

## STERILISATION OF PAINTED APPLE MOTH *TEIA ANARTOIDES* (LEPIDOPTERA: LYMANTRIIDAE) BY IRRADIATION

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### ABSTRACT

The Australian painted apple moth (*Teia anartoides*) has been the target of an eradication programme in Auckland. This has included an extensive trapping programme underpinned by moth dispersal studies. Sterilisation of males was considered essential before release to avoid exacerbating the eradication problem. Late stage male pupae were irradiated using 1.25 MeV gamma rays from a Cobalt<sup>60</sup> source, at six doses (60, 80, 100, 120, 140 and 160 Gy). No effects were measurable on male emergence or mating performance in the treated compared to control insects. Significant effects were observed in the F<sub>1</sub> generation, with increasing doses producing increased mortality. At the highest doses 100% sterility was achieved in the F<sub>2</sub> generation. Male flight in a wind tunnel was not significantly affected by irradiation at 160 Gy. Mark-release recapture experiments were successfully conducted, with the maximum recorded dispersal distance of several kilometers by irradiated sterile male moths.

**Keywords:** painted apple moth, *Teia anartoides*, biosecurity, irradiation, inherited sterility.

### INTRODUCTION

Painted apple moth, *Teia anartoides* (Lepidoptera: Lymantriidae) is an Australian insect with potential for significant economic and ecological damage in New Zealand. This is particularly due to its wide host range, including plants of importance to horticulture and forestry, as well as the natural estate. Female painted apple moths are flightless, and ballooning larvae are the main means of dispersal in this species.

The eradication programme operated by the Ministry of Agriculture and Forestry Biosecurity Authority has included a pheromone trapping programme based on female moths, in order to map the pest distribution. However, the interpretation of the results has required a better understanding of male moth dispersal.

Quantitative information on moth dispersal is valuable in the interpretation of both trap catches and pest biology (Suckling et al. 1990, 1994). Male painted apple moth catches could result from flight on either local or long distance scales, and the MAF Technical Advisory Group recognised the need for quantifying the dispersal capacity of male moths to better determine the areas for targeting eradication. However, the potential risk of feral female moths being mated led to the requirement for male sterility in advance of mark-release recapture studies of male dispersal.

Irradiation for male sterility of another Lymantriid moth, *Lymantria dispar* (gypsy moth), has been investigated for the purpose of slowing the spread of the pest in North America (Maestro 1993). While mating disruption is now used in preference to irradiation (Sharov et al. 2002), the gypsy moth programme provided invaluable background to this project, including doses of irradiation likely to be appropriate. The sterile male technique is currently being used to control codling moth (*Cydia pomonella*) in the Okenagan

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Valley of British Columbia (Anon. 2002). A combination of mating disruption and mass release of sterile codling moth males has also been investigated, using doses of 100 and 250 Gy (Bloem et al. 2001). Inherited sterility in males was greater at the higher of the two doses, but moth dispersal distance was reduced.

In New Zealand, male insect sterility has the advantage of meeting the stringent requirements of the Hazardous Substances and New Organisms Act, which seriously limits the application of many technologies with potential value for eradication (e.g. Suckling et al. 2002). This study was instigated to determine the impact of irradiation on male emergence and mating, and on genetic damage, expressed as mortality in the next two generations (inherited sterility). A key requirement of releases of irradiated males for dispersal studies is that moth fitness for dispersal should not be different from the wild type. Hence the study focussed on inherited sterility, measured at the  $F_1$  and  $F_2$  generations, achieved using low doses of radiation to reduce impacts on dispersal. Male fitness was assessed in a wind tunnel.

## MATERIALS AND METHODS

### Insects

Male and female pupae of painted apple moth were received from a colony at the HortResearch Insect Rearing Unit, Auckland. The colony was established from field-collected insects, reared on artificial diet (A.M. Barrington, pers. comm.) for one generation.

### Irradiation

Samples of 16-17 day-old male pupae were irradiated using 1.25 MeV gamma rays from a Cobalt<sup>60</sup> source. Petri dishes (9 cm diameter) were positioned on an aluminium scissor jack and irradiated in two groups of three. The dishes were periodically rotated to ensure as uniform distribution of dose as possible and were sequentially removed as the required dose was delivered. The reference point for the irradiations was taken as at the geometrical centre of the stacked containers (as appropriate for either one, two, or three containers), which was set at a distance of 55 cm from the Co<sup>60</sup> source. The entrance air kerma rate (measured free in air, with no back scatter in a 10 cm x 10 cm field at 1 m) was 19.9 mGy/s. The radiation field size in the reference plane of the dishes was approximately 11 cm x 11 cm. The dishes containing a total of 80-82 pupae per dose were irradiated for times of 3009, 4012, 5015, 6018, 7021 and 8024 s respectively, giving doses (air kerma) of 60, 80, 100, 120, 140 and 160 Gy respectively. There was an untreated 'control' group of 160 pupae.

### Assessment

Five male pupae ( $F_0$ ) were placed on tissue in a 60 mm Petri dish, and 1 female pupa (3 days old, not irradiated) was placed on the lid of a 35 ml catering portion cup. The insects were placed in a 680 ml container fitted with a lid. Fifteen females were used in each treatment (105 females in total). The containers were placed in the quarantine facilities at Lincoln (25°C), and successful emergence of male pupae was recorded. The emergent females laid  $F_1$  eggs on the catering cup lid, which was double contained. Larvae were removed and counted as they emerged (hatching larvae usually emerged the same day). Several days after all hatching larvae had been removed, the eggs were placed in the freezer until the number of unhatched eggs was counted. Sixty larvae from five females in each treatment were reared to adults on artificial diet to determine whether any  $F_2$  effects were present. The hatching success of  $F_2$  eggs (backcrossed to wild-type females) was also determined. Eight  $F_1$  males were mated to untreated females and the numbers of eggs per female and hatching rate of larvae assessed at the  $F_2$  generation. The sample size of  $F_1$  males was constrained by survivorship at the higher doses of radiation. Cumulative effects were calculated from the product of the survivorships of individual stages.

### Male fitness

Male moths (n=60) were irradiated at 160 Gy as described above, and emerged along with untreated males (n=30), before being flown individually in a wind tunnel to virgin

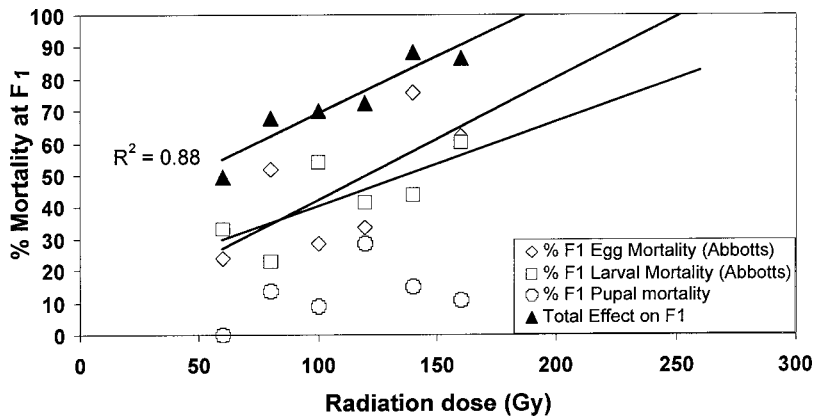
females. Males were given 2 min to respond to a calling female at the upwind end of the tunnel. Treated and control males were alternated in the tunnel. Six categories of behaviour were recorded: no response, activation, upwind flight, mid-tunnel flight, arrival or landing at the female.

#### Statistical analysis

Regression analysis was used to describe the dose-response to irradiation. The cumulative mortality at a given dose of irradiation was calculated from the product of survivorships of individual life stages. Thus, Cumulative mortality =  $100 - (\text{survivorship (eggs)} * \text{survivorship (larvae)} * \text{survivorship (pupae)})$ . In the  $F_2$  generation, this statistic only included the proportion of females ovipositing, and the survivorship (eggs) due to the high level of inherited sterility in the doses chosen. The cumulative effect was the product of the survivorship of the two generations. Abbott's (1925) correction was used for plotting the data. Chi-squared tests were used to compare flight behaviour of treated and untreated male moths in the wind tunnel.

### RESULTS AND DISCUSSION

The irradiated male moths emerged and mated normally compared to the 'control' group (Table 1). Mortality of  $F_1$  eggs in the controls was higher than desirable (24%), but a linear dose response was evident in the offspring of irradiated males (Fig. 1) in both egg and larval mortality, after Abbott's correction for the control mortality (Abbott 1925). The high control mortality was confined to the offspring of five females, with 0-1% mortality for the other ten control females. No obvious explanation is available for the poor survival of progeny from a subset of females. The effect of a possible over-correction for 'control' mortality would be to underestimate the sterility achieved in the treatments.



**FIGURE 1: Dose response of male pupae of painted apple moth to Cobalt-60 irradiation, with the effect assessed at the next generation as egg, larval and pupal mortality (corrected for control mortality, Abbott 1925).**

The highest doses gave approximately 90% sterility at the  $F_1$  generation when egg, larval and pupal effects are taken into account (Table 1). The offspring of surviving males was therefore examined in order to assess the additional sterilising effect accumulated in the  $F_2$  generation.

**TABLE 1: Influence of irradiation dose on male painted apple moth pupae ( $F_0$ ) expressed as mortality at each life stage, resulting in the cumulative mortality in offspring ( $F_1$ ). See statistical analysis for full details of calculations.**

Dose (Gy)	No. male $F_0$ pupae exposed	$F_0$ mortality (%)	Mean no. $F_1$ eggs per female	Egg mortality (%)	Larval mortality (%)	$F_1$ pupal mortality (%)	Cumulative mortality at $F_1$ (%)
0	160	0	330.1	24.3	16.7	10.4	
60	82	0	281.0	42.5	38.3	9.7	55.7
80	81	1	405.3	63.4	23.3	22.2	71.8
100	80	0	263.5	45.7	38.3	31.6	80.6
120	80	0	340.5	49.7	36.7	46.4	79.3
140	80	0	429.2	81.4	46.7	24.0	90.3
160	81	0	258.3	71.2	66.7	26.3	88.9

Three of the eight females that were not irradiated failed to lay fertile eggs, while the remainder averaged 80% hatch. This resulted in 50% the mean hatch rate from the eight females (Table 2). At the highest two doses of irradiation, no  $F_2$  eggs were laid by females mated to surviving  $F_1$  males, and at all doses there was a relatively low rate of hatch compared to the untreated controls. The cumulative effect of the irradiation, calculated as the product of the survivorships in both generations was complete or near complete sterility at all doses from 60 to 160 Gy (Table 2).

**TABLE 2: Influence of irradiation dose on male painted apple moth ( $F_0$ ), assessed as females not hatching eggs and egg mortality in offspring ( $F_2$ ). The cumulative mortality was calculated from the product of survivorships from the two generations ( $F_1$  from Table 1).**

Dose (Gy)	No. $F_1$ males	No. females not hatching eggs	Mean no. $F_2$ eggs per female	$F_2$ egg mortality (%)	Cumulative mortality at $F_1$ and $F_2$ (%)
0	8	3	378.4	51.1	
60	8	5	403.7	96.5	99.98
80	8	3	414.2	84.0	99.89
100	8	6	294.0	94.8	99.96
120	8	6	273.5	98.7	99.99
140	8	8	0	100	100.00
160	8	8	0	100	100.00

There was no statistically significant difference between the proportion of males that flew to virgin female moths in the wind tunnel ( $\chi^2=3.17$ ;  $P>0.05$ ), or in the proportion reaching lower behavioural categories. However, the sample size was low (45% success in the controls,  $n=30$ , 23% in the treated males,  $n=60$ ), and further tests would be needed to be more definitive. These preliminary results, combined with a lack of effect on emergence and mating success on the  $F_0$ , were considered indicative that sufficient fitness was present to warrant release experiments.

Release recapture experiments, centred on Waikumete Cemetery in Auckland, have confirmed the fitness of irradiated males (160 Gy) for characterising minimum dispersal

potential, with a high percentage of marked irradiated males (e.g. up to 34%) recaptured, some up to several kilometers from the release site (D.M. Suckling, unpubl. data). While no statistically significant difference in wind tunnel flight was detected, the lower proportion of successful flights by treated males was suggestive of an effect. Furthermore, lower doses of irradiation (which would still have achieved near complete sterility, Table 2) would presumably have had a lesser impact on male moth fitness (e.g. Bloem et al. 2001). It is therefore possible that the use of 160 Gy as the dose of choice for dispersal studies has led to an underestimate of the potential dispersal distance of this insect.

This study has demonstrated the potential value of inherited sterility as a technique for underpinning a biosecurity operation, by ensuring that males released for the purposes of quantifying dispersal did not contribute to the target pest population. In future, it could also prove desirable to further examine inherited sterility as a potential control tactic in eradication of this and other pest insects, particularly where the infestation is limited.

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