Radiation Physics and Chemistry 97 (2014) 370–373

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Effects of gamma irradiation on the grape vine moth, *Lobesia botrana*, mature larvae

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HIGHLIGHTS

- Effects of gamma irradiation on *Lobesia botrana* mature larvae are examined.
- Results showed that a dose of 200 Gy was sufficient to prevent adult emergence from mature larvae.
- This dose (200 Gy) is less than the suggested generic phytosanitary irradiation dose of 250 Gy for Lepidopteran larvae.
- The dose is also much lower than the maximum allowed dose for irradiation of fresh fruits and vegetables.

ARTICLE INFO

Article history:
Received 27 March 2013
Accepted 28 July 2013
Available online 8 August 2013

Keywords:
Phytosanitary irradiation
Phytosanitary treatment
Quarantine treatment

ABSTRACT

Mature 5th instars of the grape vine moth, *Lobesia botrana* (Denis and Schiffermuller) were exposed to gamma radiation dosages ranging from 50 to 250 Gy. The effects of gamma radiation on pupation, adult emergence, sex ratio and rate of development were examined. Results showed that the radiosensitivity of the grape vine moth larvae increased with increasing radiation dose. The severity of the effect, however, depends on the criterion used for measuring effectiveness; adult emergence was more severely affected than pupation. Pupation was significantly affected at 150 Gy and decreased by about 25% at 250 Gy. Adult emergence, on the other hand, was significantly affected at 100 Gy and completely prevented at 200 Gy. Probit analysis of dose mortality data for pupation and adult emergence show that the LD_{99} for preventing subsequent development to pupae and adults was 2668 and 195 Gy, respectively. In addition, the rate of development of mature larvae to the adult stage was negatively affected and sex ratio was skewed in favor of males.

1. Introduction

The grape vine moth, *Lobesia botrana* (Denis and Schiffermuller), is a key pest on grape vine, *Vitis vinifera* L., in Syria and causes millions of dollars in losses to the grape industry every year. The infestation rate in neglected orchards is over 40% and even with control, the infestation rate may reach 10% (Dawarah, 2000). The pest is also of quarantine importance in many parts of the world (Anonymous, 1992, Venette et al., 2003, Varela, 2010) and, consequently, exports to *L. botrana* free countries are impossible without effective phytosanitary treatment as immature stages of this species, particularly eggs and larvae can be transmitted with fresh fruits to importing countries.

Methyl bromide, a chemical fumigant highly affective against this pest, is being phased out globally and is supposed to be banned completely by the year 2015 (Heather and Hallman, 2008; Besri, 2010) with no other chemical to replace it at present. Although this chemical is currently exempted from quarantine uses, this could change in the future. Ionizing radiation is an alternative to methyl bromide for treating fresh agricultural products in order to overcome quarantine barriers in trade (Hallman, 2011). This requires, however, determining the irradiation dose which guarantees complete control of the irradiated insects without any unacceptable effects on the treated commodity. Effects of gamma irradiation on the grape vine moth egg stage has been reported before (Mansour and Al-Attar, 2012). Information on the effects of gamma radiation on the larval stage, however, is lacking which makes it necessary to study the sensitivity of this stage to gamma irradiation.

The objectives of this study were to provide data on the effects of gamma radiation on *L. botrana* mature larvae, as the most radioresistant larval stage, and determine the required irradiation dose for phytosanitary treatment of this pest in this stage.

2. Materials and methods

2.1. Obtaining Lobesia botrana mature 5th instars

Larvae used in this research were obtained from a colony of *L. botrana* that had been reared on an artificial medium similar to that reported by Mohamad et al. (1997) where mature larvae

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http://dx.doi.org/10.1016/j.radphyschem.2013.07.030

pupate on the dry surface of the medium. The colony originated from insects collected at several locations near the city of Sweida (Syria) in the summer of 2009. To maintain vigor, wild *L. botrana* males from the natural populations in the same area were periodically introduced into the colony every summer since. Larvae were reared in plastic trays (19 × 14 × 5 cm), each containing about 700 g of the larval rearing medium. Rearing conditions were maintained at 26 ± 1 °C, 60 ± 5% RH and 16:8 (L:D) photoperiod and, under these conditions, the larval stage requires about 20 days. When larvae finished feeding, strips of sterile white paper (25 × 1 cm) folded at one cm distance to take a zigzag form "larval holding strips", were distributed at the top of the rearing medium to collect mature larvae leaving the medium and searching for a place to spin their cocoons and pupate. The larval holding strips were removed 24 h later and prepared for irradiation studies.

2.2. Preparing mature 5th instars for irradiation

The strips of white paper holding the grape vine moth mature larvae "larval holding strips" (total of 50 larvae/rep.) were placed in Petri dishes 9 cm in diameter. The dishes containing the larvae were placed inside an insulating box and transferred to the gamma cell for irradiation.

2.3. Irradiation

Grape vine moth larvae were exposed to gamma radiation dosages in a gamma cell supplied with a Co-60 source around the cylindrical (15 × 25 cm) irradiation chamber (Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd. USR). The average dose rate at the time of irradiation was approximately 12.5 Gy/min with a dose uniformity ratio (the ratio of the max. to the min. received dose) of about 1.14 and the absorbed dose was calibrated with Fricke solution. At each dose level, five Petri dishes containing, each, 50 grape vine moth larvae, were irradiated simultaneously ($n=5$). The Petri dishes were placed at the center of the irradiation chamber and radiation dosages ranged between 50 and 250 Gy at 50 Gy increment.

2.4. Effects of gamma radiation on pupation, adult emergence and sex ratio

After irradiation, the Petri dishes containing the larvae were returned immediately to the laboratory and incubated at 26 ± 1 °C for pupation and adult emergence. The dishes were checked daily and emerging adults were removed, examined and their number and sex was recorded. Following cessation of adult emergence, the "larval holding strips" were examined and the number of dead larvae and pupae was recorded. The total number of pupae was calculated from adding the number of dead pupae to the number of emerging adults. Percentage pupation and adult emergence was calculated by dividing the number of formed pupae and emerging adults by the number of irradiated larvae.

2.5. Effects of gamma irradiation on the rate of development

Adult emergence data from the previous studies were recorded daily and tabulated at 5 day intervals. Percentage adult emergence every 5 days was calculated by dividing the number of emerging moths during the 5 day period in each treatment on the total number emerging moths in the same treatment. The date of the emergence of the 1st moth in the control was used as the starting date for recoding adult emergence.

2.6. Effects of gamma irradiation on egg laying

Emerged adults from the irradiation studies were placed separately in oviposition cages, provided with water on cotton wicks and notes were made on egg laying. In addition, notes were made on the physical appearance of the moths and any abnormalities were recorded.

3. Data analysis

Data from experiments on the effects of gamma irradiation on pupation and adult emergence were subjected to analysis of variance. Means were separated, at the 5% level of probability, by Fisher's protected least significant difference (PLSD) test. In addition, percentage pupation and adult emergence data were subjected to probit analysis. Probit analysis, including probit transformation of percentage mortality and log 10 transformation of dose, was performed using the computer program SPSS Statistics for Windows (SPSS, 2008) Inc. To examine the effects of gamma irradiation on sex ratio, data on the number of males and females resulted from irradiated larvae were subjected to Chi-square test at 5% level of significance.

4. Results

Results of studies on the effects of gamma radiation on pupation, adult emergence and sex ratio in mature 5th instar *L. botrana* larvae are presented in Table 1. The results clearly show that the radiosensitivity of *L. botrana* mature larvae increased with increasing irradiation dose. Adult emergence was more severely affected than pupation. For example, while pupation was significantly affected at 150 Gy ($F=29.12$; df=5, 24; $P<0.0001$) and decreased by only about 25% at 250 Gy, adult emergence was significantly affected at 100 Gy ($F=77.2$; df=4, 20; $P<0.0001$) and completely prevented at 200 Gy. Probit analysis of dose mortality data for pupation and adult emergence are presented in Table 2. The results of the analysis show that the LD_{99} for preventing subsequent development of irradiated mature 5th instar *L. botrana* larvae to pupae and adults was 2668 and 195 Gy, respectively. It should be pointed out, however, that although the estimated dose to prevent adult emergence (195 Gy) is in agreement with the observed dose (200 Gy), the data for adult emergence did not fit well. Consequently, it cannot be used to make predictions of efficacy at any dose.

Table 1 also presents data on the effects of gamma radiation on sex ratio. The data clearly show that sex ratio was significantly affected at 50 Gy dose ($\chi^2=11.9$, df=4, $P<0.05$); sex ratio was skewed in favor of males and, at 150 Gy dose, all emerged insects were males. In fact, even the emerging females from the 100 Gy

Table 1
Effects of gamma radiation on pupation and adult emergence in mature 5th instar *L. botrana* larvae.

Dose (Gy)	% ± SD		
	Pupation	Adult emergence	Adult males
0	96.80 ± 2.28 a	85.20 ± 4.15 a	47.6 ± 9.8 a
50	94.40 ± 3.29 a	76.40 ± 8.41 a	54.5 ± 11.09 ab
100	92.00 ± 2.45 a	38.80 ± 19.677 b	62.6 ± 6.43 b
150	86.80 ± 4.38 b	7.60 ± 3.29 c	100 ± 0.0 c
200	82.00 ± 5.29 b	0 ± 0 c	–
250	72.8 ± 3.63 c	0 ± 0 c	–

Means followed by the same letter within each column are not significantly different ($P<0.0001$, Fisher's PLSD test).

Table 2
Statistics for dose mortality probit analysis: effects of gamma irradiation on pupation and adult emergence in mature 5th instar *L. botrana* larvae.

Statistics	Pupation (95% fiducial limits)	Adult emergence (95% fiducial limits)
df	22	17
X^2	29.0	92.6
Slope $\pm$ SE	3.0 $\pm$ 0.6	8.2 $\pm$ 0.6
LD_{50} (95%)	449.1 (357.2–824.1)	101.3 (87.3–111.1)
LD_{95} (95%)	1582.9 (850.1–8974.8)	160.6 (145.4–190.0)
LD_{99} (95%)	2667.7 (1212.8–24234.2)	194.5 (169.8–251.0)

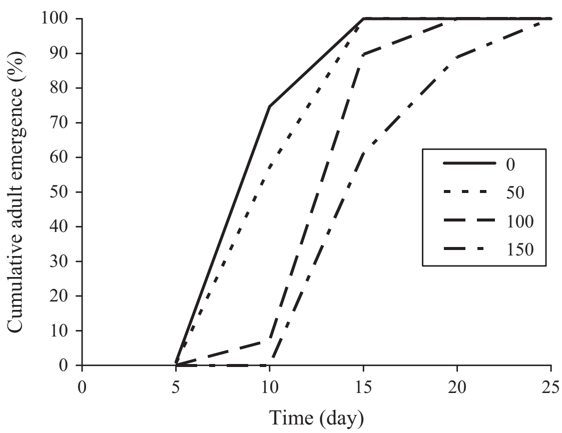

Fig. 1. Effects of gamma irradiation on the rate of development of *L. botrana* mature 5th instars into adults.

dose were short lived (about 2 days), mostly deformed and failed to lay any eggs.

Data on the effects of gamma radiation on the rate of development of *L. botrana* mature 5th instars to the adult stage are presented in Fig. 1. The Figure clearly shows that the rate of development was negatively affected by irradiation treatment and this effect increased with increasing the dose. For example, less than 8% of moths resulted from larvae exposed to 100 Gy dose emerged within the 1st 10 days of treatment in comparison with 57% and 74% of those resulting from larvae exposed to 50 and 0 (control) Gy, respectively.

5. Discussion

Phytosanitary treatments are used to disinfest agricultural products of quarantine pests so that such products can be shipped out of quarantine areas. Among other techniques, ionizing radiation is being used increasingly as a phytosanitary treatment to overcome biological barriers to trade in agricultural products (Hallman, 2011). In this report, the effects of gamma radiation on pupation, adult emergence, sex ratio, rate of development and egg laying in mature 5th instar *L. botrana* larvae are examined. In addition, the required dose for phytosanitary treatment against *L. botrana* mature larvae is discussed.

In general, the results of this study show that the radiosensitivity of *L. botrana* mature 5th instars to gamma irradiation increased with increasing radiation dose and the severity of the effect depends on the criterion used for measuring effectiveness. More specific, *L. botrana* mature larvae required a high dose when prevention of pupation was used as a criterion for measuring effectiveness; at 250 Gy about 73% of larvae pupated. This indicates that preventing *L. botrana* mature 5th instars from reaching the pupal stage requires a relatively high dose (the estimated dose is 2668 Gy). This dose may be harmful to grapes (Al-Bachir, 1999), particularly when applied commercially, where the dose uniformity ratio could be as much as 3:1 (Hallman, 2000). However, when survival to the adult stage was used, instead, as a criterion for measuring effectiveness (Hallman, 2001), the results were very promising. A dose of 200 Gy applied to *L. botrana* mature larvae completely prevented any resulting pupae from reaching the adult stage (the estimated dose is 195 Gy) and at 150 Gy dose all resulted moths were males. In fact, even females resulted from mature 5th instars exposed to a dose of 100 Gy were, mostly, deformed, short lived (about 2 days), and failed to lay any eggs. These results are in general agreement with data reported for 5th instar larvae of other examined Lepidopterans (Erdman, 1963; Estevam et al., 1995; Mansour, 2003; Ayvaz and Tuncbilek, 2006; Ayvaz et al., 2008; Hallman et al., 2013). For example, 350 Gy was reported to completely prevent adult emergence in the Indian meal moth, *Plodia interpunctella,* mature larvae (Ayvaz et al., 2008) and 200 Gy was found to completely prevent adult emergence from codling moth, *Cydia pomonella*, mature 5th instars (Mansour, 2003). In a 3rd study, Ayvaz and Tuncbilek (2006) found that 250 Gy was needed to prevent adult emergence from 5th instar Mediterranean flour moth, *Ephestia kuehniella* larvae.

Sexual differences in radiosensitivity were also noticeable. Irradiated female larvae were more susceptible to irradiation injury than males. One explanation for this phenomenon is that, in moths, males are homogametic while females are heterogametic. This means that males possess a duplicate of the sex chromosome that may make them more radioresistant (Erdman, 1963). The adaptive significance of this phenomenon, in this case, is that we may be able to reduce the irradiation dose (if desirable) to the level that guarantees complete death of females, even though such dose allows some males to emerge.

Irradiating grape vine moth mature 5th instars also caused extended developmental periods to the adult stages. Dose dependant developmental delay has been reported before for irradiated eggs and larvae of several insect species including *E. kuehniella* (Ayvaz and Tuncbilek, 2006; Mansour, 2010), *P. interpunctella* (Ayvaz et al., 2008), the gypsy moth, *Lymantria dispar* (Zubrik and Novotny, 2009), the khapra beetle, *Trogoderma granarium* (Abdel-Kaway, 1999), the mealy bug, *Dysmicoccus neobrevipes* (Doan et al., 2012) and the grape vine moth, *L. botrana* (Mansour and Al-Attar, 2012). Hallman (2000) indicated that irradiated immature insects may live longer than unirradiated controls which may be related to unbalanced hormonal system (Makee and Saour, 2003).

In summary, the results of this study provide data that contribute to developing a generic dose against Lepidopteran larvae. They also indicate that the use of gamma radiation as a phytosanitary treatment for grapes infested with *L. botrana* larvae requires a relatively low dose (200 Gy), provided that prevention of moth emergence is used as a criterion for effectiveness. In fact, this dose can even be lower if the inability to reproduce was used as a criterion for measuring effectiveness (Hallman, 2011); at 150 Gy all moths were males and females resulted from larvae exposed to 100 Gy dose failed to lay any eggs. This dose (100–200 Gy), depending on the criterion used for measuring effectiveness, is less than the suggested generic phytosanitary irradiation dose of 250 Gy for Lepidopteran larvae (Hallman et al., 2013) and much lower than the maximum allowed dose for irradiation of fresh fruits and vegetables (Anonymous, 1986).

Acknowledgments

The authors would like to thank Dr. I. Othman, Director General of the Syrian Atomic Energy Commission and Dr. F. Kurd-Ali, Head of the Department Agriculture for supporting this project. They would also like to thank Prof. G. Hallman (USDA-ARS) for his comments on an earlier version of this manuscript. This research was financially supported by the International Atomic Energy Agency, Vienna, Austria (Res. Contract No. SYR/15574).

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