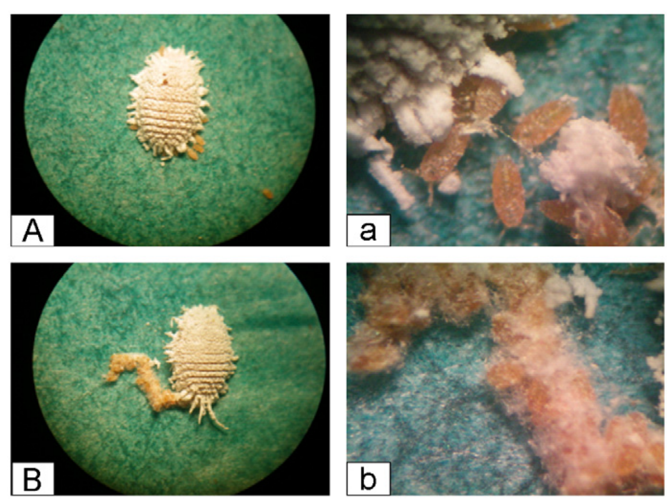

Fig. 2. Reproduction ability of *D. neobrevipes*, (A and a: None irradiated female adult and young nymphs; B and b: 200 Gy irradiated female adult and none hatched eggs).

4. Conclusions

All irradiation doses delayed the development of immature stages of *D. neobrevipes* and caused eventual death in comparison with the control.

Irradiation dosages from 100 to 250 Gy with 50 Gy increment affected on the survival ability of development stages *D. neobrevipes*. Percentage of the mortality increased with the increase of dose.

Immature stages are more sensitive to irradiation than the adult one. The pattern of tolerance to irradiation in *D. neobrevipes* was 1st instars < 2nd instars < 3rd instars < adults.

The survival rate of nymphs decreased with the increasing dose. Adults were not able to reproduce after treatment from dose of 200 Gy. Dose range between 200 and 250 Gy might be efficient to sterilize for *D. neobrevipes* instead of 400 Gy. However, the effect of irradiation on *D. neobrevipes* female adults at the estimated range needs to be carried out on large scale confirmatory tests.

Acknowledgments

We are grateful to Dr. G.J. Hallman (USDA, Agricultural Research Service, Weslaco, TX) for helpful discussions. This research is partly supported by the International Atomic Energy Agency (IAEA) under Research Contract no. 15635.

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