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Flight ability and dispersal of European grapevine moth gamma-irradiated males (Lepidoptera: Tortricidae)

George Saour

Abstract

Flight abilities and dispersal distances of males of irradiated vs. untreated European grapevine moths (*Lobesia botrana* [Denis & Schiffermüller]: Lepidoptera: Tortricidae) were assessed in a flight assessment cage and in a vineyard. Newly emerged adult male moths were either untreated or γ -irradiated either with 150 Gy or 350 Gy, and each group was marked with a different colored fluorescent dust. Males were released in a laboratory flight assessment cage (70 × 40 × 50 cm) and at the center of a vineyard. The flight assessment cage test revealed significant differences in the flight responses of irradiated and untreated *L. botrana* males to calling females during the first 2 days after the initiation of the test. The greatest percentage of non-flying males (47%) was observed in the 350 Gy-treatment, whereas no significant differences were detected in male flight ability between untreated and 150 Gy γ -irradiated male moths. Six hundred male moths were released in a vineyard with a rectangular trapping grid around a central release point, and traps were baited with a synthetic pheromone. One hundred and thirty one males (21.8%) were recaptured, with the farthest being caught 40 m from the release point. No differences were observed in male field performance between 150 Gy γ -irradiated and untreated male moths, whereas 350 Gy γ -irradiated males showed limited field dispersal when compared with either 150 Gy γ -irradiated or untreated male moths. The results, the values of several attributes (flight ability, dispersal distance and recapture rate of released males in pheromone-baited traps)—which are critical for effective population suppression by the sterile insect technique with inherited or F_1 sterility (SIT/ F_1)—were significantly decreased by increasing the radiation dose applied to *L. botrana* males from 150 Gy up to 350 Gy. The flight assessment cage proved to be a valuable tool for measuring differences in the quality of untreated and irradiated moths.

Key Words: *Lobesia botrana*; irradiation; flight assessment cage; release-recapture; vineyard; trapping

Resumen

Se evaluaron la habilidad de vuelo y distancia de dispersión de machos de la polilla europea de la vid (*Lobesia botrana* [Denis y Schiffermüller]: Lepidoptera: Tortricidae) irradiados vs no tratados en una jaula de evaluación de vuelo y en una viña. Cada grupo de adultos de las polillas macho adultos recién emergidos no tratados o γ -irradiados con 150 Gy o 350 Gy fue marcado con un polvo fluorescente de un color diferente. Los machos fueron liberados en una jaula de vuelo evaluación de laboratorio (70 × 40 × 50 cm) y en el centro de una viña. La prueba de evaluación en una jaula de vuelo reveló diferencias significativas en las respuestas de vuelo de los machos de *L. botrana* irradiados y sin tratar hacia las llamadas de las hembras durante los primeros 2 días después del inicio de la prueba. Se observó el mayor porcentaje de machos no volantes (47%) en el tratamiento de 350 Gy, mientras que no se detectaron diferencias significativas en la capacidad de vuelo de los machos entre las polillas no tratadas o γ -irradiados con 150 Gy. Seiscientos polillas macho fueron liberados en una viña con una cuadrícula de captura rectangular alrededor de un punto de liberación central, y se cebaron las trampas con una feromona sintética. Ciento treinta y un machos (21.8%) fueron recapturados, con el más lejano de ser capturado 40 m del punto de liberación. No se observaron diferencias en el desempeño de los machos en el campo entre los machos no tratados y los γ -irradiados con 150 Gy, mientras que los machos γ -irradiados con 350 Gy mostraron una dispersión de campo limitada en comparación con los machos γ -irradiados con 150 Gy o no tratados. Los resultados, los valores de varios atributos (capacidad de vuelo, distancia de dispersión y de la tasa de recuperación de los machos liberados en trampas cebadas con feromonas) - que son críticos para la supresión efectiva de la población por la técnica del insecto estéril con esterilidad heredada o de F_1 (SIT/ F_1) — se redujo significativamente mediante el aumento de la dosis de radiación aplicada a los machos de *L. botrana* de 150 Gy hasta 350 Gy. La jaula de evaluación de vuelo demostró de ser una herramienta valiosa para medir de las diferencias en la calidad de las polillas no tratadas e irradiadas.

Palabras Clave: *Lobesia botrana*; irradiación; jaula de evaluación de vuelo; liberar-recapturar; viña; atrapando

The European grapevine moth (*Lobesia botrana* [Denis & Schiffermüller]; Lepidoptera: Tortricidae) is widely distributed in southern Europe and the Mediterranean basin (Thiery & Moreau 2005) where it is a serious lepidopteran pest of vineyards. The pest recently invaded the major wine-growing regions of Argentina, Chile and California (Varela et al. 2010). Although potentially polyphagous, the species generally infests grape clusters (*Vitis vinifera* L.; Vitales: Vitaceae) and other berry fruits, which make them susceptible to the gray mold fungal pathogen (*Botrytis cinerea* Persoon: Fries [Fermaud & Le Menn]; Leotiales:

Sclerotiniaceae) (Ioriatti et al. 2011; Giner et al. 2012). The species has 2 to 4 generations each year (Pavan et al. 2006). Larvae of the first generation damage the inflorescences, and those of the following generations damage green and ripe grapes (Ifoulis & Savopoulou-Soultani 2007). *Lobesia botrana* moths are nocturnal and actively disperse during evening twilight hours (Hurtrel & Thiery 1999; Tasin et al. 2011).

Chemical insecticides remain the most widespread control method of this pest, either for economic or practical reasons, but several of these compounds possess hazardous and dangerous properties (Io-

riatti et al. 2005). Therefore, environmentally benign alternatives to pesticides that are compatible with the protection of beneficial organisms and human health are required (Sáenz-de-Cabezón Irigaray et al. 2010).

The sterile insect technique (SIT) is unique as a biological control tactic that involves the release of sterile males to control the same species. Based upon several studies, inherited sterility or F_1 sterility (SIT/ F_1), which requires the selection of a radiation dose so that irradiated females are completely sterile while irradiated males are partially sterile is regarded as the most favorable genetic method for most application against lepidopterans (Bloem et al. 1999; Carpenter et al. 2001). Consequently, over the last 10 years interest in the use of SIT/ F_1 for the suppression/eradication of economically important lepidopteran pests has significantly increased (Simmons et al. 2010; Vreysen et al. 2010; Carpenter et al. 2013).

The SIT can only be successful when sterile moths of high biological quality are released (Simmons et al. 2010). Behavioral traits—such as dispersal, response to calling females, flight propensity and ability of the released male moths—largely influence the success of the SIT in the field (Simmons et al. 2010; Vreysen et al. 2010). Moreover, bioassays to assess the responses of released males to pheromone traps are indispensable to any SIT/ F_1 program and crucial for monitoring irradiated moths throughout the target area (Vreysen et al. 2006; Carpenter et al. 2012). The advantages of inherited sterility over the fully sterile male moths in lepidopteran pest management have been widely discussed in the literature. Many authors have reported on increased quality and mating competitiveness of the released sterile moths as the dose of radiation used to induce sterility is decreased (Makee & Saour 2004; Jang et al. 2012).

In previous work, the radiation sensitivity of *L. botrana* was assessed in relation to its use in F_1 sterility programs. A dose of 150 Gy administered to female moths resulted in complete sterility of the females, whereas males that had been irradiated with 400 Gy and mated with unirradiated females retained a residual fertility of 2.7%. It is worth mentioning that noticeable reductions in fertility were also recorded when F_1 males—the F_1 progeny of unirradiated females and males that had been irradiated with 150 Gy—mated with either F_1 or untreated females (Saour 2014). In this paper, the effects of a dose of 150 and 350 Gy on *L. botrana* male flight ability and their responses to calling females were assessed in laboratory cage experiments. In addition, a field trial was conducted to assess the effect of these γ -radiation treatments on recapture rates and distances that the males dispersed.

Materials and Methods

INSECTS

The *L. botrana* insects used in these experiments were obtained from a laboratory colony that was refreshed each year with larvae collected from infested grapevines. The larvae were reared on a semi-artificial diet described by Thiery & Moreau (2005) with the following composition: 150 mL water, 3 g agar, 9 g maize flour, 11 g wheat germ, 9 g yeast, 0.9 g ascorbic acid, 0.3 g benzoic acid, 0.3 mL maize oil, 0.3 g Nipagine (methylparaben), and 0.2 g Iprodione (fungicide). Male and female adults (100 pairs) were placed in cages (30 × 60 × 30 cm) that contained a 5% sucrose solution as a source of food and bands of waxed paper (15 × 2 cm) for oviposition. The eggs were collected daily and placed in plastic boxes (15 × 12 × 6 cm) for ~5 days until egg hatch. Using a fine camel-hair brush, newly emerged larvae were transferred to smaller plastic boxes (4 × 3 × 2 cm) that contained the semi-artificial diet. Larvae were checked daily for food supply until pupation, and

once moths emerged, they were collected and transferred to a plexiglass cage (50 × 40 × 40 cm). Eggs, larvae, pupae, and adults were held at a constant temperature of 25 ± 1 °C, 60 ± 10% RH and a photoperiod of 15:8 h L:D + 1 h of dusk.

EXPERIMENTAL DESIGN

To assess the flight ability of *L. botrana* adult males, an experimental transparent plexiglass cage of 70 × 40 × 50 cm was used that had 2 compartments separated by a plexiglass partition fixed in the middle of the cage, i.e., a male moth compartment on one side, and a female one on the other side (Fig. 1). Three openings of 38 cm long × 2 cm wide were made in the partition at 15, 30, and 45 cm from the cage bottom. The openings in the female compartment were narrowed to 0.5 cm by gluing 2 sloping flanges of cardboard to the edges of the slit in the partition at an angle of 45 degrees to permit males to pass 1 way, but prevent their return. The front side of each compartment was fitted with a circular hole (15 cm diam) closed with mesh gauze for handling insects in the cage. The air flow inside the cage was controlled by 2 small electric fans located on the opposite sides of the cage and covered with a plastic mesh. The fan in the female compartment sucked in the ambient air, whereas the air in the male compartment was exhausted out of the cage. The speed of each fan was adjusted with an external voltage regulator. Water-soaked cotton wicks in Petri dishes ($n = 4$) affixed over the bottom provided moisture to the experimental cage. The inner walls of the male compartment were covered with a thin layer of white talc to prevent moths from climbing.

IRRADIATION PROCEDURES

The male moths were exposed to 2 doses of radiation (150 and 350 Gy) in a ^{60}Co gamma cell that had a cylindrical (15 × 25 cm) irradiation chamber (Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd., Russia; www.tenes.ru). The male moths were placed individually in small transparent plastic tubes (8 cm long and 1 cm diam) prior to irradiation treatment. The dose rate at the time of irradiation was 12.5 Gy/min with a dose uniformity ratio (max:min of the received dose) of about 1.14 and the absorbed dose was calibrated using Fricke dosimetry.

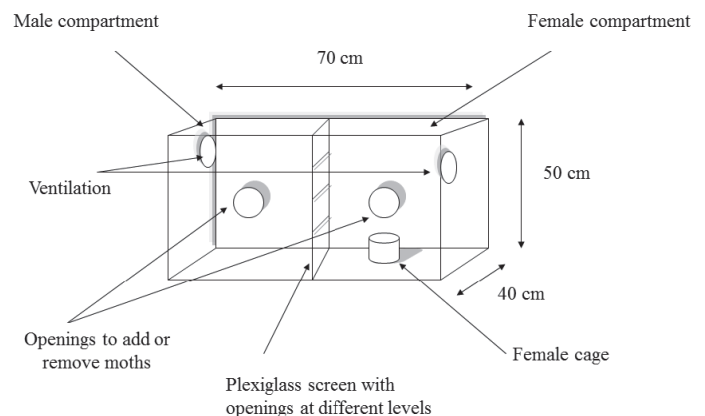


Fig 1. Schematic representation of the flight assessment cage used to measure the flight responses of *Lobesia botrana* males to calling females. In the female compartment, 2-day-old virgin females were confined inside a small cylindrical plastic mesh box with a 5% sucrose-wetted wick. Males irradiated either with 150 Gy or with 350 Gy and untreated males differentially marked with variously colored fluorescent powders were introduced into the male compartment. The number of males of each of the 3 kinds that flew through the open slit at the 45 cm height [the 2 lower openings (slits) were sealed] into the female compartment were recorded at 24, 48, 72 and 96 h. Air was drawn into the female compartment and exhausted from the male compartment.

EFFECT OF GAMMA RADIATION ON MALE FLIGHT ABILITY IN A FLIGHT ASSESSMENT CAGE

Cohorts of newly emerged (< 24 h old) untreated *L. botrana* males and males irradiated with a dose of 150 and 350 Gy (25 males for each radiation dose) were held at 4 °C for 3 min and then placed on the bottom of the male compartment. Two-day-old virgin females ($n = 5$) were confined inside a small cylindrical plastic mesh box that contained a cotton wick soaked in 5% sucrose solution. The number of males that had flown through the opening at the 45 cm height (the other 2 openings were sealed using single-strap adhesive tape) in to the female compartment was recorded after 24, 48, 72 and 96 h. Various colors of fluorescent powders (provided by the Insect Pest Control Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria), were used to mark and distinguish between the untreated and irradiated adult males. The experiment was replicated 6 times with 75 male moths per replicate.

EFFECT OF GAMMA RADIATION ON MALE DISPERSAL IN A VINEYARD

The trial was conducted in a 10-year old 0.5 ha vineyard located near Damascus in October 2012. The vineyard plantation had 250 plants/ha and was bordered on its western side by a row of cypress trees that served as a windbreak. The orchard consisted of 6 rows of grapevine plants separated by 5-m wide alleys. The grapevine plants (2 m tall) were spaced 3.5 m apart and maintained on a trellis system. The vineyard was subjected to a calendar-based chemical spray program for disease management, but received no insecticides. Males were captured by using pheromone-baited traps (Large Plastic Delta Trap, Russell IPM, United Kingdom). Thirty traps were deployed throughout the vineyard and suspended from the trellis wire. The traps were deployed according to a rectangular trapping grid around the release point (Fig. 2). Fluorescent powders of different color were used to mark and distinguish between newly emerged untreated male moths and those irradiated with a dose of 150 and 350 Gy. After marking, moths of each treatment were placed in 800-mL transparent plastic jars with a plastic mesh lid and taken to the release point. Moths were released at the base of a vine at the central point in the vineyard 1 h before sunset. To release the moths, the lid was gently removed to enable the moths to fly out. On the next day, the number of moths released was calculated by subtracting the number of moths found dead inside the jar from the initial number. All traps were checked 24, 48, 72 and 96 h after moth release. The captured moths were removed with a finely pointed forceps in the vineyard and taken to the laboratory where they were examined under ultraviolet light. Four releases were carried out with 150 *L. botrana* males per release.

DATA ANALYSIS

Male flight ability data was analyzed using analysis of variance (ANOVA) at the 5% level ($P < 0.05$). Mean comparisons were conducted with the Fisher protected least significant difference test (PLSD) at $\alpha < 0.05$ probability level (StatView Version 4.02, Abacus Concept, 1994). A normal approximation test (Z) was used to compare between percentages of total male moths recaptured.

Results

EFFECT OF GAMMA RADIATION ON MALE FLIGHT ABILITY

The number of *L. botrana* males that managed to fly into the female compartment was dependent on the received radiation dose and

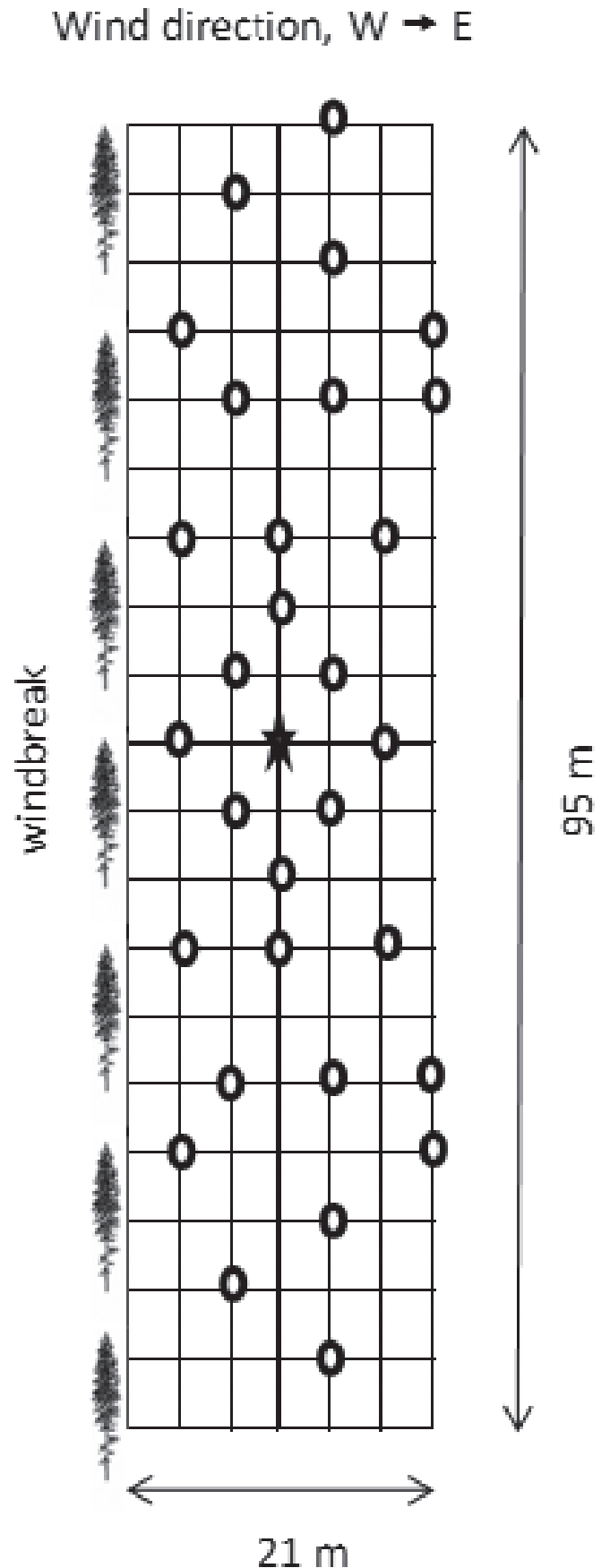


Fig. 2. Schematic representation of the vineyard and the pheromone traps positions at the experimental plot where the marked male moths were released.

on the days after the initiation of the test (Table 1). With the exception of days 3 and 4 after the initiation of the experiment, the mean percentage of moths flying to the other compartment was significantly higher for 150 as compared with 350 Gy-irradiated males ($F = 47$; $df = 2,15$; $P < 0.0001$ and $F = 33.1$; $df = 2,15$; $P < 0.0001$ for day 1 and 2, respectively), and no differences in flight ability were found between 150 Gy-irradiated and untreated male moths. The greatest flight rate was recorded during the 2nd day after the start of the trial. Males that had been irradiated with 350 Gy had a significantly greater percentage of non-flyers as compared with males that were untreated or irradiated with 150 Gy ($F = 67.2$; $df = 2,15$; $P < 0.0001$) (Table 1).

EFFECT OF GAMMA RADIATION ON MALE DISPERSAL IN A VINEYARD

The 350 Gy radiation dose negatively affected the ability of the male moths to fly and respond to pheromone-baited traps. A total of 600 moth males were released in the course of experiment. During the 4 release and trapping periods, 131 (21.8%) marked males were recaptured during 4 successive days after each release, i.e., 67 untreated males and 57 and 7 males that had been treated with 150 and 350 Gy, respectively (Table 2). At a distance of 5 m from the release point only 150 Gy-irradiated and untreated males were trapped. Three males (2 untreated and one 150 Gy-irradiated) were trapped 40 m from the release point. Most of the male moths were recaptured during the first day after release (11%). Recapture percentages of 150 Gy-irradiated male moths did not significantly differ from that of untreated males ($Z = 0.06$; $P > 0.05$ for moths recapture after 24 h of release), whereas only 7 males that had been treated with 350 Gy were recaptured out of 200 males released.

Discussion

In this study, the effect of 2 radiation doses (150 and 350 Gy) on the flight ability of male *L. botrana* was assessed in laboratory cages and under field conditions. Another feature of this study was the possibility to examine the response of irradiated males to natural, intermittently and weak (virgin females) and strong (synthetic) sex pheromone signals in laboratory and field experiments, respectively.

Laboratory bioassays to assess flight ability of Lepidoptera have included actographs (Saito 2000; Hashiyama et al. 2013), computer-linked flight mills (Schumacher 1997; Sarvary et al. 2008), wind tunnels (Suckling et al. 2011) and flight cylinders (Carpenter et al. 2012). Nonetheless, each of these techniques although effective has certain limitations. For instance, some of these approaches are complex and require advanced technological knowledge. An additional inconvenience for the flight mill was that the insect needed to be on a flight arm (i.e., tethered insect) (Taylor et al. 2010; Carpenter et al. 2012). The flight

cage developed for use in our study to evaluate the effect of radiation on *L. botrana* flight performance was original, simple and practical.

One advantage of our flight cage over the flight cylinders proposed by Carpenter et al. (2012; 2013) is that the latter did not include the interaction between male and female moths (response of males to calling females) and the cylinder test therefore mainly assessed the males' flight propensity (moths used flight to escape the cylinders) rather than their flight ability. In our flight cage it was assumed that the female sex pheromone was the main trigger for the male flight response. The males needed to detect the source of pheromone emission (virgin female), initiate vertical and horizontal flights and then cross the opening in the screen toward the calling females in the adjacent compartment.

Irradiated and untreated males showed a different response to calling females in terms of flight ability in the flight cage experiments. A dose of 150 Gy did not appear to impair virgin female pheromone perception of *L. botrana* males; since there were no significant differences in flight ability between 150 Gy-irradiated and untreated males 24 h following the initiation of the test. Significant differences in flight ability were however detected between untreated males and males irradiated with 350 Gy during the first and second day after the start of the experiment. These results are somewhat similar to those of Bloem et al. (2006) who observed reduced mobility counts of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) in laboratory bioassays using actographs after exposure to high doses of radiation.

Not unexpectedly, observations of the flight behavior of *L. botrana* males in the flight cage revealed that most of non-flyer male moths were those that had been irradiated with 350 Gy. These results confirm the negative effects of high radiation doses on the quality and flight performance of male moths and also support the use of lower doses of gamma radiation (150 Gy) when treated moths will be released in the field in any future SIT/F₁ program against the European grapevine moth.

Dispersal (mark-release-recapture) experiments were carried out in a vineyard to consolidate and validate the laboratory flight cage data and to study the effect of radiation on field dispersal. Both laboratory and field bioassays should be performed to provide feedback on quality and performance of laboratory-reared moths in any SIT/F₁ sterility program (Carpenter et al. 2013). Our experiments showed that the percentage of recaptured *L. botrana* males was significantly dependent on the applied radiation dose. Treating *L. botrana* males with a dose of 150 Gy had almost no effect on the ability of the males to orient and fly to the sex pheromone traps, whereas 350 Gy-irradiated males had very limited field dispersal when compared with either 150 Gy-irradiated or untreated male moths. These findings contribute to the mounting body of evidence documenting the benefits of lowering the dose of radiation applied to the minimum required to fully sterilize females and partially sterilize males. The data also corroborate the work of Bloem et al. (2004; 2006) who showed a negative linear relationship between treatment dose and mobility or competitiveness of sterile codling moth irradiated with various doses of gamma radiation (dose range of

Table 1. Performance of *Lobesia botrana* male moths in a flight assessment cage. Mean percentages (\pm SE) of untreated males and males γ -irradiated either with 150 Gy or with 350 Gy that succeeded in flying to the compartment with calling virgin females during 4 successive days. Also the mean percentage (\pm SE) of each of the 3 kinds of males that failed to fly into the female compartment is shown in the column farthest to the right.

Type of male	Percentage of males that flew into the compartment with calling females on the indicated day				Percentage of non-flying males
	1st day	2nd day	3rd day	4th day	
350 Gy-irradiated	11.5 \pm 1.0 C,b	19.0 \pm 1.6 B,b	16.0 \pm 1.6 BC,a	6.5 \pm 1.2 CE,a	47.0 \pm 3.3 A,a
150 Gy-irradiated	21.0 \pm 1.1 B,c	42.7 \pm 3.2 A,a	14.2 \pm 0.9 C,a	7.3 \pm 1.0 D,a	14.8 \pm 2.5 C,b
Untreated	26.5 \pm 1.2 B,a	45.0 \pm 2.4 A,a	12.3 \pm 1.6 C,a	7.1 \pm 1.1 D,a	9.1 \pm 0.9 CD,b

Means in each row followed by the same uppercase letter are not significantly different ($P < 0.05$, Fisher PLSD); means in each column for each day followed by the same lowercase letter are not significantly different ($P < 0.05$, Fisher PLSD).

Table 2. Dispersal distance and number of untreated, 150 and 350 Gy-irradiated *Lobesia botrana* male moths recaptured in pheromone traps.

Time after release (h)	Type of captured male moths	Number of moths captured								Total moths captured	Males captured (%)	Males* recaptured (%)
		5m	10m	15m	20m	25m	30m	35m	40m			
24	350 Gy	0	0	0	1	0	0	0	0	1	0.8	0.2b
	150 Gy	8	5	6	9	3	0	1	0	30	22.9	5a
	Untreated	12	4	5	9	3	1	0	1	35	26.7	5.8a
48	350 Gy	0	1	1	1	0	0	0	0	3	2.3	0.5b
	150 Gy	6	1	0	1	0	0	1	0	9	6.9	1.5a
	Untreated	7	0	1	0	0	1	1	0	11	8.4	1.8a
72	350 Gy	0	0	0	1	1	0	0	0	2	1.5	0.3b
	150 Gy	2	2	1	2	0	2	2	0	13	9.9	2.2a
	Untreated	2	1	0	3	3	2	2	1	13	9.9	2.2a
96	350 Gy	0	0	0	1	0	0	0	0	1	0.8	0.2b
	150 Gy	0	1	0	1	2	0	0	1	5	3.8	0.8ab
	Untreated	0	0	5	1	2	0	0	0	8	6.1	1.3a
Total	—	37	15	19	30	14	6	7	3	131	—	—
Males captured (%)	—	28.2	11.4	14.5	22.9	10.7	4.6	5.3	2.3	—	100	—
Males recaptured (%)	—	6.2	2.5	3.2	5.0	2.3	1.0	1.2	0.5	—	—	21.8

*Total number of captured males/total number of released males × 100. Percentages in column for each time after release followed by the same letter are not significantly different ($P < 0.05$; two-sample Z-test of proportions). 4 releases were performed, 150 *Lobesia botrana* males per release.

100–250 Gy). Moreover, our data—that showed no marked differences in recapture rates between 150 Gy-irradiated and untreated males—support and extend similar findings reported by Bloem et al. (2001) and Kumano et al. (2007) pertaining to the codling moth and the sweet potato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), respectively. They showed improved trap recaptures and dispersal ability in males of both species when treated with lower—but still adequate—doses of radiation.

In addition to the negative impact of radiation on dispersal, our data revealed that *L. botrana* males were relatively poor flyers, since the percentage of moths recaptured over 40 m from their release point did not exceed 0.5%. Most importantly, this finding is in agreement with the suggestion of Roehrich & Carles (1981) and Schmitz et al. (1996) that *L. botrana* has a limited range of dispersal.

To our knowledge, there have been no experimental data on the effect of gamma radiation on the flight ability of male *L. botrana* in laboratory or in field settings. The only published information relates to the effect of radiation on the fecundity and fertility of *L. botrana* (Saour 2014). The results presented in this paper lead us to conclude that the flight cage assessment can be considered an additional valuable tool that enabled the detection of differences in flight ability caused by irradiation and thus provided a better understanding of male *L. botrana*'s flight behavior. Moreover, the experimental set-up of our flight cage could be used as part of a simple quality control protocol to detect differences in flight ability and performance of mass-reared sterile male moths.

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