

# Radiation Biology and Inherited Sterility of Light Brown Apple Moth (Lepidoptera: Tortricidae): Developing a Sterile Insect Release Program

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**ABSTRACT** The radiation biology of two geographically isolated populations of the light brown apple moth [*Epiphyas postvittana* (Walker)] was studied in Australia and New Zealand as an initiation of a SIT/F<sub>1</sub> sterility program. Pharate and  $\leq 2$  d pre-emergence pupae were exposed to increasing radiation doses up to a maximum dose of 300 Gy. Fertility and other life history parameters were measured in emerging adults (parental) and their progeny (F<sub>1</sub>-F<sub>3</sub> adults). Parental fecundity was significantly affected by increasing irradiation dose in pharate pupae only. For both populations, parental egg fertility declined with increasing radiation. This was most pronounced for the irradiated parental females whose fertility declined at a higher rate than of irradiated males. At 250 Gy, females  $\leq 2$  d pre-emergence pupae produced few larvae and no adults at F<sub>1</sub>. No larvae hatched from 250 Gy-irradiated female pharate pupae. At 300 Gy, males still had residual fertility of 2–5.5%, with pharate pupae being the more radio-sensitive. Radiation-induced deleterious inherited effects in offspring from irradiated males were expressed as increased developmental time in F<sub>1</sub> larvae, a reduction in percent F<sub>1</sub> female survival, decreased adult emergence and increased cumulative mortality over subsequent generations. Males irradiated at  $\geq 150$  Gy produced few but highly sterile offspring at F<sub>1</sub> and mortality was  $>99\%$  by F<sub>2</sub> egg.

**KEY WORDS** *Epiphyas postvittana*, irradiation, sterile insect technique, inherited sterility, eradication

Despite progress in border management and surveillance, the likelihood of exotic moth incursions into new areas continues to be high because of increasing cross border trade. Historically, eradications of exotic Lepidoptera have been achieved through intensive use of broad-spectrum insecticides, but current social acceptance for indiscriminate use of broad-spectrum insecticides has raised concerns about toxicological effects in the environment and on human health (Brockerhoff et al. 2010). In addition, the intensive use of insecticides has led to the development of pesticide resistance (Giliomee and Riedl 1998). There is growing pressure from consumers, farmers and the community to develop new, or refine, current environmentally and socially acceptable tools for pest population suppression.

Tortricid moth larvae are among the most devastating pests of fruits and vegetables (Van der Geest and Evenhuis 1991, Vreysen et al. 2006). Their establishment in horticultural crops can result in considerable economic loss from crop damage, extra control costs and market access restrictions. Light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is a member of this family. Indigenous to south eastern Australia (Common 1970), it has gradually established in other parts of Australia, including Western Australia. Elsewhere, it is a widespread and important leafroller pest in New Zealand and is present in the British Isles, Sweden, and Hawaii and recently in California (Suckling and Brockerhoff 2010). It is widely polyphagous, feeding on hosts such as Rosales and Fabales (Suckling and Brockerhoff 2010). In Australia and New Zealand it is an important pest of apples (*Malus domestica* Borkh.), pears (*Pyrus* spp.), stonefruits, citrus, and grapes (*Vitis* spp.) (Wearing et al. 1991). Damage includes defoliation, fruit scarring, fruit drop, and an increase in susceptibility of injured plants to disease. Presence of any life stage in shipments can have adverse market access implications.

Biological control, mating disruption, and the sterile insect technique (SIT) are potential alternative tactics

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to broad-spectrum insecticides for use against exotic lepidopteran incursions. Biological control is a sustainable pest management tactic, but it is a poor strategic fit with emergency responses to new incursions because of the time taken to search for natural enemies and assess their nontarget effects before approval for release. Mating disruption using pheromones to disrupt mate finding in Lepidoptera is being widely used for area-wide pest management (Witzgall et al. 2008), as well as to slow moth spread (Sharov et al. 2002). Over large areas it is most efficient to apply this technology aerially, although this has proven to be socially unacceptable in California (Suckling and Brockerhoff 2010). The sterile insect technique (SIT) is a sustainable technology that has been used successfully against a variety of dipteran pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Krafsur 1998) and the New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Klassen and Curtis 2005). In addition, SIT may work synergistically with other socially acceptable tools that target different life stages of a pest species, such as biological control targeting eggs and larvae (Carpenter et al. 2004), biopesticides such as *Bacillus thuringiensis* (Bt), and mating disruption (Suckling and Brockerhoff 2010).

Lepidopteran species are more radio-tolerant than many other insect orders (LaChance 1985, Bakri et al. 2005) and the large dose of radiation required to achieve full sterility may reduce their competitiveness and performance in the field (Suckling et al. 2011). In spite of this disadvantage, successful operational SIT programs have been implemented against cotton pest pink bollworm *Pectinophora gossypiella* (Saunders) in California (Simmons et al. 2007), codling moth *Cydia pomonella* (L.) in British Columbia, Canada (Bloem et al. 2005), and the painted apple moth *Teia anartoides* Walker in New Zealand (Suckling et al. 2007).

One approach to circumvent the negative effects associated with the high radio-resistance of lepidopteran pests is to take advantage of inherited or  $F_1$  sterility. This genetic phenomenon among Lepidoptera is characterized by the development of dominant lethal genes in offspring from partially sterile parents. This is expressed by higher sterility than their parents, lower fecundity, longer larval development times, higher mortality, and a skewed  $F_1$  sex ratio in favor of males (North 1975, LaChance 1985, Carpenter et al. 2001a). As female moths are more sensitive to radiation than males, in species where separation of the sexes is difficult, doses can be adjusted so that females are rendered completely sterile and males only partially so for release. Male moths irradiated at substerilizing doses have reasonable flight fitness and are thus more likely to be competitive, bringing about better control than fully sterile but poorly physically competitive counterparts (Kean et al. 2008, Suckling et al. 2011). Surviving  $F_1$  progeny resulting from pairings between partially sterile males and wild fertile females have a greater population suppression potential from these radiation-induced deleterious effects (Bloem et al. 1999a). In addition, as these highly sterile

$F_1$  progeny are produced in the field, they are likely to be in developmental synchrony, thus emerging at the same time as the wild population, targeting the next generation effectively. Inherited sterility is seen as an effective and environmentally safe tactic for lepidopteran pest suppression that is useful under a variety of environmental conditions, crop production strategies, and is compatible with many pest control tactics (Carpenter 1993).

Coordinated radiation biology studies were undertaken on two geographically-isolated light brown apple moth populations from Australia and New Zealand. This was done to verify results using similar methodologies between the two laboratories. The effects of increasing doses of gamma radiation on fecundity and fertility on parental (P) pupae and radiation-induced inherited effects on subsequent progenies were assessed using standard methods as far as possible. These results can be used to initiate a full-sterility or  $F_1$  inherited sterility program against this tortricid.

## Materials and Methods

### Test Insects and Rearing Technique

**New Zealand (NZ).** The New Zealand moths were obtained from a Plant & Food Research laboratory-reared colony that was raised on a modified Singh diet (Singh 1983) at 20°C, 60% ± 5% RH, and a photoperiod of 16:8 (L:D) h. The colony was kept disease free by washing the eggs in 5% formalin and pupae in 1.5% chlorine. At the time of the trial, the colony was in its 143th generation.

**Western Australia (WA).** The light brown apple moth laboratory colony was sourced from the South Australian Research & Development Institute (SARDI) and was in its 21st generation at the time of the first trial. Eggs were washed in a solution of 0.1% sodium hypochlorite and allowed to hatch on an artificial diet as described by Singh (1983) at 20–25°C, 65–70% RH, and a daily photoperiod of 14:10 (L:D) h. Upon pupation, pupae were collected 1–2 d after initiation, washed with a 0.1% sodium hypochlorite solution and left to mature at a temperature 25°C, 70% RH, and a daily photoperiod of 14:10 (L:D) h.

**Irradiation Method.** The irradiation studies were undertaken in two different laboratories: the Department of Agriculture and Food of Western Australia (DAFWA) in Perth, and Plant & Food Research (PFR), Lincoln, New Zealand.

In Western Australia, pupae were irradiated by exposure to gamma radiation by using a Cobalt<sup>60</sup> Gammacell 220 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada). Pupae were placed in a plastic vial (3.5 cm in diameter) on top of a jug used to support the dosimeter at midpoint in the Gammacell (IAEA 2004). Dose rates were verified by Gafchromic film dosimetry, type HD-810 (ISP Technologies Inc) and decreased from 7.02 to 6.54 Gy/min over the length of the trial. Target doses (and actual dose) were 200 (208 ± 1.32), 250 (255 ± 1.59), and 300 (312 ± 1.87)

and 200 ( $201 \pm 0.52$ ), 250 ( $252 \pm 0.91$ ), and 300 ( $300 \pm 0.67$ ) for the first and second trial, respectively.

In New Zealand, pupae were placed in tissue paper and were irradiated in 90-mm by 20-mm divided petri dishes, males in one side and females in the other for specified doses. Irradiations were conducted at the National Radiation Laboratory in Christchurch by using an external beam Cobalt<sup>60</sup> Theratron unit (Atomic Energy of Canada Ltd.), which is a single radiation source that irradiates from above the target. Dose rate was 0.54 Gy/min, 55 cm from the source. To ensure that the dose was uniform across the pupae, the dishes were inverted when half the required dose had been administered, and were irradiated inverted for the second half of the irradiation.

Dose rates were verified by Gafchromic film dosimetry type HD-810. The film was placed in with the pupae from doses 0–200 at the time of irradiation. Target doses (and actual dose) were 50 ( $51 \pm 0.6$ ), 100 ( $107 \pm 1.0$ ), 150 ( $165 \pm 1.2$ ), and 200 ( $216 \pm 1.8$ ).

### Irradiation Trials

**New Zealand.** Irradiations were carried out at 50-Gy intervals from 0 to 300 Gy (seven doses). Both male and female pupae were irradiated in petri dishes at  $-2 \pm 1$  d before adult eclosion. Pupae were irradiated in batches of 50 for each dose. This was repeated for 5 wk running; each week was considered a replicate. Effects of increasing radiation dose on fertility and fecundity were investigated by mating irradiated (P) with un-irradiated (N) moths. For simplicity, in the text and results, 'P' refers to an irradiated 'parent' individual and 'N' an un-irradiated individual. Two-day-old adult male and female moths were paired in ratios of 20:10 (P ♂ × N ♀) and 10:10 (P ♀ × N ♂). Moths for a given irradiation treatment were held in one or two humidified plastic bags depending on moth age similarity, with 10% sugar water for food and waxed paper for oviposition. They were allowed to mate for seven days at 19°C. Subsequent eggs were allowed to hatch at 19°C over a period of 21 d. Up to 200 hatched  $F_n$  larvae per treatment combination per replicate were collected and allowed to develop through to adulthood. Any additional larvae were destroyed daily, to prevent egg cannibalism. For a randomly sampled subset of egg batches laid in a bag, the numbers hatching and not hatching were recorded for a maximum of 500 eggs per batch. (A batch of eggs is a clump within the bag, generally laid by the same female at a single laying time). Collected larvae were placed individually in test tubes that were one third filled with the modified Singh diet (Singh 1983) and closed with a cotton dental roll. The number of days to pupate was recorded for each individual. Upon pupation, pupae were removed from the tubes and held at 10°C for up to 2 wk to arrest development to allow subsequent larvae time to pupate, synchronizing mating trials. Pupae were separated by sex and allowed to eclose at 19°C in humidified plastic bags.

For the  $F_2$  generation, adults of both sexes from both male and female irradiated parents were mated

with un-irradiated moths of the opposite sex, giving four combinations. For the third generation ( $F_3$ ), were again mated with un-irradiated partners (to give eight generational combinations).

Methods for the second and third generations were similar to for the first generation, except that only egg numbers and mortality of larvae were recorded for the  $F_3$  generation.

**Western Australia.** Irradiation studies on the light brown apple moth were carried out over two separate trials from September 2007 to August 2008. The first experiment was conducted on pupae  $\leq 2$  d from emergence; in the second, more mature pharate pupae ( $\leq 1$  d from emergence) were used.

**Trial 1.** After results from initial radiation studies in New Zealand, Australian male and female pupae were exposed to 0, 200, 250, or 300 Gy. Approximately 100 pupae were irradiated batch wise by treatment. After irradiation, pupae were placed individually in plastic containers and left to emerge. Only moths that emerged 2 d after irradiation treatment were used. After emergence, the irradiated virgin male and female moths were left to mature for 1 d before pairing individually with un-irradiated virgin partners in 100-ml plastic containers. Between 35 and 40 individual pairings were made for each dose per sex combination. Each pair was fed by a moist (10% honey solution) dental wick inserted through a hole in the lid. Adults were held for mating and oviposition at  $20 \pm 2^\circ\text{C}$ , 60–70% RH, and a photoperiod of 14:10 (L:D) h for 8 d. Moths were then removed and the number of  $F_1$  eggs counted (parental fecundity). Eggs were left to hatch under the same conditions in the same plastic containers for another 12 d. The number of hatched eggs was recorded. During hatching, larvae were removed daily to avoid cannibalization on unhatched eggs. Depending on the larvae progeny that survived, between 2–41 larvae of each sex for each dose per irradiated sex combination were reared individually in 25-ml plastic cups on the artificial diet (Singh 1983) at  $20$ – $25^\circ\text{C}$ , 65–70% RH, and a daily photoperiod of 14:10 (L:D) h.

**Trial 2.** Pharate male and female pupae were exposed to either, 0, 150, 200, 250, or 300 Gy in batches of 100 pupae similar to trial 1. The 150 Gy dose was added because of the likelihood of increasing radiosensitivity with age (FOA/IAEA/USDA 2003). Moths that emerged within 6 hr after irradiation were used. At emergence, groups of five irradiated male or female moths from a dose were paired with five virgin un-irradiated partners under similar conditions as in trial 1. Five to seven groups were used for at each dose and were set up at the same time. Each group was placed in 100-ml plastic containers and held under similar conditions as for trial 1.

Depending on the numbers hatching, between 104 and 150  $F_1$  larvae per treatment (49 for 200 Gy irradiated females) were kept for studies of inherited effects. Larvae were pooled across the groups. For each larva retained, days to pupation, days until emergence from pupae, and sex of emerged adults were recorded. Emerged  $F_1$  adults were then out-crossed to

un-irradiated adults of the opposite gender in single pairs to assess for  $F_1$ -inherited sterility. The moths were allowed to mate, oviposit, and eggs allowed to hatch under the same conditions as described for the parental crossing. The total number of eggs laid and the number of hatching eggs per pair were recorded.

### Statistical Analysis

**New Zealand.** Fertility (percent hatch), larval survival (percent pupation), and sex ratio (percent female offspring) were analyzed. Data over all three generations for a particular measurement were analyzed together. A binomial-beta hierarchical generalized linear model (HGLM, Lee et al. 2006) approach was used. This allowed for adjustment for any carry-over effects from one generation to the next (not associated with the treatment). These effects, such as replicates (weeks), which could be a source of additional variation, were included in the analysis as random effects. The treatments (dose, irradiated sex), generation, and sex of the irradiated line at each generation, and interactions between these, were included as fixed effects. The importance of the various fixed effects was then assessed with Wald statistics (W) tested against a  $X^2$  distribution (GenStat Committee 2009).

**Western Australia.** Data from each generation were analyzed separately, because groupings of moths from the first generation were not carried over into subsequent generations (as happened in New Zealand). Thus, no adjustment for any carry-over effects from the first to the second generation could be made, and consequently, results for the second generation may be biased or more variable than if adjustment had been possible.

Days to pupation and days to emergence were analyzed using a Poisson generalized linear model with a log link (McCullagh and Nelder 1989), and percentage data (% fertility, % larval survival, % female offspring) analyzed using a binomial generalized linear model. The total number of eggs laid (fecundity) was analyzed with a negative binomial generalized linear model, which is appropriate for count data where there is some clustering. The aggregation parameter for the negative binomial distribution was estimated using an iterative Newton Raphson method (Lawless 1987) as implemented in GenStat.

For all these analyses, irradiated sex and dose were included as contrasts, along with the interaction between them. For the  $F_1$  data,  $F_1$  gender also was included, along with interactions between it and the other factors. Contrasts were assessed with F-tests (or, where there was no over dispersion,  $X^2$  tests) within an analysis of deviance.

For all sets of data, because of the varying replication for the treatment combinations, main effects of treatments (sex, dose, etc.) were tested both before and after adjusting for all other main effects. However, in the results, statistics are presented for testing after adjustment only.

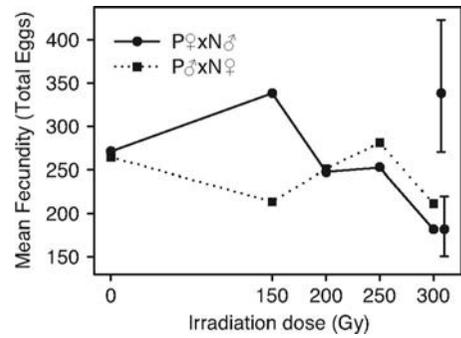


Fig. 1. Mean fecundity (total eggs produced) for *E. postvittana*, where either the female parent ( $P♀$ ) or the male parent ( $P♂$ ) was irradiated at various doses. Error bars are 95% CL for two selected means in the plot.

For all data, estimated means (counts or percentages) were obtained on the transformed scale (logit or log) along with 95% CL. These were back-transformed to the count or percentage scales. For clarity, in some figures only selected confidence limits are presented.

All analyses were carried out in GenStat (GenStat Committee 2009).

**Cumulative Mortality.** Cumulative mortality across three generations was calculated from the data for the New Zealand trial. This calculation ignored adult mortality, including only mortality of eggs (100-% hatching) and larvae. For each of five stages ( $F_1$  eggs,  $F_1$  larvae,  $F_2$  eggs,  $F_2$  larvae,  $F_3$  eggs), cumulative mortality was calculated from the means for each dose and sex combination as:

$$\text{CumulativeMortality}_i = 100$$

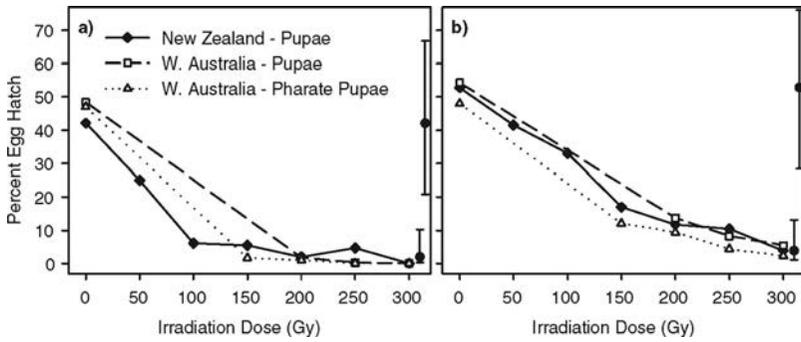
$$\times \left( 1 - \prod_{j=1}^i \text{survival}_j / 100 \right) \quad [1]$$

where  $\text{survival}_j$  is the mean survival (% hatching, % larvae pupating) at stage  $j$ . Cumulative mortality is presented graphically, and was not analyzed formally.

## Results

### Effect of Irradiation on Parental (P) Fecundity

**Western Australia.** The effect of increasing irradiation dose on fecundity (i.e., number of eggs laid) appeared to be influenced by the pupal age at the time of treatment. However, because age at treatment was not assessed directly in a single experiment, the effect cannot be formally assessed. Fecundity of pupae irradiated at  $\leq 2$  d old did not vary significantly with irradiation dose for either parental gender treated ( $F_{3,258} = 1.97$ ;  $P = 0.116$  for the dose effect and  $F_{3,258} = 1.10$ ;  $P = 0.351$  for the interaction of dose with sex). However, on average more eggs were produced from male-irradiated rather than female-irradiated pairings ( $F_{1,258} = 4.78$ ;  $P = 0.030$ ). With pharate pupae, however, (Fig. 1) fecundity declined significantly with increasing irradiation dose ( $F_{2,63} = 5.10$ ;  $P < 0.001$ ). Fecundity did not vary strongly between



**Fig. 2.** Mean fertility (% eggs hatching) of *E. postvittana* for three trials; New Zealand pupae, Western Australia pupae and pharate pupae where either the a) female parent or b) male parent was irradiated at various doses. Error bars are 95% CL for two selected means in each plot.

the treated sexes ( $F_{2,63} = 0.19$ ;  $P = 0.829$  for the overall effect and  $F_{7,63} = 0.108$ ;  $P = 0.082$  for the sex  $\times$  dose interaction).

**Effect of Irradiation on Parental (P) Fertility**

**New Zealand and Western Australia (Pupae).** For both populations, the percentage of egg hatching (fertility) declined significantly with increasing radiation dose (Fig. 2, for the overall dose effect,  $F_{3,258} = 170.77$ ;  $P < 0.001$  for the WA  $\leq$  2-d-old pupae trial;  $W_6 = 51.28$ ;  $P < 0.001$  for the NZ trial). Female pupae were more radio-sensitive than male pupae, with egg fertility declining faster as the radiation dose increased ( $F_{3,258} = 11.80$ ;  $P < 0.001$ , for the dose  $\times$  sex interaction for the Western Australia trial, and  $W_1 = 13.47$ ;  $P < 0.001$  for the sex effect for the New Zealand trial). Female crosses (P  $\times$  N  $\delta$ ) irradiated at 250 and 300 Gy had embryonic development clearly visible inside most of the egg batches for both populations. However, fewer than 5% of eggs hatched at the 250-Gy dose and none hatched at the 300-Gy dose. Hatched larvae did not survive longer than a few days. Thus, no adults were produced. Conversely, egg hatch was observed up to 300 Gy from irradiated male pupae (P  $\delta$   $\times$  N  $\delta$ ), although it was greatly reduced compared with the untreated control, with fertility of 5.4% and 3.8% in Australia and New Zealand respectively. Similar trends were observed with irradiated pharate pupae (Fig. 2).

**F<sub>1</sub>-Inherited Effects**

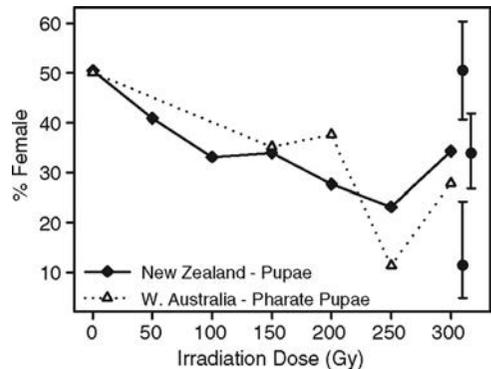
**Percentage of Female Offspring.** For the New Zealand trial and the Western Australia pharate pupae trial, the percentage of female adults among F<sub>1</sub> progeny of irradiated male parents was significantly reduced by increasing the irradiation dose ( $F_{4,32} = 6.14$ ;  $P = 0.003$  and  $F_{6,88} = 4.20$ ;  $P < 0.001$  for the dose effect for irradiated males for WA and NZ, respectively) (Fig. 3). The percentage of female pupae was as low as 23.1% (95% C. L. 16.9–30.7%) at 250 Gy in New Zealand, compared with 50.5% (95% C. L. 40.6–60.3%) with 0 Gy. In Australia with pharate pupae, 11.3% were female (95% C. L. 4.9–24.2%) at 250 Gy compared with 50.0% (95% C. L. 39.8,

60.2%) at 0 Gy. For irradiated female parents, the percentage of female progeny was close to 50% for all doses in the New Zealand trial ( $F_{4,88} = 4.20$ ;  $P = 0.729$ ) and for the Western Australia trial ( $F_{2,32} = 2.91$ ;  $P = 0.069$ ).

**Developmental Time.** Developmental time of F<sub>1</sub> progeny to the pupation stage increased with increasing radiation dose to the parents ( $X_4^2 = 8.09$ ;  $P < 0.001$ , Fig. 4a). For doses of above 200 Gy, the time to pupation of F<sub>1</sub> progeny from irradiated males (P  $\delta$   $\times$  N  $\delta$ ) was at least a day longer than in the controls. (No eggs hatched for irradiated female parents for doses above 200).

Days to eclosion was longer by between 0.9 and 2.5 d (Fig. 4b) for progeny of irradiated parents of either sex ( $X_4^2 = 3.58$ ;  $P = 0.006$ ), with little difference between irradiation doses.

**Fecundity.** The fecundity of F<sub>1</sub> adult crosses from the Western Australia irradiated pharate pupae trial (F<sub>1</sub>  $\delta$   $\times$  N  $\delta$ ; and F<sub>1</sub>  $\delta$   $\times$  N  $\delta$ ) did not differ strongly with dose ( $X_4^2 = 0.62$ ;  $P = 0.650$ ). However, for F<sub>1</sub> adult crosses from  $\leq$  2 d old irradiated P pupae, egg numbers (Fig. 5) reduced with increasing radiation dose ( $X_3^2 = 4.11$ ;  $P = 0.006$ ). This response was only weakly influenced by the gender of the F<sub>1</sub> adult from the irra-



**Fig. 3.** Percentage of *E. postvittana* progeny that were female (New Zealand and Western Australia), where the male parent was irradiated at various doses and the female parent was untreated. Error bars are 95% CL for three selected means in the plot.

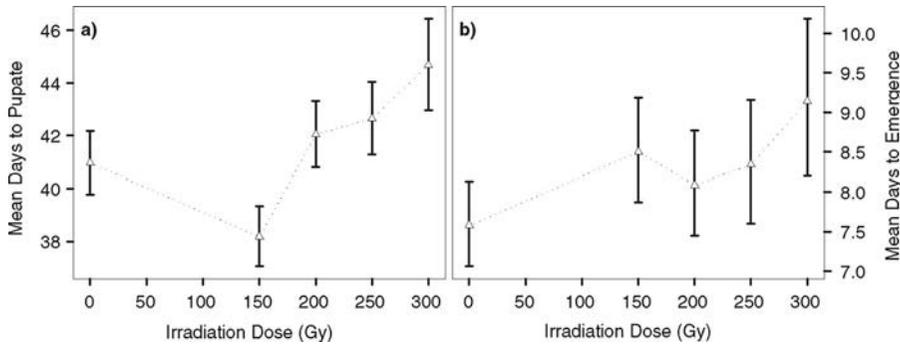


Fig. 4. Mean number of days for male offspring to a) pupation and for b) adult eclosion for *E. postvittana* progeny where the male parent was irradiated at various doses and the female parent was left untreated. Females not displayed as there was no egg hatch at 200 Gy or above. Error bars are 95% CL.

diated line ( $X^2_3 = 2.11$ ;  $P = 0.097$  for the dose  $\times$  sex interaction).

**Fertility.**  $F_2$  egg hatch from all three trials was influenced by irradiation dose administered to the parental moths ( $F_{3,280} = 83.47$ ;  $P < 0.001$  WA trial 1,  $F_{4,87} = 2.52$ ;  $P = 0.047$  WA trial 2, and  $W_6 = 42.79$ ;  $P < 0.001$  NZ trial).

For the Western Australia trials, the pattern of change with dose varied with sex of the parents and the sex of the  $F_1$  offspring (Fig. 6a,b;  $F_{1,250} = 7.11$ ;  $P = 0.008$  for the three-way interaction for trial one and  $F_{2,87} = 3.09$ ;  $P = 0.05$  for the irradiated parental sex  $\times$  dose interaction for trial 2). Fertility of the P male irradiated, male  $F_1$  offspring line appeared to be further reduced in the  $F_2$  generation, compared with the fertility of the initial irradiated P moth pupae, whether or not they were irradiated (Fig. 6a).

For the New Zealand trial, no  $F_1$  progeny survived to adulthood from irradiated parental females for the 250 and 300 Gy doses, and only one survived at the 200 Gy dose. The effect of increasing dose was otherwise fairly similar, regardless of the sex of irradiated parent (data not presented).

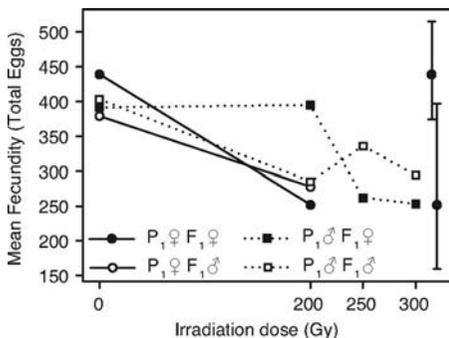


Fig. 5. Mean fecundity (total eggs produced) for *E. postvittana*, for  $F_1$  female or male progeny ( $F_1♀$ ,  $F_1♂$ ) whose female parent ( $P♀$ ) or male parent ( $P♂$ ) was irradiated at various doses, and the other parent was left untreated. No progeny were produced for  $P♀$  for 250 and 300 Gy. Error bars are confidence limits 95% for two selected means in the plot.

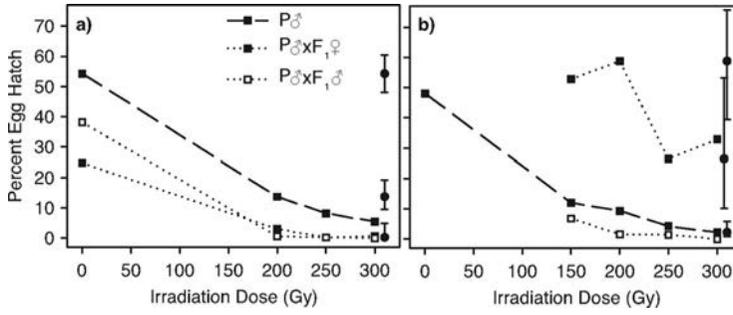
### Cumulative Mortality

**New Zealand.** Cumulative mortality at each stage (eggs and larvae) over three generations is shown in Fig. 7. Mortality for the controls (Dose = 0) was quite high, even at the first stage (Fig. 7a, above 50%), and was higher for 'irradiated' control females than for 'irradiated' males. It is unknown why this is the case and may be attributed to lab rearing or a random effect. The higher mortality of progeny of irradiated females that was apparent at the first stage (Fig. 7a,  $F_1$  egg) carried through to the second stage (Fig. 7b,  $F_1$  larvae mortality), where there were no surviving larvae at 250 Gy. By the third stage (Fig. 7c,  $F_2$  egg); at 100 Gy cumulative mortality was above 95% regardless of the sex of  $F_1$  progeny. However, at 50 Gy, the lowest dose, cumulative mortality for progeny of  $F_1$  females ( $P♀ F_1♀ = 98\%$ ,  $P♂ F_1♀ = 96\%$ ) was higher than that for progeny of  $F_1$  males ( $P♀ F_1♂ = 92\%$ ,  $P♂ F_1♂ = 90\%$ ), regardless of the sex of the irradiated parent. By the fifth stage (Fig. 7e,  $F_3$  egg), cumulative mortality was above 95% even for progeny of unirradiated parents. The high control, 0 Gy mortality multiplies up over the five stages so that even if within each stage mortality was  $\approx 50\%$ , the cumulative mortality would be 96.9% [ $100(1 - (0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5))$ ]. Cumulative mortality was above 99% for all progeny from the original moths that were irradiated with doses of 100 Gy or more.

### Discussion

Our results have shown that for both populations, an irradiation dose of 250 Gy administered to *Epiphyas postvittana* pupae induced  $>95\%$  sterility in females and  $>90\%$  sterility in males. Parental fecundity and fertility were negatively affected by increasing dose of irradiation. Surviving  $F_1$  larvae had extended development time and percent  $F_1$  female adult survival reduced with increasing radiation.

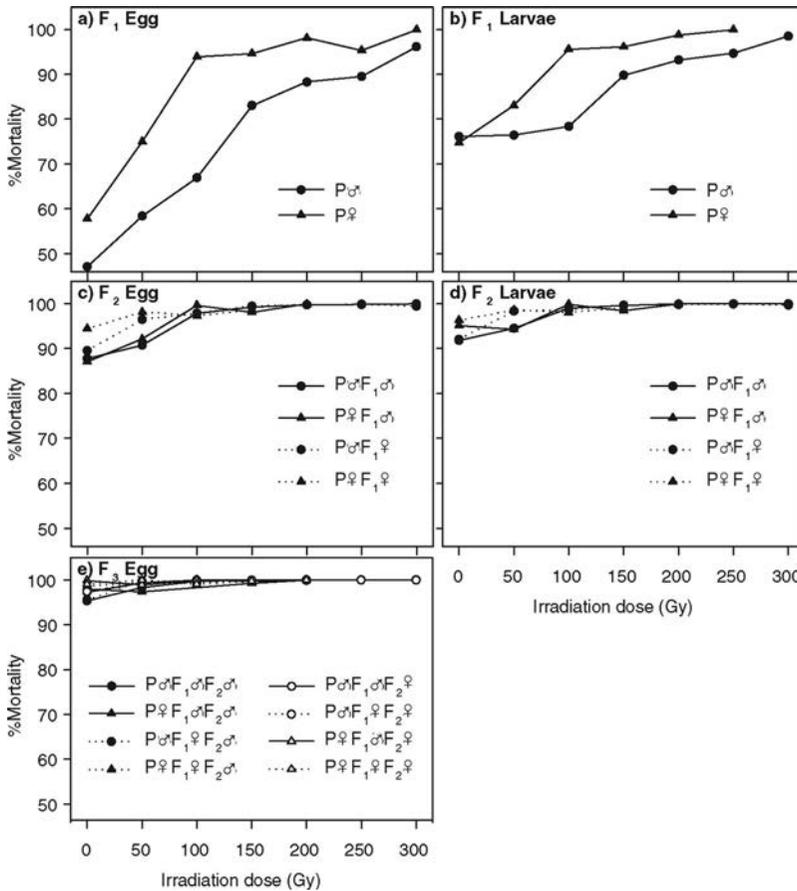
Declining parental fecundity and fertility with increasing doses of irradiation in Australia and New Zealand light brown apple moth populations were congruent with previously reported studies on other



**Fig. 6.** Mean fertility (% eggs hatching) of *E. postvittana*, for  $F_1$  eggs (from  $P♂$ ), and eggs from  $F_1$  progeny ( $F_1♀$ ,  $F_1♂$ ) where the male parent ( $P♂$ ) was irradiated at various doses, and the female parent and  $F_1$  cross was left untreated. a) pupae, b) pharate pupae, W. Australian trials. Error bars are 95% CL for two selected means in each plot. Note that there were no viable  $F_1$  adults produced from the 0 dose for trial b).

tortrics, particularly on the codling moth, *C. pomonella* (Bloem et al. 1999a and Blomefield et al. 2010) and false codling moth, *Cryptophlebia leucotreta* (Meyrick) (Bloem et al. 2003). For both populations, the female light brown apple moths were more radio-sensitive than the male, as is typical for Lepidoptera (North 1975, Carpenter et al. 1986, Bloem et al. 1999a,

Blomefield et al. 2010), although not always the case (Carpenter et al. 2001b). We observed that an irradiation dose of 250 Gy administered to parental (P) female pupae resulted in some egg hatch in both populations but, importantly, no emerged adults. Full sterility at the egg stage only occurred at 300 Gy. In comparison, this dosage of 250 Gy is higher than re-



**Fig. 7.** Cumulative mortality over three generations ( $F_1, F_2, F_3$ ) for two life stages of *E. postvittana* (egg, larvae), for various combinations of irradiated male or female parents (P) and their  $F_1$  and  $F_2$  progeny of both sexes (New Zealand data). Irradiated parents and their progeny were mated with untreated moths of the opposite sex.

ported studies with other tortricid species, where radiation doses  $\leq 200$  Gy were sufficient to induce sterility in females (Bloem et al. 1999a, 2003; IDIDAS 2007). Male pupae irradiated at 300 Gy still had a residual fertility in the range of 2–5%. Interestingly, our results suggest a greater sensitivity to radiation by pharate pupae for both parental genders than that of  $\geq 2$  d pre-emergence pupae. In two other Lepidoptera species studied, *C. leucotreta* (Bloem et al. 2003) and Mexican leafroller, *Amorbia emigratella* Busck (Follett 2008), the opposite results were found. However, our results concur with those of Suckling et al. (2004) with the painted apple moth *T. anartoides*. It is generally accepted that tolerance to radiation increases with age and developmental stage and with fruit flies physiological damage and reduced sexual fitness are greater among younger, pupae and female pupae irradiated close to emergence may produce fertile progeny (FAO/IAEA U.S. Dep Agric. 2003). The nature of this inconsistent response among Lepidoptera to irradiation with pupal age is unclear. However, it can be advantageous in an operational SIT program against the light brown apple moth, where large numbers of pupae need to be irradiated, given that it is impractical to irradiate all pupae at exactly the same developmental stage. Thus, our results suggest that irradiating older light brown apple moth pupae does not reduce sterility as strongly, and that targeting pupae in the range 0–3 d from emergence would give optimal results assuming there are some fitness costs to irradiating developmentally younger pupae.

The production of highly sterile  $F_1$  males ( $\geq 99\%$ ) at doses  $\geq 250$  Gy for both populations opens the prospect of using partially sterile male moths (inherited sterility) versus fully sterile males for release. Greater suppressive potential with partially sterile moths and more competitive moths have been demonstrated in SIT programs with other moths (Knipling 1970; Bloem et al. 1999b, 2001; Carpenter et al. 2005; Hendrik et al. 2005; Wee et al. 2005). This results from the multiplier effect from any successful mating from partially sterile males, because there can be many more  $F_1$  offspring produced than irradiated parental males released but none would be viable beyond  $F_2$  (Wee et al. 2005; Suckling et al. 2007). The skewed sex ratio in the  $F_1$  generation in favor of males at  $\approx 2:1$  (250 Gy) further promotes the release of partially sterile male moths, thus reducing the number of females at the  $F_1$  generation that could contribute to survivorship of any wild population. In many SIT operational programs, managers are reluctant to release fertile females into an area as they can mate with wild males and lay eggs. This is particularly important in low damage threshold horticultural crops, where damage by even a small number of larvae can dramatically affect the market value or access. The low survival rate of female moths observed in our trials is beneficial because of their seemingly lack of inherited deleterious effects.

These results suggest that a sterile insect technique (SIT) or  $F_1$  sterility program can be applied to control an infestation of *Epiphyas postvittana*, but these would still be reliant on complementary information such as

physical fitness (Suckling et al. 2011) and modeling of overflooding ratios (Kean et al. 2011). The challenge now is to identify the dose of radiation that would provide a balance between insect sterility and field competitiveness (Suckling et al. 2011). As there is yet no practical method of separating male and female light brown apple moths *en masse*. To avoid the risk of releasing potentially fecund and fertile females in the wild when using low doses of radiation, the appropriate dose should be  $\geq 250$  Gy, although this is most critical at the end of an eradication attempt when no surviving offspring are wanted (Knipling 1970, La-Chance 1985).

For a number of operational reasons, especially difficulties in the separation of pupae from rearing media, irradiation of codling moths and pink bollworm for SIT programs is generally done at the adult stage. However, there are advantages with pupal irradiation in terms of shipment and handling, provided a suitable and effective means of pupal separation from rearing media is developed. The potential to use size to separate male from female light brown apple moth pupae offers the possibility of reduced labor involved in a male-only release (A. M. Barrington, personal communication). Our results can be applied alone or integrated in a multi-tactic approach in an integrated eradication or area-wide management strategy against an incursion of this exotic lepidopteran, reducing the need for broad-spectrum insecticide use.

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