Phytosanitary irradiation against *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae)

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Abstract

The purpose of this research was to determine the efficacy of ionizing irradiation as a phytosanitary treatment against the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), a species having major pest status in the Indian subcontinent and elsewhere. Various ontogenetic stages of *M. hirsutus* were exposed to gamma radiation at different doses to assess the metamorphic disruptions leading to lethality and/or adult sterility. Effective treatment doses ($ED_{99.9}$) ranged from 28 to 371 Gy where induced lethality due to metamorphic arrest and prevention of adult formation were used as the measure of efficacy against the various irradiated pre-imaginal stages. Further, $ED_{99.9}$ doses ranging from 30 Gy for the radio-sensitive egg stage to 88 Gy for the N_3-female—the most resistant pre-imaginal stage—were sufficient to induce sexual sterility in the parent generation (P) adults due to a carry-over effect of sub-lethal doses administered to the pre-imaginal stages. The $ED_{99.9}$ values to induce sterility in adult females ranged from 112 to 235 Gy depending on the age of the irradiated adult females. Further, the effect of sub-sterilizing irradiation doses given to P generation adults was ascertained on the viability of their F_1 progeny. $ED_{99.9}$ doses in the range of 37–79 Gy administered to P adult females belonging to various age groups were sufficient to induce the complete prevention of F_1 female adult formation. A gamma radiation $ED_{99.9}$ dose in the range of 30–52 Gy administered to P adults—depending on which of the various age groups was irradiated—induced complete F_1 sterility. In addition, this study also examined sensory characteristics like color and weight loss, and physico-chemical features like total soluble solids, titratable acidity, pH, vitamin C and ß-carotene contents of irradiated table grapes, *Vitis vinifera* L.(Vitales: Vitaceae)—a major host of *Maconellicoccus hirsutus*—that were found to be unaffected by phytosanitary irradiation at the highest dose tested, 400 Gy. The results indicated that at least 371 Gy would be required to cause metamorphic inhibition in the most radio-resistant pre-imaginal N_3 female stage and ~240 Gy to completely sterilize the most radio-resistant adult gravid female stage. However, the phytosanitary dose could be further reduced to less than 200 Gy if the criterion of efficacy would be the prevention of the formation of F_1 N_2 nymphs. This criterion warrants pilot testing to validate the final gamma irradiation dose to be employed for phytosanitary irradiation. This range of quarantine doses would also ensure no damage to the nutritional quality of irradiated foods.

Key Words: metamorphic arrest; adult formation prevention; prevention of F_1 N_2 nymphs; pink hibiscus mealybug; phytosanitary irradiation; quarantine treatment; grape

Resumen

El propósito de esta investigación fue determinar la eficacia de la radiación ionizante como tratamiento fitosanitario contra la cochinilla rosada del hibisco, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), una especie que tiene el estatus de una plaga importante en el subcontinente indio y en otros lugares. Varios estadios ontogenéticos de *M. hirsutus* fueron expuestos a la radiación gamma en diferentes dosis para evaluar las interrupciones metamórficas que conducen a la mortalidad y/o esterilidad del adulto. El rango de las dosis eficaces ($DE_{99.9}$) de tratamiento fue de 28 hasta 371 Gy donde se utilizo la letalidad inducida debido a un paro metamórfico y a la prevención de la formación de adultos como medida de la eficacia contra diversos estadios pre-imaginales irradiados. Además, las dosis $DE_{99.9}$ entre 30 Gy para el estadio de huevo con el radio mas sensible hasta 88 Gy para la hembra N_3—con el estadio pre-imaginal más resistente—fueron suficientes para inducir la esterilidad sexual en los adultos debido a un efecto de arrastre de dosis subletales administrados a los estadios pre-imaginales. El valor $DE_{99.9}$ varia desde el 112 hasta 235 Gy para inducir la esterilidad en hembras adultas irradiadas dependiendo de la edad de la adulta irradiada. Además, se determinó el efecto de las dosis de irradiación de sub-esterilización dadas a los de adultos de la generación pariente (P) sobre la viabilidad de su progenie F_1. La dosis $DE_{99.9}$ de 37 a 79 Gy administrada a las hembras adultas P pertenecientes a diferentes grupos de edad fueron suficientes para inducir la prevención completa de la formación de hembras adultas de F_1. Una dosis $DE_{99.9}$ de radiación gamma de 30 a 52 Gy administrada a las adultas P—dependiendo de cuál de los diferentes grupos en edad fue irradiado —indujo la esterilidad completa de la F_1. Además, este estudio también examinó las características sensoriales como el color y la pérdida de peso, y las características físico-químicas como sólidos solubles totales, acidez titulable, pH, contenido de vitamina C y beta-caroteno de las uvas de mesa *Vitis vinifera* L. (Vitales: Vitaceae) irradiadas—un hospedero importante de *Maconellicoccus hirsutus*—que se encontró que no se vean afectados por la irradiación fitosanitaria a la dosis más alta probada, 400 Gy. Los resultados indicaron que al menos 371 Gy sería necesario para causar la inhibición metamórfica en el estadio N_3 más radio-resistente de la hembra pre-imaginal y ~ 240 Gy para esterilizar completamente el estadio más radio-resistente de la hembra adulta grávida. Sin embargo, la dosis fitosanitaria podría reducirse a menos de 200 Gy si el criterio de la eficacia sería la prevención de la formación de ninfas N_2 de la F_1. Este criterio merece una prueba piloto para validar la dosis final de la irradiación gamma a emplear para la irradiación fitosanitaria. Esta rango de dosis de cuarentena también aseguraría el no daño a la calidad nutricional de los alimentos irradiados.

Palabras Clave: arresto metamórfica; prevención de la formación de adultos; prevención de ninfas F_1 N_2; cochinilla rosada; irradiación fitosanitaria; tratamiento de cuarentena; uva

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The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcideae) is an economic pest of a wide variety of plants in many tropical and subtropical regions of the world (EPPO 2005). It is native to southern Asia and has spread to other parts of the world including India (Rao et al. 1993; Muralidharan & Badaya 2000). It is present, but not widespread in the Americas and is absent in Europe. In India, it is a major pest of grape, *Vitis vinifera* L. (Vitales: Vitaceae), reducing yields by as much as 50 to 100% (Meyerdirk 1996). *Maconellicoccus hirsutus* has a recorded host range of > 300 plants (APHIS 1998) and was added in 2003 to the European and Mediterranean Plant Protection Organization (EPPO) A1 action list (EPPO 2004), thus at-risk EPPO member countries are recommended to regulate *M. hirsutus* as a quarantine pest.

Maconellicoccus hirsutus and other mealybugs can be found in cracks, crevices, and other protected areas of a host. This behavior can reduce efficacy of some postharvest treatments and make inspection difficult. For example, insecticidal coatings and hot water treatment show reduced efficacy in treatment against mealybugs infesting limes because of the protection provided by the fruit's calyx (Gould & McGuire 2000). Additionally, *M. hirsutus* ovisacs can provide protection from chemicals and insulation from heat.

Irradiation is a promising phytosanitary treatment because its overall efficacy against many types of insects on several host plants has been well documented (Hallman 2011). Irradiation leaves no toxic chemical residues, and generally is less phytotoxic than heat, cold, or fumigation treatments. A phytosanitary treatment is a procedure for killing, inactivating or removing pests, or for rendering pests infertile or devitalized (IPPC 2009). The purpose of the phytosanitary treatment is to prevent an arthropod pest in a commodity being exported to a region or country—where the pest does not exist and where it could be damaging—from establishing a population therein. Generic irradiation doses can be developed to target a broad group of pests, regardless of host crop. However, research findings on the radiation tolerance of mealybugs are still too limited.

The present study was undertaken to assess the differential radio-sensitivity of various ontogenetic stages of *M. hirsutus* and to estimate an efficacious dose that could serve as a phytosanitary irradiation (PI) treatment against this pest. The information will also contribute to a generic dose against mealybugs (Hofmeyr et al. 2016). In addition, the impacts of gamma irradiation on quality characteristics of fresh table grape, a potential host for this species, were evaluated.

Materials and Methods

REARING OF THE PINK HIBISCUS MEALYBUG

The culture of *M. hirsutus* was initiated with ovipositing gravid female adults obtained from agricultural fields in New Delhi. The insect was reared on sprouted potato (*Solanum tuberosum* L.; Solanales: Solanaceae) in a rearing jar filled up to ¼th capacity with sterilized, moist soil. As *M. hirsutus* is a sexually reproducing species, about 4-5 adult pairs were placed on one sprouted potato in a culture jar. The mated female started egg laying within 6–7 d. Each female laid a cluster of 300–350 eggs, which hatched within 6–7 d. The female develops through 3 nymphal instars, and the male develops through 4 instars (Fig. 1). After hatching the crawlers (first instar nymph or N_1) showed affinity towards sprouts. When they molted to the N_2 (second instar nymph) the latter started dispersing. At this point in time, they were transferred to fresh sprouted potatoes. Likewise, on their next molting to the N_3, these nymphs were transferred to fresh food. The longevity of males was found to be relatively much shorter than that of females.

IRRADIATION TREATMENT

Irradiation of various life stages of *M. hirsutus* was conducted at the Radiobiological Unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defense, Delhi using a ^{60}Co source (Gamma–5000 irradiator, BRIT, BARC, Trombay, Mumbai, Maharashtra, India). The dose rate was 1.5–1.8 kGy/h over the span of the research. A dose range of 5–500 Gy was used for conducting radio-biological evaluations on various ontogenetic metamorphic stages of the mealybugs, including adults. Fricke dosimetry was performed on the gamma cell to establish the dose distribution and authenticate gamma doses absorbed. Dosimetry of this gamma cell was confirmed via a thermocouple chip provided by the International Atomic Energy Agency (IAEA).

Effects of Gamma Irradiation of Various Pre-imaginal Stages and Adults of *Maconellicoccus hirsutus*

Effects of Gamma Irradiation on Metamorphosis and Development: The effects of gamma irradiation were studied on the metamorphosis and development of pre-imaginal stages of *M. hirsutus* irradiated as 0–1 d-old eggs, crawlers (N_1), second instar nymphs (N_2), third instar female nymphs (N_3-♀), and third and fourth instar male nymphs (N_3♂

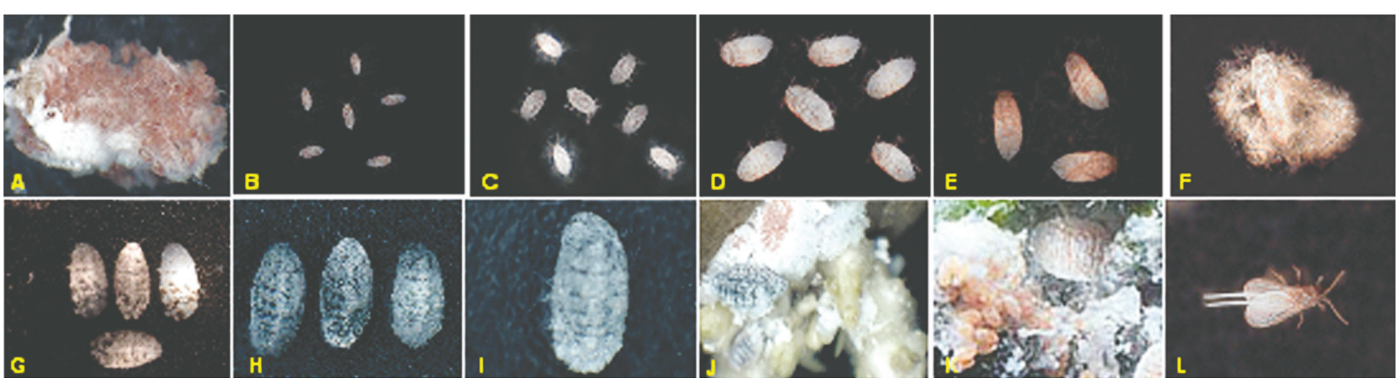

Fig. 1. Life stages of *Maconellicoccus hirsutus:* A. Eggs; B. First instar nymphs (crawlers; N_1); C. Second instar nymphs (N_2); D. Third instar female nymphs (N_3-♀); E. Third instar male nymphs (N_3-♂); F. Fourth instar male nymph (N_4-♂); G. Female adults 0–1d-old; H. Pre-gravid female adults 3–4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

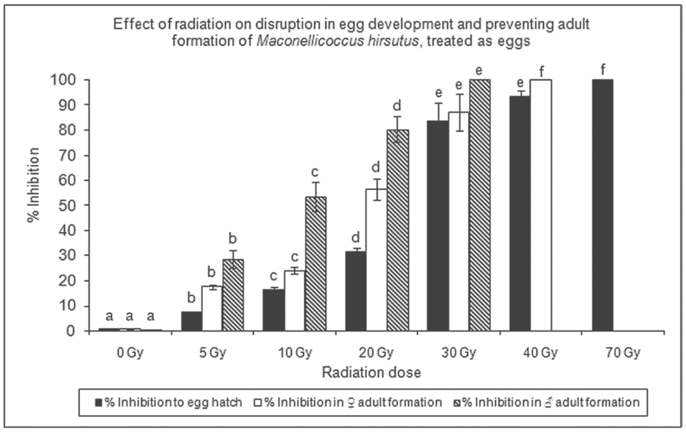

Fig. 2. Prevention of egg hatch and adult of *Maconellicoccus hirsutus* irradiated as eggs. Since no male progeny were produced at ≥ 40 Gy and neither male nor female progeny at 70 Gy, the corresponding bars are absent. Means followed by the same letter within each of the 3 molts are not significantly 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, $n = 10$. Fifty eggs within each treatment constituted 1 replicate.

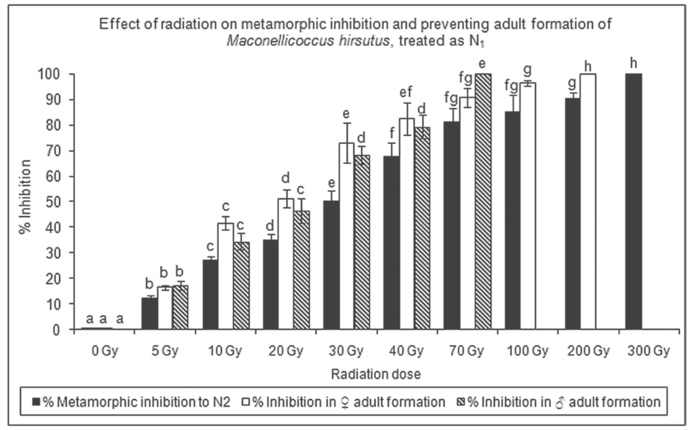

Fig. 3. Prevention of N_2 nymphs and adults of *Maconellicoccus hirsutus* irradiated as N_1 nymphs. Since no male progeny were produced at doses ≥ 100 Gy and no female progeny at 300 Gy, the corresponding bars are absent. Means followed by same letter within each of the 3 molts are not significantly 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, $n = 10$. Fifty N_1 nymphs within each treatment constituted 1 replicate.

and N_4♂, respectively) (Fig. 1) with a dose range of 5–500 Gy. Non-irradiated controls (0 Gy) were also included. About 50 eggs, a cohort of 50 N_1 crawlers, and a cohort of 12–15 nymphs of each of the remaining immature stages each constituted 1 replicate. The forms in each of these replicates were evaluated daily for further metamorphosis and adult formation in response to irradiation.

Effect of Gamma Irradiation of Pre-imaginal Stages on the Reproduction of Adult Progeny: The sterilizing potential of adults derived from irra-

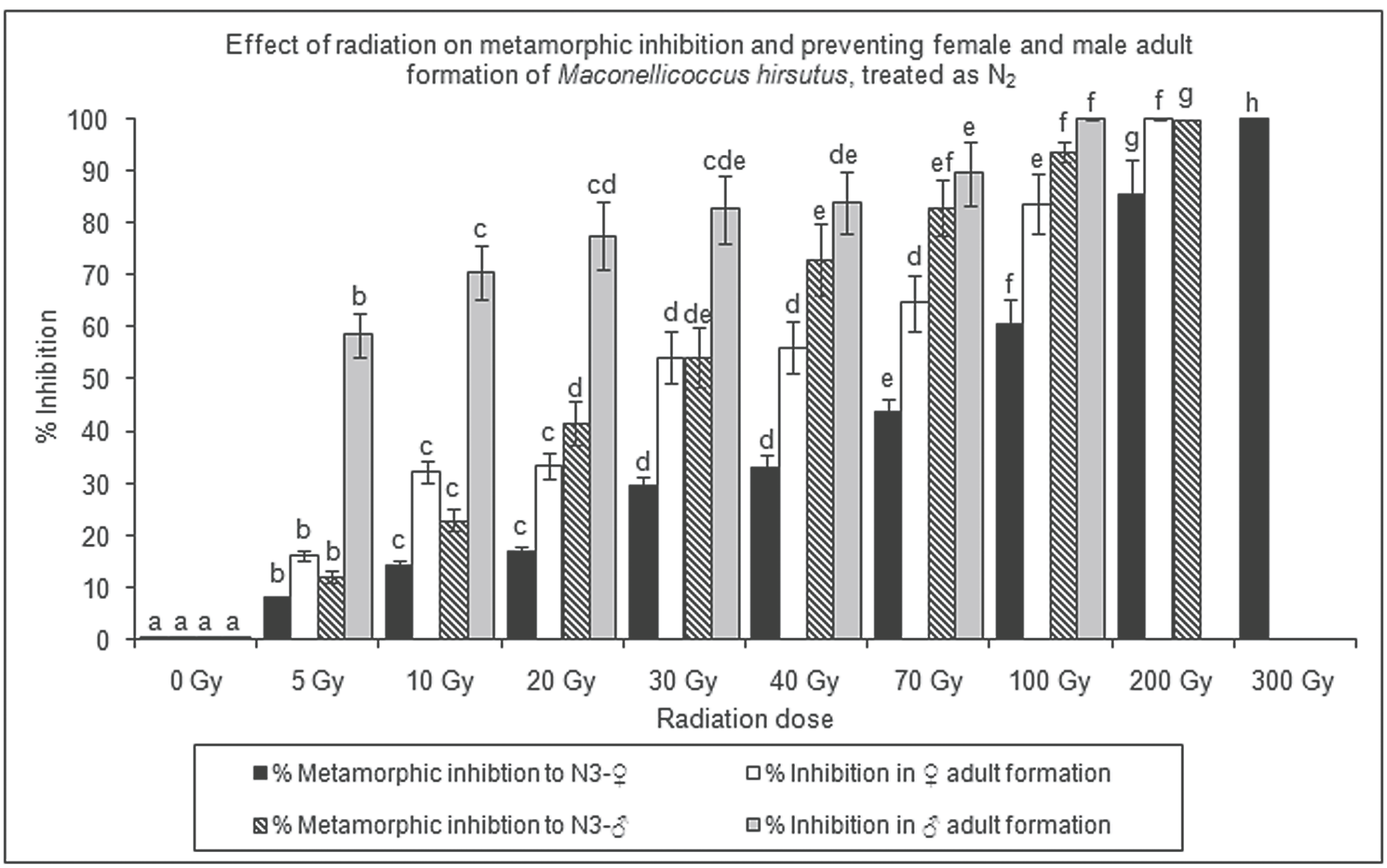

Fig. 4. Prevention of N_3 nymphs and adults of *Maconellicoccus hirsutus* irradiated as N_2 nymphs. Since no male progeny at ≥ 200 Gy and no female progeny at 300 Gy were produced, the corresponding bars are absent. Means followed by same letter within each of the 4 molts are not significantly different 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.

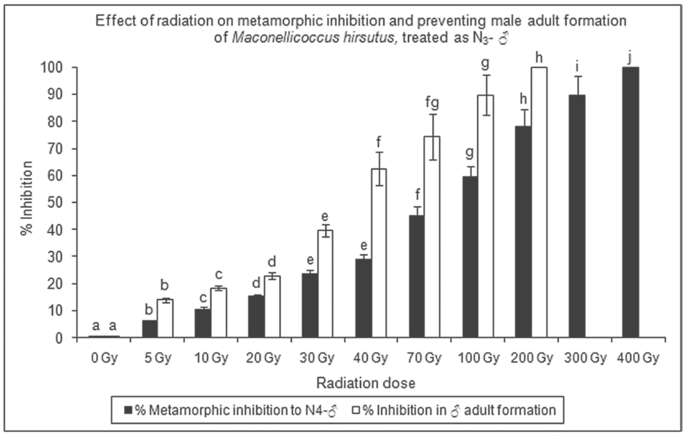

Fig. 5. Prevention of N_4 nymphs and adults of *Maconellicoccus hirsutus* irradiated as N_3-♂ nymphs. Since no male adult was produced at ≥ 300 Gy, the corresponding bars are absent. Means followed by same letter within each of the 2 molts are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Observations from 15 N_3-♂ nymphs within each treatment constituted 1 replicate).

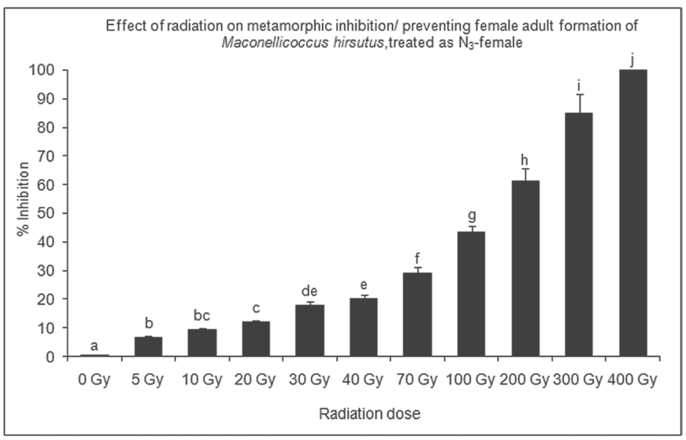

Fig. 6. Prevention of adult of *Maconellicoccus hirsutus* irradiated as N_3-♀ nymphs. Means followed by same letter are not significantly different at $P <$ 0.05 level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates, n = 10. Observations from 15 N_3-♀ nymphs constituted 1 replicate.

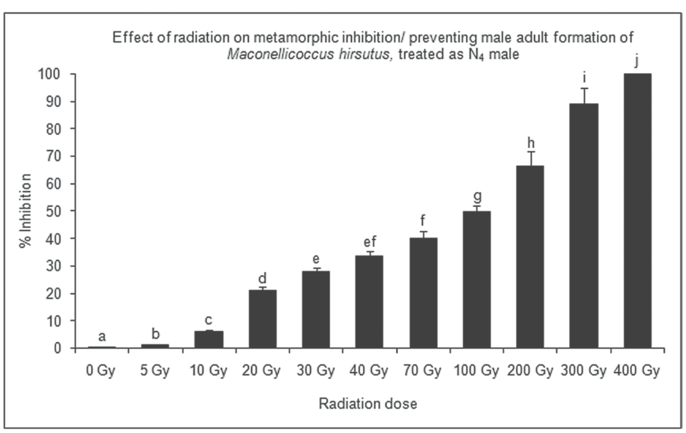

Fig. 7. Prevention of adults of *Maconellicoccus hirsutus* irradiated as N_4-♂ nymphs. Means followed by same letter are not significantly different at $P <$ 0.05 level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates, n = 10. Observations from 15 N_4- ♂ nymphs constituted 1 replicate.

diated pre-imaginal stages was evaluated daily. Reproduction of survivors of irradiated eggs, N_1, and N_2 was assessed via self-crosses (I♀ × I♂), as the sexual gender could not be distinguished in those stages. Older nymphal stages were placed with unirradiated members of the opposite sex (U♀ × I♂ and I♀ × U♂) when they became adults. Reproduction was evaluated from a mean of 7 individual pairs per replicate. Effective doses (ED) for 50, 90, and 99.9% (i.e., ED_{50}, ED_{90}, and $ED_{99.9}$) inhibition of metamorphic development, adult formation, and sterility in resultant adults from irradiated pre-imaginal stages were estimated with regression analysis.

Effect of Gamma Irradiation of P Adults on their own Reproduction: 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 members of the opposite sex. Oviposition and egg viability in mating crosses of each of the irradiated imaginal stages in different age groups were ascertained, and 7 individual pairs constituted a replicate.

Effect of Gamma Irradiation of P Adults on the Reproduction of their F_1 Progeny: The effect of gamma irradiation was further evaluated on the development of F_1 adults derived from parents irradiated with 5–200 Gy as adults. A cohort of 50 eggs was taken per replicate for assessing development and reproduction of the F_1 progeny in each treatment. The ED_{50}, ED_{90}, and $ED_{99.9}$ of F_1 male and F_1 female adult formation and for inducing sterility in F_1 adults as a consequence of irradiation of the parent generation were estimated via regression analysis.

Physicochemical and Nutritional Quality Analysis of Irradiated Table Grapes

A range of physicochemical features (weight loss, color change, acidity, and pH) and vitamin C and β-carotene as nutritional indices of grapes subjected to radiation doses in the range 200-400 Gy and stored for 7 d at 5 °C were examined. The percentage weight loss of the irradiated grapes following 1 wk of storage at 5 °C was calculated. The color properties of the grapes were measured by using a X-Rite CA22 Spectrophotometer (X-Rite Inc., Grand Rapids, Michigan, USA).Color was recorded using the CIE-Lab, 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 were determined using an Abbemat refractometer (Anton Paar Instruments, Seelze-Letter, Germany) at 20 ºC and expressed in percentage by mass of total soluble solids (DGHS 2005). Acidity was determined by titrating a fruit sample diluted with distilled water using a 0.1 N NaOH solution using a phenolphthalein indicator. The percentage of titratable acidity as citric acid was calculated (AOAC 2012), and pH was determined by a pH meter (Orion-420, ThermoScientific, Waltham, Massachusetts, USA) using homogenized grapes (DGHS 2005). Vitamin C was extracted and titration performed using 2,6-dichlorophenol indophenol dye (AOAC 2012). β-Carotene was extracted and the quantification was carried out by high performance liquid chromatography (Shimadzu Scientific Instruments, Kyoto, Japan) and detected by absorbance at 436 nm. The concentration of carotene in the samples was determined by comparing the sample area of the peak with the standard β-carotene.

STATISTICAL ANALYSES

The data obtained in the above experiments 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 with probit analysis (PoloPlus, Petaluma, California) of the dose-response data. The data were subjected to appropriate analysis of variance (ANOVA). A probability of $P < 0.05$ was

Table 1. Prevention of molting to the subsequent stage in *Maconellicoccus hirsutus* by gamma irradiation of the various pre-imaginal stages.

Pre-imaginal stage irradiated and the blocked succeeding stage	Estimated dose[a] (Gy) to prevent 50, 90, and 99.9% from reaching the succeeding stage			Linear regression equation	Slope
	ED_{50}	ED_{90}	$ED_{99.9}$		
Egg to N_1	26 ± 2a	51 ± 4a	57 ± 5a	y = 1.626x + 6.836 R^2 = 0.832	1.626 ± 0.13a
N_1 to N_2	57 ± 5c	194 ± 16c	228 ± 18c	y = 0.291x + 33.34 R^2 = 0.658	0.291 ± 0.024cd
N_2 to N_3-♂	42 ± 3b	104 ± 8b	119 ± 9b	y = 0.646x + 22.96 R^2 = 0.795	0.646 ± 0.05b
N_2 to N_3-♀	105 ± 7d	224 ± 15c	253 ± 17c	y = 0.337x + 14.44 R^2 = 0.913	0.337 ± 0.022bc
N_3♂ to N_4♂	141 ± 10e	303 ± 22d	343 ± 25d	y = 0.247x + 15.10 R^2 = 0.892	0.247 ± 0.018d
N_3♀ to Adult ♀	168 ± 12e	331 ± 24	371 ± 27d	y = 0.245x + 8.927 R^2 = 0.975	0.245 ± 0.018d
N_4♂ to Adult ♂	150 ± 11e	316 ± 22d	357 ± 25d	y = 0.24x + 13.81 R^2 = 0.914	0.241 ± 0.017d
F-value	*F* = 81.2	*F* = 48.5	*F* = 36.9		*F* = 21.7

[a]ED_{50}, ED_{90}, and $ED_{99.9}$ are the effective doses that induced 50%, 90%, and 99.9% mortality, respectively. Means followed by same letter within a column are not significantly different at $P < 0.05$ level (ANOVA, df = 6,63 followed by LSD post-test). Number of replicates, $n = 10$. Fifty eggs, or 50 neonates for the N_1, and 12–15 nymphs each for the N_2, N_3♂, N_3♀ and N_4♂ instars constituted a replicate.

considered significant. Percentage data was transformed using arcsine √x value before ANOVA. Means were separated at the 5% significance level by least significant difference (LSD) test among the different treatments (SPSS 22.0, Armonk, New York, USA).

Results

EFFECT OF GAMMA RADIATION ON THE STAGES OF *MACONELLICOCCUS HIRSUTUS*

Doses of Radiation Applied to Various Pre-imaginal Stages that Inhibited Metamorphic Change

Doses of 30, 40, and 70 Gy applied to 0–1 d-old eggs completely inhibited the formation of adult males and females and egg hatch, respectively (Fig. 2). Females were more radiotolerant than males as measured by inhibition of development of irradiated eggs and instars. Doses of 70, 200, and 300 Gy applied to the N_1 completely inhibited formation of adult males, adult females, and the N_2, respectively (Fig. 3). Doses of 100, 200, 200, and 300 Gy applied to the N_2 completely inhibited formation of adult males, adult females, N_3

Table 2. Doses of gamma radiation of the various pre-imaginal stages of *Maconellicoccus hirsutus* required to prevent 50, 90 or 99.9% of the irradiated nymphs from developing into adults.

Pre-imaginal stage irradiated to prevent adult formation	Estimated dose[a] (Gy) to prevent 50, 90, and 99.9 % from reaching adult stage			Linear regression equation	Slope
	ED_{50}	ED_{90}	$ED_{99.9}$		
Egg to adult ♂	12.2 ± 0.7a	24.6 ± 1.5a	27.7 ± 1.7a	y = 3.229x + 10.52 R^2 = 0.9509	3.229 ± 0.19a
Egg to adult ♀	18.5 ± 1.5b	33.9 ± 2.8b	37.7 ± 3.1b	y = 2.591x + 2.157 R^2 = 0.9857	2.591 ± 0.21b
N_1 to adult ♂	25.4 ± 1.4c	54.2 ± 3.1c	61.3 ± 3.5c	y = 1.391x + 14.67 R^2 = 0.9126	1.391 ± 0.07c
N_1 to adult ♀	27.7 ± 2.1c	96.0 ± 7.2d	113 ± 8.5d	y = 0.586x + 33.75 R^2 = 0.6698	0.586 ± 0.043d
N_2 to adult ♂	38.0 ± 3.0d	79.5 ± 6.2d	90 ± 7d	y = 0.626x + 48.9 R^2 = 0.4932	0.626 ± 0.048d
N_2 to adult ♀	54.9 ± 4e	144 ± 12e	167 ± 13e	y = 0.447x + 25.51 R^2 = 0.8322	0.447 ± 0.036e
N_3♂ to adult ♂	59.2 ± 4e	140 ± 9e	159 ± 11e	y = 0.497x + 20.59 R^2 = 0.7826	0.497 ± 0.034de
N_3♀ to adult ♀	168 ± 12f	331 ± 24f	371 ± 27f	y = 0.2449x + 8.9272 R^2 = 0.9748	0.245 ± 0.018f
N_4♂ to adult ♂	150 ± 11f	316 ± 22f	357 ± 23f	y = 0.241x + 13.814 R^2 = 0.9142	0.241 ± 0.017f
F-value	*F* = 242.8	*F* = 157.9	*F* = 132.8		*F* = 216.7

[a]ED_{50}, ED_{90} and $ED_{99.9}$ are the effective doses that prevented 50%, 90%, and 99.9%, respectively, from reaching the adult stage. Means followed by same letter within a column are not significantly different at $P < 0.05$ level (ANOVA, df = 8,81, 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.

Table 3. Reproductive performance—number of eggs laid and percent that hatched—of *Maconellicoccus hirsutus* females irradiated while they were still in various pre-imaginal stages. When the adults used in the crosses had developed from either irradiated eggs, irradiated N_1 nymphs, or irradiated N_2 nymphs, the irradiated individuals were self-crossed. However, when the adults used in the crosses had developed from irradiated last instars (N_3 female, N_3 and N_4 males), then the irradiated individuals were each mated with a non-irradiated counterpart of the opposite sex.

Stage of parent irradiated and Cross; (I = irradiated, U = non-irradiated)	Reproductive parameters	Radiation doses applied to parent generation										F-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	100 Gy	200 Gy	300 Gy	
Egg (I♀ × I♂)	Eggs laid per female	306.5 ± 35.2a	287.1 ± 22.3ab	230.4 ± 16.7b	145.4 ± 15.3c	0d	—	—	—	—	—	*F* = 245.8; df = 4,45
	Egg hatch (%)	95.7 ± 0.83a	82.7 ± 3.4b	72.9 ± 3.28b	30.4 ± 2.6c	0d	—	—	—	—	—	*F* = 108.1; df = 4,45
N_1 (I♀ × I♂)	Eggs laid per female	377.6 ± 17.8a	316.4 ± 22.9ab	292.6 ± 19.7bc	257.4 ± 22.7c	179.6 ± 14.5d	81.9 ± 4.2e	0f	—	—	—	F = 134.6; df = 6,63
	Egg hatch (%)	98.2 ± 0.42a	86.1 ± 3.7ab	78.9 ± 4.2b	54.3 ± 2.4c	24.4 ± 1.6d	0e	—	—	—	—	*F* = 295.4; df = 5,54
N_2 (I♀ × I♂)	Eggs laid per female	367.2 ± 13.7a	348.5 ± 18.6a	319.1 ± 21.6a	253.8 ± 15.1b	193.0 ± 8.4c	118.4 ± 5.7d	98.2 ± 4.5e	0f	—	—	F = 714.7; df = 7,72
	Egg hatch (%)	96.5 ± 0.67a	88.1 ± 2.6ab	83.1 ± 3.2b	60.9 ± 3.7c	39.8 ± 2.1d	20.3 ± 1.4e	0f	—	—	—	*F* = 239.9; df = 6,63
N_3-♂ (U♀ × I♂)	Eggs laid per female	349.7 ± 21.4a	342.3 ± 18.7a	339.7 ± 21.5a	330.6 ± 23.4b	221.0 ± 18.6c	179.2 ± 14.6d	105.6 ± 9.8e	73.4 ± 6.3f	0g	—	F = 664.2; df = 8,81
	Egg hatch (%)	97.1 ± 0.47a	87.4 ± 4.6ab	80.2 ± 2.4b	65.4 ± 2.9c	52.5 ± 2.6d	23.6 ± 2.0e	0f	—	—	—	*F* = 218.2; df = 6,63
N_3-♀ (U♀ × I♂)	Eggs laid per female	385.4 ± 29.8a	354.5 ± 26.9ab	335.1 ± 27.5ab	296.9 ± 20.3b	244.1 ± 18.9c	192.3 ± 13.7d	128.6 ± 10.4e	90.6 ± 6.5f	36.5 ± 2.7g	0h	F = 824.8; df = 9,90
	Egg hatch (%)	98.8 ± 0.36a	90.3 ± 2.7b	85.6 ± 2.6b	71.9 ± 3.6c	50.8 ± 2.4d	31.2 ± 2.1e	12.9 ± 1.5f	0g	—	—	*F* = 400.6; df = 7,72
N_4-♂ (U♀ × I♂)	Eggs laid per female	355.2 ± 23.1a	350.6 ± 20.6a	325.8 ± 25.9ab	308.9 ± 18.9b	224.5 ± 19.4c	197.8 ± 12.4d	164.9 ± 13.8e	82.1 ± 4.5f	47.9 ± 2.4g	0h	F = 561.4; df = 9,90
	Egg hatch (%)	95.8 ± 0.68a	87.2 ± 4.1ab	81.1 ± 3.3b	66.2 ± 2.9c	55.4 ± 2.6d	25.6 ± 1.3e	3.8 ± 0.6f	0g	—	—	*F* = 293.3; df = 7,72

Means ± SE followed by same letter within a row 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 table are back transformations. Number of replicates = 10. Average data of 7 pairs of adult females and males within each treatment regimen constituted 1 replicate.

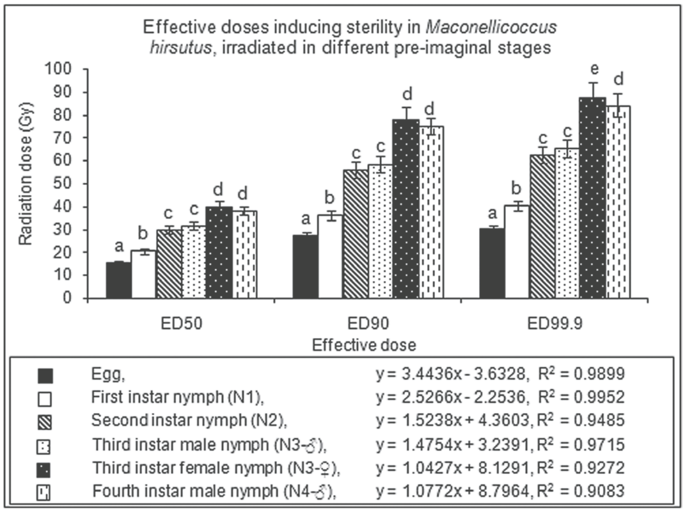

Fig. 8. Estimated doses for preventing 50, 90, and 99.9% hatch of F_1 eggs in *Maconellicoccus hirsutus* irradiated in different pre-imaginal stages. Means followed by same letter within a sterility level are not significantly different at P < 0.05 level (ANOVA followed by LSD post-test). Number of replicates, n = 10.

males, and N_3 females, respectively (Fig. 4). Doses of 200 and 400 Gy to N_3 males were required to completely inhibit formation of adult males and N_4 (pupal cocoon) males, respectively (Fig. 5), while 400 Gy was required to prevent N_3 females as well as N_4 males from reaching the adult stage (Figs. 6 and 7).

Table 1 presents estimated doses to prevent molting to the subsequent stage when various pre-imaginal stages of *M. hirsutus* were irradiated. In general doses increased as stage of development that was irradiated increased, and the molting of males was prevented with lower doses than the molting of females. The increase of the dose between ED_{50} and $ED_{99.9}$ varied about 2.2-fold to prevent eggs from becoming first instars to 4-fold to prevent first instars from becoming second instars. The $ED_{99.9}$ to prevent eggs from becoming first instars was 57 ± 5 Gy, while that to prevent the N_3♀ from becoming adults was 371 ± 27 Gy.

Doses of Radiation Applied to Pre-imaginal Stages that Prevented Adult Formation

Table 2 presents estimated doses applied to the various pre-imaginal stages that prevented development to proceed to the formation to the adult stage. In general, the dose decreased as the number of molts between the stage irradiated and the adult stage increased, and males were prevented from reaching the adult stage at lower doses than females.

Sterility when Irradiated in Different Pre-imaginal Stages

Table 3 presents the numbers of eggs laid and percentages of F_1 eggs that hatched at various doses when *M. hirsutus* were irradiated in different pre-imaginal stages. The dose required to prevent egg hatch increased as the irradiated stage increased (Fig. 8), as demonstrated by a significant interaction between age of mealybug and irradiation dose for inducing prevention of egg hatch (F = 8.42; df = 5, 35 for stages; F = 14.7; df = 5,35 for doses; F = 6.14; df = 25,35; P < 0 .05 among interactions). Fig. 8 presents the estimated doses for inducing 50, 90, and 99.9 % prevention of egg hatch for the various pre-imaginal stages.

Table 4. Reproductive performance—number of eggs laid and percent that hatched—of *Maconellicoccus hirsutus* irradiated as adults in different age groups and crossed with non-irradiated members of the opposite sex.

Imaginal stage irradiated	Reproductive parameters	Radiation doses												*F*-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy	
Freshly formed male (0-1d-old)	Eggs laid per female	368.2 ± 38.7a	352.0 ± 26.1a	348.7 ± 28.1a	332.6 ± 24.6a	325.2 ± 27.0a	319.6 ± 21.3a	294.1 ± 13.8a	148.6 ± 8.2b	95.8 ± 4.9c	0d	—	—	F = 895.1; df = 9,90
	Egg hatch (%)	98.7 ± 0.24a	93.1 ± 1.7ab	87.2 ± 3.4b	76.3 ± 3.2c	49.7 ± 3.0d	35.4 ± 1.6e	16.7 ± 1.1f	0g	—	—			*F* = 406.2; df = 7,72
Freshly formed female (0-1d-old)	Eggs laid per female	354.6 ± 24.9a	349.8 ± 26.7a	347.9 ± 34.9a	335.2 ± 27.4a	329.5 ± 21.4 a	315.9 ± 26.3a	297.9 ± 23.8 ab	199.3 ± 17.5 b	121.0 ± 9.9 c	70.4 ± 4.9 d	0e	—	F = 989.4; df = 10,99
	Egg hatch (%)	97.6± 0.46a	90.8 ± 2.23b	83.2 ± 3.02b	67.3 ± 2.2c	56.8 ± 3.0d	3 8.3 ± 1.6e	19.2 ± 1.2f	7.1 ± 0.84g	0h	—			*F* = 397.8; df = 8,81
Pre-gravid female (3-4 d-old)	Eggs laid per female	374.4 ± 22.8a	372.9 ± 37.5a	330.1 ± 23.2ab	305.9 ± 29.8ab	289.1 ± 28.3bc	274.3 ± 14.4 cd	253.7 ± 17.3cd	218.9 ± 18.1 cd	203.9 ± 10.5 d	109.2 ± 10.3 e	73.0 ± 6.2 f	41.0 ± 4.4 g	F = 734.8; df = 11,108
	Egg hatch (%)	97.5 ± 0.37a	91.7 ± 1.85b	88.6 ± 4.61bc	79.4 ± 2.3c	66.0 ± 4.1d	48.0 ± 2.9e	21.8 ± 1.4f	10.7 ± 0.82g	0h	—			*F* = 362.4; df = 8,81
Gravid female (6-7 d-old)	Eggs laid per female	372.2 ± 17.2a	360.6 ± 22.3a	347.3 ± 28.5ab	343.7 ± 20.4 ab	339.1 ± 24.2 ab	334.8 ± 20.9 ab	305.8 ± 15.8 ab	303.6 ± 12.8 ab	286.9 ± 10.7 c	198.9 ± 17. 8 de	176.4 ± 12.32 e	158.8 ± 10.5 e	F = 527.6; df = 11,108
	Egg hatch (%)	96.8 ± 0.4a	93.3 ± 1.52ab	90.6 ± 2.26bc	82.0 ± 3.7c	70.0 ± 2.3d	56.8 ± 2.6e	22.5 ± 1.9f	14.2 ± 1.6g	6.4 ± 0.18h	0i			*F* = 385.2; df = 9,90

Means ± SE followed by same letter within a row 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 table are back transformations. Number of replicates = 10. Average data of 7 pairs of adult females and males within each treatment regimen constituted a replicate.

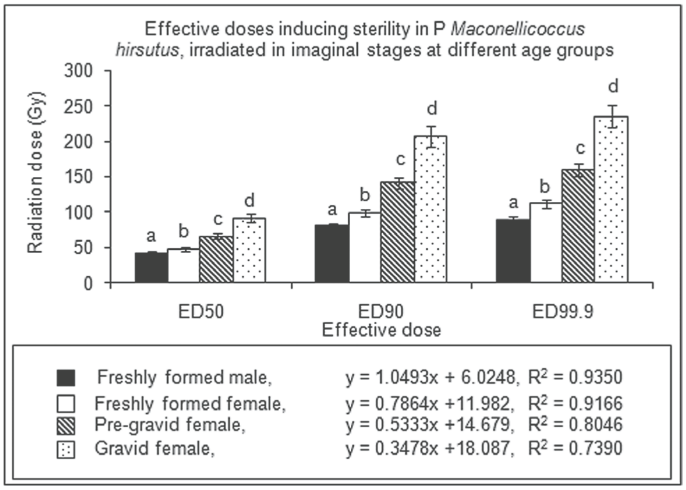

Fig. 9. Estimated doses for preventing 50, 90, and 99.9% hatch of F_1 eggs when parent generation *Maconellicoccus hirsutus* 0–1d-old adult males or adult females of 3 age groups were irradiated. Means followed by the same letter within a sterility level are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates, $n = 10$.

Radiation Induced Sterility in Parent (P) Generation when Irradiated in Various Imaginal Stages

Table 4 presents the numbers of eggs laid and percentages of F_1 egg hatch observed when *M. hirsutus* adults of 3 ages were irradiated with various doses and then paired with non-irradiated members of the opposite sex. Hatch of F_1 eggs was totally prevented by irradiating the female parent ≤ 4 d-old with 200 Gy, and with 300 Gy for those 6–7 d-old. Estimated doses to result in 50, 90, and 99.9 % prevention of F_1 egg hatch are given in Fig. 9. The upper 95% limit for the $ED_{99.9}$ (99.9 %) for the most tolerant stage, i.e., gravid females, was near 250 Gy.

Parent (P) Irradiation-induced Impact on Metamorphosis and Development in F_1 Progeny

Prevention of development of the F_1 N_2 from crawlers (N_1) could be considered as the threshold for efficacy of phytosanitary irradiation treatments against the pink hibiscus mealybug in order to reduce the gamma radiation dose (~300 Gy) that would be required to cause sterility (0% egg hatch) in the gravid female (Table 4). In this case the effective doses for 50%, 90% and 99.9% metamorphic inhibition of molting of F_1 first instar nymphs to second instar nymphs were computed as 73.5 Gy, 148.4 Gy and 166.9 Gy, respectively, when the most radio-resistant 6–7 d-old gravid female parents were irradiated (Table 5).

Data on F_1 survival to the adult stage when adults of different ages in the parent generation were irradiated are presented in Table 6. Males were more radio-susceptible than females. Prevention of F_1 adults was accomplished with relatively low doses; thus, in the most radiotolerant age, 6-7 d-old females, this was accomplished with 100 Gy.

Estimated doses to result in 50, 90, and 99.9 % prevention of the F_1 adult are given in Fig. 10. The upper 95% limit for the $ED_{99.9}$ (99.9 %) for the most tolerant stage, gravid females, is near 80 Gy.

Radiation Induced Sterility in F_1 Adult Progeny of Parents Irradiated as Adults

Prevention of oviposition (infecundity) by F_1 adults was accomplished with quite low doses applied to parent generation adults. Complete F_1 sterility—manifested as 0% egg hatch was achieved at 60 Gy, and coincidentally this dose also caused complete infecundity in the most tolerant stage, 6–7 d-old parent females (Table 7). Estimated doses to achieve 50, 90, and 99.9 % prevention of hatching of F_2 eggs laid by F_1 adults when parent adults of different ages were irradiated are given in Fig. 11. The upper 95 % limit of the estimated dose for 99.9 % F_1 sterility, i.e., infecundity, of the most tolerant parent stage, gravid females, was 55 Gy.

PHYSIO-CHEMICAL AND NUTRITIONAL QUALITY OF TABLE GRAPES AFTER GAMMA IRRADIATION

There were no statistically significant differences (at the 95% level) in any of the physio-chemical or nutritional parameters examined between non-irradiated table grapes and those irradiated with 200 or 400 Gy and then held at 5 °C for 7 d (Table 8).

Discussion

Radiation doses necessary to kill insect pests within 1 or 2 d of irradiation are generally too large to tolerate for most fresh agricultural produce. However, relatively smaller doses (e.g., 50–350 Gy), which are tolerated by many—if not most—fresh commodities, can prevent successful development or reproduction of the pest, and, therefore, prevent sustained infestations. Even smaller doses may prevent F_1 progeny either from developing into adults or prevent their reproduc-

Table 5. Estimated doses of gamma radiation required to prevent metamorphosis of F_1 first instars into second instars in *Maconellicoccus hirsutus* irradiated as adults in different age groups.

	Estimated dose[a] (Gy) for preventing F_1 N_1 from molting to N_2			
Age of adult irradiated	ED_{50}	ED_{90}	$ED_{99.9}$	Regression equation
Freshly formed male (0-1d-old)	37.3 ± 2.2a	75.9 ± 4.6a	85.4 ± 5.1a	$y = 1.038x + 11.26$ $R^2 = 0.879$
Freshly formed female (0-1d-old)	45.4 ± 2.5b	125.6 ± 6.2b	145.4 ± 7.5b	$y = 0.499x + 27.31$ $R^2 = 0.641$
Pre-gravid female (3-4 d-old)	49.6 ± 2.9b	128.8 ± 7.4b	148.3 ± 8.6b	$y = 0.505x + 24.96$ $R^2 = 0.681$
Gravid female (6-7 d-old)	73.5 ± 4.9c	148.4 ± 11.2c	166.9 ± 11.7c	$y = 0.534x + 10.75$ $R^2 = 0.867$
F-value	$F = 204.1$	$F = 212.2$	$F = 242.1$	for slope: $F = 261.4$

[a]ED_{50}, ED_{90}, and $ED_{99.9}$ are the effective doses applied to each age group of P generation adult that prevent 50%, 90%, 99.9% of F_1 N_1 from molting to F_1 N_2. Means followed by same letter within a column are not significantly different at $P < 0.05$ level (ANOVA, df = 3,36, followed by LSD post-test). Number of replicates = 10. Fifty F_1 N_1 nymphs for each treatment regimen constituted 1 replicate.

Table 6. Percent survival to adulthood of the F_1 progeny of parent generation *Maconellicoccus hirsutus* adults that were irradiated at 3 different ages. Either the female or male parent of these F_1 progeny had been irradiated as an adult belonging to 1 of 3 different age groups, and then crossed with a non-irradiated member of the opposite sex.

Age group of the irradiated parent	Sex of F_1 adults formed	Percent survival to adulthood[a] of the F_1 progeny of a parent irradiated as an adult								*F*-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	100 Gy	
Freshly formed male (0-1 d-old)	Male	23.7 ± 1.3a	20.3 ± 1.25a	12.6 ± 1.2b	8.8 ± 0.7c	5.6 ± 0.2c	0d	—	—	*F* = 41.3 df = 5,54
	Female	63.6 ± 2.6a	48.5 ± 1.9b	27.1 ± 1.5c	19.4 ± 1.3d	12.6 ± 0.9e	2.5 ± 0.1f	0g	—	*F* = 60.5 df = 6,63
Freshly formed female (0-1 d-old)	Male	21.3 ± 1.2a	15.9 ± 1.2b	13.8 ± 1.1b	8.3 ± 1.15c	2.6 ± 0.2d	0e	—	—	*F* = 65.2 df = 5,54
	Female	64.7 ± 2.1a	57.5 ± 2.2a	38.8 ± 1.6b	24.7 ± 1.9c	18.9 ± 0.8d	0e	—	—	*F* = 67.9 df = 5,54
Pre-gravid female (3-4 d-old)	Male	23.3 ± 1.1a	21.3 ± 1.03a	17.6 ± 1.1b	9.3 ± 0.4c	4.2 ± 0.3d	0e	—	—	*F* = 32.7 df = 5,54
	Female	68.0 ± 2.2a	52.3 ± 2.3b	48.0 ± 2.2b	35.6 ± 1.4c	10.2 ± 0.5d	5.4 ± 0.2e	0f	—	*F* = 83.4 df = 6,63
Gravid female (6-7 d-old)	Male	24.7 ± 1.3a	22.6 ± 1.1a	18.7 ± 1.12b	13.3 ± 1.2c	8.3 ± 0.4d	3.9 ± 0.17e	0f	—	*F* = 46.8 df = 6,63
	Female	67.9 ± 2.1a	60.3 ± 2.3ab	52.9 ± 2.7bc	47.3 ± 2.1c	28.9 ± 2.1d	9.1 ± 0.7e	3.1 ± 0.1f	0g	*F* = 98.3 df = 7,72

[a]Means ± SE followed by same letter within a row are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in table are back transformations. Number of replications = 10. Fifty F_1 N_1 nymphs for each treatment regimen constituted 1 replicate.

tion. These 2 outcomes may be accepted as end points of PI although they allow for much development beyond the stage at which the insects were irradiated.

Phytosanitary treatments must prevent all stages of viable regulated pests that may be present in exported commodities from transiting outside of quarantined areas, i.e., pests in irradiated commodities must be incapable of establishing a population at the destination of the shipment. Phytosanitary security of a treatment must be confirmed for all of these stages, and if adequate control is demonstrated for the most tolerant stage that could be present, no matter if it is not the most common stage present, it will suffice for all other less tolerant stages.

Radiotolerance usually increases with the progression of metamorphosis. Therefore, for any pest species, such as a mealybug, that may occur on exported commodities as adults, the actively reproducing adult would be the most tolerant stage (Hallman et al. 2010), and this observation was greatly substantiated with this study of *M. hirsutus*.

PI differs from all other commercial treatments in 1 important technical consideration, i.e., that the end point of the treatment is not acute mortality but prevention of further biological development and reproduction (Hallman 2011), so that the forms of the pests still alive cannot establish a population at the destination of the shipment. The measure of efficacy when adults are irradiated is often prevention of significant F_1 development after irradiation of parent adults. Prevention of oviposition by the parent generation or hatch of the eggs they lay often requires excessive doses because the eggs may be largely developed, and thus able to hatch, by the time they would be irradiated under commercial conditions. Prevention of the molt of the F_1 first instar to the second instar is usually obtained with a reasonable dose applied to the parent generation, as was observed in this study. Therefore, a feasible phytosanitary treatment against *M. hirsutus* would be a dose that completely prevents the formation of F_1 second instars when gravid females are irradiated; and the present study indicates that this dose would be ~200 Gy. Large-scale confirmatory testing is required to confirm a dose that would completely prevent the formation of F_1 second instars when a large number of gravid adults were irradiated. The margin of safety with a dose of 200 Gy would be great because even 100 Gy applied to the parent generation would prevent all F_1 progeny from developing to the adult stage.

The dose for PI may depend on the age of the most tolerant stage. Hence, in the present study, the radio-sensitivities of different age groups of female adults, 0–1d, 3–4 d (pre-gravid) and 6–7 d-old (gravid) females, were compared to identify the most tolerant female age and the dose to control this mealybug species. It was suggested the dose required to prevent F_1 egg hatch would provide a considerable margin of security for PI of commodities (Hallman et al. 2013). Irradiated females were crossed with normal males to study the reproductive potential of parent female adults, metamorphic development of F_1 progeny to adulthood and reproductive performance of resulting F_1 adults. The present study demonstrated that the irradiated pre-gravid female *M. hirsutus* (3-4-d old) irradiated with 5–500 Gy and then crossed with normal males, were able to lay F_1 eggs, but complete mortality occurred during embryonic development in eggs laid by reproducing irradiated females irradiated with 200–500 Gy. Reduced proportions of F_1 males and females survived from P pre-gravid females irradiated with 5–30 Gy as compared to the control, but the reproductive capacity of these F_1 progeny was greatly reduced because they were 22–77% sterile.

In the present investigation, *M. hirsutus* females were irradiated with 5–500 Gy when 6–7 d-old and gravid and then crossed with normal males; and they were able to lay F_1 eggs, but complete mortality occurred during embryonic development of eggs laid by those females

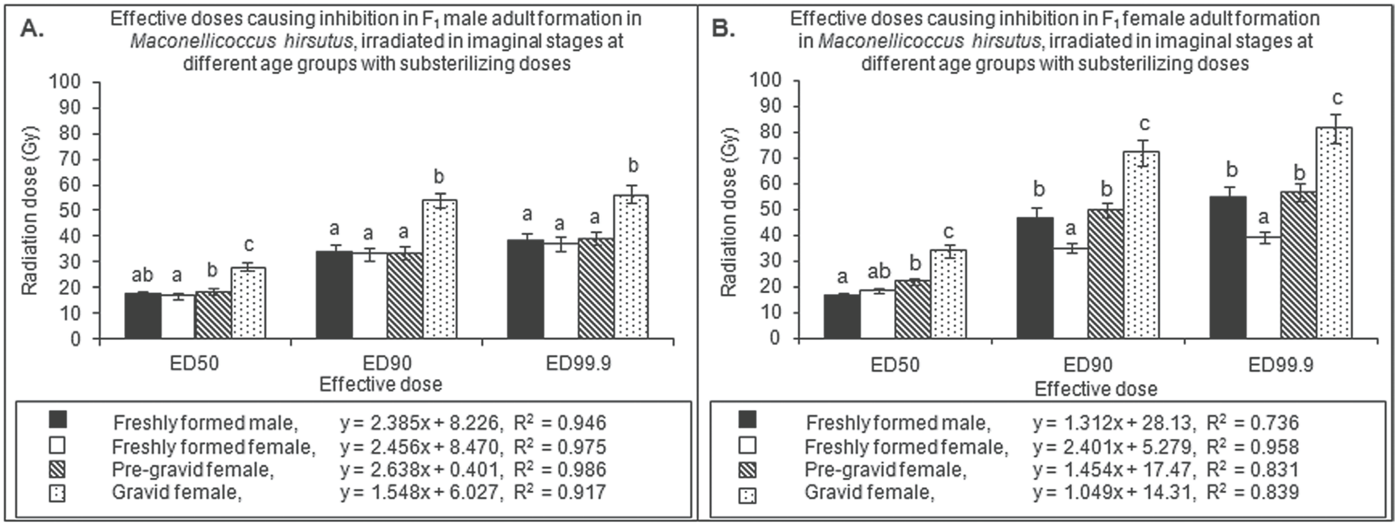

Fig. 10. Estimated doses for preventing 50, 90, and 99.9% F_1 male or female adults from forming in *Maconellicoccus hirsutus* when parent generation adults of different age groups were irradiated and then crossed with non-irradiated members of the opposite sex. A: Prevention of male adult; B: Prevention of female adult. Means followed by same letter within a level of prevention of adult formation are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates, $n = 10$.

irradiated with 300–500 Gy. Present results seemed to be in consonance with that of Dohino et al. (1997), who reported that adult female *Pseudococcus comstocki* (Kuwana) irradiated with 200–600 Gy produced eggs, but their eggs did not hatch except at 200 Gy. Jacobsen & Hara (2003) reported that adult female *M. hirsutus* produced eggs at all doses evaluated, but the proportion that oviposited decreased by > 85% as doses increased. They found that hatchability of the eggs was reduced to 1.2% when adult females were irradiated with 100 Gy and was absent with doses ≥ 250 Gy. Dohino & Masaki (1995) found that 200 Gy did not completely prevent the hatch of eggs laid by irradiated adults of *P. comstocki* (a sexually reproducing mealybug species). Some of these hatchlings developed to the adult stage, but did not reproduce.

Our findings indicate that among different age groups of *M. hirsutus* adult females, the gravid female was the most radio-tolerant.

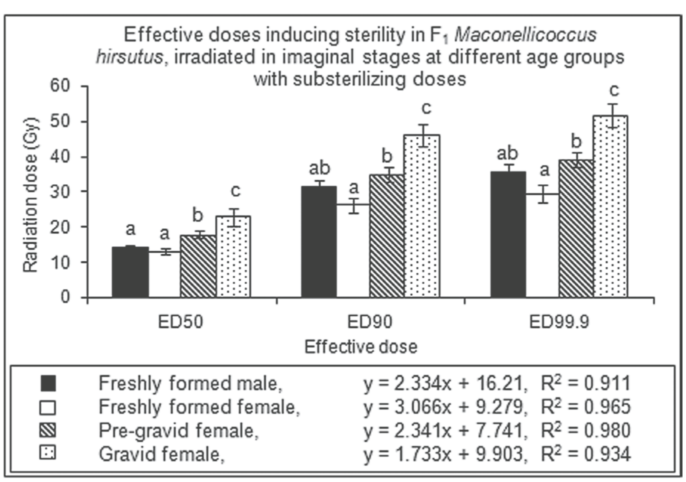

Fig. 11. Estimated doses for preventing 50, 90, and 99.9% F_2 egg hatch in *Maconellicoccus hirsutus* when parent generation adults of different age groups were irradiated and then crossed with members of the opposite sex, followed by the self crossing of F_1 adults. Means followed by same letter within a level of sterility are not significantly different at $P < 0.05$ level (ANOVA followed by LSD post-test). Number of replicates, $n = 10$.

Mohan et al. (2012) suggested that the mealybug genetic system possesses very efficient DNA repair machinery that ensures protection of the many tiny chromosome fragments produced by irradiation and—via rapid healing of broken chromosome ends—prevents them from fusing to one another. Therefore, it might be concluded that when the most radio-tolerant adult stage is irradiated, mortality may subsequently occur in different developmental stages of the progeny. Feldmann (1978) observed that dominant lethal mutations were partly expressed during embryogenesis and especially during critical developmental phases. One prevalent vulnerable phase was the egg-larva boundary stage (Hadorn 1951); the larvae often succeeded in tearing the egg membrane and crawling out, but then succumbed to death. Other prevalent mortality stages of dominant lethal gene expression could be early organ differentiation (von Dittrich 1968) and very young, just hatched larvae.

For the broad group of mealybug species, the possible putative generic PI dose in the review by Hallman (2011) was 250 Gy when measure of efficacy was prevention of reproduction of the adult. In the present study on *M. hirsutus*, the $ED_{99.9}$ value for inducing 99.9% sterility was calculated as 235 Gy to prevent the most radio-tolerant gravid female adult to produce eggs that hatch. However regulatory entomologists may wish to keep in mind that in the cases of arthropods whose chromosomes multiple have "diffuse centromeres", chromosomal damage caused by irradiation is inherited by the next generation in which it induces a greater level of sterility than in the treated parent; and radiation doses that are less than fully sterilizing in the irradiated parent often result in total sterility in their F_1 progeny.

NUTRITIONAL QUALITY OF IRRADIATED FRESH TABLE GRAPES

The primary objective of PI is the prevention of the distribution of invasive species through infested commodities. Therefore, it is imperative that the host commodities tolerate the irradiation treatment and maintain their quality. Physicochemical quality analysis of grape showed no evident change in irradiated fresh table grapes at 200–400 Gy in comparison to unirradiated grapes in the present study. Titratable acidity, juice pH as well as total soluble solids were measured to provide an overview of grape maturity (market quality).

Table 7. Reproductive performance—number of eggs laid and percent that hatched—of *Maconellicoccus hirsutus* F_1 progeny of parents involved in matings in which 1 parent was irradiated with a sub-sterilizing dose in the range of 0–60 Gy. The irradiated parent belonged to 1 of the 3 age groups and the non-irradiated parent belonged to the same age group.

Parent adult stage irradiated (Age group)	Reproductive parameters	Radiation doses applied to P generation adult females							*F*-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	60 Gy	
Freshly formed male (0-1 d-old)	Eggs laid per female	339.4 ± 23.8a	327.1 ± 28.3a	235.8 ± 17.9b	96.7 ± 6.2c	45.3 ± 2.7d	0e	—	F = 472.5; df = 5,54
	Egg hatch (%)	97.8 ± 0.7a	69.1 ± 3.2b	46.9 ± 1.1c	26.0 ± 1.3d	12.1 ± 0.6e	0f	—	*F* = 274.7; df = 5,54
Freshly formed female (0-1 d-old)	Eggs laid per female	383.4 ± 23.5a	291.1 ± 27.8ab	228.9 ± 19.2b	152.1 ± 16.5c	76.2 ± 4.1d	0e	—	F = 422.6; df = 5,54
	Egg hatch (%)	97.0 ± 0.5a	63.3 ± 2.8b	56.0 ± 2.2b	30.1 ± 1.4c	0d	—	—	*F*= 257.9 df = 4,45
Pre-gravid female (3-4 d-old)	Eggs laid per female	349.8 ± 29.9a	335.2 ± 30.1a	297.3 ± 19.5a	205.8 ± 15.2b	126.4 ± 11.3c	0d	—	F = 398.2; df = 5,54
	Egg hatch (%)	95.0 ± 0.5a	73.9 ± 3.6b	57.9 ± 1.8c	43.7 ± 1.7d	21.6 ± 1.2e	0f	—	*F* = 214.9 df = 5,54
Gravid female (6-7 d-old)	Eggs laid per female	361.2 ± 27.7a	343.7 ± 31.4a	304.1 ± 23.1a	237.3 ± 18.8b	205.8 ± 15.6b	87.4 ± 3.4c	0d	F = 484.6; df = 6,63
	Egg hatch (%)	94.8 ± 1.2a	76.5 ± 4.9b	69.0 ± 2.6b	53.1 ± 2.2c	26.3 ± 1.5d	7.1 ± 0.43e	0f	*F* = 337.1 df = 5,54

Means followed by same letter within a row 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 table are back transformed. Number of replicates = 10. Average data of 7 pairs within each treatment regimen constituted a replicate.

Table 8. Physiochemical and nutritional parameters of table grapes irradiated with 0, 200 and 400 Gy after 7 d of storage at 5 °C. None of the values within each column differed significantly from each other at $P < 0.05$ (ANOVA, $n = 5$; df = 2,12).

	Food quality parameters								
		Color characteristics							
Radiation doses	Weight loss (%)	'L' value	'a' value	'b' value	Total soluble solids (%)	Titratable acidity (%)	pH	Vitamin C (mg/100 g)	β-carotene (mg/100 g)
0Gy	3.93 ± 0.33	31.0 ± 2.77	-3.10 ± 0.19	8.4 ± 0.51	13.4 ± 0.97	0.73 ± 0.010	3.43 ± 0.03	0.37 ± 0.03	1.73 ± 0.14
200Gy	4.50 ± 0.38	32.6 ± 1.23	-2.93 ± 0.23	8.6 ± 1.18	14.1 ± 0.62	0.76 ± 0.005	3.53 ± 0.08	0.33 ± 0.03	1.97 ± 0.32
400Gy	4.61 ± 0.41	33.4 ± 0.95	-2.36 ± 0.27	9.2 ± 1.17	14.9 ± 0.60	0.76 ± 0.006	3.57 ± 0.08	0.33 ± 0.03	2.13 ± 0.24
F-value	*F* = 0.56	*F* = 1.50	*F* = 2.7	*F* = 0.56	*F* = 3.33	*F* = 2.13	*F* = 3.86	*F* = 2.77	*F* = 2.34

Total soluble solids would measure the sugar in grapes that could be used as an indicator of maturity. As total soluble solids increases, the juice pH rises, and the titratable acidity declines which make the grapes sweet and mature. Hence, acidity is important for flavor balance and a low pH leads to more stable color and inhibits microbial spoilage (CRCV 2005). The vitamins of importance are identified as vitamin C and carotene, since they are among the more radiation sensitive vitamins and a significant percentage of our dietary intake of these vitamins comes from fresh fruits and vegetables (FSANZ 2014). In the present study, there was no significant change in content of vitamin C and β-carotene of irradiated grape at 200–400 Gy, which is the probable phytosanitary dose range against the pink hibiscus mealybug. Present findings are in agreement with others that report no significant changes in irradiated grape (Cantos et al. 2001; Kang et al. 2012; Kim et al. 2014).

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