Gamma Irradiation as a Phytosanitary Treatment of *Bactrocera tau* (Diptera: Tephritidae) in Pumpkin Fruits

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ABSTRACT The fruit fly *Bactrocera tau* (Walker) is an important quarantine pest that damages fruits and vegetables throughout Asian regions. Host commodities shipped from infested areas should undergo phytosanitary measures to reduce the risk of shipping viable flies. The dose–response tests with 1-d-old eggs and 3-, 5-, 7-, 8-d-old larvae were initiated to determine the most resistant stages in fruits, and the minimum dose for 99.9968% prevention of adult eclosion at 95% confidence level was validated in the confirmatory tests. The results showed that 1) the pupariation rate was not affected by gamma radiation except for eggs and first instars, while the percent of eclosion was reduced significantly in all instars at all radiation dose; 2) the tolerance to radiation increased with increasing age and developmental stage; 3) the estimated dose to 99.9968% preventing adult eclosion from late third instars was 70.9 Gy (95% CL: 65.6–78.2, probit model) and 71.8 Gy (95% CL: 63.0–87.3, logit model); and iv) in total, 107,135 late third instars cage infested in pumpkin fruits were irradiated at the target dose of 70 Gy (62.5–85.0, Gy measured), which resulted in no adult emergence in the two confirmatory tests. Therefore, a minimum dose of 85 and 72 Gy, which could prevent adult emergence at the efficacy of 99.9972 and 99.9938% at the 95% confidence level, respectively, can be recommended as a minimum dose for phytosanitary treatment of *B. tau* in any host fruits and vegetables under ambient atmospheres.

KEY WORDS *Bactrocera tau*, phytosanitary irradiation, gamma radiation, irradiation, pumpkin

Introduction

The fruit fly *Bactrocera tau* (Walker) is a primary pest that damages fruits and vegetables throughout South Asian countries (India, Sri Lanka, Bangladesh, and Bhutan), to Southeast Asian countries (Thailand, Laos, Myanmar, Pakistan, Singapore, Cambodia, Malaysia, Vietnam, Philippines, and Indonesia), and the Far East Asian region including Taiwan and South China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hubei, Hong Kong, Sichuan, Yunnan, and Zhejiang; White and Elson-Harris 1992, Akhtaruzzaman et al. 1999, Thanaphum and Thaenkham 2003, Prabhakar et al. 2009, Hu et al. 2010). Host plants of *B. tau* are Anacardiaceae, Cucurbitaceae, Elaeocarpaceae, Moraceae, Myrtaceae, Oxalidaceae, Rutaceae, Sapotaceae, and Solanaceae (Mahfuza et al. 2011). The fly appears to show a preference for attacking the fruits of Cucurbitaceae, such as melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita maxima* Duchesne), angled luffa [*Luffa acutangula* (L.) Roxb.], and bitter gourd (*Momordica charantia* L.; CAB International [CABI] 2009). Like other fruit flies, *B. tau* damages fruits and vegetables by laying eggs under the skin. The eggs hatch into larvae feeding in the flesh of the fruits or vegetables. Infested fruits and vegetables become rotten and inedible or drop to the ground prematurely, thus causing considerable production losses (Li et al. 2006). In China, the production losses caused by *B. tau* were 21–34% in *Siraitia grosuenorii* Swingle and 21.3–31.8% in pumpkin (Deng 1992, Liu et al. 2005). In Indonesia, the fruit loss of passion fruit (*Passiflora edulis* Sims) was estimated as high as 40% (Hasyim et al. 2008). Therefore, this fruit fly has been regarded as a potential high risk pest by importing countries, such as the United States, Australia, New Zealand, and Japan (Ohno et al. 2008, CABI 2009, Biosecurity Australia 2011, Hossain et al. 2011).

Currently, fumigation, temperature (cold and heat), and irradiation are the main phytosanitary treatment measures that are commonly used for postharvest disinfestations of fruit flies in fruits and vegetables (Heather and Hallman 2008). Fumigation with methyl bromide is the most common type of insect disinfestation treatment, but leaves residues after the fumigation (Bell 2000). Irradiation is a promising phytosanitary treatment that is increasing in use worldwide. Advantages over other treatments include tolerance by the vast majority of fresh commodities, ability to treat in final packaging in pallet loads, and lack of residues (Hallman

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2011). Usually, prevention of emergence of normal looking adults and not larval mortality is the objective of irradiation treatments of tephritids. The International Plant Protection Convention (IPPC) approved the generic dose of 150 Gy for Tephritidae, citing the 11 studies that may lead to 99.9968% prevention of normal-looking adult eclosion from third instars at a 95% confidence level (Hallman 2012). However, to save resources and reduce the potential negative effect of an irradiation treatment on commodities, the effective dose for any treatment should be made as low as possible (Torres and Hallman 2007). Although, Hossain et al. (2006) developed the dose required to kill immature stages of *B. tau* in fruits, the minimum dose for prevention of adult emergence was not identified and should be established to facilitate the application of irradiation as a phytosanitary treatment of fruits and vegetables.

The objective of this research was to compare the radiation tolerance in eggs and instars of *B. tau* that infest host fruits and vegetables, and determine the minimum absorbed dose that provides quarantine security of late third instars in pumpkin fruits at the $ED_{99.9968}$ (effective dose) with 95% confidence level. This required conducting dose–response tests to determine the most tolerant stage infested in fruits, and treating a minimum of 93,636 late third instars (the most tolerant stage) with no adult emergence in the confirmatory tests (Couey and Chew 1986, IPPC 2003, 2007).

Materials and Methods

Insects Rearing. Test insects were originally collected from pumpkin fruits that were infested with *B. tau* in a pumpkin field in Guangzhou city, Guangdong Province, China, in 2011 and 2012. Late third instars of *B. tau* that emerged out of pumpkin fruits were transferred to moist sand for pupariation in laboratory. One day before adult eclosion, the pupae were placed in rearing cages (60 by 60 by 120 cm), and the adults were fed with fresh papaya pulp and a solid mixture of sucrose and hydrolyzed yeast (3:1) (Li et al. 2014). Eggs were colleted by placing pumpkin pulp slices (2–3 mm in thickness) in Petri dishes in the adults rearing cage, and transferred to the surface of pumpkin slices (~1 cm in thickness) that placed in the rearing boxes. Larvae were allowed to develop in pumpkin pulp till late third instars emerged (8 d in the rearing room), then pupariated in moist sand and subsequently emerged. All the stages were reared under 24–26°C, 50–70% relative humidity (RH), with a photoperiod of 14:10 (L:D) h in the rearing room.

Experiment Design. *Egg Collection and Larvae Rearing.* To minimize variation in egg age, eggs laid by adults within 2 h were divided on to the black filter papers with 100 individual eggs on each, and then transferred to the pumpkin slices (~1 cm in thickness) that were placed on the bottom of rearing cups (plastic, 6 cm in diameter and 5 cm in height). New pumpkin pulp was added for *B. tau* larvae feeding and development to the required stages.

Dose–Response Tests on Eggs. To conduct dose–response test, the ISPM #18 recommended using at least five dose levels and a control for each developmental stage, with a minimum of 50 individuals, where possible, for each of the doses and a minimum of three replicates (IPPC 2003). The 1-d-old eggs (in the rearing cups with pumpkin slices) were irradiated at a series doses between 7 and 49 Gy at 7-Gy increments; each dose was replicated three times (three cups).

Dose–Response Tests on Larvae. According to the lifespan of *B. tau* developed under laboratory condition at 25°C and 60–70% RH on pumpkin (Singh et al. 2010), the first, second, third, and late third instars used in the dose–response tests were prepared by placing eggs on pumpkin slices within rearing cup (100 eggs per cup) for the development of 3, 5, 7, and 8 d, respectively. All larval stages were irradiated at the target dose of 14 (for first and second instars), 21, 28, 35, 42, 49, 56, and 63 Gy (for third and late third instars); each dose was replicated three times (three cups).

Confirmatory Tests on Late Third Instars. To confirm the minimum dose to provide quarantine security at the 99.9968% level of prevention of adult emergence (95% confidence level), a target dose of 70 Gy (based on the probit analysis) was applied to the pumpkins infested with late third instars (8 d old). The confirmatory tests were repeated until the cumulative number of insects treated exceeded 100,000. On each of the confirmatory tests, 60 pumpkins (1.5–2 kg, water-washed surface) were placed in two adult cages for 2 h to allow for oviposition. The infested pumpkins were placed in 10-litre plastic boxes for 8 d, where a nylon net was used for preventing the escape of larvae.

Irradiation Treatment. *Irradiator.* All irradiation treatments were conducted at the National Institute of Metrology Research Irradiator, Beijing, China, where a 1.5×10^{15} Bq Colbalt-60 source of gamma radiation was located 5 m below the surface of the platform used for radiation treatment. Reference standard and routine dosimetry were done with the Fricke system (ASTM E1026-13, 2002). This dosimetry system was calibrated in accordance with the international standard ISO/ASTM 51261 (2002) and ASTM E1026-13 (2002), and the uncertainty of the measured value was calculated according to ISO/ASTM 51707 (2002).

Gamma Radiation in the Dose–response Tests. All the eggs and larvae reared in pumpkin pulps designated to be irradiated at the same dose were subjected to gamma radiation simultaneously, where the rearing cups were placed 100 cm from the source by randomly placing every three cups in a row. Halfway through the treatment, the irradiator was stopped and the cups were each turned 180° to give a more uniform exposure for each radiation dose. The absorbed dose was monitored by placing 12 Fricke dosimeters (National Institute of Metrology, Beijing, China) in the rearing cups for the 56-Gy treatments; the dose rate monitored was 6.1 Gy/min with the dose uniformity ratio (maximum/minimum) of 1.16.

Gamma Radiation in the Confirmatory Tests. Every five pumpkins infested with 8-d-old larvae were placed in one plastic box (20 by 33 by 23 cm) for radiation in the two confirmatory tests. On each of the treatment dates (September 2012 and March 2013), 1 box was selected as control and the other 11 boxes were exposed to gamma radiation simultaneously on a platform surrounding the source, where the boxes were placed within 100 cm from the source. The irradiator was stopped and the boxes were rotated 180° after half of the total exposure time to give a more uniform exposure. In each confirmatory test, five Fricke dosimeters (two in the center and three on the surface of pumpkin) were included in every fifth box to measure dose variation.

Rearing After Irradiation. After irradiation, the eggs and immature larvae were reared with pumpkin pulp in the rearing room to allow for the maturation of larvae. All the emerging late third instars were transferred to moist sand for pupariation and adult eclosion. The number of late third instars, pupae, and adults were finally checked 3 wk after the late third instars emerged from the pumpkin pulp.

Data Analysis. Dose–response data on the number of late third instars, as well as the percentage of pupariation and ecolosion in each dose and control, were subjected to one-way analysis of variance (ANOVA), means were separated by Tukey's multiple comparison tests (Data Processing System [DPS] 2010). To make comparison of radiation tolerance between life stages, dose–response data on percent mortality to adult stage for emerging late third instars were arcsine-transformed to improve normality and subjected to linear regression after analysis of covariance (ANCOVA) using the Tukey model. Data used in the analyses included any radiation dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality (Follett 2004, DPS 2010). For each replicate, mortality values <100% were adjusted for control mortality using Abbott's formula (Abbott 1925). To estimate the minimum dose for 99.9968% prevention of adult emergence for conducting confirmatory test, the dose–mortality data were subjected to probit analysis (probit and logit model) using the PoloPlus program (LeOra Software 2002). For the confirmatory tests, the level of confidence associated with treating a number of insects with 0 survivors is given by the equation,

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

where *Pu* is the acceptable level of survivorship (as a proportion) and *n* is the number of test insects (Couey and Chew 1986). Confidence level was calculated for the number of treated *B. tau* late third instars assuming the efficacy of 99.9968% that was normally required by the treatment of fruit flies (Hallman 1999, Follett and Neven 2006), and the mortality proportion $(1 - Pu)$ was calculated by equation (2) assuming the confidence level at 95%.

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

Results

Radiation Effect on the Development to Pupae. The number of late third instars of *B. tau* developing out of irradiated eggs and larvae at all doses did not significantly decreased as compared with their controls ($P > 0.05$), indicating that the development to late third instars was not strongly affected by radiation up to 63 Gy (Table 1). The percentage of pupariation was significantly decreased in eggs ($F_{7,16} = 3.8$; $P = 0.0126$) and first instars ($F_{7,16} = 3.5$; $P = 0.0179$) as compared with their controls, but not significantly affected by radiation doses up to 63 Gy in other instars (Table 1). To compare the tolerance between life stages, the percentage of pupariation in Table 1 were corrected with their control mortality and subject to ANOVA. The corrected percentage of pupariation from eggs and first instars were significantly lower than that in other instars when irradiated at 35 Gy ($F_{4,10} = 6.0$; $P = 0.01$), 42 Gy ($F_{4,10} = 6.3$; $P = 0.0086$), or 49 Gy ($F_{4,10} = 6.2$; $P = 0.0089$), respectively. Thus, the lowest mean percentage of pupariation at the radiation doses >35 Gy in eggs indicated that it was the most susceptible stage, and first instars were more susceptible than other instars when prohibition of pupariation was used as an indicator of effectiveness.

Prevention of Adult Emergence. *Percentage of Adult Eclosion.* The percentage of adult eclosion was significantly decreased as dose increased for eggs ($F_{7,16} = 165.2$; $P < 0.0001$), first instars ($F_{7,16} = 131.7$; $P < 0.0001$), second instars ($F_{7,16} = 114.4$; $P < 0.0001$), third instars ($F_{7,16} = 583.6$; $P < 0.0001$), and late third instars ($F_{7,16} = 64.6$; $P < 0.0001$) (Table 1). No eggs or first instars developed to adults at a radiation dose of 35 Gy, no second instars developed to adults at 49 Gy, and no third instars or late third instars developed to adults at 56 Gy (Table 1), indicating that the minimum dose to 100% prevention of adult eclosion increased with increasing age and developmental stage.

Linear Regression. The dose–response data on prevention of adult ecolosion from emerging late third instars analyzed by ANCOVA showed mortality at all stages, and the radiation dose had significant interaction ($F_{9,68} = 4.8$; $P = 0.0018$). Therefore, all radiation doses affected the survival of different developmental stages of *B. tau*, with the percentage mortality to adult stage increased with increasing radiation dose. Linear regression was used to test whether slopes were significantly different from 0 (significant effect of radiation dose), and to predict a radiation dose needed to prevent adult eclosion in *B. tau*. Slopes were positive and significant for eggs and all instars ($P < 0.0001$), indicating that the tolerance to radiation increased with increasing age and developmental stage (Table 2). The late third instars were predicted to require the highest radiation dose to prevent 100% adult emergence (estimated at 52.0 Gy), whereas the eggs were predicted to require the lowest dose to prevent 100% adult emergence (estimated at 33.1 Gy; Table 2).

Probit Analysis. The parameters from the results of probit analysis (probit and logit model), including slope, heterogeneity (chi-square divided by degrees of freedom), and estimated minimum absorbed dose to 99% (ED_{99}) and 99.9968% ($ED_{99.9968}$) prevention of adult emergence, are presented in Table 3. The estimated ED_{99} and $ED_{99.9968}$ analyzed by the probit were

Table 1. Maturation of *B. tau* eggs and instars after irradiation

Stage	Dose (Gy)	No. treated (mean ± SE)	No. emerging third instars (mean ± SE)	% pupariation[a] (mean ± SE)	% adult eclosion[b] (mean ± SE)
Egg (1 d)	0	100 ± 0.0	77.3 ± 1.9a	97.8 ± 0.4a	91.5 ± 1.9a
	7	100 ± 0.0	69.3 ± 2.9a	95.7 ± 2.4a	91.2 ± 5.8a
	14	100 ± 0.0	64.3 ± 3.9a	87.9 ± 5.6ab	73.3 ± 6.4b
	21	100 ± 0.0	64.7 ± 5.8a	91.2 ± 2.7ab	18.1 ± 3.2c
	28	100 ± 0.0	66.7 ± 1.8a	92.5 ± 1.9ab	1.5 ± 0.9d
	35	100 ± 0.0	71.3 ± 5.6a	87.5 ± 1.9ab	0.0 ± 0.0d
	42	100 ± 0.0	64.3 ± 4.7a	85.6 ± 2.5ab	0.0 ± 0.0d
	49	100 ± 0.0	69.3 ± 3.5a	80.5 ± 3.0b	0.0 ± 0.0d
L_1 (3 d)	0		75.7 ± 5.8a	96.8 ± 1.0a	89.7 ± 3.9a
	14		76.7 ± 0.3a	95.6 ± 1.8ab	84.3 ± 4.8a
	21		76.7 ± 5.2a	94.1 ± 1.1ab	61.9 ± 6.5b
	28		80.3 ± 3.4a	86.2 ± 3.4b	7.6 ± 4.6c
	35		73.7 ± 2.3a	87.6 ± 2.8ab	0.0 ± 0.0c
	42		77.3 ± 4.4a	90.3 ± 2.5ab	0.0 ± 0.0c
	49		73.3 ± 2.9a	87.7 ± 1.0ab	0.0 ± 0.0c
	56		76.3 ± 2.9a	93.1 ± 2.2ab	0.0 ± 0.0c
L_2 (5 d)	0		72.7 ± 2.7a	99.1 ± 0.9a	92.7 ± 3.2ab
	14		73.0 ± 2.6a	98.5 ± 0.9a	97.0 ± 1.7a
	21		66.3 ± 9.8a	92.2 ± 3.8a	80.9 ± 3.1b
	28		84.3 ± 1.5a	95.7 ± 1.4a	60.4 ± 11.1c
	35		76.7 ± 2.7a	95.5 ± 1.7a	6.6 ± 0.9d
	42		68.0 ± 4.5a	94.6 ± 0.8a	1.1 ± 1.1d
	49		83.3 ± 0.9a	92.4 ± 2.1a	0.0 ± 0.0d
	56		79.0 ± 4.6a	91.6 ± 0.9a	0.0 ± 0.0d
L_3 (7 d)	0	79.0 ± 1.5	79.0 ± 1.5a	99.2 ± 0.8a	92.5 ± 1.3a
	21	77.3 ± 3.4	77.3 ± 3.4a	97.8 ± 0.5a	89.1 ± 1.9a
	28	71.0 ± 3.5	71.0 ± 3.5a	95.2 ± 2.2a	59.5 ± 3.9b
	35	69.3 ± 1.2	69.3 ± 1.2a	99.0 ± 1.0a	7.7 ± 1.8c
	42	73.7 ± 3.8	73.7 ± 3.8a	97.8 ± 0.4a	0.4 ± 0.4c
	49	76.3 ± 2.6	76.3 ± 2.6a	95.7 ± 1.5a	0.4 ± 0.4c
	56	71.0 ± 1.7	71.0 ± 1.7a	97.7 ± 0.9a	0.0 ± 0.0c
	63	73.0 ± 4.5	73.0 ± 4.5a	95.4 ± 0.9a	0.0 ± 0.0c
Late L_3 (8 d)	0	88.3 ± 3.2	88.3 ± 3.2a	96.9 ± 0.5a	91.7 ± 0.8a
	21	80.0 ± 1.5	80.0 ± 1.5a	98.3 ± 0.5a	88.3 ± 2.2a
	28	80.3 ± 2.7	80.3 ± 2.7a	89.9 ± 2.2a	49.7 ± 13.2b
	35	85.3 ± 3.5	85.3 ± 3.5a	96.5 ± 0.6a	18.4 ± 3.1c
	42	86.3 ± 2.6	86.3 ± 2.6a	97.3 ± 0.7a	5.7 ± 1.9c
	49	85.3 ± 1.3	85.3 ± 1.3a	94.9 ± 3.1a	0.4 ± 0.4c
	56	74.7 ± 2.6	74.7 ± 2.6a	98.7 ± 0.8a	0.0 ± 0.0c
	63	77.0 ± 9.6	77.0 ± 9.6a	97.9 ± 0.6a	0.0 ± 0.0c

Within a stage, means followed by the same letter a, b, or c within a column are not significantly difference ($P > 0.05$; Tukey's multiple comparison test);

[a] % pupariation, No. pupae/No. emerging third instars ×100.

[b] % adult eclosion, No. adult/No. pupae × 100.

much closer to that estimated by the logit model, and the small value of heterogeneity in probit model (0.08–1.69) and logit model (0.13–2.72) indicated that the estimations had good fit to the data. In addition, the estimated ED_{99}, which was very close to the dose which produce 100% mortality in dose–response tests (Tables 1 and 3), increased from eggs to late third instars, indicating the resistance in *B. tau* to radiation increased with increasing age and developmental stage. Thus, the late third instars likely to be found in fruits were determined to be the most tolerant stage in fruits.

Confirmatory Tests. Confirmatory tests were conducted to validate the estimated dose to 99.9968%, preventing adult eclosion from late third instars (the most resistant stage in harvested fruits) of *B. tau*, where a dose of 70 Gy was selected as target dose according to the estimation of probit analysis (Table 3). No adults emerged from 107,135 late third instars developed in the pumpkin fruits, whereas the percentage of adult eclosion in the control was >90% (Table 4). Actual absorbed doses measured by dosimetry ranged from 62.0 to 71.7 Gy in the first and 65.3 to 85.0 Gy in the second confirmatory test (Table 4), resulting in the dose uniformity ratio (maximum/minimum) of 1.16 and 1.30, respectively.

Table 2. Linear regressions on mortality to adult stage when eggs and larval stages of *B. tau* were irradiated at 7–63 Gy

Stage	Observations	y-intercept (mean ± SE)	Slope (mean ± SE)	R^2	Predicted dose for 100% mortality (Gy)
Egg	15	−25.78 ± 6.00	3.50 ± 0.26	0.9340	33.1
L_1	12	−62.43 ± 11.81	4.43 ± 0.46	0.9028	34.4
L_2	15	−56.08 ± 9.39	3.30 ± 0.32	0.8931	44.3
L_3	18	−43.13 ± 11.64	2.66 ± 0.29	0.8414	50.1
Late L_3	18	−44.21 ± 7.78	2.58 ± 0.19	0.9179	52.0

Regression analysis used data on prevention adult eclosion from emerging late third instars in Tables 1. The percentage mortality to adult stage was corrected for control mortality using Abbott's formula and arcsine-transformed. Extrapolated values for 100% mortality are used for comparing stage-specific tolerance to irradiation, not to suggest a treatment dose to prevent adult eclosion.

Discussion

To identify the most resistant stage, the relationship between dose and response for each stage of an insect is determined using a dose–response test (IPPC 2003). In general, tolerance in *B. tau* to radiation increased with increasing age and developmental stage when comparing the efficacy on preventing pupariation and eclosion. The fact that a radiation dose of >35 Gy produced the lowest pupariation rate and 100% prevention eclosion from irradiated 1-d-old eggs indicates that it is more sensitive to radiation than other stages (Table 1). Moreover, the linear regression and probit analysis are ordinarily used to analyze data on dose–mortality (preventing adult eclosion from third instars) after arcsine or probit transformation of mortality, these extrapolated values are presented to illustrate relative differences in stage-specific response and tolerance to irradiation, and the $ED_{99.9968}$ may be used to suggest a treatment dose to prevent adult eclosion (Hallman and Martinez 2001, Follett 2004). The lowest dose of 33.1 Gy to 100% prevention adult eclosion (Table 2) and the lowest value of ED_{99} (Table 3) also indicate that the 1-d-old eggs are the most radio-sensitive stage. When comparing the radiation effects on preventing adult eclosion, the predicted dose to 100% mortality to adult stage and the value of ED_{99} and $ED_{99.9968}$ increased from eggs to late third instars, indicating that the sequence of the tolerance to radiation in the stages of *B. tau* is as: late third instars > third instars > second instars > first instars > eggs. Therefore, the late third instars (8 d) were determined to be the most tolerant stage in pumpkin fruits. This is in agreement with the extensive review of the irradiation treatments literatures by Hallman et al. (2010) that the most developed stage is invariably the most radio-tolerant when a common measure of efficacy is used. Fruit fly third instars are the most tolerant stage in their host fruits when conducting radiation tests (Hallman and Loaharanu 2002). However, *B. tau* late third instars (8 d) were a little

Table 3. Estimating the minimum absorbed dose for prevention of adult eclosion from irradiated eggs and larval instars of *B. tau*

Stage	Analyzing model	Slope ± SE[a]	Intercept ± SE[a]	ED_{99} (95% CI)	$ED_{99.9968}$ (95% CI)	Heterogeneity
Egg	Probit	10.06 ± 0.74	−12.52 ± 0.96	29.9 (28.1, 32.5)	43.9 (39.4,50.6)	0.08
	Logit	0.399 ± 0.034	−7.15 ± 0.67	29.4 (27.9, 31.6)	43.9 (40.3, 48.9)	0.13
L_1	Probit	15.20 ± 1.24	−20.76 ± 1.72	33.0 (29.7, 41.9)	42.5 (35.7, 65.1)	3.66
	Logit	0.443 ± 0.039	−10.06 ± 1.00	33.5 (32.0, 35.9)	46.5 (43.2, 51.3)	0.80
L_2	Probit	18.56 ± 1.62	−27.47 ± 2.43	40.8 (38.2, 45.7)	50.8 (45.4, 62.1)	3.35
	Logit	0.421 ± 0.036	−12.71 ± 1.25	41.0 (39.5, 43.2)	54.7 (51.2, 59.6)	0.64
L_3	Probit	13.16 ± 1.00	−19.42 ± 1.52	44.9 (41.1, 52.4)	60.1 (51.7, 79.9)	1.69
	Logit	0.314 ± 0.023	−9.48 ± 0.72	44.8 (41.2, 51.3)	63.1 (55.3, 78.0)	1.94
Late L_3	Probit	10.64 ± 0.61	−15.70 ± 0.93	49.4 (47.2, 52.1)	70.9 (65.6, 78.2)	0.98
	Logit	0.249 ± 0.015	−7.54 ± 0.50	48.8 (44.7, 55.4)	71.8 (63.0, 87.3)	2.72

[a] Mean ± SE; heterogeneity means chi-square divided by degrees of freedom.

Table 4. Large-scale confirmatory tests irradiating *B. tau* late third instars in pumpkin fruits

Date of radiation	No. pumpkin	Dose monitored (Gy)		No. emerging third instars[a]	No. pupae	No. adults
		Max	Min.			
Sept. 2012	55	71.7	62.0	48,700	47,806	0
(Control)	5	0	0	4,429	4,360	4,092
Jan. 2013	55	85.0	65.3	58,435	58,037	0
(Control)	5	0	0	6,951	6,828	6,425

[a] The late third instars were collected from pumpkin fruits within 1 d after irradiation.

more tolerant than third instars (7 d) in our research (Tables 2 and 3). Therefore, *B. tau* late third instars should be treated to validate the efficacy as the most resistant stage should be tested in the confirmatory tests even if it is not the most common one occurring in the commodity (IPPC 2003, 2007).

Efficacy of an irradiation treatment against tephritids is measured by the prevention of the eclosion of adults capable of flight when irradiated as third instars inside fruit (Hallman and Loaharanu 2002, Follett and Armstrong 2004). In the confirmatory test, the target dose was ordinarily based on the value of $ED_{99.9968}$ estimated by statistical analysis of the dose–response data, where probit analysis was widely used for a number of fruit flies (Heather et al. 1991, Mansour and Franz 1996, Hallman and Thomas 2010, Bustos et al. 2004). Therefore, the estimated dose of 70.9 Gy (probit model) and 71.8 Gy (logit model), that could control the most resistant stage of *B. tau* at the efficacy of 99.9968% with the 95% confidence level (Table 3), should be applied to *B. tau* late third instars in the confirmatory tests. Hence, a radiation dose of 70 Gy was selected as target dose to conduct the confirmatory tests. In total, 48,700 and 58,435 late third instars developed in pumpkin fruits were irradiated in the two confirmatory tests, and as a result there was no adult eclosion (Table 4). Therefore, the probit analysis was validated. This research, performed with almost all fully developed third instars, was a worst-case test of the fly's ability to survive an irradiation treatment. Most *B. tau* in pumpkin can be expected to be less developed than late third instars, and thus more susceptible to irradiation. Therefore, assuming a required efficacy of 99.9968%, the confidence level (C) calculated by equation (1) is: $C = 1 - (1 - 0.000032)^{107135}$, then our confidence level was 96.8% that the true survival of *B. tau* was <0.000032. The most rigorous standard used for confirming the efficacy of an irradiation treatment is "probit 9" at the 95% confidence level (Hallman and Loaharanu 2002). Probit 9 represents the effective dose to achieve a result at the 99.9968 percentile ($ED_{99.9968}$). In our research, the mortality proportion $(1 - Pu)$ in *B. tau* late third instars calculated by equation (2) was 99.9972% at the 95% confidence level when counting all the irradiated late third instars in the two confirmatory tests. The maximum absorbed dose in the confirmatory tests may be the minimum dose required for the approved treatment (Heather 2002, IPPC 2003, Hallman et al. 2010). Therefore, a dose of 85 Gy (Table 4) is suggested as the minimum dose for treatment of *B. tau* in fruits and vegetables. This research shows that irradiation treatments at a minimum absorbed dose of 85 Gy provides quarantine security against *B. tau* to the highest degree demanded of a commercial phytosanitary treatment, $ED_{99.9968}$ at the 95% confidence level. These results accordingly support the assertion that relatively low doses of radiation can serve as phytosanitary treatments against many tephritids (Hallman, 1999), and supports the proposal by IPPC (2009) of a generic dose of 150 Gy for all eggs and larvae of the family of Tephritidae on all host commodities.

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 and Weinert 2009, Hallman et al. 2010), so that when 85 Gy is sought, some of the load could receive up to <200 Gy. Almost all hosts of the pest would tolerate this treatment applied on a commercial scale (ICGFI 1994). Even avocado, which has low tolerance to fumigation and temperature treatment but tolerates about 100–200 Gy (Thomas 2001), might have a viable treatment against *B. tau* with 85 Gy. Therefore, a minimum dose of 85 Gy, that could acquire the controlling (preventing adult emergence) efficacy of 99.9972% at the 95% confidence level, can be recommended for the phytosanitary treatment of *B. tau* on all shipped fruits and vegetables. In addition, some countries accept the efficacy of 99.99% mortality to adult stage at the 95% confidence level. The mortality proportion of *B. tau* late third instars in the first confirmatory test calculated by equation (2) was 99.9938% at the 95% confidence level, where the highest dose of 71.7 Gy was measured

(Table 4). Therefore, a minimum dose of 72 Gy could be also recommended for the phytosanitary treatment of *B. tau* in the commodity under ambient atmospheres.

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References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Akhtaruzzaman, M., M. Z. Alam, and M. A. Sardar. 1999. Identification and distribution of fruit flies infesting cucurbits in Bangladesh. Bangladesh J. Entomol. 9: 93–101.

ASTM E1026-13. 2002. Standard practice for using the Fricke dosimetry system. West Conshohocken, PA.

Bell, C. 2000. Fumigation in the 2first century. Crop Prot. 19: 563–569.

Bustos, M. E., W. Enkerlin, J. Reyes, and J. Tolrdo. 2004. Irradiation of Mangoes as a postharvest quarantine treatment for fruit flies. J. Econ. Entomol. 97: 286–292.

Biosecurity Australia. 2011. Revised conditions for importing fresh mango fruit from India, final report. Department of Agriculture, Fisheries and Forestry, Canberra. (http://www.daff.gov.au/__data/assets/pdf_file/0011/1901567/Mangoes_from_India-Final_revised_conditions.pdf).

Couey, H. M., and V. Chew. 1986. Confidence limits and sample size in quarantine research. J. Econ. Entomol. 79: 887–890.

(CABI) CAB International. 2009. Invasive species compendium pest: *Bactrocera tau*. (http://www.cabi.org/isc/datasheet/8741).

Deng, Y. P. 1992. Biological characteristics and control measure of *Bactrocera tau* in Grosvenor momordica fruit orchard. Plant Prot. 18: 24–25.

(DPS) Data Processing System. 2010. User's guide. Version 13.5. Hangzhou RuiFeng Information Technology Co., Lt. Hangzhou, China.

Follett, P. A. 2004. Irradiation to control insects in fruits and vegetables for export from Hawaii. Radiat. Phys. Chem. 71: 161–164.

Follett P. A., and J. W. Armstrong. 2004. Revised irradiation doses to control Melon fly, Mediterranean fruit fly, and Oriental fruit fly (Diptera: Tephritidae) and a generic dose for Tephritid fruit flies. J. Econ. Entomol. 97: 1254–1262.

Follett, P. A., and L. G. Neven. 2006. Current trends in quarantine entomology. Annu. Rev. Entomol. 51: 359–385.

Follett, P. A., and E. Weinert. 2009. Comparative radiation dose mapping of single fruit type and mixed-fruit boxes for export from Hawaii. J. Food Process. Preserv. 33: 231–244.

Hallman, G. J. 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. Postharvest Biol. Technol. 16: 93–106.

Hallman, G. J. 2011. Phytosanitary applications of irradiation. Compr. Rev. Food Sci. Food Saf. 10: 143–151.

Hallman, G. J. 2012. Generic phytosanitary irradiation treatments. Radiat. Phys. Chem. 81: 861–866.

Hallman, G. J., and L. R. Martinez. 2001. Ionizing irradiation quarantine treatment against Mexican fruit fly (Diptera: Tephritidae) in citrus fruits. Postharvest Biol. Tech. 23: 71–77.

Hallman, G. J., and P. Loaharanu. 2002. Generic ionizing radiation quarantine treatments against fruit flies (Diptera: Tephritidae) proposed. J. Econ. Entomol. 95: 893–901.

Hallman, G. J., and D. B. Thomas. 2010. Ionizing radiation as a phytosanitary treatment against fruit flies (Diptera: Tephritidae): efficacy in naturally versus artificially infested fruit. J. Econ. Entomol. 103: 1129–1134.

Hallman, G. J., M. L. Nichole, J. L. Zettler, and I. C. Winborne. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. J. Econ. Entomol. 103: 1950–1963.

Heather, N. W. 2002. Generalised quarantine disinfestation research protocol, pp. 171–178. *In* Irradiation as a phytosanitary treatment of food and agricultural commodities. IAEA-TEC-DOC-1427.

Heather, N. W., and G. J. Hallman. 2008. Pest Management and Phytosanitary Trade Barriers, p. 257. CABI Press. United Kingdom.

Heather, N. W., R. J. Corcoran, and C. Banos. 1991. Disinfestation of mangoes with gamma irradiation against two Australian fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 84: 1304—1307.

Hossain M. A., M. A. Wadud, S. A. Khan, and M. S. Islam. 2006. Dose mortality response on different developmental stages of fruit fly, *Bactrocera tau* (Walker) to gamma radiation. Nucl. Sci. Appl. 15: 108–112.

Hossain M. A., G. J. Hallman, A. S. Khan, and M. S. Islam. 2011. Phytosanitary irradiation in South Asia. J. Entomol. Nematol. 3: 44–53.

Hu, F., G. N. Zhang, F. X. Jia, W. Dou, and J. J. Wang. 2010. Morphological characterization and distribution of *Antennal sensilla* of six fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 103: 661–670.

(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.

(IPPC) International Plant Protection Convention. 2009. ISPM #28, Annex 7: Irradiation treatment for fruit flies of the family Tephritidae (generic). Food and Agricultural Organization, Rome, Italy.

ISO/ASTM 51261. 2002. Standard guide for selection and calibration of dosimetry systems for radiation processing. ASTM 51261-2002. American Society for Testing and Materials, West Conshohocken, PA.

ISO/ASTM 51707. 2002. Standard guide for estimating uncertainties in dosimetry for radiation processing. ISO/ASTM 51707-2002. American Society for Testing and Materials, West Conshohocken, PA.

LeOra Software, 2002. PoloPlus. A user's guide to probit or logit analysis. Version 0.03. LeOra Software, Berkeley, CA.

Li, L., T. Liu, B. S. Li, F. H. Zhang, S. J. Dong, and Y. J. Wang. 2014. Toxicity of phosphine fumigation against Bactrocera (Zeugodacus) tau (Walker) at low temperature. J. Econ. Entomol. 107: 601–605.

Li, X. Z., Y. H. Liu, and Z. L. Wang. 2006. Biology and management of *Bactrocera* (Zeugodacus) *tau* (Walker). Plant Prot. 32: 146–150.

Liu, L. H., Y. H. Liu, B. Zhou, and Y. Q. Zhang. 2005. Studies on damage and quantity dynamics of *Bactrocera tau* Walker in different host fields. J. Southwest. Agric. Univ. (Nat. Sci.) 27: 176–179.

Mahfuza, K., T. B. Rashid, and A. J. Howlader. 2011. Comparative host susceptibility, oviposition, and colour preference of two polyphagous tephritids: *Bactrocera cucurbitae* (Coq.) and *Bactrocera tau* (Walker). Res. J. Agric. Biol. Sci. 7: 343–349.

Mansour, M., and G. Franz. 1996. Gamma radiation as a quarantine treatment for the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 89: 1175–1380.

Ohno, S., Y. Tamura, D. Haraguchi, and T. Kohama. 2008. First detection of the pest fruit fly, *Bactrocera tau* (Diptera: Tephritidae), in the field in Japan: evidence of multiple invasions of Ishigaki Island and failure of colonization. Appl. Entomol. Zool. 43: 541–545.

Prabhakar, C. S., P. Sood, P. K. Mehta, and A. Choudhary. 2009. Distribution and developmental biology of fruit flies infesting cucurbits in north-western Himalaya. J. Insect Sci. 22: 300–308.

Singh, S. K., D. Kumar, and V. V. Ramamurthy. 2010. Biology of *Bactrocera* (*Zeugodacus*) *tau* (Walker) (Diptera: Tephritidae). Entomol. Res. 40: 259–263.

Thanaphum, S., and U. Thaenkham. 2003. Relationships of forms within the *Bactrocera tau* (Walker) (Diptera: Tephritidae) taxon based on heat shock protein 70 cognate sequences. Ann. Entomol. Soc. Am. 96: 44–53.

Thomas, P. 2001. Irradiation of fruits and vegetables, pp. 213–240 *In* R. Molins (ed.), Food Irradiation Principles and Applications. Wiley Interscience, New York, NY.

Torres, R. Z., and G. J. Hallman. 2007. Low-dose irradiation phytosanitary treatment against Mediterranean fruit fly (Diptera: Tephritidae). Fla. Entomol. 90: 343–347.

White, I. M., and M. M. Elson-Harris. 1992. Fruit flies of economic significance: their identification and bionomics. Centre for Agriculture and Biosciences International, Wallingford, United Kingdom.

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