

# Phytosanitary irradiation of Jack Beardsley mealybug (Hemiptera: Pseudococcidae) females on rambutan (Sapindales: Sapindaceae) fruits

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## Abstract

The Jack Beardsley mealybug, *Pseudococcus jackbeardsleyi* (Gimpel & Miller) (Hemiptera: Pseudococcidae), is a quarantine pest of fruits, vegetables, flowers and seedlings. Host commodities shipped from infested areas should undergo phytosanitary measures to reduce the risk of shipping viable mealybugs. Large-scale confirmatory tests on late stage female *P. jackbeardsleyi* (the most tolerant stage) irradiated with the target dose of 150 Gy were conducted to determine the minimum absorbed dose for the use of irradiation as a phytosanitary treatment. No F<sub>1</sub> generation 2nd stage nymphs emerged from an estimated 118,520 potato (*Solanum tuberosum* L.; Solanales: Solanaceae) reared females irradiated with 133.5–164.1 Gy, or 49,290 pumpkin (*Cucurbita* sp.; Cucurbitales: Cucurbitaceae) reared females irradiated with 131.3–166.0 Gy, resulting in an efficacy of 99.9975% (potato reared) and 99.9939% (pumpkin reared) and 99.9982% (all females) at the 95% confidence level. Quality determinations on ‘Rongrien’ rambutan (*Nephelium lappaceum* L.; Sapindales: Sapindaceae) fruits—the favorite host of *P. jackbeardsleyi*—were conducted 5, 10 and 15 days after gamma radiation at doses of 300, 600, 900, and 1,200 Gy with storage at 10 °C. Irradiation did not induce significant changes in ascorbic acid content, total acidity, total soluble solids, soluble protein, and weight loss. However, storage time had a significant negative effect on the chemical and nutritional contents and weight loss. A dose of 166 Gy is recommended as the minimum dose for phytosanitary treatment against the Jack Beardsley mealybug. ‘Rongrien’ rambutan can tolerate a dose of 900 Gy but at 1,200 Gy its sensory attributes are significantly affected.

Key Words: *Pseudococcus jackbeardsleyi*; Jack Beardsley mealybug; irradiation; phytosanitary irradiation; rambutan; quality assessment

## Resumen

La cochinilla de Jack Beardsley, *Pseudococcus jackbeardsleyi* (Gimpel & Miller) (Hemiptera: Pseudococcidae), es una plaga cuarentenaria para frutas, hortalizas, flores y plantas. Los productos que son hospederos enviados desde las zonas infestadas deben ser sometidos a medidas fitosanitarias para reducir el riesgo de envío de cochinillas viables. Se realizaron pruebas confirmatorias de gran escala sobre las hembras más maduras de *P. jackbeardsleyi* (el estadio más tolerante) a la dosis objetivo de 150 Gy para determinar la dosis absorbida mínima para el uso de la irradiación como tratamiento fitosanitario. No emergieron ninfas del segundo estadio de la generación F<sub>1</sub> de un estimado de 118,520 hembras criadas sobre papas (*Solanum tuberosum* L.; Solanales: Solanaceae) e irradiadas a 133.5–164.1 Gy, o 49,290 hembras criadas sobre calabaza (*Cucurbita* sp.; Cucurbitales: Cucurbitaceae) e irradiadas a 131.3–166.0 Gy, lo que resulta en una eficacia del 99,9975% (criadas sobre papa) y 99,9939% (criadas sobre calabaza) y 99,9982% (todas hembras) en el nivel de confianza del 95%. Se realizó una determinación de calidad en frutos de rambután (*Nephelium lappaceum* L.; Sapindales: Sapindaceae) ‘Rongrien’ (hospedero favorito de *P. jackbeardsleyi*) 5, 10 y 15 días después de la radiación gamma en dosis de 300, 600, 900, y 1,200 Gy y una temperatura de almacenamiento de 10 °C. La irradiación no indujo cambios significativos en el contenido de ácido ascórbico, acidez total, sólidos solubles totales, proteínas solubles, y pérdida de peso. Sin embargo, el tiempo de almacenamiento tuvo un efecto negativo significativo en la química y contenido nutricional y la pérdida de peso. Se recomienda una dosis de 166 Gy que es la dosis mínima para el tratamiento fitosanitario contra la cochinilla harinosa Jack Beardsley. El rambután ‘Rongrien’ puede tolerar una dosis de 900 Gy, pero en 1,200 Gy sus características externas se ven afectados de manera significativa.

Palabras Clave: *Pseudococcus jackbeardsleyi*; cochinilla Jack Beardsley; irradiación; irradiación fitosanitaria; rambután; evaluación de calidad

The Jack Beardsley mealybug, *Pseudococcus jackbeardsleyi* (Gimpel & Miller) (Hemiptera: Pseudococcidae), is reported on a diverse array of fruits, vegetables, and ornamentals from 88 genera in 38 plant families (CABI 2001). Some major hosts include custard apple (*Annona* spp.; Magnoliales: Annonaceae), tomato (*Lycopersicon* spp.; Solanales: Solanaceae), banana (*Musa* spp.; Zingiberales: Musaceae), rambutan (*Nephelium lappaceum* L.; Sapindales: Sapindaceae), durian

(*Durio zibethinus* Murray; Malvales: Bombacaceae), pineapple (*Ananas comosus* (L.) Merr.; Poales: Bromeliaceae) and rose mallows (*Hibiscus* spp.; Malvales: Malvaceae) (Ben-Dov et al. 2006). The Jack Beardsley mealybug is widespread throughout the Americas, parts of Oceania and Asia including Taiwan, India, Indonesia, Malaysia, Philippines, Thailand, Singapore, Sri Lanka and Vietnam (Ben-Dov et al. 2006; Tokihiro 2006; Anura et al. 2012; Mani et al. 2013) and Cote d’Ivoire, Africa

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(N'Guessan et al. 2014) The mealybugs are usually found in protected areas of the host such as under the leaves, in the axils of leaves and in cracks and crevices on the trunk. They may also occur on developing fruit branches and fruits (Gimpel & Miller 1996; CABI 2001) resulting in a decrease of fruit quality (Williams 2004; Kondo et al. 2008; Jiao et al. 2011). Although *P. jackbeardsleyi* has never been reported as a serious pest, the numbers of hosts of economic importance that it can infest as well as its ability to expand its geographic range make it an ideal candidate as a pest of the future (CABI 2001). It can be injurious in the absence of suitable natural enemies (Williams & Watson 1988). The mealybugs were often intercepted on rambutan, durian, pineapples and other fruits in entry-exit ports in China. Consequently it is considered as a pest of quarantine importance and should be subjected to a phytosanitary treatment when imported (Jiao et al. 2011).

Irradiation is the best tolerated treatment among those used as phytosanitary measures for fresh commodities. Most horticultural crops harvested at commercial maturity can tolerate the minimum doses required against arthropod pests (Heather & Hallman 2008). Though relatively new, the commercial use of phytosanitary irradiation (PI) is rapidly growing worldwide. A minimum dose of 400 Gy is often used for insect pests other than fruit flies (Hallman 2011). For mealybugs, the objective of PI is usually to inhibit reproduction but not to cause acute larval mortality (Jacobsen & Hara 2003; Doan et al. 2012). To save resources and reduce the potential negative effect of a PI treatment on commodities, the effective dose for any treatment should be made as low as possible (Torres & Hallman 2007). In a previous dose-response test, a minimum dose of 133.7 Gy (95% confidence interval: 117.2–168.0 Gy) was estimated using the probit model (dose was not transformed) to prevent 99.9968%  $F_1$  generation 2nd instar nymph emergence from irradiated female adults, which is the most tolerant stage of *P. jackbeardsleyi* (Shao et al. 2013). To validate the minimum absorbed dose that provides quarantine security, large-scale confirmatory tests were conducted on a minimum of 93,616 irradiated late stage females that resulted in no  $F_1$  generation 2nd instar nymph emergence (Couey & Chew 1986; IPPC 2003, 2007).

Acceptance of irradiation by fruit growers, packers, and consumers requires knowledge of its effect on nutritional, chemical, and sensory characteristics of the irradiated fruits (Terri et al. 2002). The quality of American rambutan was assessed for the variety 'R134' irradiated at 250 Gy and for the variety 'R167' irradiated at 250, 750 and 900 Gy. The results showed that doses up to 900 Gy had no significant effect on the chemical, physical, or sensory attributes of 'R167' rambutan after 9 d of storage (Moy et al. 1999; Follett & Sanxter 2000; Terri et al. 2002). There is currently no information available on the physiological response to irradiation of the 'Rongrien' rambutan, a variety from Thailand that China imports in large quantities. This is why the impact of irradiation on the quality of 'Rongrien' rambutan was also evaluated in this research.

## Materials and Methods

### INSECTS REARING

The *P. jackbeardsleyi* mealybug was originally intercepted from rambutan fruits imported from Thailand at the Shenzhen entry-exit port in 2012, and the mealybugs were reared in a laboratory on sprouting potato (*Solanum tuberosum* L.; Solanales: Solanaceae) at  $24 \pm 2$  °C, 50–70% RH with a photoperiod of 14:10 h L:D. There were 4 development stages including nymphs (1st, 2nd and 3rd instars) and adults. The life span is  $34.8 \pm 1.3$  d under laboratory conditions (Shao et al. 2013). To prepare the late stage females used in the confirmatory tests, gravid females were placed onto potatoes or pumpkins for a period of

5 d for laying eggs, and the  $F_1$  generation developed for another 30 d under laboratory conditions.

### IRRADIATION TREATMENT

**Irradiator.** All irradiation treatments were conducted at the National Institute of Metrology Research Irradiator, Beijing, China, with a Cobalt-60 source of  $1.5 \times 10^{15}$  Bq. The dose rate was 5.0–5.9 Gy/min at 100 cm from the source. The Institute uses the Fricke system (ASTM E1026-13, 2002) for routine dosimetry.

**Gamma Irradiation of Late Stage Females.** Late stage females of *P. jackbeardsleyi* reared on potato or pumpkin (*Cucurbita* sp.; Cucurbitales: Cucurbitaceae) in plastic boxes (approximately 1.6 L, 2 tubers or fruits per box) were exposed to gamma radiation. The boxes were placed 100 cm from the source. For a more uniform dose distribution, the boxes were rotated 180° at mid-exposure. Every fifth box contained 2 Fricke dosimeters used to measure dose variations. A dose of 150 Gy was selected as target dose based on the estimation of the required dose by probit analysis.

**Gamma Irradiation of Rambutan.** Refrigerated 'Rongrien' rambutan fruits imported from Thailand and stored at 10 °C in a climatic chamber (Binder KBF720, Germany) to ensure constant conditions were loaded in plastic file boxes (9 × 25 × 30 cm). The boxes were placed 100 cm from the sources and irradiated at 300, 600, 900, and 1,200 Gy. Each dose (treatment) was replicated 3 times. The boxes were rotated 180° at mid-exposure. A dose uniformity ratio (DUR) of 1.16 was obtained according to the dosimeter readings.

**Rearing of Mealybug after Irradiation.** The irradiated potatoes and pumpkins were transferred to new plastic boxes (20 × 30 × 18 cm) and kept in a rearing room. Potatoes or pumpkins were restocked as necessary for optimum development of the mealybugs to  $F_1$  generation 2nd instar nymphs. To avoid cross contamination, untreated controls were reared in a separate rearing room. The number of female adults and  $F_1$  generation 1st and 2nd instar nymphs that developed were checked in each treatment and the control.

### QUALITY ASSESSMENT OF RAMBUTAN

**Instrumental and Chemical Analysis.** The quality of 'Rongrien' rambutan stored at 10 °C and 90% RH was assessed 5, 10 and 15 d after irradiation. The following characteristics were examined: changes in total ascorbic acid (Vitamin C), percentage acidity (acidity), total soluble solids (TSS), soluble protein and weight loss. The analysis was performed in triplicate for all treatments and replicates.

The content of ascorbic acid (reduced form) was measured after extraction from the fruit pulp by Tris-HCl buffer solution. It was measured with a UV-spectrophotometer (U-3310, Hitachi Co., Japan) at 265 nm (Yu et al. 2006; Dong et al. 2013).

For soluble protein, the centrifuged pulp juice was stained with Coomassie brilliant blue and measured with a UV-spectrophotometer (U-3310, Hitachi Co., Japan) at 595 nm.

The acidity of the centrifuged pulp juice was measured for each treatment and the control with a fruit acidity meter (GMK-708, G-won Co., Korea), and the TSS with a digital refractometer (GMK-701R, G-won Co., Korea).

**Sensory Evaluation.** A 20-member panel was constituted with staff members and students of the Chinese Academy of Inspection and Quarantine who participated in a 1-h training session to become familiar with sensory evaluation using a hedonic scale. The taste evaluation performed 5 d after irradiation included sweetness, firmness, and composite taste; the rating scale being: 3 = excellent, 2 = acceptable, 1 = off-flavors and 0 = highly distasteful. Panelists evaluated samples under white light in individual booths. Distilled water was provided for

oral rinsing between samples. The order of sample presentation to the panelists was balanced and randomized.

## DATA ANALYSIS

To determine the target dose for conducting confirmatory tests on *P. jackbeardsleyi* late stage females, the dose-mortality data on percentage mortality of  $F_1$  generation 2nd instar nymphs from 1st instars reported by Shao et al. (2013) were newly analyzed with the normal probit model (dose transformed) and logit model (dose non-transformed) by using PoloPlus (LeOra Software 2002). The mortality data were also arcsine transformed to improve normality and subjected to linear regression after ANCOVA by using the Tukey model (DPS 2010; Zhan et al. 2014). The results of the analyses are presented in Table 1.

For the confirmatory tests, the level of confidence associated with treating a number of mealybugs with zero survivors is given by the equation (1),

$$C = 1 - (1 - Pu)^n \quad (1)$$

in which  $C$  is the confidence level,  $Pu$  is the acceptable level of survivorship (as a proportion) and  $n$  is the number of test insects (Couey & Chew 1986). The confidence level was calculated for the number of treated *P. jackbeardsleyi* late stage females assuming an efficacy of 99.9968%, and the mortality proportion ( $1 - Pu$ ) was calculated using equation (2) for a confidence level of 95% (Follett 2008):

$$1 - Pu = (1 - C)^{1/n} \quad (2)$$

For the analysis of contents of vitamin C, TSS, acidity, soluble protein and weight loss of rambutan fruits, two-way analysis of variance (2-way ANOVA) was used to analyze the effects of irradiation treatments, storage time (5, 10 and 15 d) and interaction effect of irradiation treatment and storage time in a randomized complete block design. Means separation were done using Tukey model at  $P \leq 0.05$  (DPS 2010). Data on sensory evaluation were subjected to one-way ANOVA and means were separated by Tukey's multiple comparison tests (DPS 2010).

## Results

### LARGE-SCALE CONFIRMATORY TESTS TO PREVENT 99.9968% OF $F_1$ GENERATION 2 ND INSTAR NYMPH EMERGENCE

Large-scale confirmatory tests were conducted to validate the estimated dose to prevent 99.9968% of  $F_1$  generation 2nd instar nymphal emergence from late stage females—the most resistant stage in harvested fruits of *P. jackbeardsleyi*—where a dose of 150 Gy was selected as the target dose based on the estimation by pro-

bit analysis (Table 1). No  $F_1$  generation 2nd instar nymph emerged from an estimated 118,520 late females reared on potato, or 49,290 late females reared on pumpkin, whereas, the mortality of 2nd instar nymphs that developed from neonates in the control was at most 2% (Table 2). The actual absorbed doses measured by Fricke dosimeter ranged from 133 to 164 Gy for potato reared females, and 131 to 166 Gy for pumpkin reared females (Table 2). The DUR was 1.23 in the first, 1.25 in the second, and 1.19 in the third confirmatory tests (Table 2).

### QUALITY EVALUATION OF IRRADIATED RAMBUTAN FRUITS

When measuring the interaction effects of irradiation doses and storage time on the chemical and nutritional contents and weight loss of rambutan fruit irradiated at various doses, no significant difference was found for the content of Vitamin C ( $F = 0.1$ ;  $df = 8,30$ ;  $P = 0.9261$ ), acidity ( $F = 0.8$ ;  $df = 8,30$ ;  $P = 0.6352$ ), TSS ( $F = 0.6$ ;  $df = 8,30$ ;  $P = 0.7564$ ), soluble protein ( $F = 2.2$ ;  $df = 8,30$ ;  $P = 0.0517$ ) and weight loss ( $F = 0.7$ ;  $df = 8,30$ ;  $P = 0.6985$ ). The effects of the various irradiation doses and storage durations are presented below.

**Effects of Various Irradiation Doses.** Comparisons of the objective effects of irradiation at 300, 600, 900 and 1,200 Gy on the attributes of rambutan are presented in Figs. 1–5. There were no significant differences between the irradiated samples and the controls regarding Vitamin C ( $F = 1.5$ ;  $df = 4,30$ ;  $P = 0.2345$ ) (Fig. 1), TSS ( $F = 3.0$ ;  $df = 4,30$ ;  $P = 0.0898$ ) (Fig. 2), acidity ( $F = 2.5$ ;  $df = 4,30$ ;  $P = 0.0635$ ) (Fig. 3), soluble protein ( $F = 0.9$ ;  $df = 4,30$ ;  $P = 0.5077$ ) (Fig. 4) and weight loss ( $F = 1.8$ ;  $df = 4,30$ ;  $P = 0.2159$ ) (Fig. 5), indicating that the irradiation treatments had no significant objective effects on these attributes.

**Effects of Storage Time.** The post-irradiation storage time (5, 10 and 15 d) had a significant influence on Vitamin C content ( $F = 66.7$ ;  $df = 2,30$ ;  $P < 0.0001$ ) (Fig. 1), TSS content ( $F = 9.9$ ;  $df = 2,30$ ;  $P = 0.0068$ ) (Fig. 2), acidity ( $F = 31.0$ ;  $df = 2,30$ ;  $P < 0.0001$ ) (Fig. 3), soluble protein content ( $F = 23.1$ ;  $df = 2,30$ ;  $P < 0.0001$ ) (Fig. 4) and percentage weight loss ( $F = 49.5$ ;  $df = 2,30$ ;  $P < 0.0001$ ) (Fig. 5). The contents of Vitamin C and TSS and acidity after either 10 or 15 d were significantly lower than after 5 d (Figs. 1–3). The amount of soluble protein decreased significantly from the 5th day to the 15th day (Fig. 4), while the percentage weight loss significantly increased with storage time (Fig. 5).

**Sensory Evaluation.** The results from the 20-member panel who evaluated the taste characteristics are presented in Table 3. The results showed that irradiation of rambutan fruits at 1,200 Gy produced significant reductions in the scores for sweetness ( $F = 6.1$ ;  $df = 4,95$ ;  $P = 0.0002$ ), firmness ( $F = 4.1$ ;  $df = 4,95$ ;  $P = 0.0039$ ) and flavor ( $F = 7.0$ ;  $df = 4,95$ ;  $P = 0.0001$ ). For all other doses, no significant difference was detected. 'Rongrien' rambutan fruits can be irradiated with doses ranging up to 900 Gy.

**Table 1.** Minimum absorbed dose required to prevent the emergence of  $F_1$  generation 2nd instar nymphs from irradiated late females of *Pseudococcus jackbeardsleyi*. Estimates were made by 4 methods of analysis.

| Analysis model               | Slope $\pm$ SE <sup>a</sup> | Intercept $\pm$ SE <sup>a</sup> | ED <sub>99.9968</sub> (Gy)<br>(95% CI) | Dose for 100% mortality<br>(Gy) | Heterogeneity            |
|------------------------------|-----------------------------|---------------------------------|--|---------------------------------|--------------------------|
| Probit, normal               | 12.11 $\pm$ 0.72            | -22.71 $\pm$ 1.36               | 160.9<br>(135.9, 216.2)                | —                               | 2.18                     |
| Probit, dose non-transformed | 0.70 $\pm$ 0.004            | -5.37 $\pm$ 0.29                | 133.7 Gy<br>(117.2, 168.0)             | —                               | 7.50                     |
| Logit, dose non-transformed  | 0.13 $\pm$ 0.008            | -9.7 $\pm$ 0.63                 | 156.8<br>(124.5, 292.7)                | —                               | 8.62                     |
| Linear regression            | 0.77 $\pm$ 0.09             | -11.21 $\pm$ 7.48               | —                                      | 133.7                           | 0.8415 (R <sup>2</sup> ) |

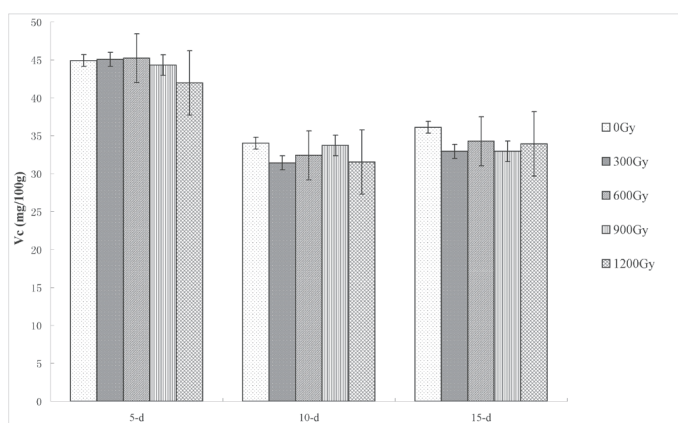
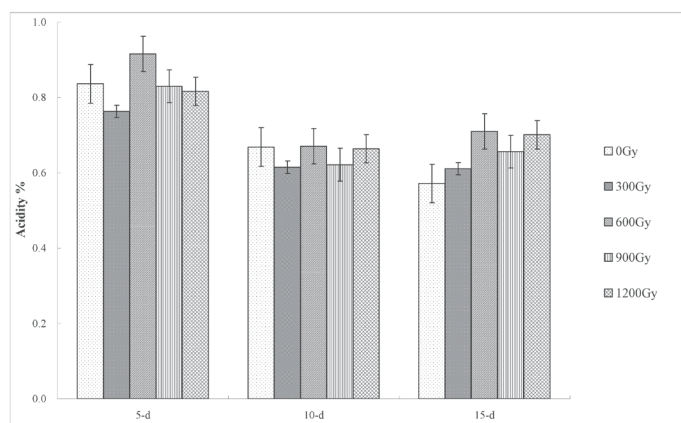
<sup>a</sup>Mean  $\pm$  SE.

CI means the confidence interval.

Heterogeneity was estimated by dividing Chi-square by the degrees of freedom.

**Table 2.** Results of large-scale confirmatory tests on prevention of  $F_1$  generation progeny by irradiating late *Pseudococcus jackbeardsleyi* females reared either on potato or on pumpkin.

| Date of radiation      | Dose monitored |            | No. fruits                 | No. late females | Developed to $F_1$ generation |                    |
|------------------------|----------------|------------|----------------------------|------------------|-------------------------------|--------------------|
|                        | Max.           | Min.       |                            |                  | No. of neonates               | No. of 2nd instars |
| Mar. 2013<br>(control) | 164.1<br>0     | 133.5<br>0 | 212 potatoes<br>6 potatoes | 118,520<br>3,500 | 189,600<br>192,600            | 0<br>188,860       |
| Jan. 2014<br>(control) | 164.6<br>0     | 131.3<br>0 | 120 pumpkins<br>8 pumpkins | 22,340<br>1,620  | 23,600<br>122,500             | 0<br>122,300       |
| Apr. 2014<br>(control) | 166.0<br>0     | 140.0<br>0 | 134 pumpkins<br>8 pumpkins | 26,950<br>1,560  | 31,200<br>132,800             | 0<br>130,200       |

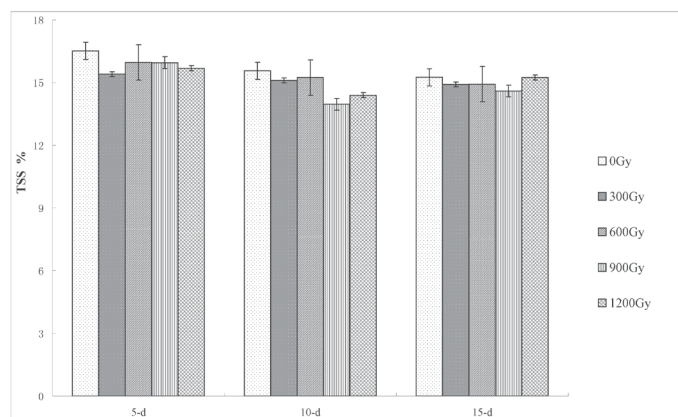
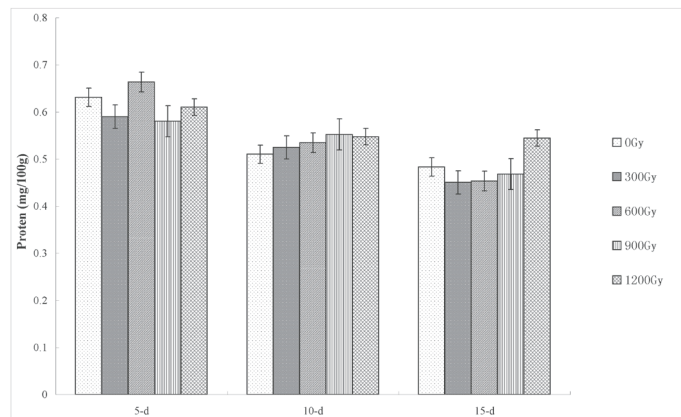
**Fig. 1.** Vitamin C contents of 'Rongrien' rambutan fruits irradiated at 0, 300, 600, 900 and 1,200 Gy and stored at 10 °C. Vitamin C content was determined at 5, 10 and 15 d post-irradiation.**Fig. 3.** Percentage acidity in 'Rongrien' rambutan fruits irradiated at 0, 300, 600, 900 and 1,200 Gy and stored at 10 °C. Percentage acidity of the fruit was determined at 5, 10 and 15 d post-irradiation.

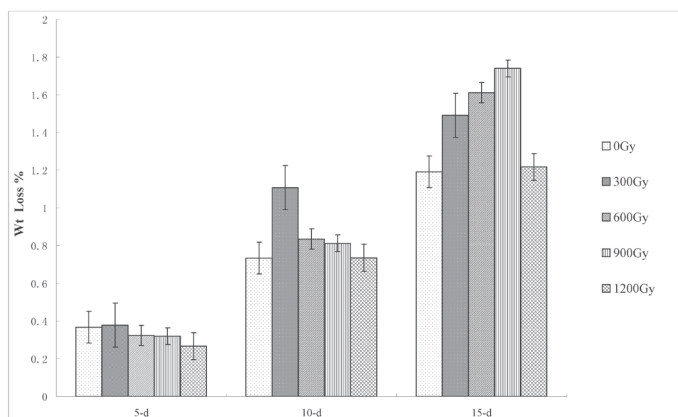
## Discussion

The most radiation tolerant stage of *P. jackbeardsleyi* was the late female stage (Shao et al. 2013), which was the focus of our trials to validate the efficacy for PI even if the late female is not the most prevalent stage occurring in the commodity (IPPC 2003, 2007). In other experiments, the efficacy of the PI treatment against mealybug females was usually measured by the prevention of reproduction, such as preventing the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green, from laying eggs at 250 Gy (Jacobsen & Hara 2003), or preventing hatching of  $F_1$  generation eggs of *Planococcus minor* Maskell at 150–250 Gy

(Ravuiwasa et al. 2009) and *Dysmicoccus neobrevipes* Beardsley at 200–250 Gy (Doan et al. 2012), or preventing the emergence of *D. neobrevipes*  $F_1$  generation adults at 150 Gy (Kang et al. 2011). A minimum dose of 250 Gy was proposed as a generic dose for PI treatment of all mealybugs (Hallman 2011). Because the minimum dose for preventing 100% hatch of  $F_1$  eggs of late stage *P. jackbeardsleyi* females was > 200 Gy (the highest dose used in this investigation) (Shao et al. 2013), we adopted the prevention of emergence of 2nd instar  $F_1$  generation nymphs as the criterion for PI treatment efficacy in this research study.

The most rigorous standard used for confirming the efficacy of a PI treatment is "probit 9" at the 95% confidence level (Hallman & Lo-

**Fig. 2.** Total soluble solids (TSS) in 'Rongrien' rambutan fruits irradiated at 0, 300, 600, 900 and 1,200 Gy and stored at 10 °C. Total soluble solids were determined at 5, 10 and 15 d post-irradiation.**Fig. 4.** Soluble protein contents of 'Rongrien' rambutan fruits irradiated at 0, 300, 600, 900 and 1,200 Gy and stored at 10 °C. Soluble protein content was determined at 5, 10 and 15 d post-irradiation.



**Fig. 5.** Percent weight loss of 'Rongrien' rambutan fruits irradiation at 0, 300, 600, 900 and 1,200 Gy and stored at 10 °C. Percentage weight loss of the fruit was determined at 5, 10 and 15 d post-irradiation.

aharanu 2002). Probit 9 represents the effective dose (ED) needed to meet the criterion of efficacy at the 99.9968th percentile ( $ED_{99.9968}$ ). At the 95% confidence level, this requires that no survivor occurs when at least 93,616 individuals are treated (Couey & Chew 1986). The target doses applied in the large-scale confirmatory tests are ordinarily based on the value of  $ED_{99.9968}$  estimated by the probit or logit analysis of dose-response data, and this has been validated in a number of PI experiments for several species of tephritid fruit flies (Mansour & Franz 1996; Hallman & Thomas 1999; Bustos et al. 2004), the oriental fruit moth *Grapholita molesta* Busck (Hallman 2004), and the peach fruit moth *Carposina sasakii* Matsumura (Zhan et al. 2014). Hence, a radiation dose of 150 Gy was set as target dose, because it was close to the  $ED_{99.9968}$  predicted by normal probit model (161 Gy) and the logit model (157 Gy) but higher than by linear regression (134 Gy) and by probit analysis with the doses not transformed (134 Gy) (Table 1). This dose was applied to an estimated 118,520 late stage females on potato, and no 2nd instar nymph emerged in the  $F_1$  generation; and thus the probit analysis was validated. Assuming a required efficacy of 99.9968%, the confidence level (C) in the confirmatory test calculated by formula (1) is  $C = 1 - (1 - 0.000032)^{118520}$  was 97.7%, and the true survival of *P. jackbeardsleyi* on potatoes was less than 0.000032. We normally assume that at the confidence level  $C = 0.95$ , the mortality proportion  $(1 - Pu)$ , when calculated by formula (2), is 99.9975%. As pumpkin is one of the major hosts of *P. jackbeardsleyi* and is often used as a rearing substrate for mealybugs, a confirmatory test on an estimated 49,290 late stage females on pumpkin was also conducted. The mortality proportion calculated by formula (2) was

**Table 3.** Sensory evaluation of 'Rongrien' rambutan fruits by a 20-member panel at 5 d after irradiation of the fruits with various doses of  $\gamma$ -rays.

| Dose (Gy) | Sensory characteristics |                |                 |
|-----------|-------------------------|----------------|-----------------|
|           | Firmness                | Sweetness      | Composite taste |
| 0         | 2.55 ± 0.69 a           | 2.45 ± 0.83 a  | 2.50 ± 0.61 a   |
| 300       | 2.20 ± 0.77 a           | 2.35 ± 0.67 a  | 2.30 ± 0.80 a   |
| 600       | 2.00 ± 0.56 ab          | 2.05 ± 0.69 ab | 2.20 ± 0.62 a   |
| 900       | 2.25 ± 0.64 a           | 2.00 ± 0.65 ab | 2.00 ± 0.56 ab  |
| 1,200     | 1.55 ± 0.69 b           | 1.60 ± 0.82 b  | 1.45 ± 0.76 b   |

Scale for ratings: 3 = excellent, 2 = acceptable, 1 = off-flavors, and 0 = highly distasteful. Mean values followed by the same letter within a column are not significantly different ( $P > 0.05$ ; Tukey's multiple comparison test).

99.9939% at the 95% confidence level (Table 2). The results confirm the conclusion proposed by Hallman et al. (2010) that the host does not affect the efficacy of irradiation on arthropod pests and that all host plants can be used for rearing materials. Furthermore, the mortality proportion calculated by formula (2) is 99.9982% at the 95% confidence level when counting all the irradiated females in the confirmatory tests (Table 2). The maximum absorbed dose used in the confirmatory tests should be the minimum dose required for the approved treatment (Heather 2002; IPPC 2003; Hallman et al. 2010). Therefore, a dose of 166 Gy is suggested as the minimum dose for irradiation as a phytosanitary treatment against *P. jackbeardsleyi* on any commodity.

Besides being effective against the target pest, a phytosanitary treatment should not degrade the quality of a commodity to the point that it is no longer marketable (Hallman 2000). Our quality assessment demonstrated that irradiation doses up to 1,200 Gy showed no significant changes in the chemical and nutritional quality (vitamin C, TSS, acidity, and soluble protein) of 'Rongrien' rambutan up to 15 d of storage. Our findings for the acidity and sugar levels are in line with the results of studies on other fruits: Hawaiian rambutan 'R167', papaya (*Carica papaya* L.; Brassicales: Caricaceae) and orange (*Citrus × sinensis* [L.] Osbeck; Sapindales: Rutaceae) (Terri et al. 2002), mango (Mitchell et al. 1992), grapefruit (*Citrus × paradisi* McFad.; Sapindales: Rutaceae) (Moshonas & Shaw 1982), lemon (*Citrus × limon* L.) Burm.f.; Sapindales: Rutaceae) and mandarin (*Citrus reticulata* Blanco; Sapindales: Rutaceae) (Mitchell et al. 1992), muskmelon (*Cucumis melo* L.; Cucurbitales: Cucurbitaceae) (Lalaguna 1998), strawberry (*Fragaria × ananassa* Duchesne; Rosales: Rosaceae) (Yu et al. 1995) and apple (*Malus domestica* Borkh.; Rosales: Rosaceae) (Bhushan & Thomas 1998). Storage duration has more impact than irradiation at any dose on Vitamin C, acidity, TSS and protein contents of rambutan fruits (Figs. 1–4). Decreases in nutritional contents during ripening are attributed to the decomposition of nutrients as the fruit undergoes senescence (Terri et al. 2002). A 20-member panel found that irradiation doses up to 900 Gy caused only minor changes in the sensory quality of rambutan fruits after 5 d of storage (Table 3). These results are in agreement with those of other studies on irradiated rambutan in which they remained acceptable up to 750–800 Gy (Terri et al. 2002) or 900 Gy (Moy et al. 1999) for variety 'R167' after 9 d of storage.

When irradiation is applied on a commercial scale the maximum dose absorbed by a load can be 1.5 or 2 times the minimum dose applied (Follett & Weinert 2009; Hallman 2011). If 166 Gy is the required minimum dose, parts of the load will receive up to 330 Gy. Almost all hosts of the pest would tolerate this treatment applied on a commercial scale (ICGFI 1994). This suggests that a PI treatment with the minimum absorbed dose of 166 Gy would be practical as a phytosanitary treatment schedule for controlling *P. jackbeardsleyi* on all commodities in international trade.

In conclusion, large-scale confirmatory tests at the target dose of 150 Gy (131–166 Gy measured) applied to the most tolerant stage (late female) of *P. jackbeardsleyi* resulted in the emergence of no  $F_1$  generation 2nd instar nymphs from an estimated 118,520 late stage females reared on potato or 49,290 late females reared on pumpkin. The efficacy was 99.9975% (potato-reared) and 99.9939% (pumpkin-reared) and 99.9982% (potato and pumpkin) at 95% confidence level. Quality evaluation on 'Rongrien' rambutan fruits demonstrated that duration of storage has more effects on quality parameters than irradiation up to doses of 1,200 Gy. The irradiated samples showed no significant changes in the nutritional and chemical characteristics studied, i.e., vitamin C, SST, acidity and soluble protein) and weight loss. 'Rongrien' rambutan tolerated irradiation

up to 900 Gy without significant reduction in sensory quality. Therefore, a radiation dose of 166 Gy is recommended as the minimum irradiation dose for the phytosanitary treatment of the Jack Beardsley mealybug infesting any host commodities.

## Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine Treatments. This research was supported by IAEA Research Contract no. 15633, Chinese AQSIQ Annual Special Project on Animal and Plant Quarantine Treatment (2015), and by Research Project 2015BAD08B02.

## References Cited

- Anura UG, Sirisena I, Watson GW, Hemachandra KS, Wijayagunasekara HNP. 2012. Mealybugs (Hemiptera: Pseudococcidae) introduced recently to Sri Lanka, with three new country records. *Pan-Pacific Entomologist* 88(3): 365-367.
- ASTM E1026-13. 2002. Standard practice for using the Fricke dosimetry system. West Conshohocken, PA.
- Bell C. 2000. Fumigation in the 21st century. *Crop Protection* 19: 563-569.
- Ben-Dov Y, Miller DR, Gibson GAP. 2006. ScaleNet, Life Histories. <http://www.sel.barc.usda.gov/scalenet/lifehist.htm>.
- Bhushan B, Thomas P. 1998. Quality of apples following gamma irradiation and cold storage. *International Journal of Food Sciences and Nutrition* 49: 485-492.
- Bustos ME, Enkerlin W, Reyes JR, Toledo J. 2004. Irradiation of mangoes as a postharvest quarantine treatment for fruit flies. *Journal of Economic Entomology* 97(2): 286-292.
- CABI (CAB International). 2001. *Crop Protection Compendium*. Wallingford, UK: CAB International.
- Couey HM, Chew V. 1986. Confidence limits and sample size in quarantine research. *Journal of Economic Entomology* 79: 887-890.
- Doan TT, Nguyen TK, Vo TKL, Cao VC, Tran TTA, Nguyen HHT. 2012. Effects of gamma irradiation on different stages of mealybug *Dysmicoccus neobrevipes* (Hemiptera: Pseudococcidae). *Radiation Physics and Chemistry* 81: 97-100.
- Dohino T, Masaki S, Takano T, Hayashi T. 1997. Effects of electron beam irradiation on sterility of Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Homoptera: Pseudococcidae). *Research Bulletin of the Plant Protection Service Japan* 33: 31-34.
- Dong SJ, Liu T, Li L, Wang YJ, Gao XW. 2013. Improvement of Vc UV spectrophotometric direct determination in fruits and vegetables. *Food Science and Technology* 38(3): 268-271, 275.
- DPS (Data Processing System). 2010. User's guide. Version 13.5. Hangzhou RuiFeng Information Technology Co., Lt. Hangzhou, China.
- Follett PA. 2007. Postharvest phytosanitary radiation treatment: Less-than-probit 9, generic dose, and high dose applications, pp. 425-433 *In* Vreyssen MJB, Robinson AS, Hendrichs J [eds.], *Area-Wide Control of Insect Pests*. Springer, Dordrecht, The Netherlands.
- Follett PA. 2008. Effect of irradiation on Mexican leafroller (Lepidoptera: Tortricidae) development and reproduction. *Journal of Economic Entomology* 101 (3): 710-715.
- Follett PA, Neven LG. 2006. Current trends in quarantine entomology. *Annual Review of Entomology* 51: 359-385.
- Follett PA, Weinert E. 2009. Comparative radiation dose mapping of single fruit type and mixed-fruit boxes for export from Hawaii. *Journal of Food Processing and Preservation* 33: 231-244.
- Follett PA, Sanxter SS. 2000. Comparison of rambutan quality after hot forced-air and irradiation quarantine treatments. *HortScience* 35(7): 1315-1318.
- Gimpel WF, Miller DR. 1996. Systematic analysis of the mealybugs in the *Pseudococcus maritimus* complex (Homoptera: Pseudococcidae). *Contributions on Entomology International* 2: 1-163.
- Hallman GJ. 2000. Expanding radiation quarantine treatments beyond fruit flies. *Agricultural and Forest Entomology* 2(2): 85-95.
- Hallman GJ. 2004. Ionizing irradiation quarantine treatment against oriental fruit moth (Lepidoptera: Tortricidae) in ambient and hypoxic atmospheres. *Journal of Economic Entomology* 97(3): 824-827.
- Hallman GJ. 2011. Phytosanitary applications of irradiation. *Comprehensive Reviews in Food Science and Food Safety* 10: 143-151.
- Hallman GJ, Loaharanu P. 2002. Generic ionizing radiation quarantine treatments against fruit flies (Diptera: Tephritidae) proposed. *Journal of Economic Entomology* 95: 893-901.
- Hallman GJ, Thomas DB. 1999. Gamma irradiation quarantine treatment against blueberry maggot and apple maggot (Diptera: Tephritidae). *Journal of Economic Entomology* 92 (6): 967-973.
- Hallman GJ, Thomas DB. 2010. Ionizing radiation as a phytosanitary treatment against fruit flies (Diptera: Tephritidae): Efficacy in naturally versus artificially infested fruit. *Journal of Economic Entomology* 103 (4): 1129-1134.
- Hallman GJ, Levang-Brilz NM, Zettler JL, Winborne IC. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. *Journal of Economic Entomology* 103(6): 1950-1963.
- Heather NW. 2002. Generalised quarantine disinfestation research protocol, pp. 171-178 *In* *Irradiation as a phytosanitary treatment of food and agricultural commodities*, IAEA-TEC-DOC-1427, Vienna, Austria.
- Heather NW, Hallman GJ. 2008. Phytosanitation with ionizing radiation, pp. 132-152 *In* *Pest Management and Phytosanitary Trade Barriers*. CAB International, Wallingford, UK.
- Huang F, Li WD, Li XQ, Bei YW, Lin WC, Lu YB, Wang BK. 2014. Irradiation as a quarantine treatment for the solenopsis mealybug, *Phenacoccus solenopsis*. *Radiation Physics and Chemistry* 96: 101-106.
- ICGFI (International Consultative Group on Food Irradiation). 1994. Irradiation as a quarantine treatment of fresh fruits and vegetables. ICGFI Document No.17. Vienna, Austria.
- IPPC (International Plant Protection Convention). 2003. ISPM #18: Guidelines for the use of irradiation as a phytosanitary measure. Food and Agricultural Organization, Rome, Italy.
- IPPC (International Plant Protection Convention). 2007. ISPM #28: Phytosanitary treatments for regulated pests. Food and Agricultural Organization, Rome, Italy.
- Jacobsen CJ, Hara AH. 2003. Irradiation of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) for phytosanitation of agricultural commodities. *Journal of Economic Entomology* 96(4): 1334-1339.
- Jiao Y, Yu DJ, Xu L, Chen ZL, Lou DF, Kang L. 2011. The interception of Jack Beardsley mealybug on wax-apple imported from Thailand. *Plant Quarantine* 25(4): 63-65.
- Kang FF, Wei YD, Cheng Y, Niu CJ, Yin B. 2011. Primary study of gamma ray irradiation effect on *Dysmicoccus neobrevipes* Beardsley. *Plant Quarantine* 25(5): 25-27.
- Kondo T, Andrea AVN, Erika VVN. 2008. Updated list of mealybugs and putoids from Colombia (Hemiptera: Pseudococcidae and Putoidea). *Bulletin of the Museum of Entomology at the University of Valle* 9(1): 29-53.
- Lalaguna F. 1998. Response of 'Galia' muskmelons to irradiation as a quarantine treatment. *HortScience* 33: 118-120.
- LeOra Software. 2002. *ProPlus*. A user's guide to probit or logit analysis. Version 0.03. LeOra Software, Berkeley, CA.
- Mani M., Joshi S, Kalyanasundaram M, Shivaraju C, Krishnamoorthy A, Asokan R, Rebijith, KB. 2013. A new invasive Jack Beardsley Mealybug, *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) on papaya in India. *Florida Entomologist* 96(1): 242-245.
- Mansour M, Gerald F. 1996. Gamma radiation as a quarantine treatment for the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* 89(5): 1175-1180.
- Mitchell GE, McLauchlan RL, Isaacs AR, Williams DJ, Nottingham SM. 1992. Effect of low dose irradiation on composition of tropical fruits and vegetables. *Journal of Food Composition and Analysis* 5: 291-311.
- Moshonas MG, Shaw PE. 1982. Irradiation and fumigation effects on flavor, aroma and composition of grapefruit products. *Journal of Food Science* 47: 958-964.
- Moy JH, Paull RE, Bian X, Chung R, Wong L. 1999. Quality of tropical fruit irradiated as a quarantine treatment, pp. 45-53 *In* Moy JH, L. Wong [eds.], *The use of irradiation as a quarantine treatment of food and agricultural commodities*. Proceedings of a Workshop, Honolulu, Hawaii, 10-12 Nov 1997.
- N'Guessan PW, Watson GW, Brown JK, N'Guessan FK. 2014. First record of *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) from Africa, Côte D'Ivoire. *Florida Entomologist* 97(4): 1690-1693.
- Ravuiwasa KT, Lu KH, Shen TC, Hwang SY. 2009. Effects of irradiation on *Planococcus minor* (Hemiptera: Pseudococcidae). *Journal of Economic Entomology* 102(5): 1774-1780.
- Shao Y, Ren LL, Liu YJ, Wang YJ, Jiao Y, Wang QL, Zhan GP. The preliminary results of the impact on the development and reproduction of Jack Beardsley mealybug irradiated with Cobalt-60 gamma rays. *Plant Quarantine* 27(6): 51-55.

- Terri DB, Reitmeier AC, Moy JH, Mosher GA, Taladriz L. 2002. Sensory quality and nutrient composition of three Hawaiian fruits treated by X-irradiation. *Journal of Food Quality* 25: 419-433.
- Tokihiko G. 2006. List of mealybugs (Homoptera: Pseudococcidae) intercepted by Japanese plant quarantine mainly from areas without a record of distribution. *Japan Research Bulletin of the Plant Protection Service* 42: 59-61.
- Torres RZ, Hallman GJ. 2007. Low-dose irradiation phytosanitary treatment against Mediterranean fruit fly (Diptera: Tephritidae). *Florida Entomologist* 90: 343-347.
- Williams DJ. 2004. Mealybugs of Southern Asia. Southdene Sdn Bhd, Kuala Lumpur, Malaysia. pp. 667-671.
- Williams DJ, Watson GW. 1988. The scale insects of the tropical South Pacific Region. Part 2. The Mealybugs (Pseudococcidae). CAB International, Wallingford, UK. 260 pp.
- Yu L, Reitmeier CA, Gleason ML, Nonnecke GR, Olson DG, Gladon RJ. 1995. Quality of electron beam irradiated strawberries. *Journal of Food Science* 60: 1084-1087.
- Yu RL, Huang WF, Chen F, Wu XY, Huang SY. 2006. Determination of vitamin C from local product fruits of Guangxi by spectrophotometry. *Studies of Trace Elements and Health* 2(2): 32-33.
- Zhan GP, Li BS, Gao MX, Liu B, Wang YJ, Liu T, Ren LL. 2014. Phytosanitary irradiation of peach fruit moth (Lepidoptera: Carposinidae) in apple fruits. *Radiation Physics and Chemistry* 103: 153-157.