Towards phytosanitary irradiation of *Paracoccus marginatus* (Hemiptera: Pseudococcidae): Ascertaining the radiosensitivities of all life stages

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Abstract

The present investigation was carried out to create a scientific foundation on which to establish a phytosanitary irradiation regimen against the quarantine pest, the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae). All of the ontogenetic stages of *P. marginatus* were irradiated with a range of doses of gamma radiation intended to completely prevent reproduction either by inducing sterility of the adults or by metamorphic arrest of the immatures stages. Effective gamma doses ($ED_{99.9}$) ranged from 26 Gy for the egg—the most sensitive pre-imaginal stage—to 258 Gy for the N_3-female—the most resistant pre-imaginal stage in which induced lethality—caused by metamorphic arrest to prevent adult formation—was used as the measure of efficacy against the various irradiated pre-imaginal stages. Further, an $ED_{99.9}$ dose in the range of 27–62 Gy applied to various immature stages was sufficient to induce reproductive sterility in parent (P) generation adults as a consequence of carry-over effects induced by sub-lethal irradiation of the pre-imaginal immature stages. $ED_{99.9}$ doses applied to adult females for the induction of complete sterility were in the range of 56–165 Gy depending on the age of the irradiated adult females. In addition, sub-sterilizing doses administered to P generation adults were efficacious in inducing either metamorphic arrest or reproductive sterility in their F_1 progeny. The prevention of development of F_1 N_2 nymphs from F_1 N_1 crawlers—another important threshold for efficacy of phytosanitary irradiation—was feasible with the $ED_{99.9}$ dose of 91 Gy administered to the most radio-tolerant stage of P generation, i.e., gravid females. $ED_{99.9}$ values in the range of 35–62 Gy administered to P generation adult females belonging to different age groups were sufficient to completely prevent the formation of F_1 adult females. $ED_{99.9}$ sub-sterilizing gamma radiation doses in the range of 27–37 Gy given to P adult males and females—irradiated in different age groups—were sufficient to induce complete F_1 sterility. Further, irradiation with either 200 Gy or 400 Gy did not degrade the sensory indices of papaya, *Carica papaya* L. (Brassicales: Caricaceae)—a major host of *P. marginatus*—such as color and weight, and vital physico-chemical attributes, thus assuring phytosanitary irradiation as an acceptable method safeguarding this fresh food. The present investigation indicated that gamma radiation in the range of 165–258 Gy applied against *P. marginatus* could be used as an effective and safe phytosanitary irradiation measure, because these doses induce lethality in developing stages, sterility in adults of this pest and do not degrade the quality of papaya fruits as a human food. Since the magnitude of the adult sterilizing dose was less than the dose causing pre-imaginal metamorphic disruption, a gamma dose of 200 Gy might be considered as an effective phytosanitary treatment to enable infestation-free agricultural commerce with respect to this quarantine pest.

Key Words: papaya mealybug; metamorphic inhibition; adult formation; reproductive sterility; quarantine treatment; papaya quality

Resumen

Se realizó la presente investigación para crear una base científica sobre la que se puede establecer un régimen de irradiación fitosanitaria contra la plaga de cuarentena, la cochinilla de la papaya, *Paracoccus marginatus* Williams y Granara de Willink (Hemiptera: Pseudococcidae). Se irradiaron todos los estadios ontogenéticos de *P. marginatus* con un rango de dosis de radiación gamma con el intento de impedir totalmente la reproducción, ya sea mediante la inducción de esterilidad de los adultos o por el arresto metamórfico de los estadios de inmaduros. Las dosis eficaces de gamma ($ED_{99.9}$) variaron de 26 Gy para el huevo—el estadio más sensible pre-imaginal—a 258 Gy para la hembra N_3—el estadio pre-imaginal más resistente en el que la letalidad inducida—causada por el arresto metamórfico para prevenir la formación del adulto—se utilizó como una medida de la eficacia frente a los diversos estadios pre-imaginales irradiados. Además, una dosis $ED_{99.9}$ en el intervalo de 27 a 62 Gy aplicada a los estadios inmaduros fue suficiente para inducir la esterilidad reproductiva en los adultos de la generación pariente (P) como consecuencia de efectos de arrastre inducidos por irradiación subletal de la de los estadios pre- imaginales de inmaduros. Las dosis $ED_{99.9}$ aplicadas a las hembras adultas para la inducción de esterilidad completa fueron en el rango de 56 a 165 Gy dependiendo de la edad de las hembras adultas irradiadas. Además, las dosis de sub-esterilización administradas a los adultos de generación P fueron eficaces en la inducción de la detención metamórfica o la esterilidad reproductiva en su progenie F_1. La prevención del desarrollo de las ninfas F_1 N_2 del primer estadio F_1 N_1—otro umbral importante para la eficacia de la irradiación fitosanitaria—fue factible con la dosis de 91 Gy $ED_{99.9}$ administrada al estadio más radio tolerante de las hembras grávidas de generación P. Los valores $ED_{99.9}$ en el rango de 35 a 62 Gy administrados a las hembras adultas de generación P pertenecientes a diferentes grupos de edad fueron suficientes para evitar completamente la formación de hembras adultas de F_1. Las dosis $ED_{99.9}$ de gamma esterilizantes en el rango de 27 a 37 Gy dado a los adultos machos y hembras P—irradiaron en diferentes grupos de edad—fueron suficientes para inducir la esterilidad completa de la F_1. Además, la irradiación, ya sea con 200 o 400 Gy no degradaba los índices sensoriales de la papaya, *Carica papaya* (Brassicales: Caricaceae)—un hospedero importante de *P.marginatus*,—tales como el color y la pérdida de peso, y los atributos físico-químicas vitales, así asegurando la irradiación fitosanitaria como un

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método aceptable para la salvaguardia de este alimento fresco. La presente investigación indica que la radiación gamma en el rango de 165 a 258 Gy aplicada contra *P. marginatus* podría ser utilizada como una medida eficaz y segura irradiación fitosanitaria, debido a que estas dosis induce letalidad en los estadios de desarrollo, la esterilidad en los adultos de esta plaga y no hacer degradar la calidad de los frutos de papaya como alimento humano. Puesto que la magnitud de la dosis de esterilización de adultos era menos de la dosis que causa la interrupción metamórfico pre-imaginal, una dosis gamma de 200 Gy puede ser considerada como un tratamiento fitosanitario eficaz para permitir el comercio agrícola libre de infestación con respecto a esta plaga de cuarentena.

Palabras Clave: cochinilla harinosa de la papaya; inhibición metamórfica; formación de adultos; esterilidad reproductiva; tratamiento de cuarentena; calidad de la papaya

The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is an exotic sucking pest reported to be a native of Mexico and/or Central America (Williams & Granara de Willink 1992), and its invasions have been recorded in many countries (Matile-Ferrero et al. 2000). The pest is polyphagous in nature having a wide host range and is reported to infest tropical fruits besides papaya including avocado (*Persea americana* Mill.; Laurales: Lauraceae), citrus (*Citrus* spp. Sapindales: Rutaceae), mango (*Mangifera indica* L.; Sapindales: Anacardiaceae), pomegranate (*Punica granatum* L.; Myrtales: Lythraceae), and, sugar apple (*Annona squamosa* L.; Magnoliales: Annonaceae), many field and vegetable crops including bean (various genera in Fabales: Fabiceae), cassava (*Manihot esculenta* Crantz; Malpighiales: Euphorbiaceae), corn (*Zea mays* L.; Poales: Poaceae), cotton (*Gossypium* spp.; Malvales: Malvaceae), eggplant (*Solanum melongena* L.; Solanales: Solanaceae), pepper (*Capiscum* spp.; Solanales: Solanaceae), pea (*Pisum sativum* L.; Fabales: Fabaceae), tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae), and sweet potato (*Ipomoea batatas* (L.) Lam.; Solanales: Solanaceae), and some ornamental crops, various ornamental crops including acacia (*Racosperma* sp.; Fabales: Fabaceae), pogoda flower (*Clerodendrum paniculatum;*Lamiales: Lamiaceae), sea grape (*Coccoloba* sp.; Caryopyllales: Polygonaceae), and hibiscus (*Hibiscus* sp.; Malvales: Malvaceae), and weeds such as Santa Maria feverfew (*Parthenium hysterophorus* L.; Asterales: Asteraceae), and wireweed (*Sida* sp.; Malvales: Malvaceae) (Miller et al. 1999; Miller & Miller 2002). In India, the occurrence of this pest was reported for the first time in Coimbatore area of Tamil Nadu during 2008 on the papaya, *Carica papaya* L. (Brassicales: Caricaceae), (Muniappan et al. 2008), and its infestations were observed on 84 plant species belonging to 35 families including 10 seriously economically important species (Mahalingam et al. 2010; Thangamalar et al. 2010; Tanwar et al. 2010; Selvaraju & Sakthivel 2011). Papaya mealybug has attained a status of major coccid pest in India due to its polyphagous nature coupled with its high reproductive capacity.

Infestation of the mealybug appears as clusters of cotton-like masses with long waxy filaments on the above-ground portion of plants. Immature and adult stages of *P. marginatus* suck the sap of the plant, induce chlorosis, produce honey dew that results in sooty mold, which interferes with photosynthesis and weakens the plant. Therefore, the yield and quality of agricultural commodities are impaired, and quarantines erected against *P. marginatus* impede international trade. Therefore, Follett (2009) urged greater focus on irradiation studies on *P. marginatus* and other of mealybug species to develop a generic dose against this major group of quarantine pests.

Phytosanitary irradiation is an effective procedure for nullifying the capacity of a pest species that infests an exported commodity to become established at a destination where it is not wanted (Hallman 2011; IPPC 2009). Susceptibility to gamma radiation (radiosensitivity) is known to be different between and within the developmental stages of the same species (Cogburn et al. 1973; Burditt et al. 1989; Ozyardimci et al. 2006). As all developmental stages of the insect are usually present postharvest in an agro-commodity, it is important to know the radiosensitivity of each developmental stage of the insect. This information can be used to determine the most radio-tolerant life stage, and the effective irradiation dose for control of the pest (IAEA/IDIDAS 2006).

The present study was conducted to determine the radiosensitivities of all life stages of *P. marginatus* with the aim of formulating an effective phytosanitary irradiation (PI) regimen against this tropical mealybug species. These findings will be crucial in establishing a generic dose against mealybugs. In addition, the nutritional and physico-chemical qualities of irradiated papaya—a major host of *P. marginatus*—were investigated to determine whether phytosanitary irradiation would impair the fruit's nutritional value and/or the organoleptic quality and consumer acceptability.

Materials and Methods

REARING OF THE PAPAYA MEALYBUG

The culture of *P. marginatus* was initiated with ovipositing gravid female adults obtained from Indian Agriculture and Research Institute (IARI), New Delhi. *Paracoccus marginatus* was reared on sprouted potatoes (*Solanum tuberosum* L.; Solanales: Solanaceae) each in a culturing jar filled up to 1/4th capacity with moist sterilized soil. Since *P. marginatus* is a sexually reproducing species, about 4–5 pairs of adults were fed on the sprouted potato in a culture jar. The mated females started egg laying within 6–7 d. Each female oviposited a cluster of 200–300 eggs, which hatched within 7–8 d. An egg destined to become a female adult develops through 3 successive nymphal instars to reach adulthood, while an egg destined to become a male adult develops through 3 successive nymphal instars, a pupa—but which we designated as N_4-♂—to reach adulthood (Fig. 1). After each nymphal molt, the mealybugs were transferred to the fresh culture jars that had been replenished with the fresh food.

DOSIMETRY AND IRRADIATION OF THE VARIOUS LIFE STAGES OF *P. MARGINATUS*

Irradiation of each of the various ontogenetic stages of *P. marginatus* was conducted with the dose rate of 1.19–1.48 kGy/h at the Radiobiological Unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defence, Delhi using the ^{60}Co source, positioned in the Gamma cell-5000 (Gamma –5000 irradiator, BRIT, BARC, Trombay, Mumbai, Maharashtra, India). Doses in the range of 5–500 Gy were applied to test the radio-biological responses of each immature stage as well as different genders of adults of this mealybug species. Fricke dosimetric measurements were made to establish the dose distribution. Fricke dosimetry was performed on the gamma cell to establish the absorbed dose values and the absorbed dose distribution. The results of the Fricke dosimetry of this gamma cell were confirmed via thermocouple chips provided by the International Atomic Energy Agency (IAEA) to authenticate the validity of gamma dose ad-

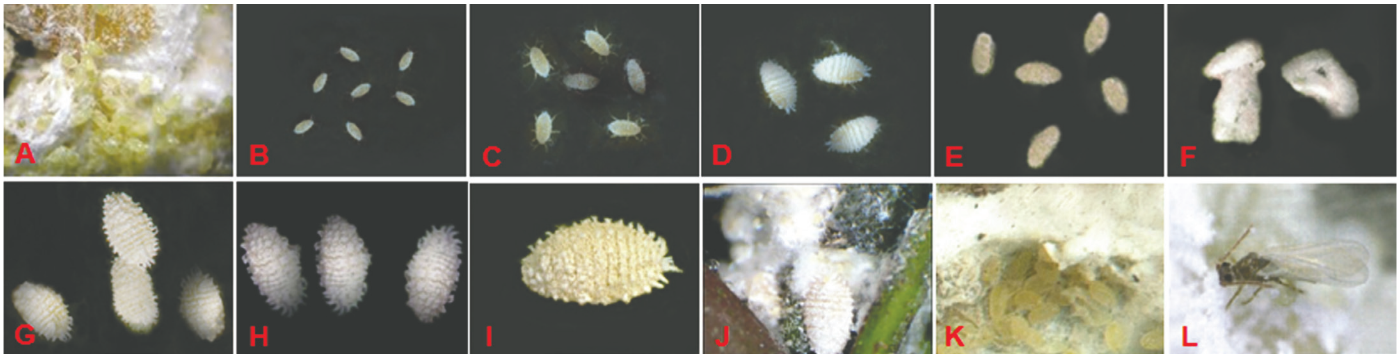

Fig. 1. Life stages of *Paracoccus marginatus:* A. Eggs; B. Crawlers or first instar nymphs (N_1); C. Second instar nymphs (N_2); D. Third instar female nymphs (N_3-♀); E. Third instar male nymphs (N_3-♂); F. Fourth instar male nymph (N_4-♂); G. Freshly formed female adults 0–1 d-old; H. Pre-gravid female adults 3–4 d-old; I. Gravid female adult 6–7 d-old; J. Gravid female along with ovisac; K. Crawlers emerging from ovisac; and L. Male adult.

ministered at given dose rate. After the samples were irradiated, the samples and the control were immediately transferred to plastic jars containing fresh food.

EFFICACY OF IRRADIATION OF PRE-IMAGINAL STAGES IN BLOCKING METAMORPHOSIS

The bio-efficacy of gamma irradiation was ascertained on the metamorphosis of pre-imaginal stages of *Paracoccus marginatus* irradiated as eggs, crawlers (N_1), second instar nymphs (N_2), third instar female nymphs (N_3-♀), third instar male nymphs (N_3-♂), and fourth instar male nymphs (N_4-♂), respectively, with doses in the 5–300 Gy range and a non- irradiated control (0 Gy) as a reference. About 50 eggs, a group of 50 N_1 as crawlers, and a cohort of 12-15 nymphs of each of the remaining immature stages constituted 1 replicate. Biological observations on each replicate of each stage following irradiation were done daily with respect to metamorphosis including adult formation.

EFFICACY OF IRRADIATION OF PRE-IMAGINAL STAGES IN NULLIFYING FECUNDITY AND/OR FERTILITY OF THE ADULTS

The sterilizing potential of adults developed from irradiated pre-imaginal stages was monitored daily. Reproduction of survivors of irradiated eggs, N_1, and N_2—which do not display sexual dimorphism—was evaluated by self-crossing (viz., I♀ × I♂). The subsequent pre-imaginal stages—which do display sexual dimorphism—were placed with unirradiated members of the opposite sex (U♀ × I♂ and I♀ × U♂) as soon as they became adults. Egg laying and fertility were assessed from an average of 7 pairs of adults per replicate.

Effective doses (ED) of 50, 90, and 99.9%, i.e., ED_{50}, ED_{90}, and $ED_{99.9}$ with respect to (i) the inhibition of metamorphosis of each irradiated pre-imaginal stage into the subsequent pre-imaginal stage(s), or into adults, and (ii) reproductive sterility of these adults were estimated by regression analysis.

EFFICACY OF IRRADIATION OF PARENTAL GENERATION ADULTS IN BLOCKING EGG LAYING AND/OR EGG HATCH

Freshly formed adult males (0–1d-old) and adult females of 3 age groups—freshly formed females 0–1d-old, pre-gravid females 3–4d-old, and gravid females 6–7d-old (Fig. 1)—were irradiated and crossed with normal member of the opposite sex. Number of eggs laid and hatch in mating crosses of each of the irradiated imaginal stages in different age groups were assessed, and a mean of data from 7 pairs of adults constituted 1 replicate.

EFFICACY OF IRRADIATION OF P GENERATION ADULTS IN BLOCKING METAMORPHOSIS AND REPRODUCTION OF THEIR F_1 PROGENY

The efficacy of various doses of irradiation was also ascertained on the development and reproduction of the F_1 progeny derived from the parent generation irradiated as adults with 5–100 Gy. A cohort of 50 F_1 eggs was taken per replicate for assessing the metamorphosis of pre-imaginal stages, and the reproduction of F_1 adults. Seven pairs of adults constituted 1 replicate in each treatment for F_1 reproduction.

The ED_{50}, ED_{90}, and $ED_{99.9}$ doses for inducing P_1 sterility, and physiological disruption in F_1 progeny, and inducing F_1 sterility as a consequence of irradiation of the parent generation were estimated by regression analysis.

PHYSICOCHEMICAL AND NUTRITIONAL QUALITY ANALYSIS OF IRRADIATED PAPAYA

Physicochemical features (weight, color, acidity, and pH) and vitamin C and β-carotene as nutritional indices of papaya exposed to radiation doses of 200 and 400 Gy and stored for seven days at 5 °C were estimated. The percentage weight loss of the irradiated papaya following 1 wk of storage at 5 °C was calculated. The color characteristics of the papaya were measured at a X-Rite CA22 Spectrophotometer(X-Rite Inc., Grand Rapids, Michigan, USA). Color was recorded by using the scale of the Commission Internationale de l'Eclairage, i.e., CIELAB scale, where L* value indicates lightness, a* value indicates chromaticity on a green (-) to red (+) axis, b* value indicates chromaticity on a blue (-) to yellow (+) axis (Ranganna 1986). Total soluble solids (TSS) were determined by an Abbemat refractometer (Anton Paar Instruments, Seelze-Letter, Germany) at 20 °C and expressed in percentages by mass of total soluble solids (DGHS 2005). Acidity was determined by titrating a fruit sample—diluted with distilled water—with a 0.1 N NaOH solution with phenolphthalein indicator. The percentage of titratable acidity as citric acid was calculated (AOAC 2012). The pH of homogenized papaya flesh was determined using a pH meter (Orion-420, Thermo Scientific, Waltham, Massachusetts, USA) (DGHS 2005). Quantitative analysis of vitamin C was done by extraction and titration using 2,6-dichlorophenol indophenol dye (AOAC2012). β-Carotene was extracted, then purified by high performance liquid chromatography (Shimadzu Scientific Instruments, Kyoto, Japan), and quantified spectroscopically by recording absorbance at 436 nm. The amount of the β-carotene present in the papaya samples was determined by comparing the sample area of the peak with the β-carotene standard (AOAC2012).

STATISTICAL ANALYSES

The experiments in this study were replicated 10 times. For computing the ED_{50}, ED_{90}, and $ED_{99.9}$ with respect to metamorphic inhibition, and preventing adult formation, the regression analysis was done after the dose response study. Prevention of reproduction was analyzed by probit analysis (PoloPlus, Petaluma, California) of the dose-response data. The data were subjected to analysis of variance (ANOVA), as appropriate. A probability of $P < 0.05$ was considered significant. Percentage data were transformed using the arcsine √x value before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test among the different treatments (SPSS 22.0, Armonk, New York, USA).

Results

EFFICACY OF IRRADIATION OF PRE-IMAGINAL STAGES IN BLOCKING METAMORPHOSIS

The effects of irradiation in blocking the metamorphosis of each pre-imaginal stage into successive pre-imaginal stage(s), or into the adult were recorded. Radiation adversely affected the embryonic development of embryos irradiated as 0–1 d-old eggs. Doses ≥ 30 and ≥ 40 Gy given to 0–1 d-old eggs completely prevented the metamorphosis of the pre-imaginal stages into the adult, and completely prevented egg hatch, respectively (Fig. 2). Doses ≥ 40, ≥ 100, and ≥ 200 Gy administered to N_1 crawlers completely inhibited metamorphosis into adult males, adult females, and N_2 nymphs, respectively (Fig. 3). Further, irradiation of N_2 nymphs caused complete inhibition of metamorphosis at ≥ 200 Gy, and completely prevented adult formation at ≥ 70 Gy (Fig. 4). The dose of 200 Gy administered to N_3-♂ nymphs was enough to completely inhibit the formation of N_4-♂ nymphs (pupal cocoons) and consequently to adult males (Fig. 5), while 300 Gy was required to prevent N_3-♀ nymphs (Fig. 6) as well N_4-♂ nymphs (Fig. 7) from reaching the adult stage. Clearly, the male nymphs appeared to be more radiosensitive than the female nymphs as indicated by the inhibition by irradiation of their metamorphosis into the successive pre-imaginal stage.

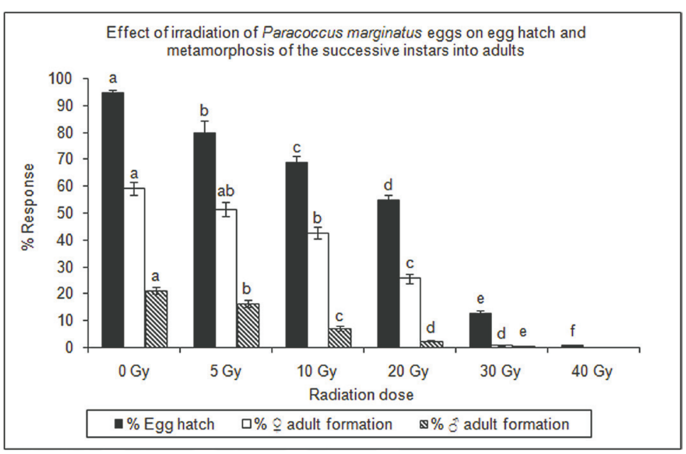

Fig. 2. Egg development and adult formation of *Paracoccus marginatus* irradiated as eggs. Irradiation of 0–1 d-old eggs with ≥ 30 and ≥ 40Gy completely prevented adult formation and egg hatch, respectively; therefore, the corresponding bars are absent in this figure. Means followed by the same letter within each of the 3 physiological processes (egg hatch, male formation, female formation) are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Fifty eggs within each treatment constituted 1 replicate.

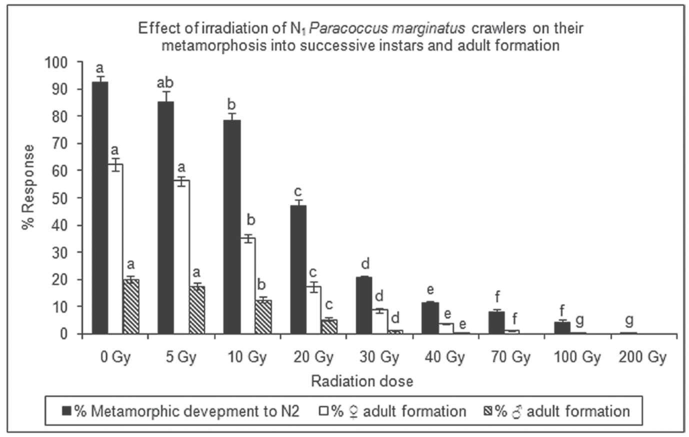

Fig. 3. Effect of irradiation of N_1 *Paracoccus marginatus* nymphs on their metamorphosis into N_2 nymphs and into adult males and females. Since, doses ≥ 40 Gy, ≥ 100 Gy, and ≥ 200 Gy completely prevented metamorphosis into adult males, adult females, and N_2 nymphs, respectively; therefore, the corresponding bars are absent in this figure. Means followed by same letter within each of the 3 molts are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Fifty N_1 nymphs within each treatment constituted 1 replicate.

EFFICACY OF IRRADIATION OF PRE-IMAGINAL STAGES IN NULLIFYING FECUNDITY AND/OR FERTILITY OF THE ADULTS

The numbers of eggs laid (Fig. 8) and percentage of F_1 egg-hatch (Fig. 9) were reduced in a dose dependent manner when the various pre-imaginal stages were irradiated, and the successive adults self-crossed (viz., I♀ × I♂) if the irradiated stages were the egg, N_1 and N_2 nymphs, or out-crossed to the non-irradiated sex (viz., U♀ × I♂ and I♀ × U♂) if the more advanced pre-imaginal stages—i.e., N_3 males, N_3 females, and N_4 males—were irradiated. The older developmental stages were the more radioresistant stages. For instance, a dose of 20 Gy allowed only 14.5, 40.2 and 53.5% fertility in the adults that had developed from the egg, N_1 and N_2 progeny of self-crosses, respectively. In comparison, fertility— as measured by F_1 eggs laid by adults derived from 20 Gy-treated N_3 and N_4 males, and N_3 females crossed with normal counterparts—was 40.2, 58.9 and 62.5%, respectively (Fig. 9).

Table 1 presents effective doses—i.e., ED_{50}, ED_{99}, and $ED_{99.9}$ applied to various pre-imaginal stages of *P. marginatus*—to prevent metamorphosis to the successive stage, to prevent adult formation, and to induce reproductive sterility in the adults that were formed. In general, the dose required to block metamorphosis increased as the pre-imaginal instar that was irradiated increased, and the formation of male adults was prevented with lower doses than the formation of female adults. The increase of the dose between ED_{50} and $ED_{99.9}$ varied about 2.0-fold to prevent eggs from becoming first instars to 3.2-fold to prevent first instars from becoming second instars. The $ED_{99.9}$ to prevent eggs from becoming first instars was 38.8± 2.2 Gy, while that to prevent the N_3-♀ from becoming adults was 258.4 ± 15.9 Gy.

Radioresistance increased as the number of molts increased between the developing stage irradiated and the adult stage of *P. marginatus*; hence, radioresistance increased in the successive instars. $ED_{99.9}$ doses that prevented male adult formation from irradiated eggs, N_1, N_2, N_3-♂ and N_4-♂ were found to be 26, 35, 54, 156, and 248 Gy, respectively. Similarly, $ED_{99.9}$ doses that prevented female adult formation from irradiated eggs, N_1, N_2, and N_3-♀ were 30, 74, 117, and 258 Gy, respectively, and these doses were larger than those required to prevent male adult formation (Table 1).

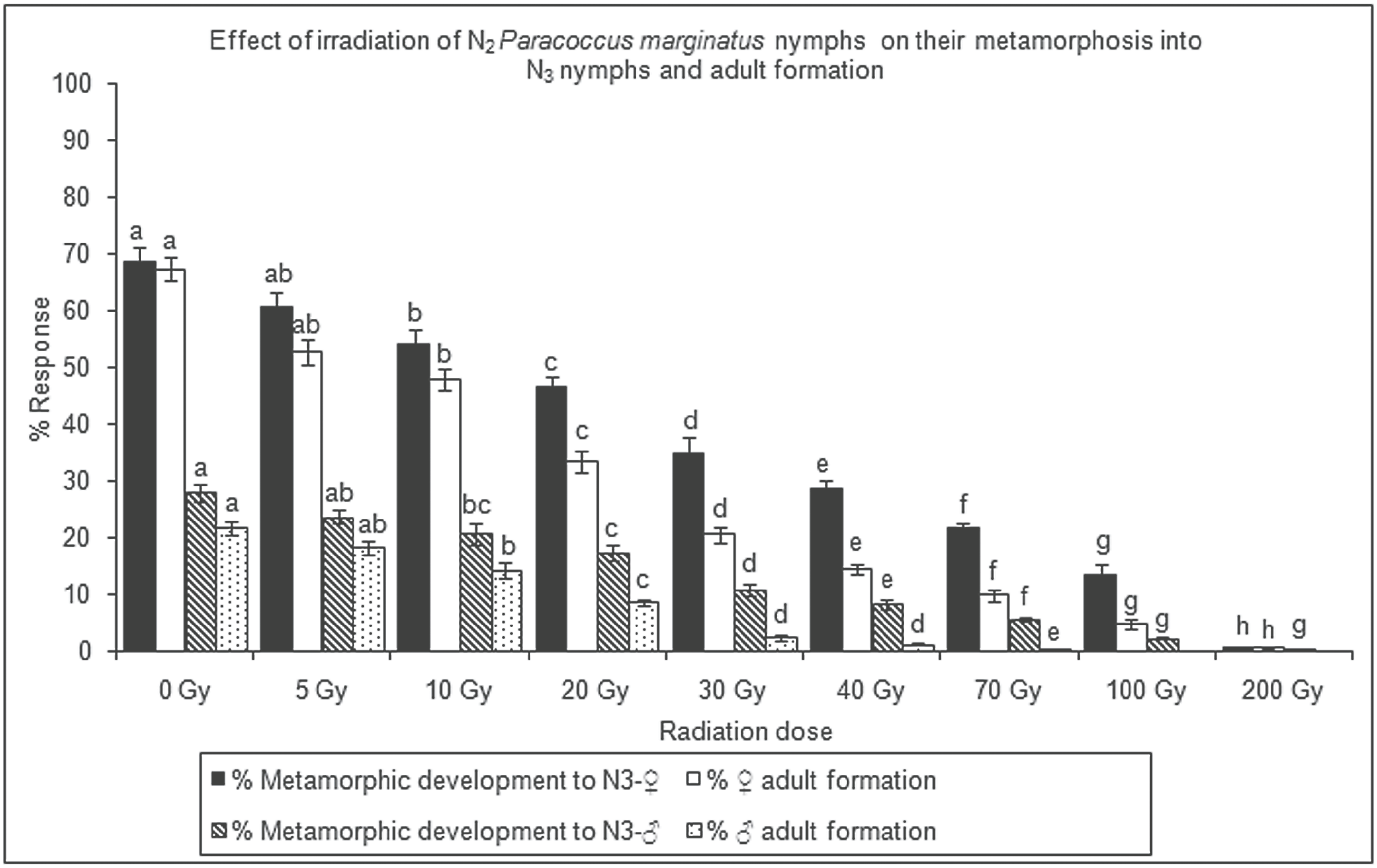

Fig. 4. Effect of irradiation of *Paracoccus marginatus* N_2 nymphs on their metamorphosis into N_3 nymphs and into adult males and females. Irradiation of N_2 nymphs caused complete inhibition of metamorphosis into N_3 nymphs and prevented the formation of adult females at 200 Gy, and completely prevented formation of adult males at ≥ 70 Gy; therefore, the corresponding bars are absent in this figure. Means followed by same letter within each of the 4 molts are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Fifteen N_2 nymphs within each treatment constituted 1 replicate.

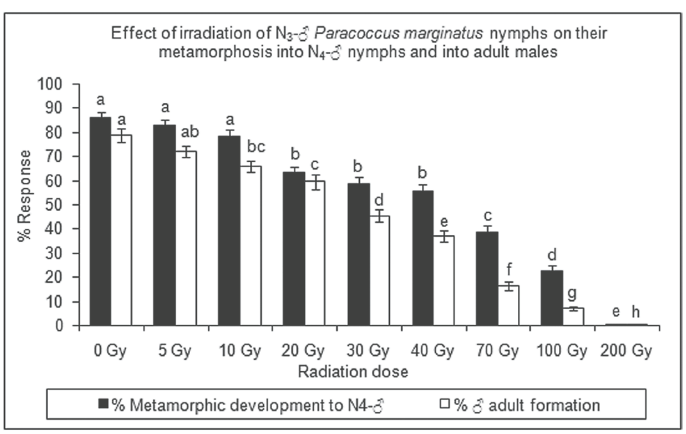

Fig. 5. Effect of irradiation of *Paracoccus marginatus* N_3-♂ nymphs on their metamorphosis into N_4-♂ nymphs (pupal cocoons) and into adult males. The dose of 200 Gy completely prevented N_3-♂ nymphs from metamorphosing into N_4-♂ nymphs, and consequently into adult males. Means followed by same letter within each of the 2 molts are not significantly different at the $P < 0.05$ level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in the graph are back transformed. Number of replicates = 10. Observations from 15 N_3-♂ nymphs within each treatment constituted 1 replicate.

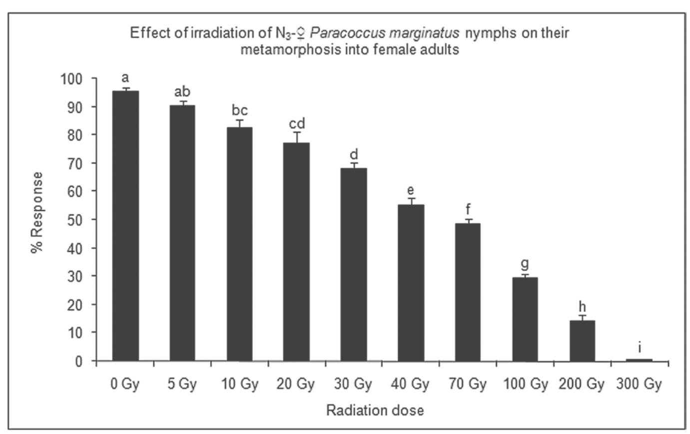

Fig. 6. Effect of irradiation of *Paracoccus marginatus* N_3-♀ nymphs on their metamorphosis into female adults. The dose of 300 Gy completely prevented to N_3-♀ nymphs from metamorphosing into adult females. Means followed by same letter are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Observations from 15 N_3-♀nymphs constituted 1 replicate.

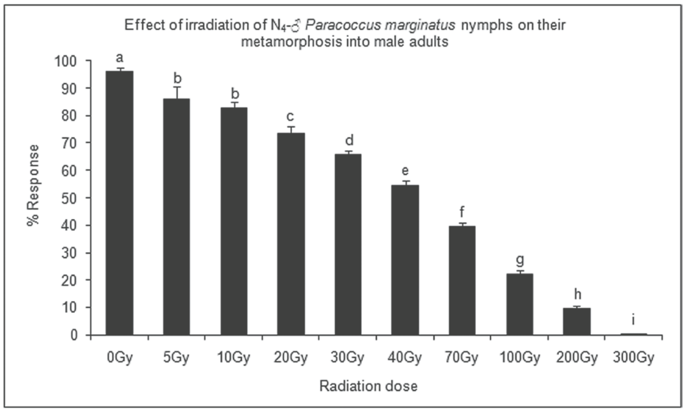

Fig. 7. Effect of irradiation of N_4-♂ *Paracoccus marginatus* nymphs on their metamorphosis into male adults. Means followed by same letter are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Observations from 15 N_4- ♂ nymphs constituted 1 replicate.

When various pre-imaginal stages were irradiated with doses that allowed some adult females to be formed, then the dose that also prevented their eggs from hatching increased as the irradiated pre-imaginal stage increased. A dose of 62.4 Gy applied to any pre-imaginal stage induced complete sterility of the F_1 eggs of all females that were formed (Table 1). Therefore, 62.4 Gy applied to pre-imaginal stages of *P. marginatus* meets one of the crucial requirements of phytosanitary irradiation.

EFFICACY OF IRRADIATION OF P GENERATION ADULTS IN BLOCKING EGG LAYING AND/OR EGG HATCH

Parent generation male adults were relatively more radiosensitive than their female counterparts because 99.9% sterility was induced with 37.9 Gy applied to males that were mated to non-irradiated females. In contrast to induce this same level of sterility in females, at least 55.5 Gy has to be applied to females that were mated to non-irradiated males (Table 2). There was a dose dependent decrease in fertility of eggs laid at doses in the range of 5–40 Gy in 0–1-d-old male adults ($F = 327.5$; df = 5,54; $P < 0.05$), at 5–70 Gy in 0–1-d-old females ($F = 338.5$;df = 6,63; $P < 0.05$), 5–100 Gy in pre-gravid 3–4-d-old females ($F = 342.1$; df = 7,72; $P < 0.05$); and in 5–200 Gy irradiated gravid 6–7-d-old female adults ($F = 249.5$; df = 8,81; $P < 0.05$), respectively. The females laid no eggs in crosses in which either a freshly formed 0–1 d-old male or a freshly formed 0–1 d-old female was irradiated with 200 Gy, or in crosses involving a pre-gravid female 3–4 d-old irradiated with 400 Gy (Fig. 10). Only non-viable eggs were produced in crosses involving either freshly formed 0–1 d-old adult males irradiated with 40 Gy, or freshly formed 0–1 d-old females irradiated with 70 Gy, or pre-gravid 3–4 d-old females irradiated with 100 Gy, or gravid 6–7 d-old females irradiated with 200 Gy (Fig. 11). The results demonstrate that the radioresistance of females was increased with age in terms of the doses required to block either egg laying or egg hatch (Figs. 10 and 11).

EFFICACY OF IRRADIATION OF P GENERATION ADULTS IN BLOCKING METAMORPHOSIS AND REPRODUCTION OF THEIR F_1 PROGENY

Data on F_1 transformation into the adult stage when P adults of different ages were irradiated are presented in Figs. 12, 13. When par-

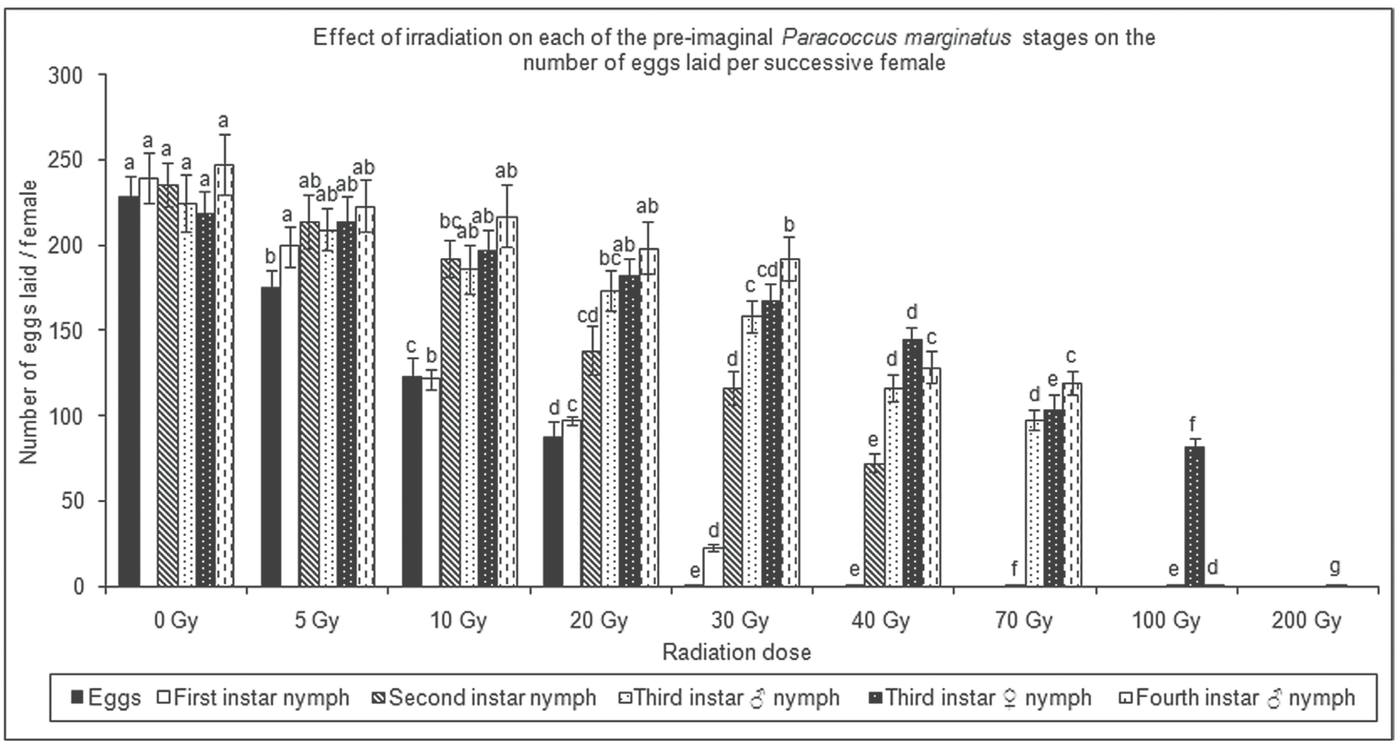

Fig. 8. Oviposition by *Paracoccus marginatus* females derived from crosses in which one or both parents were irradiated in 1 of the various pre-imaginal stages. Adults derived from irradiated eggs, and N_1 & N_2 nymphs were self-crossed, whereas the adults from N_3 onwards were out-crossed to their non-irradiated counterparts. Females did not lay any eggs if they had developed from eggs irradiated with 30 Gy, or from N_1 nymphs irradiated with 40 Gy, or from N_2 nymphs irradiated at 70 Gy, or if they were non-irradiated females mated to adults transformed out of N_3 & N_4 males irradiated at 100 Gy, or if the adult females had developed out of N_3 female nymphs irradiated with 200 Gy and mated to non-irradiated males. In these cases, the corresponding bars are absent in the figure. Means followed by same letter within a single dose regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average data of 7 mated females in each regimen constituted 1 replicate.

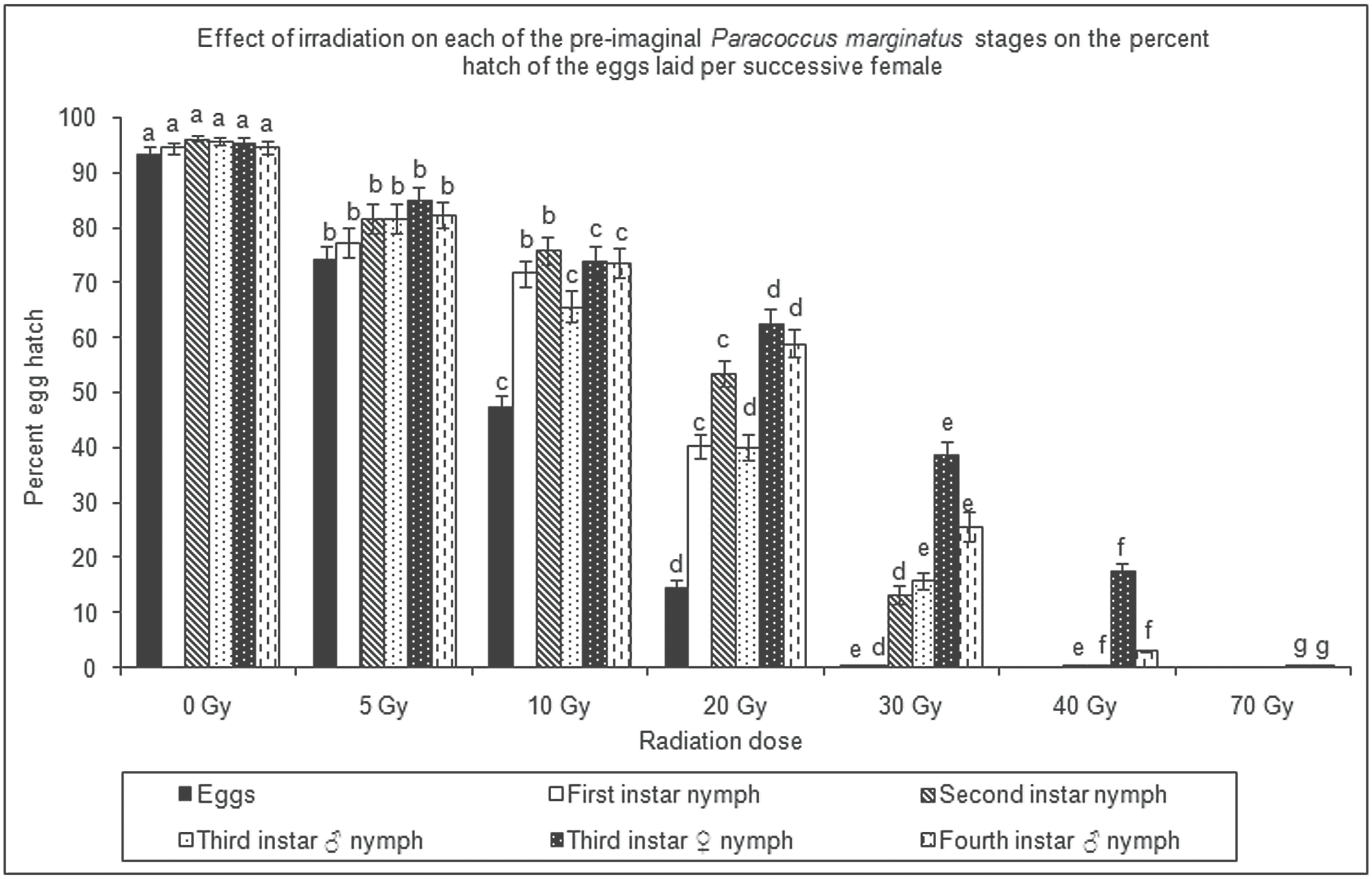

Fig. 9. Fertility of *Paracoccus marginatus* adult females that had been irradiated in different pre-imaginal stages. Adults derived from either irradiated eggs, or N_1, or N_2 nymphs were self-crossed, whereas the adults derived from N_3 onwards were out-crossed with their non-irradiated counterparts. Only non-viable eggs were produced by adult females if they derived from either eggs, or from either N_1 nymphs irradiated with 30 Gy, or from N_2 nymphs irradiated with 40 Gy; or by non-irradiated females mated to adults from N_3 males irradiated with 40 Gy, or by non-irradiated females mated to adults from N_4 males irradiated with 70 Gy, or by adults from N_3 females irradiated with 70 Gy and mated to non-irradiated males. In these cases, the corresponding bars are absent. Means followed by same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average percent egg hatch obtained from 7 pairs of adults in each regimen constituted 1 replicate.

ent generation adults of different age groups were irradiated and then crossed with non-irradiated members of the opposite sex, no F_1 adult males were produced in crosses in the 30 Gy treatment that involved either freshly formed 0–1 d-old adult males, or freshly formed 0–1 d-old females, or pre-gravid 3–4 d-old females. Moreover in the 40-Gy treatment even gravid 6–7 d-old females failed to produce F_1 adult males (Fig. 12). No F_1 adult females were produced in crosses in the 40-Gy treatment that involved either irradiated freshly formed 0–1 d-old adult males, or irradiated freshly formed 0–1 d-old females. Moreover in the 70-Gy treatment crosses involving irradiated pre-gravid 3–4 d-old females and irradiated gravid 6–7 d-old females failed to produce F_1 adult females (Fig. 13). These data demonstrate the adult males were more radio-susceptible than adult females. Complete prevention of both F_1 male and female adults was accomplished with the relatively low dose of 70 Gy when applied to 6–7d-old gravid females— the most radiotolerant stage.

Irradiation of parent generation adults of different age groups with certain doses prevented the F_1 females in self-crosses from laying F_2 eggs. F_1 females laid no eggs in the 30 Gy treatment involving either irradiated freshly formed 0–1 d-old males, or irradiated freshly formed 0–1 d-old females, nor in the 40-Gy treatment involving either irradiated pre-gravid females 3–4 d-old, or irradiated gravid 6–7 d-old females (Fig. 14).

In the 30 Gy treatment to 0–1 d-old freshly formed females, 3–4 d-old pre-gravid females, and 6–7 d-old gravid females—which were crossed with untreated males, followed by self-crosses of F_1 adults—laid 0, 29, 94 F_2 eggs per female (Fig. 14), and the corresponding percentages of hatch of F_2 eggs laid by the F_1 females derived from treated 3–4 d-old pre-gravid females, and 6–7 d-old gravid females were 0 and 16.8%, respectively (Fig. 15). Further, complete F_1 sterility was achieved at 40 Gy when applied to the most tolerant stage, 6–7 d-old P females that did not lay any eggs at this dose (Fig. 14).

Table 2 presents the effects of irradiating P generation *Paracoccus marginatus* adults of various age groups and crossing them with non-irradiated members of the opposite sex. Specifically, Table 2 presents effective doses for inducing sterility in P generation adults, for blocking the metamorphosis of their F_1 N_1 progeny into N_2 nymphs, for blocking metamorphosis of their pre-imaginal progeny into adult males or females, and for inducing F_1 sterility. In general, as the age of the irradiated P adults advanced, the effective doses for blocking metamorphosis and inducing sterility in F_1 progeny increased. The $ED_{99.9}$ to induce sterility in 6-7 d-old P females—the most radio-resistant imaginal stage—was 164.7 ± 9.3 Gy, whereas the $ED_{99.9}$ doses—applied to P adults—to induce sterility in F_1 adults and block F_1 adult formation were 37.3 and 61.5 Gy, respectively. Further, the $ED_{99.9}$ dose to block the metamorphosis of F_1 N_1 crawlers into N_2 nymphs— a crucially im-

Table 1. Prevention of pre-imaginal metamorphosis, adult formation, and induction of reproductive sterility in *Paracoccus marginatus* by gamma irradiation of each of the various pre-imaginal stages.

Desired effect	Pre-imaginal stage irradiated and the target stage to be prevented	Estimated dose[a] (Gy) to prevent 50, 90, and 99.9% from reaching the succeeding pre-imaginal stage or the adult, or inducing sterility			Regression equation	Slope
		ED_{50}	ED_{90}	$ED_{99.9}$		
Inhibition of metamorphosis to the succeeding stage	Egg to N_1	19.3 ± 1.1a	34.9 ± 1.9a	38.8 ± 2.2a	y = 2.5637x + 0.3972 R^2 = 0.9756	2.5637 ± 0.13a
	N_1 to N_2	34.4 ± 1.8b	96.2 ± 4.8b	111.5 ± 5.9b	y = 0.6471x + 27.727 R^2 = 0.6408	0.6471 ± 0.03b
	N_2 to N_3-♂	41.2 ± 2.1c	105.2 ± 5.3b	121.1 ± 6.2b	y = 0.6246x + 24.247 R^2 = 0.7953	0.6246 ± 0.03b
	N_2 to N_3-♀	60.0 ± 3.3d	146.9 ± 8.6c	168.4 ± 9.4c	y = 0.4603x + 22.348 R^2 = 0.8335	0.4603 ± 0.02c
	N_3♂ to N_4♂	78.4 ± 5.7c	157.7 ± 11.5c	177.3 ± 13.1c	y = 0.5044x + 10.443 R^2 = 0.9224	0.5044 ± 0.03c
	N_3♀ to Adult♀	104.8 ± 6.5f	227.9 ± 14.2d	258.4 ± 15.9d	y = 0.325x + 15.913 R^2 = 0.8716	0.325 ± 0.02d
	N_4♂ to Adult♂	93.3 ± 6.2ef	217.3 ± 14.6d	247.9 ± 15.6d	y = 0.3227x + 19.876 R^2 = 0.827	0.3227 ± 0.02d
	F-value	*F* = 73.2* df = 6,63	*F* = 65.4* df = 6,63	*F* = 53.9* df = 6,63		*F* = 26.7* df = 6,63
Prevention of adult formation	Egg to adult ♂	12.2 ± 1.1a	23.7 ± 2.1a	26.5 ± 2.0a	y = 3.4914x + 7.006 R^2 = 0.8948	3.4914 ± 0.30a
	Egg to adult ♀	14.0a ± 0.75a	26.9 ± 1.5ab	30.1 ± 1.6ab	y = 3.1002x + 6.483 R^2 = 0.9796	3.1002 ± 0.16ab
	N_1 to adult ♂	16.9 ± 1.5bc	31.7 ± 2.4b	35.3 ± 2.8b	y = 2.7059x + 4.1468 R^2 = 0.9471	2.7059 ± 0.21b
	N_1 to adult ♀	15.3 ± 1.1ab	62.2 ± 4.2c	73.8 ± 5.1c	y = 0.8539x + 36.881 R^2 = 0.6102	0.8539 ± 0.05d
	N_2 to adult ♂	20.7 ± 1.7c	47.9 ± 4.1d	54.6 ± 4.3d	y = 1.4737x + 19.349 R^2 = 0.7907	1.4737 ± 0.11c
	N_2 to adult ♀	32.5 ± 2.3d	100.3 ± 7.1e	117.1 ± 8.3e	y = 0.59x + 30.809 R^2 = 0.7152	0.59 ± 0.04e
	N_3♂ to adult ♂	48.8 ± 2.9c	134.8 ± 8.1f	156.0 ± 9.3f	y = 0.4653x + 27.27 R^2 = 0.7515	0.4653 ± 0.02f
	N_3♀ to adult ♀	104.8 ± 6.5f	227.9 ± 14.2g	258.4 ± 16.1h	y = 0.325x + 15.913 R^2 = 0.8716	0.325 ± 0.02g
	N_4♂ to adult ♂	93.3 ± 6.2f	217.3 ± 14.5g	247.9 ± 16.6g	y = 0.3227x + 19.876 R^2 = 0.827	0.3227 ± 0.02g
	F-value	*F* = 156.9* df = 8,81	*F* = 187.5* df = 8,81	*F* = 192.5* df = 8,81		*F* = 125.3* df = 8,81

[a]ED_{50}, ED_{90}, and $ED_{99.9}$ are the effective doses that induced 50%, 90%, and 99.9% inhibition of metamorphosis to the subsequent stage, or prevention of transformation into the adult, or the induction of reproductive sterility. Means followed by same letter within a column are not significantly different at $P < 0.05$. level (ANOVA followed by LSD post-test). Number of replicates, $n = 10$. Fifty eggs, 50 neonates for the N_1, and 12–15 nymphs each for the N_2, N_3♂, N_3♀ and N_4♂ instars constituted 1 replicate for blocking metamorphosis to the succeeding pre-imaginal stage, and prevention of adult formation; whereas for the induction of sterility, the average data obtained from 7 pairs within each treatment regimen constituted 1 replicate.

Table 1. (Continued) Prevention of pre-imaginal metamorphosis, adult formation, and induction of reproductive sterility in *Paracoccus marginatus* by gamma irradiation of each of the various pre-imaginal stages.

Desired effect	Pre-imaginal stage irradiated and the target stage to be prevented	Estimated dose[a] (Gy) to prevent 50, 90, and 99.9% from reaching the succeeding pre-imaginal stage or the adult, or inducing sterility			Regression equation	Slope
		ED_{50}	ED_{90}	$ED_{99.9}$		
Reproductive sterility	Egg (I♀ x I♂)	12.7 ± 0.62a	24.4 ± 1.4a	27.3 ± 1.5a	y = 3.414x + 6.4084 R^2 = 0.9578	3.414 ± 0.19a
	N_1(I♀ x I♂)	16.0 ± 0.81b	28.3 ± 1.6b	31.4 ± 1.8a	y = 3.2501x - 2.2538 R^2 = 0.9825	3.2501 ± 0.16ab
	N_2 (I♀ x I♂)	19.6 ± 1.1c	34.9 ± 1.7c	38.7 ± 1.9b	y = 2.603x - 1.081 R^2 = 0.9799	2.603 ± 0.14bc
	N_3-♂ (U♀ x I♂)	21.1 ± 1.2d	37.3 ± 2.1c	41.4 ± 2.3b	y = 2.4642x - 2.1224 R^2 = 0.9844	2.4642 ± 0.13c
	N_3-♀ (I♀ x U♂)	28.9 ± 1.6e	55.7 ± 3.2d	62.4 ± 3.6c	y = 1.4919x + 6.7571 R^2 = 0.9432	1.4919 ± 0.07d
	N_4-♂ (U♀ x I♂)	21.8 ± 1.1d	48.5 ± 2.5d	55.1 ± 3.1c	y = 1.4963x + 17.374 R^2 = 0.8142	1.4963 ± 0.08d
	F-value	*F* = 18.6* df = 5,54	*F* = 22.4* df = 5,54	*F* = 27.6* df = 5,54		*F* = 16.5* df = 5,54

[a]ED_{50}, ED_{90} and $ED_{99.9}$ are the effective doses that induced 50%, 90%, and 99.9% inhibition of metamorphosis to the subsequent stage, or prevention of transformation into the adult, or the induction of reproductive sterility. Means followed by same letter within a column are not significantly different at $P < 0.05$. level (ANOVA followed by LSD post-test). Number of replicates, $n = 10$. Fifty eggs, 50 neonates for the N_1, and 12–15 nymphs each for the N_2, N_3♂, N_3♀ and N_4♂ instars constituted 1 replicate for blocking metamorphosis to the succeeding pre-imaginal stage, and prevention of adult formation; whereas for the induction of sterility, the average data obtained from 7 pairs within each treatment regimen constituted 1 replicate.

portant parameter of phytosanitary irradiation—was found to be 91.1 Gy, which is considerably less than that determined for *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Seth et al. 2016 a) and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) (Seth et al. 2016b).

PHYSIO-CHEMICAL AND NUTRITIONAL QUALITY OF PAPAYA AFTER GAMMA IRRADIATION

Irradiation of papaya fruits with 200 Gy and 400 Gy did not significantly affect any of the level of important physico-chemical and nutritional indices at 7 d after treatment and storage at 5 °C (Table 3).

Discussion

After the papaya mealybug, *P. marginatus,* a quarantine surface pest, was discovered on Hawaii-produced papaya, the commercial treatment dose—in the absence of irradiation studies on *P. marginatus* (Follett 2009)—was increased to 400 Gy to prevent rejections. Hallman (2003) suggested that normal growth, development or reproduction of this and other organisms might be prevented by lower doses of irradiation, whereas the high doses of irradiation needed to kill insects quickly, would in many cases also damage the quality of the irradiated commodity. The goal of phytosanitary irradiation is to prevent the development or reproduction of the target pest so that it cannot establish a new infestation at a destination where the pest does not occur (Follett 2006a).Therefore, the present study was aimed to study the radiation sensitivities of all life stages of *P. marginatus* in order to establish an effective phytosanitary irradiation regimen below 400 Gy.

Prevention of the metamorphosis of irradiated pre-imaginal stages of *P. marginatus* to successive stages and the inhibition of their survival up to the adult stage were measured to assess the radiation bioefficacy. Survival of the growing nymph up to subsequent stages and/or eventual formation of adult stage were inversely proportional to the gamma radiation. In relation to the differences in tolerance between insect stages towards irradiation, Bergonie & Tribondeau (1959) stated that the degree of susceptibility to radiation is proportional to the activity level of cell division and the level of cell differentiation. Therefore, immature stages undergoing active cell division are more sensitive to radiation than the adult stage of most insect taxa in which somatic cell division has ceased, and cell division is restricted to the germline.

The measure of efficacy of irradiation is prevention of development and/or reproduction, rather than acute mortality (Hallman & Thomas 2010). Effective doses for 99.9% mortality in terms of inhibiting metamorphosis to the next successive stage in *P. marginatus* ranged from 39 Gy for the most radiosensitive egg stage to 258 Gy for the most radioresistant stage, the latter being the N_3-♀. Radiosensitivity of irradiated pre-imaginal stages of *P. marginatus* decreased as nymphs increased in age. Further, the present study revealed that the effective doses for 99.9% sterility in adults derived from irradiated pre-imaginal stages ranged from 27 Gy for eggs to 62 Gy for N_3 females. Complete infecundity in adults derived from irradiated pre-imaginal stages, except the N_3 female, was induced by 100 Gy. In this study complete infecundity in the N_3 female was induced by the next highest dose of 200 Gy. Our results were in consonance with that of Jacobsen & Hara (2003) who suggested the dose of 100 Gy could be used as a sterilizing dose for eggs, crawlers and later nymphal stages of *M. hirsutus*. Ravuiwasa et al. (2009) reported that adults that survived from irradiated immature stages of *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) laid eggs but none of them hatched at doses ≥ 150 Gy.

Table 2. Effects of irradiating P generation *Paracoccus marginatus* adults of various age groups, and crossing them with non-irradiated members of the opposite sex: effective doses for inducing sterility in P generation adults, for blocking the metamorphosis of their N_1 progeny into N_2 nymphs, for blocking metamorphosis of their pre-imaginal progeny into adult males or females, and for inducing F_1 sterility.

Desired effect	Age of imaginal stage irradiated		Estimated dose (Gy)[a] to induce sterility in P adults, block F_1 metamorphosis, block F_1 adult formation and induce F_1 sterility			Regression equation	Slope
			ED_{50}	ED_{90}	$ED_{99.9}$		
Sterility in P generation	Freshly formed male (0-1d-old) (U♀ x I♂)		18.8 ± 0.98a	34.1 ± 1.8a	37.9 ± 1.99a	y = 2.6205x + 0.5258 R^2 = 0.9872	2.6205b ± 0.13a
	Freshly formed female (0-1d-old) (I♀ x U♂)		23.2 ± 1.3b	49.1 ± 2.7b	55.5 ± 3.1b	y = 1.5453x + 14.025 R^2 = 0.817	1.5453 ± 0.08b
	Pre-gravid female (3-4 d-old) (I♀ x U♂)		33.4 ± 1.9c	71.5 ± 3.9c	80.9 ± 4.7c	y = 1.0506x + 14.832 R^2 = 0.833	1.0506 ± 0.05c
	Gravid female (6-7 d-old) (I♀ x U♂)		67.6 ± 3.3d	145.4 ± 8.2d	164.7 ± 9.3d	y = 0.5141x + 15.228 R^2 = 0.8189	0.5141 ± 0.02d
			F = 59.8* df = 3,36	*F* = 99.7* df = 3,36	*F* = 123.6* df = 3,36		*F* = 94.6* df = 3,36
Prevention of molt of F_1 N_1 to F_1 N_2	Freshly formed male (0-1d-old) (U♀ x I♂)		16.8 ± 0.92a	32.5 ± 1.9a	36.4a ± 2.5	y = 2.553x + 7.043 R^2 = 0.971	2.553 ± 0.175a
	Freshly formed female (0-1d-old) (I♀ x U♂)		21.2 ± 1.5b	47.7 ± 2.9b	54.3 ± 3.4b	y = 1.506x + 18.11 R^2 = 0.790	1.506 ± 0.084b
	Pre-gravid female (3-4 d-old) (I♀ x U♂)		30.3 ± 1.9c	69.5 ± 4.5c	79.2 ± 5.9c	y = 1.021x + 19.06 R^2 = 0.792	1.021 ± 0.065c
	Gravid female (6-7 d-old) (I♀ x U♂)		42.3 ± 2.4d	81.4 ± 3.7d	91.1 ± 5.9d	y = 1.022x + 6.8 R^2 = 0.950	1.022 ± 0.069c
			F = 176.5* df = 3,36	*F* = 191.1* df = 3,36	*F* = 234.9* df = 3,36		*F* = 252.9* df = 3,36
Inhibition of F_1 male and female adult formation	Freshly formed male (0-1 d-old)	F_1 male	13.0 ± 0.91a	23.9 ± 1.6a	26.6 ± 1.8a	y = 3.6504x - 2.4421 R^2 = 0.9642	3.6504a ± 0.27
		F_1 female	14.5 ± 0.80ab	30.4 ± 1.6bc	34.3 ± 1.8b	y = 2.5138x + 13.481 R^2 = 0.9213	2.5138bc ± 0.13
	Freshly formed female (0-1 d-old)	F_1 male	14.6 ± 1.1ab	26.1 ± 2.1ab	29.0 ± 2.3a	y = 3.4586x - 0.4243 R^2 = 0.9814	3.4586a ± 0.27
		F_1 female	15.0 ± 0.68ab	31.2 ± 1.5c	35.2 ± 1.6bc	y = 2.4816x + 12.528 R^2 = 0.9403	2.4816c ± 0.11
	Pre-gravid female (3-4 d-old)	F_1 male	15.5 ± 1.1b	28.0 ± 1.9ab	31.1 ± 2.2ab	y = 3.196x - 0.3849 R^2 = 0.9759	3.196 ± 0.22ab
		F_1 female	19.1 ± 1.5bc	38.8 ± 2.8c	43.6 ± 3.3c	y = 2.0347x + 11.016 R^2 = 0.946	2.0347 ± 0.15d
	Gravid female (6-7 d-old)	F_1 male	21.0 ± 1.5c	36.2 ± 2.7c	40.0 ± 3.1bc	y = 2.6238x - 5.217 R^2 = 0.983	2.6238 ± 0.19bc
		F_1 female	27.7 ± 1.9d	54.8 ± 3.7d	61.5 ± 4.3d	y = 1.4761x + 8.985 R^2 = 0.9275	1.4761 ± 0.10c
	F-value		*F* = 64.8* df = 7,72	*F* = 57.4* df = 7,72	*F* = 44.9* df = 7,72		*F* = 73.8* df = 7,72

[a]ED_{50}, ED_{90}, and $ED_{99.9}$ are the effective doses applied to each age group of P generation adults that induced 50%, 90%, 99.9% responses (inducing sterility in P adults, prevention of F_1 adult formation and sterility in F_1 adults). Means followed by same letter within a column for each feature are not significantly different at *P*< 0.05 level (ANOVA followed by LSD post-test). Number of replicates = 10. For sterility, average data from seven pairs of P and F_1 adults within each regimen constituted one replicate; for prevention of F_1 adults formation, a cohort of 50 F_1 eggs from each treatment regimen constituted 1 replicate.

Table 2. (Continued) Effects of irradiating P generation *Paracoccus marginatus* adults of various age groups, and crossing them with non-irradiated members of the opposite sex: effective doses for inducing sterility in P generation adults, for blocking the metamorphosis of their N_1 progeny into N_2 nymphs, for blocking metamorphosis of their pre-imaginal progeny into adult males or females, and for inducing F_1 sterility.

Desired effect	Age of imaginal stage irradiated	Estimated dose (Gy)[a] to induce sterility in P adults, block F_1 metamorphosis, block F_1 adult formation and induce F_1 sterility			Regression equation	Slope
		ED_{50}	ED_{90}	$ED_{99.9}$		
Sterility in F_1 generation	Freshly formed male (0-1 d-old) (F_1 self cross)	11.6 ± 0.52a	24.1 ± 1.1a	27.2 ± 1.2a	y = 3.1945x + 12.912 R^2 = 0.9287	3.1945 ± 0.14a
	Freshly formed female (0-1 d-old) (F_1 self cross)	12.3± 0.51ab	24.8 ± 1.2a	27.9 ± 1.7a	y = 3.2012x + 10.51 R^2 = 0.9547	3.2012 ± 0.14a
	Pre-gravid female (3-4 d-old) (F_1 self cross)	14.0 ± 0.67b	27.0 ± 1.3a	30.2 ± 1.8a	y = 3.0856x + 6.5572 R^2 = 0.9749	3.0856 ± 0-17a
	Gravid female (6-7 d-old) (F_1 self cross)	16.9 ± 0.89c	33.3 ± 1.8b	37.3 ± 1.9b	y = 2.4394x + 8.7211 R^2 = 0.9651	2.4394 ± 0-12b
	F-value	*F* = 23.9* df = 3,36	*F* = 37.2* df = 3,36	*F* = 45.5* df = 3,36		*F* = 17.6* df = 3,36

[a]ED_{50}, ED_{90}, and $ED_{99.9}$ are the effective doses applied to each age group of P generation adults that induced 50%, 90%, 99.9% responses (inducing sterility in P adults, prevention of F_1 adult formation and sterility in F_1 adults). Means followed by same letter within a column for each feature are not significantly different at *P*< 0.05 level (ANOVA followed by LSD post-test). Number of replicates = 10. For sterility, average data from seven pairs of P and F_1 adults within each regimen constituted one replicate; for prevention of F_1 adults formation, a cohort of 50 F_1 eggs from each treatment regimen constituted 1 replicate.

When the adult stage can occur in or on the commodity, and because it tends to be the most radiotolerant stage, the measure of efficacy is the level of sterility induced by irradiation (Follett et al. 2007). Our study showed sterilization of *P. marginatus* females requires a larger dose than sterilization of males; the $ED_{99.9}$ for inducing sterility in 0-1 d male adults was 38 Gy in comparison to 55–165 Gy in females—depending on their age. Similarly, Vercosa et al. (1993) reported that the *Panstrongylus megistus* (Burmeister) (Hemiptera: Reduviidae) males were more radiosensitive than females with respect to the induction of sterility.

Radiation sensitivity in adult females further depends on their age at the time of irradiation. In fact, the present study on radiosensitivity of different age groups of female adults demonstrated that the 6–7 d-old gravid female was the most tolerant stage of the papaya mealybug. *Paracoccus marginatus* gravid 6–7 d-old females crossed with non-irradiated males, could still oviposit 63 F_1 eggs/female—a 75% reduction with respect to the unirradiated control—at 500 Gy, but 0% egg hatch was observed at 200 Gy.

Because irradiation results in cumulative damage to organisms over time, smaller doses are generally required to achieve fatal effects later in development. Therefore, even if some F_1 individuals managed to hatch from eggs, they would most likely die in subsequent stages (Hallman et al. 2013). Similarly, in the present study, a reduced proportion of F_1 adults survived from irradiated P gravid females—the most radio-resistant adult stage—compared to the unirradiated control. We found that the $ED_{99.9}$ for P generation *P. marginatus* gravid females to block formation of F_1 male and F_1 female progeny was 44 and 62 Gy, respectively. Moreover, complete reproductive inviability was induced with 40 Gy, which caused completely prevented oviposition. Follett (2006b) reported that *Pseudaulacaspis pentagona* (Targioni) (Hemiptera: Diaspididae) adults with eggs that were irradiated with 60–90 Gy were able to lay eggs, which developed into egg-laying F_1 adults, but at 120–150 Gy reproduction was almost inhibited.

In many groups of quarantined arthropods (Acari, Homoptera, Hemiptera, Thysanoptera, and Coleoptera) all stages including actively reproducing adults may be present. Therefore, in these cases, the only possible option for an irradiation quarantine treatment is complete sterility (Hallman 1998). Scant information of the kind needed to develop generic quarantine doses exists on the radiation-susceptibility of important pest groups, such as molluscs, mealybugs and scales (Homoptera: Coccoidea), thrips (Thysanoptera), and eriophyid mites (Hallman 1998). Therefore, data from the present study could be included in the development of generic and specific doses for pests and pest groups of quarantine importance. This would contribute to reducing technical trade barriers and to facilitating international trade in agricultural produce.

Hallman et al. (2013) asserted that a dose as small as 350 Gy might be sufficient for the lepidopteran pest group if results of more large-scale studies were available or the measure of efficacy were extended beyond prevention of F_1 egg hatch, but data to defend measures of efficacy beyond F_1 egg hatch are scarce. Phytosanitary irradiation of *P. marginatus,* could be achieved with 165–258 Gy dose range in order to prevent the metamorphosis of the most radiotolerant pre-imaginal stage, the N_3-female, and reproduction of the most radiotolerant imaginal stage, the gravid female. Even the smaller dose of ~100 Gy was sufficient to prevent 99.9% of F_1 N_1 crawlers from metamorphosing into N_2 nymphs, which is an important threshold phytosanitary irradiation parameter for other mealybug species (Seth et al. 2016 a, b).

NUTRITIONAL QUALITY OF PAPAYA SUBJECTED TO PHYTOSANITARY IRRADIATION

In order for a phytosanitary irradiation regimen to achieve commercial acceptance, it must not degrade product quality (Moy & Wong,

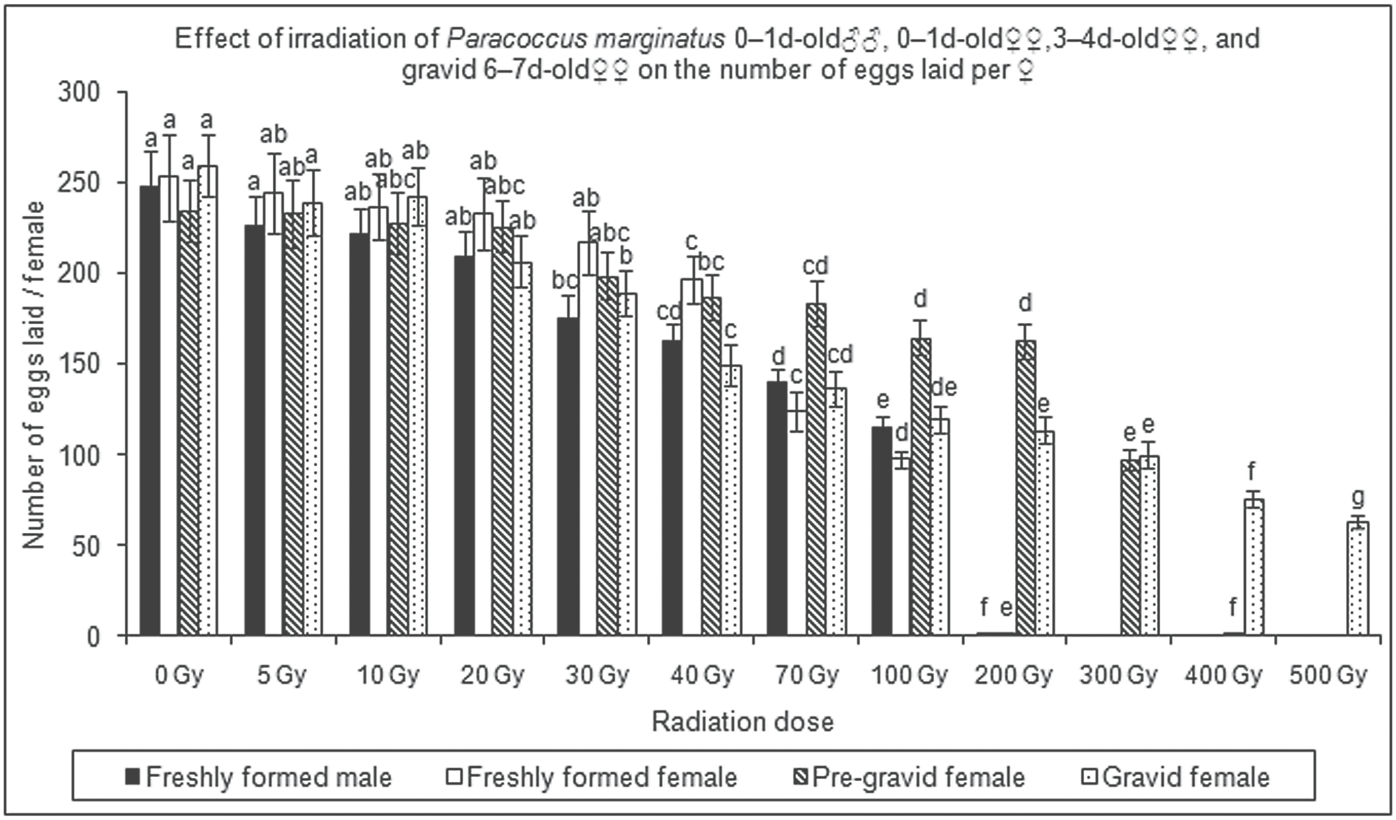

Fig. 10. Number of F_1 eggs oviposited per *Paracoccus marginatus* female after either they or their male mates were irradiated. Adult 0–1 d-old males or adult females of 3 age groups—i.e., freshly formed females 0–1 d-old, pre-gravid females 3–4 d-old, and gravid females 6–7 d-old—were irradiated with doses in the range of 0–500 Gy. Irradiated individuals were crossed with non-irradiated members of the opposite sex. The females laid no eggs in crosses in which either a freshly formed 0–1 d-old male or a freshly formed 0–1 d-old female was irradiated with 200 Gy, or in crosses involving a pre-gravid female 3–4 d-old irradiated with 400 Gy. In these cases the corresponding bars are absent. Means followed by the same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average data of 7 mated females in each regimen constituted 1 replicate.

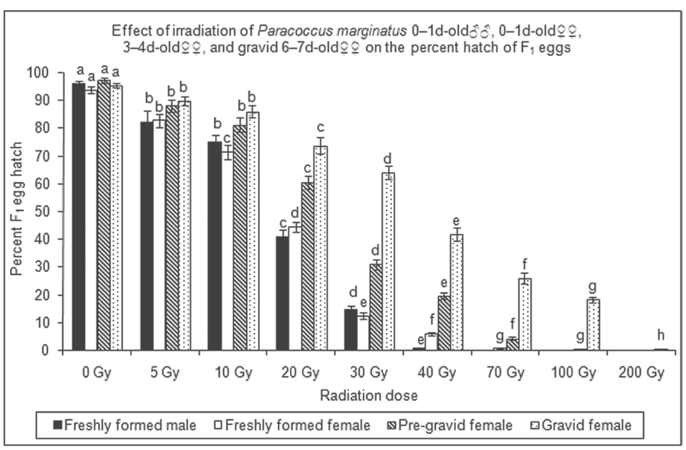

Fig. 11. Percent of F_1 egg hatch in *Paracoccus marginatus*, when the parent generation individuals were irradiated as either freshly formed 0–1 d-old adult males or adult females of 3 age groups—freshly formed 0–1 d-old females, pre-gravid 3–4 d-old females, and gravid 6–7 d-old females—and crossed with non-irradiated members of opposite sex. Only non-viable eggs were produced in crosses involving either freshly formed 0–1 d-old adult males irradiated with 40 Gy, or freshly formed 0–1 d-old females irradiated with 70 Gy, or pre-gravid 3–4 d-old females irradiated with 100 Gy, or gravid 6–7 d-old females irradiated with 200 Gy. In these cases the corresponding bars are absent. Means followed by the same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average percent egg hatch in 7 pairs of adults in each regimen constituted 1 replicate.

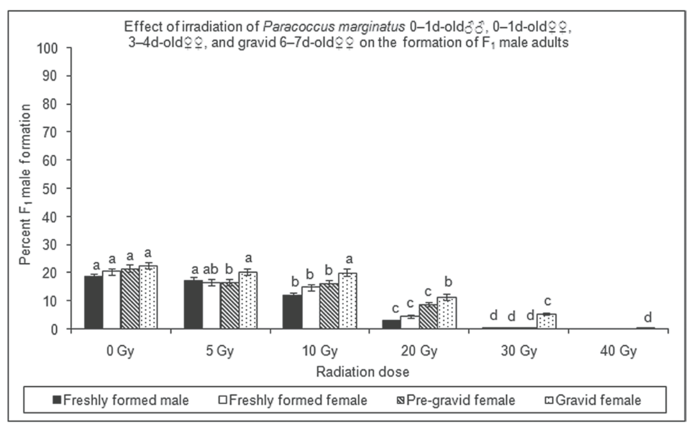

Fig. 12. Percent F_1 male adult formation in *Paracoccus marginatus*, when parent generation adults of different age groups were irradiated and then crossed with non-irradiated members of the opposite sex. No F_1 adult males were produced in crosses in the 30 Gy treatment that involved either freshly formed 0–1 d-old adult males, or freshly formed 0–1 d-old females, or pre-gravid 3–4 d-old females. Moreover in the 40 Gy treatment even gravid 6–7 d-old females failed to produce F_1 adult males. In these cases the corresponding bars are absent. Means followed by same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. A cohort of 50 eggs from each regimen constituted one replicate.

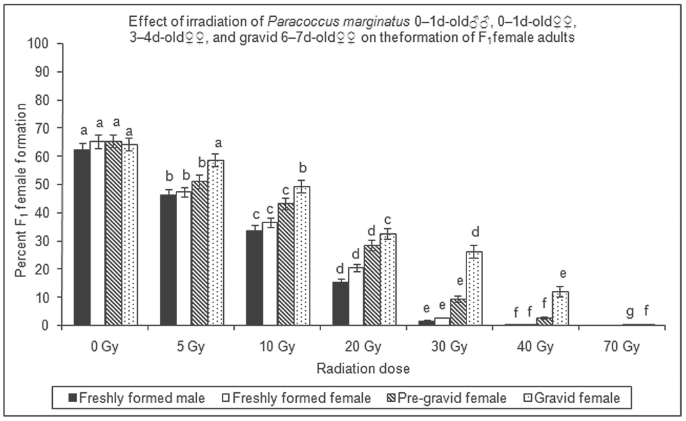

Fig. 13. Percent F_1 female adult formation in *Paracoccus marginatus*, when parent generation adults of different age groups were irradiated and then crossed with non-irradiated members of the opposite sex. No F_1 adult females were produced in crosses in the 40 Gy treatment that involved either irradiated freshly formed 0–1 d-old adult males, or irradiated freshly formed 0–1 d-old females. Moreover in the 70 Gy treatment, crosses involving irradiated pre-gravid 3–4 d-old females and irradiated gravid 6–7 d-old females failed to produce F_1 adult females. In these cases the corresponding bars are absent. Means followed by same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. A cohort of 50 eggs from each regimen constituted 1 replicate.

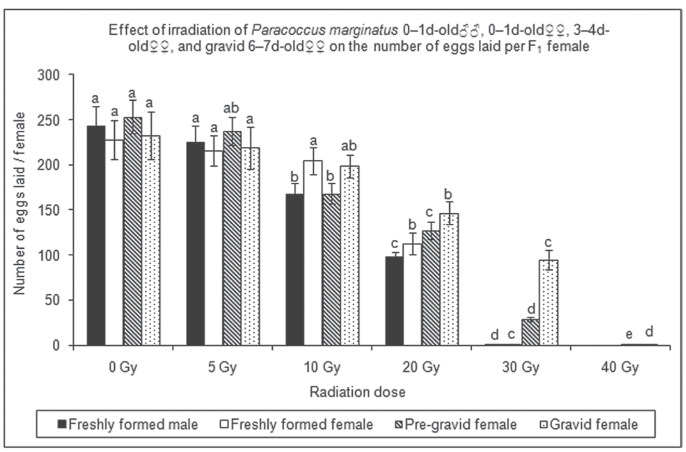

Fig. 14. Number of F_2 eggs laid by *Paracoccus marginatus*, F_1 females when parent generation adults of different age groups were irradiated and then crossed with members of the opposite sex, followed by the self-crossing of F_1 adults. No eggs were laid in the 30 Gy treatment by F_1 female descendants of irradiated freshly formed 0–1 d-old males and irradiated freshly formed 0–1 d-old females, nor in the 40 Gy treatment by F_1 female descendants of either irradiated pre-gravid females 3–4 d-old or irradiated gravid 6–7 d-old females. In these cases the corresponding bars are absent. Means followed by same letter within a regimen are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average data of 7 mated females in each regimen constituted 1 replicate.

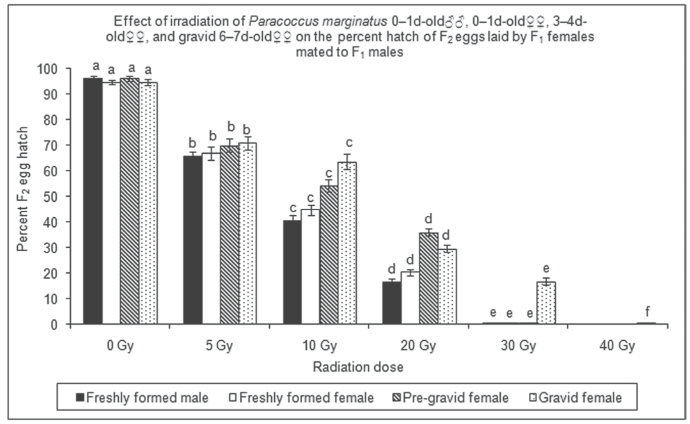

Fig. 15. Percent hatch of F_2 eggs of *Paracoccus marginatus*, when parent generation adults of different age groups were irradiated and then crossed with members of the opposite sex, followed by the self-crossing of F_1 adults. None of the eggs hatched that were laid in the 30 Gy treatment by F_1 descendants of irradiated pre-gravid females 3–4 d-old females, nor in the 40 Gy treatment by F_1 descendants of irradiated gravid females 6–7 d-old females. In these cases the corresponding bars are absent. Means followed by same letter within a level of sterility are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates = 10. Average percent egg hatch in 7 pairs of adults in each regimen constituted 1 replicate.

2002). Present study showed that irradiation with 200 or 400 Gy did not cause any apparent loss in weight and change in color of treated papayas as compared to the control. Miller & McDonald (1999) did not report any weight loss of papaya at 675 Gy. Pimentel & Walder (2004) reported no change in color of papaya irradiated with 750 Gy. Further, our results indicated that irradiation had no evident effect on the percentage of total soluble solids, titratable acidity and pH in irradiated papaya. Similar findings on papaya were reported regarding no impact of irradiation on total soluble solids by D'Innocenzo & Lajolo (2001), and on pH by Pimentel & Walder (2004). Likewise, Miller & McDonald (1999) found little or no effect of irradiation on total soluble solids and pH of treated papaya. The present study did not show any apparent radiation induced changes in vitamin C and β-carotene content as compared to the control. Similarly, Purwanto & Maha (1998) did not observe significant changes in level of vitamin C and β-carotene of papaya irradiated with 500 Gy. Therefore, we are confident that a dose in the range of 200–400 Gy will not degrade the nutritional quality, appearance and weight of papaya fruits after harvest.

Based on the data generated in this study and the above discussion we recommend that 200 Gy be considered as an effective and safe phytosanitary irradiation dose to facilitate the infestation free agricultural trade of commodities that are hosts of *P. marginatus*.

Table 3. Physico-chemical and nutritional qualities of irradiated papaya following storage for 7 d at 5 °C. None of the values in the columns differed significantly from each other at $P < 0.05$ (ANOVA, $n = 5$; df = 2,12).

	Fruit quality factors								
		Color characteristics[§]							
Radiation dose	Weight loss (%)	'L' value	'a' value	'b' value	Total soluble solids (%)	Titratable acidity (%)	pH	Vitamin C (mg/100g)	β-Carotene (mg/100g)
0 Gy	6.56 ± 0.72	56.2 ± 2.29	13.5 ± 3.51	45.8 ± 1.99	10.51 ± 0.52	0.17 ± 0.03	5.23 ± 0.03	33.9 ± 2.81	2.20 ± 0.67
200 Gy	6.79 ± 1.15	56.2 ± 1.50	11.9 ± 2.02	46.3 ± 1.50	9.12 ± 0.49	0.13 ± 0.03	5.20 ± 0.06	31.5 ± 2.20	1.92 ± 0.25
400 Gy	8.30 ± 1.42	55.1 ± 2.45	11.5 ± 2.41	45.7 ± 3.39	10.60 ± 0.80	0.17 ± 0.03	5.17 ± 0.12	32.5 ± 1.90	2.30 ± 0.31
F-value	*F* = 2.76	*F* = 3.23	*F* = 2.97	*F* = 1.82	*F* = 2.14	*F* = 2.62	*F* = 2.88	*F* = 1.82	*F* = 2.48

[§]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).

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