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# Development, reproduction and sexual competitiveness of *Conopomorpha sinensis* (Lepidoptera: Gracillariidae) gamma-irradiated as pupae and adults

Haohao Fu, Fu-wei Zhu, Yue-ye Deng, Qun-fang Weng\*, Mei-ying Hu\* and Tian-zhu Zhang

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## Abstract

Male and female litchi stem-end borers, *Conopomorpha sinensis* (Lepidoptera: Gracillariidae) were  $\gamma$ -irradiated in a  $^{60}\text{Co}$  source during several periods of their pupal development and as newly emerged adults. When mature pupae, the most suitable stage for irradiation, received increasing  $\gamma$ -radiation doses, the emergence rate, flight ability and adult longevity were increasingly diminished. Females that emerged from mature pupae irradiated with 200, 250, and 300 Gy did not oviposit any eggs when mated with either non-irradiated males (UM) or treated males (TM), indicating that 200 Gy was a sufficient dose for inducing complete sterility in females. The hatch rates of eggs oviposited by non-irradiated females (UF) mated with males irradiated with either 200, 250 or 300 Gy were 31, 13.5 and 0.67%, respectively. However, if the father—parental generation (P)—of the male in the cross, UF  $\times$  F<sub>1</sub>M, had been irradiated with either 200, 250 or 300 Gy, then the percent hatch of the eggs produced was either  $9.37 \pm 1.68$ , 0 or 0%, respectively; which indicated that 250 and 300 Gy applied to P generation males resulted in complete sterility in F<sub>1</sub> males. The sexual competitiveness (C) of male litchi stem-end borers that had been irradiated with 250 Gy ranged between 0.53 and 0.62 as measured by ratio tests in laboratory cages with UF:UM:TM ratios of 1:1:1, 1:1:3 and 1:1:5. In a field cage experiment, a mean C value of 0.48 was observed when males were treated with 250 Gy. The C values obtained suggest that males treated with 250 Gy could compete adequately with wild litchi stem-end borers both under laboratory and field cage conditions to warrant pilot scale field tests.

Key Words: litchi stem-end borer; irradiation; sterile insect technique; F<sub>1</sub> sterility; fecundity; fertility; inherited sterility; sexual competitiveness

## Resumen

Machos y hembras del barrenador del tallo de litchi, *Conopomorpha sinensis* (Lepidoptera: Gracillariidae) fueron  $\gamma$ -irradiados en una fuente de  $^{60}\text{Co}$  durante varios períodos de su desarrollo pupal y adultos recién emergidos. Cuando pupas maduras, el estadio más adecuado para la irradiación reciben dosis crecientes de  $\gamma$ -radiación, la tasa de emergencia, la capacidad de vuelo y la longevidad de adultos fueron disminuidos cada vez más. Las hembras que emergieron de las pupas maduras irradiadas con 200, 250 y 300 Gy no ovipositaron huevos cuando se aparearon con machos no tratados (MNT) o machos tratados (MT), lo que indica que 200 Gy fue una dosis suficiente para inducir la esterilidad completa en las hembras. La tasa de eclosión de huevos depositados por las hembras no tratadas (HNT) se aparearon con machos irradiados con 200, 250 o 300 Gy fueron 31, 13 y 0.67%, respectivamente. Sin embargo, si el padre—de la generación parental (P)—del macho en el cruce de HNT  $\times$  F<sub>1</sub>M, había sido irradiado con 200, 250 o 300 Gy, entonces el porcentaje de los huevos producidos que eclosionaron fue  $9.37 \pm 1.68$ , 0 o 0%, respectivamente; que indica que 250 y 300 Gy aplicada a los machos de la generación P resultó en la esterilidad completa de machos F<sub>1</sub>. La competitividad sexual (C) de los machos del barrenador del tallo de litchi que habían sido irradiados con 250 Gy fue de 0.53 a 0.62, medida por la prueba de ratio en jaulas de laboratorio con el ratio de HNT: MNT de 1:1:1, 1:1:3 y 1:1:5. En un experimento de jaula en el campo, se observó un promedio de valor C de 0.48 cuando los machos fueron tratados con 250 Gy. Los valores C obtenidos sugieren que los machos tratados con 250 Gy podrían competir adecuadamente con barrenadores del tallo de litchi salvajes, tanto en condiciones de laboratorio y de campo de la jaula para justificar las pruebas de campo a escala piloto.

Palabras Clave: barrenador del tallo de litchi; irradiación; técnica del insecto estéril; esterilidad F<sub>1</sub>; fecundidad; fertilidad; esterilidad heredada; competitividad sexual

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Litchi, *Litchi chinensis* Sonn. (Sapindales: Sapindaceae), has been cultivated in China for over 3,500 years. It is indigenous to subtropical southern China (Zhao et al. 2007; Wang et al. 2011). Litchi—having high economic value—is considered the most profitable rare fruit in China (Yang 2006; Jiang et al. 2006), where it is cultivated on 588,000 ha giving a total annual yield of 1.65 million tonnes. This production in southern China accounts for 72.5% of the world's production area and 61.1% of the world's production (Liu et al. 2009). The litchi stem-end

borer, *Conopomorpha sinensis* (Bradley) (Lepidoptera: Gracillariidae), is an important pest of litchi and damages the fruit, new shoots and young leaves, causing fruit drop and withering of the inflorescence and new shoots (FAO 2002).

The larvae live inside the fruit and are consequently difficult to control using traditional insecticide-based methods (Tsang et al. 2011). Farmers who produce litchi organically are not allowed to use synthetic insecticides, which makes it very difficult to manage this lepidopteran

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pest (Zeng et al. 2007). Currently the export of fresh litchi fruit is very challenging (Jiang et al. 2006) because of import restrictions on litchi with pesticide residues imposed as “green trade” —fresh and unprocessed fruit and vegetable—barriers of many importing countries (Fang et al. 2007).

The sterile insect technique (SIT) has been successfully used against a number of pest insect species such as the New World screw-worm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), a tsetse fly, *Glossina austeni* Newstead (Diptera: Glossinidae), the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), and the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Dyck et al. 2005). While the SIT gained its initial reputation in eradication programs, it also may be used as a part of area-wide integrated pest management (AW-IPM) strategies for the suppression, containment, and prevention of pests (Hendrichs et al. 2005). The SIT depends greatly on the production of good quality sterile male insects that are released at regular intervals into the target area. Quality is assessed through a series of assays to monitor certain parameters, such as survival, mate location, ability to transfer sterile sperm to females, egg hatch to assess the rate of induced sterility, flight ability and sexual competitiveness (Barry et al. 2003; Dyck et al. 2005; Sotero et al. 2007; Helinski & Knols 2008).

Exposing litchi fruits infested with larvae of *C. sinensis* to doses of gamma irradiation up to 400 Gy as part of quarantine treatments indicated that an estimated dose of 254 Gy (220–289 Gy, 95% fiducial limits) caused 99.5% larval mortality (Hu et al. 1998). However, to date, no studies have been conducted on the radiation dose requirement for use of the SIT against this pest. Therefore, the objectives of this study were to assess the effect of gamma irradiation on the quality and sexual competitiveness of *C. sinensis* in laboratory and field cages, and to define an optimal sterilizing dose for use in control programs based on the use of inherited sterility.

## Materials and Methods

### SOURCES OF INSECTS AND METHOD OF REARING

Litchi stem-end borers were collected as larvae from infested fruits or as pupae from fallen leaves in litchi orchards from Zengcheng in Guangdong Province and from Haikou city in Hainan Province. Fresh ‘March Red’ or ‘Fizixiao’ litchi fruit obtained from local supermarkets were used in this study.

To establish the colony, infested fruits placed in a box were covered with paper with folds. After the larvae emerged from the fruits, they sequestered themselves in the creases of this paper cover and pupated. The pupae were placed in a rearing cage, where they emerged and mated. In order to collect eggs, fresh fruits of litchi and longan, *Dimocarpus longan* Lour. (Sapindales: Sapindaceae), were wrapped with specially designed tissue paper and placed in the cage with gravid females at a high relative humidity (see Tsang & Liang 2007; Wang et al. 2008). The females oviposited their eggs on the paper covering the fruits. Every 48 h the papers with eggs were removed and replaced with new tissue paper. Litchi and longan fruits and the tissue papers containing eggs were placed in petri dishes each lined with a moistened filter paper at the bottom and placed in an incubator at  $26 \pm 1$  °C and  $73 \pm 5\%$  RH. After the eggs hatched, the larvae penetrated into the fruits, where they developed. In due time 5th instar larvae emerged from the fruits, completed their development and pupated

in the folds of the paper, which covered them. The colony was reared for 3 consecutive generations in order to provide various life stages for the experiments.

### IRRADIATION

A  $^{60}\text{Co}$  source (Nordion Inc., Ottawa, Ontario, Canada) located at the Guangzhou Furui High-Energy Technology Co., in Guangzhou was used for the radiation treatments. The dose rate at the time of the irradiation was 3.2 Gy/min.

### DETERMINATION OF THE BEST DEVELOPMENTAL STAGE TO IRRADIATE FOR THE SIT

To assess the effect of irradiation on each development stage of litchi stem-end borer, a dose of 250 Gy was administered to 100 adult insects and to groups of 100 pupae of various ages (young, middle aged and mature or late stage). The adult emergence rates of the irradiated cohorts were recorded and the emerged adults were fed a 5% litchi honey solution. Mortality of adult insects in each cohort was recorded daily. The longevity (L) of insects in each treatment was calculated by the following formula:

$$L = \frac{\sum_{i=1}^n Z_i}{T}$$

where  $Z_i$  = the number of dead adults in  $i$  days,  $n$  = the day that all adults had died, and  $T$  = the number of adults that successfully emerged from the pupae.

### EMERGENCE, FLIGHT ABILITY AND LONGEVITY OF ADULTS THAT EMERGED FROM PUPAE IRRADIATED WITH VARIOUS DOSES WHEN EITHER YOUNG, MIDDLE-AGED OR MATURE

Pupae were sorted into groups of young, middle-aged and mature pupae based on color, eyespot and duration since the onset of pupation. A pupa became mature in 5–6 d after the onset of pupation, and it had brown and waxy body, black compound eyes, and black striped wings. The middle-aged and young pupal periods were about 3–4 d and 1–2 d after the onset of pupation, respectively. The young pupa had a pale green to pale yellow body and milky white compound eyes with a black spot.

One hundred uniformly sized pupae of each of the above cohorts were each irradiated with doses ranging from 50 to 300 Gy with an interval of 50 Gy. In order to measure flight ability (percent of fliers), the irradiated pupae were allowed to emerge in a cylinder (100 mm diam, 200 mm height), which was coated on the inside with talcum powder to prevent emerged adults from crawling out. A cage (60 × 60 × 60 cm) containing 3 of the above cylinders was placed in an incubator at  $26 \pm 1$  °C,  $75 \pm 5\%$  RH and 12:12 h L:D photoperiod. Each dose treatment was replicated 3 times. The number of dead pupae (A), not fully emerged pupae (B), deformed adults (C), and adult non-fliers (D) were recorded. The percent emergence (E) and percent fliers (F) were calculated using the following 2 equations:

$$E = (100-A-B)/100$$

$$F = (100-A-B-C-D)/100$$

To assess longevity, 50 male adults that emerged from pupae irradiated with each of the various doses were selected for each treatment and placed in a cage (50 × 40 × 50 cm) and maintained at  $26 \pm 1$  °C and  $75 \pm 5\%$  RH. The adults were fed a 5% litchi honey solution. Each treatment

was replicated 3 times. The number of dead adults was recorded every 24 h and their longevity was calculated using the formula given above.

#### STERILITY OF ADULTS EMERGING FROM MATURE PUPAE IRRADIATED WITH VARIOUS DOSES

Adults that emerged from irradiated mature pupae were separated by sex after 12 h and mated with non-irradiated individuals in 3 combinations: non-irradiated females with treated males (UF × TM), treated females with non-irradiated males (TF × UM) and treated females with treated males (TF × TM). The UF × UM combination served as the control. Every combination contained 30 adult pairs, which were placed in cages each 50 × 40 × 50 cm that were kept in an incubator at 26 ± 1 °C, 75 ± 5% RH, and 12:12 h L:D photoperiod. Each combination was replicated 3 times. As a substrate for oviposition, fresh litchi fruits wrapped in tissue paper were placed in the cages and replaced every 48 h. Adults were fed on 5% litchi honey solution. The number of eggs oviposited on the tissue paper covering the litchi fruits by the parental (P), and first filial (F<sub>1</sub>) generations were counted under a binocular microscope and the corresponding hatch rates of eggs were recorded.

#### SEXUAL COMPETITIVENESS OF 250 GY IRRADIATED MALES USING VARIOUS MATING RATIOS IN LABORATORY CAGES

Adults that emerged from mature pupae irradiated with 250 Gy were separated by sex within 12 h. Thirty treated males were placed with 30 non-irradiated females in 3 ratios (UF:UM:TM), i.e., 1:1:1, 1:1:3 and 1:1:5. For each ratio the experiment was replicated 3 times, although the FAO/IAEA/USDA manual recommends 10 replicates per treatment. After the experiment, the female moths were maintained as described above. The number of oviposited eggs and egg hatch were recorded. The expected egg hatch was calculated using the following formula (Fried 1971):

$$\text{Expected egg hatch} = \frac{H_o + H_s}{N + S}$$

where  $H_o$  = egg hatch of mating between non-irradiated females and males (= 85.33%),  $H_s$  = egg hatch of mating between non-irradiated females and irradiated males (= 13.54%),  $N$  = the number of non-irradiated males, and  $S$  = the number of irradiated males

Laboratory evaluations of sexual competitiveness (C) of male treated with 250 Gy were carried out using the following formula (Fried 1971):

$$C = \frac{H_o - E_c}{E_c - H_s} \div \frac{S}{N}$$

where  $E_c$  = egg hatch in competitive mating, and  $\frac{S}{N}$  = ratio of sterile males and non-irradiated males.

#### SEXUAL COMPETITIVENESS OF MALES IRRADIATED WITH 250 GY IN FIELD CAGES

Three litchi trees were selected in an orchard and each was covered with a nylon-screen cage (3 × 3 × 3 m). One hundred adult males that emerged from pupae irradiated with 250 Gy were placed in the field cage together with 100 UF and 100 UM to obtain an UF:UM:TM ratio of 1:1:1. The number of oviposited eggs and the number that hatched were recorded. These data were used to calculate the sexual competitiveness (C) of the irradiated males using the above formula of Fried, but the variance of the competitiveness index (Fried) was not

calculated directly from the replications, but by the use of the more elaborate technique of Hooper et al. (1981).

#### STATISTICAL ANALYSIS

Data were analysed using a two-way ANOVA and a regression analysis using SPSS Version 19.0 (SPSS Inc., Chicago, IL, USA). Hatch rate values were arcsine transformed. Means were separated by Duncan's Multiple Range Test ( $P = 0.05$ ).

## Results

#### EFFECTS OF IRRADIATION OF YOUNG, MIDDLE-AGED OR MATURE PUPAE AND YOUNG ADULTS ON EMERGENCE AND LONGEVITY

Application of 250 Gy to either young, middle-aged or mature pupae resulted in emergence rates of 46, 81 and 84%, respectively. The emergence rate of irradiated young pupae was significantly less than the rates for middle-aged and mature pupae. However the emergence rates of irradiated middle-aged and mature pupae did not differ significantly. (In another experiment the percent of adult emergence from mature pupae irradiated with 250 Gy was only 76.4 ± 1.4%). Adults lived significantly longer if they had been irradiated as mature pupae or newly emerged adults than those irradiated as middle-aged or young pupae. The longevity of adults that were irradiated either as mature pupae or as newly emerged adults did not differ significantly (Table 1).

#### EFFECTS OF VARIOUS IRRADIATION DOSES APPLIED TO PUPAE ON THE EMERGENCE, FLIGHT ABILITY AND LONGEVITY OF ADULTS

Adult emergence from irradiated mature pupae decreased with increasing radiation dose (Tables 2 and 3). Whereas non-irradiated control pupae showed an emergence rate of > 93%, emergence rates of pupae irradiated with 250 and 300 Gy were reduced to 76 and 66%, respectively. Probit analysis of our data showed that the irradiation doses required to induce 50 and 90% non-emergence were 461 and 1161 Gy, respectively (Table 3).

The flight ability of both males and females were similarly affected by irradiation dose with decreasing proportions of adults flying out of the cylinder with increasing radiation dose. The proportion of fliers of adults that emerged from irradiated mature pupae was related to dose according to the equation,  $Y = -8.777 + 3.357 \ln X$ , where  $Y$  = probit failure to fly out of the cylinder and  $X$  = irradiation dose. Thus probit analysis of our data showed that the irradiation doses required to induce 50 and 90% non-fliers were 412 and 991 Gy, respectively (Table 3).

Although the longevity of adult male moths decreased progressively with increasing radiation doses between 50 and 250 Gy—as de-

**Table 1.** Effect of pupal age at the time of  $\gamma$ -irradiation with 250 Gy on the adult emergence fraction and adult longevity of *Conopomorpha sinensis*.

Stage	Emergence fraction ± SE	Longevity ± SE (days)
Young pupae	0.46 ± 0.014 b	8.15 ± 0.674 c
Middle-aged pupae	0.81 ± 0.014 a	10.32 ± 0.67 b
Mature pupae	0.84 ± 0.014 a	13.77 ± 0.67 a
Newly emerged adults	—	13.93 ± 0.67 a

Note: Multiple comparisons by Tukey HSD. Means followed by the same letter in columns do not differ statistically ( $P \leq 0.05$ ).

**Table 2.** Emergence rate, flight ability and longevity of *Conopomorpha sinensis* irradiated as mature pupae with various doses of  $\gamma$ -rays.

Dose (Gy)	% Emergence	% Fliers	Longevity (days)
Untreated	93.1 $\pm$ 1.1	81.9 $\pm$ 1.4	16.3 $\pm$ 1.2a
50	83.6 $\pm$ 4.9	80.1 $\pm$ 1.1	15.9 $\pm$ 1.3a
100	91.8 $\pm$ 0.4	80.5 $\pm$ 0.9	15.1 $\pm$ 1.2ab
150	86.4 $\pm$ 4.1	75.2 $\pm$ 3.1	14.3 $\pm$ 1.9ab
200	82.2 $\pm$ 0.6	70.1 $\pm$ 2.0	13.4 $\pm$ 2.0ab
250	76.4 $\pm$ 1.4	63.6 $\pm$ 0.9	11.6 $\pm$ 1.0ab
300	66.3 $\pm$ 1.6	53.4 $\pm$ 1.7	10.3 $\pm$ 1.8b

Note: Means followed by same letter in columns (DMRT) do not differ statistically ( $P \leq 0.05$ ).

scribed by  $Y = -0.2x + 16.88$ —their longevities were not significantly different from the control. Only when mature pupae were irradiated with 300 Gy, was longevity significantly reduced (Table 2).

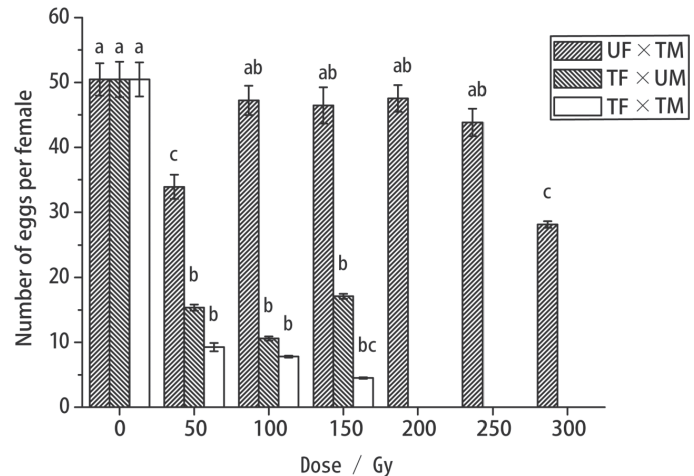
#### FECUNDITY AND STERILITY OF ADULTS EMERGING FROM MATURE PUPAE IRRADIATED WITH VARIOUS DOSES

The results (Fig. 1) show that the fecundity (number of eggs oviposited) of females in the cross UF  $\times$  TM was not significantly affected by doses up to 250 Gy (with the exception of the 50 Gy treatment group, which is a puzzling anomaly). Fecundities of the crosses TF  $\times$  UM and TF  $\times$  TM decreased significantly with increasing radiation dose, and females that were irradiated with 200, 250, and 300 Gy and mated with either an UM or a TM produced no eggs.

The mortality of eggs from the cross UF  $\times$  TM increased with increasing radiation dose, so that with a dose of 200, 250 and 300 Gy the percentages of hatch were  $31.0 \pm 5.7$ ,  $13.5 \pm 6.9$  and  $0.7 \pm 0.6$ , respectively (Table 4). However, with regard to inherited sterility, egg hatch of 9.4 and 0% were obtained from eggs oviposited by  $F_1$  generation descendants of the parental (P) generation cross UF  $\times$  TM in which the grandfathers had been irradiated with either 200 or 250 Gy, respectively. Also there were no  $F_1$  generation descendants of the cross, UF  $\times$  TM, when the parent (P) generation males (grandfathers) had been treated with 300 Gy (Table 5).

#### SEXUAL COMPETITIVENESS IN LABORATORY CAGES OF MALES IRRADIATED WITH 250 GY USING SEVERAL STERILE: NON-IRRADIATED MALE RATIOS

The observed and expected percentages of egg hatch for different sterile:non-irradiated male ratios and the derived competitiveness values of irradiated litchi stem-borer males are presented in Table 6. As the ratio of radio-sterilized males increased, the percent of egg hatch decreased. Egg hatch in the group with an UF:UM:TM ratio of 1:1:1 ( $60.5 \pm 2.0\%$ ) was greater than that of UF  $\times$  TM mating ( $13.5 \pm 6.9\%$ ) (Table 6). The C-values in the range of 0.53–0.62—that were derived in the 3 tests each with a different ratio—indicated that males treated with 250 Gy could adequately compete with wild males.



**Fig. 1.** Fecundity of P generation *Conopomorpha sinensis* adults that emerged from pupae irradiated at various  $\gamma$ -radiation doses ranging from 50 to 300 Gy. UF = non-irradiated females, TF = treated females, UM = non-irradiated males, TM = treated males. Different letters above bars indicate statistically significant differences within each combination (DMRT,  $P = 0.05$ ).

#### SEXUAL COMPETITIVENESS IN FIELD CAGES OF MALES IRRADIATED WITH 250 GY USING A UF:UM:TM RATIO OF 1:1:1

In the field cage studies that used a UF:UM:TM ratio of 1:1:1, fecundity was low with only 12.9 eggs oviposited per female (Table 7). This was significantly lower than in the laboratory with the UF  $\times$  TM cross (43.8 eggs/female; Fig. 1). Although the average egg hatch ( $62.3 \pm 1.9\%$ ) was similar to that obtained in the laboratory study ( $60.5 \pm 2.0\%$ , Table 6), the derived C value of males in field cages was only slightly less than that obtained in the laboratory, i.e., 0.48 versus 0.53, respectively (Tables 6 and 7).

## Discussion

The unique genetic phenomena responsible for inherited sterility (IS) in Lepidoptera, as compared with full sterility in the parent generation, provide multiple advantages for pest control. When partially sterile males of the P generation are released and mate with virgin wild females, the radiation-induced deleterious effects on the chromosomes and genes are inherited by the  $F_1$  generation. As a result, egg hatch in the  $F_1$  generation is reduced and the resulting offspring are both highly sterile and predominately male. Numerous studies have been carried out on inherited sterility with lepidopteran pests (Dyck et al. 2005).

In this study, the older pupae were more radiation resistant than young or middle-aged pupae, and therefore, mature pupae were selected as the best stage for radiation. With increasing radiation dose, the emergence rate, percentage of fliers and the adult moth longevity decreased progressively. The high dose of 1,161 Gy would inhibit emergence of 90% of mature pupae, whereas 991 Gy would prevent

**Table 3.** Effects of  $\gamma$ -irradiation of mature pupae on adult emergence and flight ability of *Conopomorpha sinensis*.

	Coefficients of probit regression $^*Y = a + b \ln X$				
	a	b	$x^2$	LC <sub>50</sub> (95% FL)	LC <sub>90</sub> (95% FL)
Emergence rate	-8.505	3.193	1.822	461 (402–564)	1161 (870–1796)
Rate of flying out of cylinder	-8.777	3.357	0.798	412 (366–488)	991 (767–1453)

FL = fiducial limits.

$^*Y$  = probit mortality or probit failure to fly out of cylinder,  $X$  = irradiation dose.

**Table 4.** Hatch rates of eggs oviposited by female *Conopomorpha sinensis* and mated with males in various combinations. Radiation treatments (50–300 Gy) were given in the pupal stage.

Combination	Egg hatch (%)						
	Untreated	50 Gy	100 Gy	150 Gy	200 Gy	250 Gy	300 Gy
UF × UM	85.3 ± 1.45						
UF × TM		71.3 ± 1.7	61.1 ± 2.0	52.5 ± 14.5	31.0 ± 5.6	13.5 ± 6.9	0.7 ± 0.6
TF × UM		70.2 ± 9.1	46.1 ± 2.0	37.3 ± 11.0	—	—	—
TF × TM		73.1 ± 6.4	25.8 ± 2.5	18.8 ± 0.7	—	—	—

Note: UF = non-irradiated females, TF = treated females, UM = non-irradiated males, TM = treated males, — no eggs were oviposited.

**Table 5.** Percent hatch of eggs oviposited by *Conopomorpha sinensis* females in crosses involving either F<sub>1</sub> males and/or F<sub>1</sub> females.

Dose (Gy)	Percent hatch of eggs oviposited by first filial generation (F <sub>1</sub> ) (%)		
	UF × F <sub>1</sub> M	F <sub>1</sub> F × UM	F <sub>1</sub> F × F <sub>1</sub> M
Untreated	74.7 ± 2.6	74.7 ± 2.6	74.7 ± 2.6
200	9.4 ± 1.7	—	—
250	0	—	—
300	—	—	—

Note: UF = non-irradiated females, UM = non-irradiated males, F<sub>1</sub>F = first filial generation females of UF × TM; F<sub>1</sub>M = first filial generation males of UF × TM. Each value is the mean of 3 replicates. “—” indicates that no data could be obtained because the F<sub>1</sub> descendants needed for these crosses could not be produced at the indicated radiation doses.

90% of adult moths from flying out of the cylinder. Boshra & Mikhael (2006) found that a 1,000 Gy dose prevented the emergence of both sexes of the dried fruit moth, *Ephesia calidella* (Guenée) (Lepidoptera: Pyralidae). Al-Zahaby et al. (1997) found that 1,000 Gy was the lethal dose for older pupae of the almond moth, *Ephesia cautella* (Walker) (Lepidoptera: Pyralidae). The results of these studies are very similar to those we obtained with the *C. sinensis* in our study.

The longevity of adults emerging from pupae irradiated with 300 Gy significantly declined compared with non-irradiated adults. Similar results have been reported by many authors. Thus Ahmed et al. (1985) found that the life span of the Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) adults of both sexes were shortened as the dose increased. Etman et al. (1991) reported that males of the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), irradiated with 500 Gy had a significantly shorter life span than the non-irradiated control males. These results confirm that male lepidopteran longevity is inversely correlated with radiation dose.

Reproduction of lepidopteran females is usually more sensitive to irradiation than reproduction of males of the same species. This allows the radiation dose to be adjusted to suit program requirements when programs take advantage of inherited sterility. In our experiments with *C. sinensis*, fecundity significantly decreased when females were irradiated irrespective of whether they mated with normal or irradiated males. Indeed no eggs were oviposited when fe-

males were irradiated with doses of 200, 250, and 300 Gy and mated with either with non-irradiated or irradiated males, and therefore, 200 Gy is the lowest dose needed to fully sterilize litchi stem-end borer females. The hatch rate of eggs oviposited by non-irradiated females that had mated with males irradiated with 250 Gy was only 13.5%. In contrast when F<sub>1</sub> males from the combination UF × TM—in which the male had been irradiated with 250 Gy—mated with non-irradiated females all of their eggs failed to hatch. This shows that the genetic effects of irradiation are greater on F<sub>1</sub> males—which were not irradiated—than on their irradiated fathers. Thus with regard to litchi stem-borer males we could achieve full sterility in the F<sub>1</sub> generation with a dose of 250 Gy. Consequently 250 Gy can be regarded as the optimum dose for controlling this pest.

The sexual competitiveness index, C, normally fluctuates between 0 and 1 (Ito et al. 1982; Iwahashi et al. 1983). The value of 1 indicates an equivalent level of competitiveness between the irradiated and the wild males; values close to 0 indicate overwhelmingly superior competitiveness of the wild male (Fried 1971). The C value of litchi stem-borer males irradiated with 250 Gy using different TM:UM mating ratios in laboratory cages fluctuated between 0.53 and 0.62, whereas the mean C value of males irradiated with 250 Gy in field cages was 0.48. This indicates that partially sterile male litchi stem end borers were about half as competitive as non-irradiated males. Boshra & Mikhael (2006) reported that the competitiveness index, C, for irradiated *E. calidella* males that were irradiated with 100, 150 and 200 Gy at the ratio UF:UM;TM 1:1:1 were 0.95, 0.83 and 0.82, respectively, indicating that the irradiated males were very competitive with their non-irradiated counterparts. Saour & Makee (1996) studied the effect of gamma irradiation with doses ranging from 50 to 450 Gy on the male potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), and they found that 150 Gy induced partial sterility without a significant reduction in mating competitiveness.

This study also contributed to phytosanitary irradiation (PI) treatments against Lepidoptera that may pupate in shipped commodities. The argument for a generic PI dose of 400 Gy against Lepidoptera by Hallman et al. (2013) contains no data from the family Gracillariidae. In this regard, the present study shows that ≥ 200 Gy applied to both sexes of mature pupae of *C. sinensis* resulted in no hatching of F<sub>1</sub> generation eggs (Fig. 2), which supports the 400 Gy proposal, while adding another family and another species to its support.

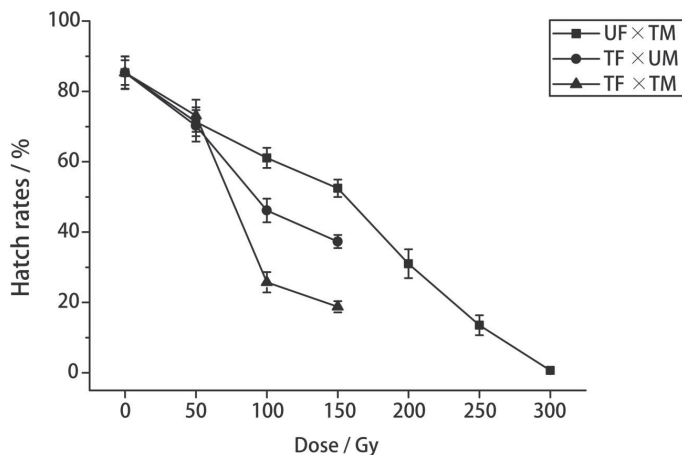
**Table 6.** Sexual competitiveness in laboratory cages of *Conopomorpha sinensis* males irradiated with 250 Gy and allowed to compete for female mates with non-irradiated males mated in several mating ratios.

Mating ratio: UF: UM: TM	No. of adults	Egg hatch (%)	Expected egg hatch (%)	Competitiveness index (C)
1:1:1	30:30:30	60.5 ± 2.0a	72.9	0.54 ± 0.07a
1:1:3	30:30:90	40.3 ± 3.0b	51.5	0.59 ± 0.11a
1:1:5	30:30:150	31.1 ± 1.2c	40.1	0.62 ± 0.05a

Note: Hatch rate data was arcsine transformed before analysis. Mean values followed by the same letters are not significantly different (DMRT) at P = 0.05 level.

**Table 7.** Sexual competitiveness in field cages of *Conopomorpha sinensis* males irradiated with 250 Gy and allowed to compete for female mates with non-irradiated males mated in a 1:1:1 mating ratio.

Replicate	No. of eggs laid by per female	Egg hatch (%)	Competitiveness index (C)
1	11.1	65.7	0.37
2	15.4	59.1	0.57
3	12.3	62.1	0.47
Mean ± SE	12.9 ± 1.3	62.3 ± 1.9	0.47 ± 0.057



**Fig. 2.** Mean ( $\pm$  SE) mortality of eggs oviposited by *Conopomorpha sinensis* females involved in 3 different crosses. The upper curve shows the percentage hatch of eggs from the cross, UF  $\times$  TM in which males were  $\gamma$ -irradiated as mature pupae with doses ranging from 50 and 300 Gy. The middle curve shows the corresponding results from the cross, TF  $\times$  UM, however females irradiated with 200–300 Gy did not oviposit any eggs. The lower curve shows the corresponding results when both parents were irradiated.

In conclusion, our competitiveness studies in laboratory and field cages indicated that male litchi stem-end borer irradiated with 250 Gy had adequate competitiveness in comparison with non-irradiated males for application of the SIT against the litchi stem-end borer. This will now have to be validated in open field trials.

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