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Author: Saour, George

Source: Journal of Insect Science, 14(8) : 1-10

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.014.08>

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Sterile insect technique and F_1 sterility in the European grapevine moth, *Lobesia botrana*

George Saour

Department of Biotechnology, Atomic Energy Commission of Syria, P. O. Box 6091, Damascus, Syria

Abstract

Newly emerged adults of the European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), were irradiated with various doses of gamma radiation and crossed to unirradiated counterparts of the opposite sex. Fecundity was decreased when unirradiated females were mated with either 300- or 350-Gy-irradiated males. Adult males that were irradiated with 400 Gy and mated with unirradiated females retained a residual fertility of 2.7%. The radiation dose at which irradiated females were found to be 100% sterile when mated with unirradiated males was 150 Gy. The inherited effects in the F_1 progeny of irradiated male parents were examined at 100, 150, and 200 Gy. Fecundity and fertility of the F_1 progeny of males irradiated with 150 Gy and inbred or crossed with irradiated and unirradiated moths were also recorded. A significant reduction in fertility was observed when F_1 males mated with either F_1 or unirradiated females. According to sterility index, F_1 females who mated with F_1 males had greater sterility than when F_1 females were crossed to 150-Gy-irradiated males. Based upon the results of this study, 150 Gy of gamma radiation would be the optimal dose to use in a sterile insect technique and F_1 sterility program against *L. botrana*.

Keywords: gamma radiation, inherited sterility, pest management, radiation biology

Abbreviations: SIT, sterile insect technique

Correspondence: ascientific@aec.org.sy

Editor: Oliver Martin was editor of this paper.

Received: 4 April 2012 **Accepted:** 11 August 2012 **Published:** 23 January 2014

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ISSN: 1536-2442 | Vol. 14, Number 8

Cite this paper as:

Saour G. 2014. Sterile insect technique and F_1 sterility in the European grapevine moth, *Lobesia botrana*. *Journal of Insect Science* 14:8. Available online: <http://www.insectscience.org/14.8>

Introduction

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermuller) (Lepidoptera: Tortricidae), is a polyphagous insect that develops on more than 200 plant species from various families. It is one of the most serious pests in Mediterranean and southern European area vineyards (Ifoulis and Savopoulou-Soultani 2004). Recently, *L. botrana* was inadvertently introduced to Japan and has been reported from Chile, Argentina, and the Napa Valley in California (USA) (Varela et al. 2010). *Lobesia botrana* adults appear in vineyards in early spring. First-generation larvae feed on inflorescences, whereas 2nd and 4th generations damage the green, ripening, and ripe berries.

Several control tactics have been evaluated against *L. botrana*, including the use of insecticides, biological control agents, and mating disruption methods (Ifoulis and Savopoulou-Soultani 2004; Irigaray et al. 2005; Akyol and Aslan 2010). It is well documented that the widespread use of insecticides is highly dangerous in sensitive ecosystems (Yadav 2010). However, in most vineyards *L. botrana* is still controlled by using broad-spectrum chemical insecticides (Irigaray et al. 2010; Ioriatti et al. 2011).

Research on the radiobiology of insects demonstrated that Lepidoptera and Homoptera are radiation-resistant orders and require a radiation dose much higher than that of other insect orders classed as radiation-sensitive (Barkri et al. 2005). A major difference between these two groups of insect orders is that the former group has a diffuse centromere (holokinetic) and the latter has a localized centromere (monokinetic) (Murakami and Imai 1974). It is believed that the centromere difference could play a major role in radiation

sensitivity (Carpenter et al. 2005). However, it was suggested that possible molecular mechanisms responsible for the high radio-resistance in Lepidoptera might include an inducible cell recovery system and a DNA repair probe (LaChance and Graham 1984). Another genetic phenomenon found in Lepidoptera is that females are more sensitive to radiation than are males of the same species, and inherited sterility in irradiated lepidopteran males is caused mainly by induced translocations that lead to the production of genetically unbalanced gametes in F₁ individuals, resulting in their sterility (Tothova and Marec 2001).

The use of autocidal control methods (i.e., sterile insect technique (SIT) and the phenomenon of inherited sterility or F₁ sterility in Lepidoptera) represents an environmentally and medically benign option that offers great potential for managing *L. botrana*. The advantages of F₁ sterility over the completely sterile insect in Lepidoptera pest control were largely discussed by several authors (lower doses of radiation used to induce F₁ sterility increased the quality and competitiveness of the released moths) (Makee and Saour 2004; Carpenter et al. 2005; Tate et al. 2007; Soopaya et al. 2011; Jang et al. 2012). Moreover, inherited sterility has been demonstrated in a number of economically important Lepidoptera. Sterile moths are currently being applied as part of operation area-wide integrated pest management programs for the following three lepidopteran pests: the codling moth, *Cydia pomonella*, in western Canada, the false codling moth, *Thaumatotibia* (=Cryptophlebia) *leucotreta*, in South Africa, and the cactus moth, *Cactoblastis cactorum*, in the USA and Mexico (Hight et al. 2005; Bloem et al. 2007; Carpenter et al. 2007).

There are no published data concerning the effects of gamma radiation or the use of SIT

against *L. botrana*. Therefore, the purpose of this study was to examine the effect of various doses of gamma radiation on the fecundity and fertility of *L. botrana* when insects were inbred or crossed with unirradiated mates. Moreover, the minimum dose at which females were 100% sterile when mated with fertile males was determined. Based on the results of the first set of experiments on the parental generation, three doses were chosen for documentation of inherited sterility effects in this species. Mortality during development, sex ratio distortion, and fecundity and fertility of the F₁ generation produced from irradiated males and unirradiated females were also determined. The results obtained are discussed in the context of using SIT/F₁ sterility as a species-specific pest control tactic that could be used to eradicate or prevent further geographic range expansion of *L. botrana*.

Materials and Methods

Insects

Insects used in the experiments were obtained from a laboratory stock culture, which was renewed each year with field collected *L. botrana* larvae from infested grapevine. The larvae were reared on a semi-artificial diet as described in Thiery and Moreau (2005) at 25 ± 1° C, 60 ± 10% RH, with a photoperiod of 15:8 L:D and 1 hr of dusk. Male and female adults (100 pairs) were placed in a large cage (30 x 60 x 30 cm), furnished with bands of waxed paper (15 x 2 cm) onto which females could oviposit, and provided with a source of food (5% sucrose solution). The oviposited eggs were collected daily and incubated in plastic boxes (15 x 12 x 6 cm) for ~5 days until the eggs hatched. Using a fine brush, newly hatched larvae were transferred to small plastic boxes (4 x 3 x 2 cm) containing semi-artificial diet. Larvae were checked daily until pupation, and the same procedure as described

before was followed.

Effect of gamma radiation on adult fertility and fecundity

Lobesia botrana pupae were removed from the small rearing boxes and placed in small transparent plastic tubes (length: 8 cm, diameter: 1 cm) and allowed to emerge at the above-mentioned rearing conditions. Newly emerged virgin adults (< 24 hr old) were sexed and exposed to gamma radiation. A Cobalt⁶⁰ gamma-cell (Issledova gamma Irradiator, Techsnabexport, www.tenex.ru) with a dose rate of 16.6 Gy/min was used to administer doses of 50, 100, 150, 200, 300, 350, and 400 Gy. The absorbed dose was measured using an alcoholic chlorobenzene dosimeter. After irradiation, each irradiated moth was placed in a transparent plastic Petri dish (9 cm diameter) with an unirradiated adult of the opposite sex (n = 45/petri dish, 1♀ and 1♂ for each tested dose). A control group was handled in the same way as irradiated moths but was not exposed to gamma radiation. Thus, three types of crosses were made at each dose (irradiated ♀ by unirradiated ♂, unirradiated ♀ by irradiated ♂, and unirradiated ♀ by unirradiated ♂ (control)). A 5% sucrose solution was provided as a food source to each moth pair in small cups fixed inside the Petri dish with a cotton wick soaked in the sugar solution. The moths were allowed to mate and lay eggs until the females died. *Lobesia botrana* does not require the stimulus of a host plant to initiate mating and oviposition, thus the inner Petri dish surface served as the oviposition substrate. Females were dissected to determine their mating status by identifying the presence of spermatophores in the bursa copulatrix. Eggs deposited by each female were held separately at the previously described conditions to allow for complete egg development and larval eclosion. The fecundity (number of eggs laid per female) and fertility (number of

eggs that hatched) were counted per pair at each tested dose. Each female that failed to oviposit fewer than 10 eggs throughout the entire experimental period was discarded in order to homogenize the variance. Sterility was expressed as the percentage of hatched eggs. The test consisted of three replicates of each cross at each dose of radiation, each consisting of 15 moth pairs.

Effect of gamma radiation on developmental time, mortality, and sex ratio of F₁ progeny

Newly emerged adult males (< 24 hr old) were irradiated at sub-sterilizing doses of 100, 150, and 200 Gy (n = 45 males for each dose). At each examined dose, a group of newly emerged adult males were taken as a control. Irradiated and control males were paired individually with newly emerged females and left together until death. The oviposited eggs were collected, counted, and left to determine percentage of hatched eggs. One hundred to 150 newly hatched larvae were taken from irradiated and control groups. These larvae were fed singly on adequate artificial diet pieces (~5 g/larva). The developmental time of the F₁ progeny from egg hatching to adult emergence, the number of emerged adults, and the sex ratio were determined. The tests were repeated so that there were a total of three replications with 15 pairs per replicate for each dose.

Fertility and fecundity of F₁ progeny of 150-Gy-irradiated males inbred or crossed to irradiated and unirradiated moths

Newly emerged *L. botrana* males were irradiated with a dose of 150 Gy and crossed to virgin unirradiated females. The deposited eggs were collected and allowed to hatch, and sterility and fecundity in the F₁ generation were calculated. F₁ neonates from 150-Gy-irradiated males crossed with unirradiated fe-

males were placed on artificial diet pieces. Pupae were collected and all emerging F₂ adults were either inbred or paired singly with 150-Gy-irradiated or unirradiated counterparts of the opposite sex (n = 45 pairs for each cross). Moths were allowed to mate and lay eggs. Eggs were collected and incubated. The sterility and fecundity in the F₂ generation were calculated. Longevity for the F₁ and F₂ pairs was recorded. Moreover, the percentage of sterility index was calculated using the formula of Toppozada et al. (1966):

$$\% \text{ Sterility} = [1 - (F_t \times Fe_t / F_c \times Fe_c)] \times 100$$

where F_t = fecundity of treated females; Fe_t = fertility of treated females; F_c = control fecundity; and Fe_c = control fertility. The experiment was conducted three times for each cross with 15 pairs per replicate.

Statistical analysis

All statistical analyses were performed using Stat-View 4.02 version (Abacus Concept 1994) at the 5% level ($p < 0.05$). An ANOVA was carried out to evaluate the differences between the means. Significant ANOVAs were followed by the protected least significant method. Student's *t*-test was used to evaluate the differences between two treatments. Differences in the sex ratio were determined with a chi-square test.

Results

Effect of gamma radiation on adult fertility and fecundity

Figure 1 presents the relationship between the applied doses of gamma radiation and the percentage of hatched eggs when *L. botrana* virgin males and females were irradiated and crossed to unirradiated counterparts of the opposite sex. The hatchability of the control eggs (~80%) was different from that of the

Table 1. Effects of gamma radiation on developmental time, mortality (mean ± SE), and sex ratio of the F₁ progeny of the male parents of *Lobesia botrana* when they were irradiated at different doses. Larvae were reared at 25° C with a photoperiod of 16:8 L:D.

Dose (Gy)	Initial no. larvae	Developmental time (days)	Mortality ^a (%)	F ₁ sex ratio ^b (F:M)
100	150	26.1 ± 0.3a	30.5 ± 0.6a	1:1.51a
Control	150	23.6 ± 0.2b	20.1 ± 0.4b	1:1.12b
150	150	26.6 ± 0.3a	28.9 ± 0.6a	1:1.63a
Control	150	24.0 ± 0.3b	18.6 ± 0.5b	1:1.11b
200	100	27.0 ± 0.4a	28.9 ± 0.5a	1:1.70a
Control	100	24.3 ± 0.3b	15.8 ± 0.3b	1:1.18b

Means within a column for each applied dose followed by the same letter are not significantly different at $p < 0.05$ (Student t-test). ^a Number of unemerged adults per initial number of tested larvae. ^b Ratios within a column for each applied dose followed by the same letter are not significantly different at $p < 0.05$ (Chi-square test). F, female; M, male. Mean of three replicates for each dose, 15 moth pairs per replicate.

Table 2. Means (± SE) of hatched eggs, number of eggs/female, moth longevity, and sterility index when *Lobesia botrana* males were irradiated with 150 Gy and crossed to fertile counterparts (parental generation (P₁)), and when F₁ adults resulting from irradiated male crossed to unirradiated female were inbred or crossed to 150-Gy-irradiated and unirradiated moths.

Generation	Crosses	Mean egg hatch (%)	Mean no. eggs/female	Mean moth longevity (days)		Sterility index (%)
				♂	♀	
Parental (P ₁)	Irradiated ♂ x Unirradiated ♀	38.9 ± 1.6b	71.8 ± 4.0a	12.2 ± 0.4a	12.9 ± 0.4a	54.3
	Unirradiated ♂ x Unirradiated ♀	81.9 ± 2.3a	74.7 ± 3.4a	13.0 ± 0.5a	12.8 ± 0.6a	-
F ₁	F ₁ ♂ x Unirradiated ♀	19.6 ± 2.5bc	42.1 ± 2.1c	10.2 ± 0.4b	12.3 ± 0.7a	86.9
	F ₁ ♂ x F ₁ ♀	10.2 ± 1.1d	37.7 ± 2.1c	10.1 ± 0.4b	10.0 ± 0.4b	93.9
	150 Gy-Irradiated ♂ x F ₁ ♀	15.1 ± 1.9cd	66.7 ± 2.7b	12.3 ± 0.4a	10.3 ± 0.4b	84.0
	Unirradiated ♂ x F ₁ ♀	24.6 ± 2.9b	72.8 ± 2.4ab	12.6 ± 0.3a	10.2 ± 0.3b	71.6
	Unirradiated ♂ x Unirradiated ♀	82.9 ± 1.8a	76.1 ± 1.7a	12.8 ± 0.3a	12.7 ± 0.4a	-

Means within a column for each generation followed by the same letter are not significantly different at $p < 0.05$ (Fisher PLSD). % sterility index = $[1 - (F_i \times F_{et} / F_c \times F_{ec})] \times 100$. F_i = fecundity of irradiated females; F_{et} = fertility of irradiated females; F_c = control fecundity; and F_{ec} = control fertility. Mean of three replicates for each cross, 15 moth pairs per replicate.

irradiated moths, irrespective of the applied dose and the gender irradiated (e.g., between 0 and 100 Gy, $t = 15.9$, $df = 31$, $p < 0.0001$, when an irradiated male was mated with an unirradiated female). A residual fertility of 2.7% was recorded when 400-Gy-irradiated males were mated with unirradiated females.

The percentage of hatched eggs sharply declined when irradiated females were paired with unirradiated males ($F = 2367.1$ $df = 2$, 102; $p < 0.0001$), indicating that the dose effect was greater for irradiated females than for irradiated males. Thus, the dose-response curve illustrates that *L. botrana* females became completely sterile with a dose between 100 and 150 Gy (Figure 1).

There was no noticeable difference between the mean number of eggs laid by females that mated with unirradiated males (control) and females that mated with males irradiated at 50, 100, and 200 Gy. In contrast, at 300 and 350 Gy the mean number of eggs per female was significantly lower than that of the control ($F = 48.6$; $df = 5$, 66; $p < 0.0001$) (Figure 2).

Effect of gamma radiation on developmental time, mortality, and sex ratio of F₁ progeny

The mean developmental time of F₁ progeny of irradiated male parents was significantly higher than that of the control (at 200 Gy, $t = 5.8$; $df = 59$; $p < 0.0001$). However, the developmental time of the F₁ progeny of 100-Gy-irradiated male parents did not differ from that of F₁ progeny of 150 and 200-Gy-irradiated

sires. The mean percentage of mortality of the F_1 progeny of irradiated male parents was higher than that of the control (at 100 Gy, $t = 13.4$; $df = 49$; $p < 0.0001$). When *L. botrana* males were irradiated with 100, 150 and 200 Gy, the percent mortality of their F_1 progeny was similar. By irradiating the males with 100, 150, and 200 Gy, the ratio of their F_1 males to F_1 females was significantly higher than that of the F_1 progeny of the control ($X^2 = 9.1$, $df = 1$, $p < 0.05$). However, sex ratios of F_1 progeny did not differ significantly among the irradiated treatments.

Fecundity and fertility of F_1 progeny of 150-Gy-irradiated males crossed to irradiated and unirradiated moths

Fecundity and percentage of hatched eggs of *L. botrana* parental generation was significantly affected by the dose of radiation used (150 Gy). However, adult longevity was not affected by the applied dose (Table 2). F_1 females crossed to unirradiated males had residual fertility comparable to that obtained from 150-Gy-irradiated males mated with unirradiated females. A significant reduction in the percent of hatched eggs with high sterility index was recorded when F_1 males were crossed to their female siblings ($F = 200.2$; $df = 4$, 131; $p < 0.0001$). In F_1 generation crosses, the fecundity of crosses between F_1 males and unirradiated females and F_1 males and F_1 females was significantly lower than that of the other crosses tested ($F = 65.9$; $df = 4$, 127; $p < 0.0001$). The mean longevity of F_1 adults was significantly lower than their irradiated parents (e.g., for male moths, $F = 8.8$; $df = 4$, 127; $p < 0.0001$).

Discussion

Lobesia botrana adults responded to increasing doses of gamma radiation with a decline in female fecundity and male fertility. Fur-

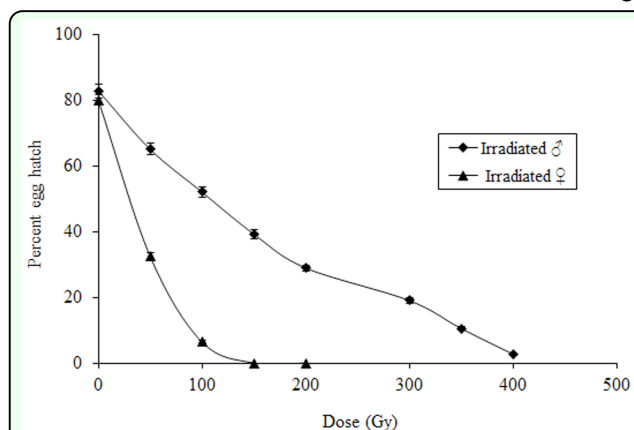


Figure 1. Mean percentage of hatched eggs when *Lobesia botrana* adults were treated with gamma radiation and crossed to unirradiated counterparts of the opposite sex. Bars indicate standard errors. Mean of three replicates for each dose, 15 moth pairs per replicate. High quality figures are available online.

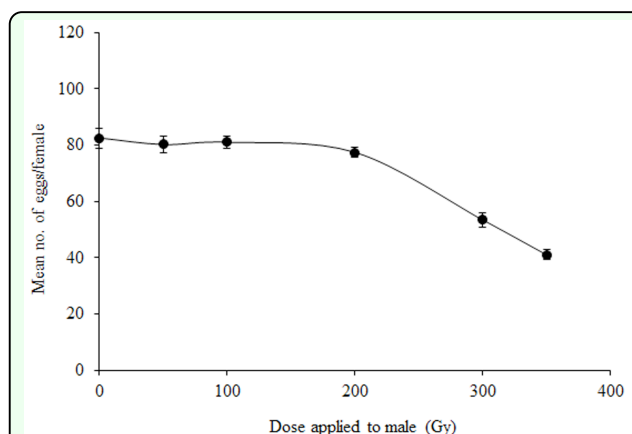


Figure 2. Effect of gamma radiation on fecundity (mean number of eggs per female) of *Lobesia botrana* females mated with irradiated males. Bars indicate standard errors. Mean of three replicates for each dose, 15 moth pairs per replicate. High quality figures are available online.

thermore, it was found that females were more radiosensitive than were males, reduction in female fecundity was greater when they were crossed with males treated at high doses, and F_1 progeny from irradiated males were less fertile than their irradiated parents. These findings are in agreement with those reported for other Lepidoptera species (Saour and Makee 1997; Carpenter et al. 2001; Makee and Saour 2004). However, comparing the results of *L. botrana* male fertility with those obtained from *C. pomonella* (Bloem et al. 2007) and the *T. leucotreta* (Carpenter et al. 2007), we found that *L. botrana* was more ra-

dioreistant than these two tortricid species (400 vs 350 Gy to obtain full sterility). However, *L. botrana* was less resistant to radiation than the potato tuber moth, *Phthorimaea operculella* (Saour and Makee 1997), and *C. cactorum* (400 vs 500 Gy) (Carpenter et al. 2001).

Successful application of SIT/F₁ in Lepidoptera is achieved by selecting a treatment dose of radiation that fully sterilizes females, to avoid increasing host plant damage, while only partially sterilizing males to maintain mating competitiveness and produce F₁ progeny highly sterile when mated with feral females (Tate et al. 2007). According to the results of our study, the dose of radiation providing these attributes in *L. botrana* was found to be 150 Gy. At this radiation dose, the fertility of irradiated males mated with unirradiated females was reduced by ~61%, while only an average of 20% of eggs hatched for F₁ males that mated with unirradiated females. Moreover, there was no impact of radiation on the parental generation female fecundity, and females that mated with irradiated males laid nearly as many eggs as did the controls.

The effects of radiation and inherited sterility on the reproduction of *L. botrana* were similar to those described for other species of Lepidoptera, i.e., reduced survival of larvae, the delay in the developmental time from F₁ neonate to adult, and the shift of sex ratio in favor of males in the F₁ generation. However, the sex ratio distortion of the F₁ progeny in favor of males was one of the benefits of inherited sterility.

There was a skewed sex ratio in favor of males for F₁ *L. botrana* adults. These results are in agreement with those reported previously for *P. operculella* (Makee and Saour 1997), *Spodoptera litura* (Ramesh et al. 2002), and

C. leucotreta (Hofmeyr et al. 2005). On the other hand, Carpenter et al. (2001) did not detect a skewed sex ratio in favor of male offspring for the *C. cactorum* F₁ adults. It is worth noting that the fecundity of F₁ females obtained in our study from male parents irradiated with 150 Gy and mated with unirradiated males was not significantly different from that of unirradiated moths. This finding is similar to those reported by Makee and Saour (1997) and Bloem et al. (2003) in F₁ progeny of *P. operculella* and *C. leucotreta*, respectively.

In all current SIT/F₁ sterility programs against Lepidoptera, both males and females are mass-reared, irradiated, and then released into the targeted area because no practical method is available to separate the adult moths by gender (Bloem et al. 2007; Blomefield et al. 2011). Moreover, the irradiated moths are released continuously from the beginning of the season, thus the possibility of crosses involving 150-Gy-irradiated males with F₁ females and F₁ males with their female counterparts could occur. The results of our study showed that the fertility of unirradiated males crossed to F₁ females did not differ significantly from that of the cross between F₁ males mated with unirradiated females, which suggests that *L. botrana* F₁ females inherited the deleterious effects from their irradiated male parents. High values of unhatched eggs and sterility index were obtained when F₁ males were mated with either F₁ or unirradiated females and when 150-Gy-irradiated males were mated with F₁ females.

The results presented here provide a starting point for developing SIT/F₁ sterility program against *L. botrana*.

Acknowledgements

Thanks are due to Dr. I. Othman (General Director) and Dr. N. Mirali for their help and support. Technical and financial assistance was provided in part by contract no. 15038 of the International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

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