Integrated Protection in Viticulture
IOBC-WPRS Bulletin Vol. 154, 2020
pp. 113-120

Wine-baited traps to evaluate the efficacy of the mating disruption technique against *Lobesia botrana* in Douro Demarcated Region

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Abstract: Wine-baited traps as tools for assessing effectiveness of the mating disruption technique (MD) against *Lobesia botrana* were used in vineyards of the Douro Demarcated Region. More specifically, this type of "food trap" was tested to: i) monitor the second and third flights of *L. botrana* in plots under MD; (ii) investigate the influence of uncultivated habitats and/or neighbouring MD-free vineyards on the pest occurrence in MD-treated plots; (iii) detect the mating status of the females captured. As expected, this type of trap was capable of capturing both males and females of *L. botrana* and other moths, despite the presence of specific clouds of synthetic pheromones, proving to be a valid basic and complementary tool to better understand and improve the MD against European grapevine moth.

Key words: adult monitoring, ethology, IPM, landscape, Tortricidae, vineyard, wine-based bait

Introduction

In the Douro Demarcated Region (DDR), the use of mating disruption technique (MD) against the European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Tortricidae), has been increasing considerably in the last 20 years. However, the results obtained have not always been satisfactory due to several constrains, such as the orography and the fragmentation of the landscape of DDR, the high summer temperatures, the higher voltinism of the species and, its high biotic potential, and not least in importance, the high susceptibility of some grape varieties to the pest infestation and damage (Carlos et al., 2010; 2013 a). Moreover, the adult population monitoring system, traditionally based on the use of pheromone traps for male captures, shows

some constraints in giving good indications about the pest evolution in plots where MD is used. The assumption that, if males cannot find the pheromone traps, they cannot even locate the females and therefore mate with them, it is not entirely true. In fact, the amount of synthetic pheromone necessary to interrupt the male orientation to the traps can be less than the quantity necessary to interrupt the orientation towards the females (Ioriatti et al., 2011). In this sense, zero captures in the pheromone traps is considered a "necessary but insufficient" indicator of effective MD (Ioriatti et al., 2011).

In the DDR it has happened several times that damages have been observed in vineyards in which catches had not been detected or remained at extremely low levels (Carlos et al., 2014; Gonçalves et al., 2014). Three phenomena may explain this fact: (i) the immigration of fertilized *L. botrana* females from neighbouring plots without MD application; (ii) the occurrence of casual encounters between males and females of *L. botrana*; (iii) and the accounting of damages due to other Lepidoptera pests that cause similar symptomatology. Under these circumstances, the use of "food traps" to monitor *L. botrana* using wine as bait may be advantageous. Thus, these traps: (i) do not interfere with the pheromone cloud nor are they affected by it (Thiéry, 2011); ii) are able to capture females and are useful for studies on their fertility and to predict the beginning of the oviposition period (Thiéry, 2011; Thiéry et al., 2006); and (iii) are not specific to *L. botrana*, allowing to monitor other moth species, such as *Ephestia unicolorella* subsp. *woodiella* Richards and Thomson and *Cadra figulilella* (Gregson) (Pyralidae, Phycitinae), recently found infesting grapevine clusters in DDR (Carlos et al., 2013 b), whose damages can be sometimes confused with those of *L. botrana*.

Under these conditions, a study was held in two wine farms from DDR aimed to: (i) evaluate the usefulness of wine-baited traps to monitor the second and third flights of *L. botrana* in plots under MD; (ii) examine the mating status of the captured females of *L. botrana*; (iii) and investigate the influence of uncultivated habitats and/or neighbouring MD-free vineyards on the moth occurrence in MD-treated plots.

Material and methods

Field work was carried out during the growing season of 2019, in two wine farms from DDR: Quinta do Vallado (in Baixo Corgo sub-region) and Quinta de S. Luiz (in Cima Corgo sub-region). During the study period, mean temperatures were: 19.1 °C in May, 19.2 °C in June, 24.0 °C in July and 23.6 °C in August in Baixo Corgo; and 19.5 °C in May, 20.0 °C in June, 25.5 °C in July and 24.8 °C in August in Cima Corgo (ADVID, 2019).

MD dispensers, loaded with 380 mg of pheromone, were installed in the experimental MD vineyards, in the beginning of March, at a dose of 400 dispensers/ha. In Quinta do Vallado the experimental MD vineyard was surrounded by a non-MD vineyard, while in Quinta de S. Luiz, it was surrounded by a woodland and a non-MD vineyard (Figure 1). In Quinta do Vallado, MD and non-MD vineyards were sprayed once, with chlorpyrifos-methyl, against *Scaphoideus titanus* Ball (Cicadellidae), in the beginning of July. In Quinta de S. Luiz the non-MD vineyard was sprayed twice, with chlorpyrifos-methyl, against *L. botrana* (one spray in each of the 1st and 2nd generations of the pest).

Wine-baited traps, type DROSOSAN with large holes (1 cm Ø) opened in the side nets, were installed in mid-May in MD plots, neighboring non-MD vineyards and non-crop habitats, and separated from each other by about 50 m. Traps were filled with a water-alcoholic solution of 50 % red wine, and were checked every 3-4 days until the end of September. Till July 15th, 5 % of borax were also added to allow insect conservation. *L. botrana* adults were counted, separated by sex and females were dissected to check their mating status (Figure 2).

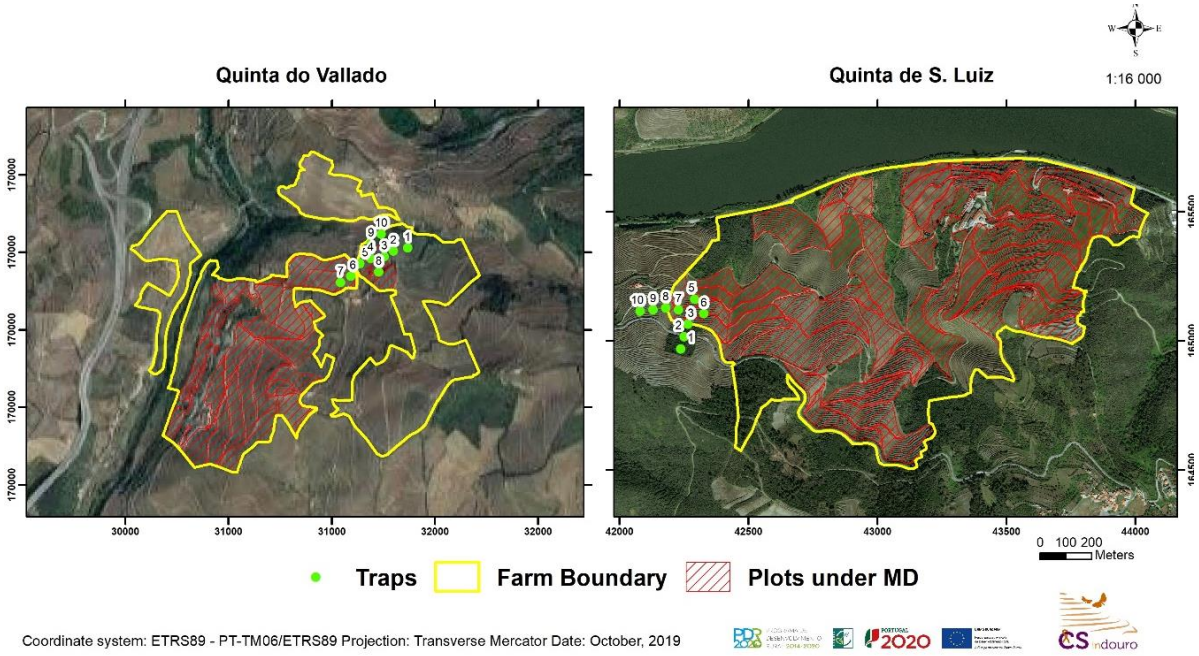

Figure 1. Sites under study where the vineyard area under mating disruption (MD) (red shading) and location of traps (green points) are indicated.

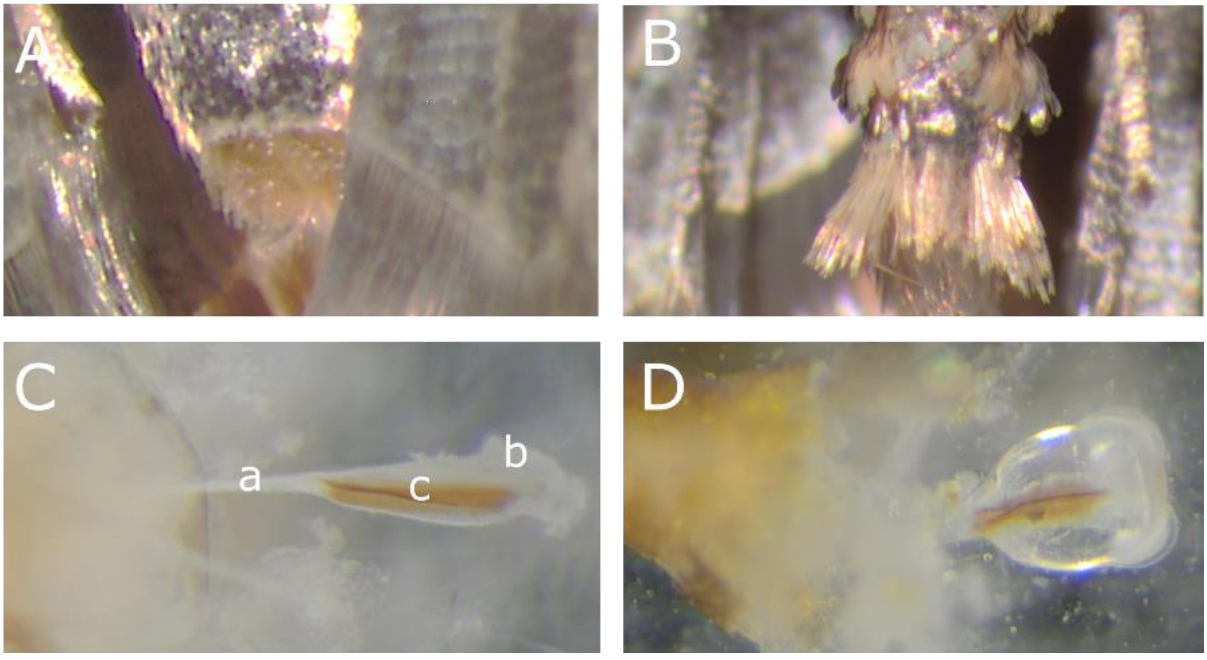

Figure 2. *Lobesia botrana*: distal abdominal part of a female (A) and of a male (B); bursa copulatrix without (C) and with spermatophora (D); corpus bursae (a), ductus bursae (b) and signum (c)

A full spatial distribution based on total captures of both sexes in traps, and on the percentage of mated females was performed through geostatistical method Inverse Distance

Weighting (IDW) in order to obtain a general frame of *L. botrana* presence at a spatial level. Analysis was performed in ArcGIS (version 10x., ESRI) using Spatial Analyst Extension, with Geostatistical Analyst Wizard (statistical analysis were done only for the 3rd flight data, due to the low number of adults captured in the 2nd flight in both study sites).

The land-use types in buffers (50 to 100 m) around each trap were mapped, categorized and accounted for (area in ha and %) using ArcGIS (version 10x., ESRI). As reviewed by Ioriatti et al. (2011), although males can fly a few meters over the vegetation to exploit air streams for longer distance migrations, females are generally less dispersive, and have not been observed to fly further than approximately 100 m. For the analysis, four categories were considered: (i) vineyards; (ii) woodlands and scrublands combined; (iii) olive groves; (iv) perennial and temporary crops combined and hereafter named other crops.

For the 3rd flight of *L. botrana*, Spearman's rank correlations were used to examine the relationship between the total captures, and the captures of each sex in each trap from the MD area, and the proportion of different land-use types around each trap, at different buffers. For greater robustness, data from the two farms were analyzed together.

Results and discussion

The total number of captured *L. botrana* adults was 109 in Quinta do Vallado and 32 in Quinta de S. Luiz. The number of captures during the 2nd flight was very low (5 in Quinta do Vallado and 12 in Quinta de S. Luiz). Since the mean temperature occurred in the second and third flight, which strongly impairs the wine fermentation, were closed, it's presumed that the lower captures occurred during the second flight were due to the use of borax that may have interfered with the volatile emission of the bait.

On the basis of checks carried out every 3-4 days, the beginning of the 3rd flight was recorded in July 29th in Quinta de S. Luiz and August 1st in Quinta do Vallado. In Quinta de S. Luiz, this date coincided with the day predicted by the degree-day model for that farm for the beginning of the 3rd flight, while in Quinta do Vallado it occurred six days after the predicted date (Carlos et al., 2018).

In the 3rd flight, in both farms, the number of females captured was higher than that of the males (Table 1), producing a sex-ratio (F/M) of 2.6 for Quinta do Vallado and 1.8 for Quinta de S. Luiz.

In both farms, both sexes were captured in higher number in non-MD vineyard plots than in MD vineyards (Table 1). In Quinta de S. Luiz, no captures were obtained in MD vineyards, possibly as a result of the reduced pest pressure, considering that MD has been applied there since 2001 and has covered almost all of the vineyard for several years. In the non-MD vineyard, even with the insecticide treatment against *L. botrana*, the number of adults was higher comparatively to those obtained in MD vineyard. In Quinta do Vallado, the high number of captures obtained in the MD vineyard border (Figure 3, Table 1), suggests the possible migration of adults, particularly mated females (Figure 4) to MD plots from non-MD vineyard plots. While in Quinta de S. Luiz, all the females captured in both neighboring MD-free vineyards and uncultivated habitats were mated, in Quinta do Vallado the percentage of mated females ranged between 83.3 % in the border of MD vineyard and 97.9 % in non-MD vineyard (Table 1). Thus, this result seems to show a low efficacy of wine-baited traps to capture virgin female. On the other hand, it should be mentioned that the presence of matted females in MD plots cannot always be seen as inefficacy of the treatment. In fact, in addition to prevention of mating, MD treatments can also result in a delay of mating, being this fact considered an indirect method by which MD works to control pest populations (Torres-Villa et al., 2002; Mori

and Evenden, 2013). Females have a limited time to mate, mature eggs, and find suitable oviposition hosts, and a delay in mating can have large consequences on female fitness and in the viablility of the offspring (Torres-Villa et al., 2002; Mori and Evenden, 2013).

Table 1. Mean number of males and females of *Lobesia botrana* captured per trap and percentage of mated females, in the surveyed locations (inside and in the borders of MD vineyards, non-MD vineyards, and non-crop habitats).

		Vineyard			Non-crop habitat
		Under MD	**Border MD**	**Non-MD**	
Males (nº)	Quinta do Vallado	0.6 ± 0.4	3.0	6.0 ± 1.7	-
	Quinta de S. Luiz	0.0	0.0	2.3 ± 2.3	0.0
Females (nº)	Quinta do Vallado	3.6 ± 0.9	5.0	13.3 ± 3.7	-
	Quinta de S. Luiz	0.0	0.0	3.0 ± 1.0	1.0 ± 0.0
Mated females (%)	Quinta do Vallado	95.0	83.3	97.9	-
	Quinta de S. Luiz	-	-	100.0	100.0

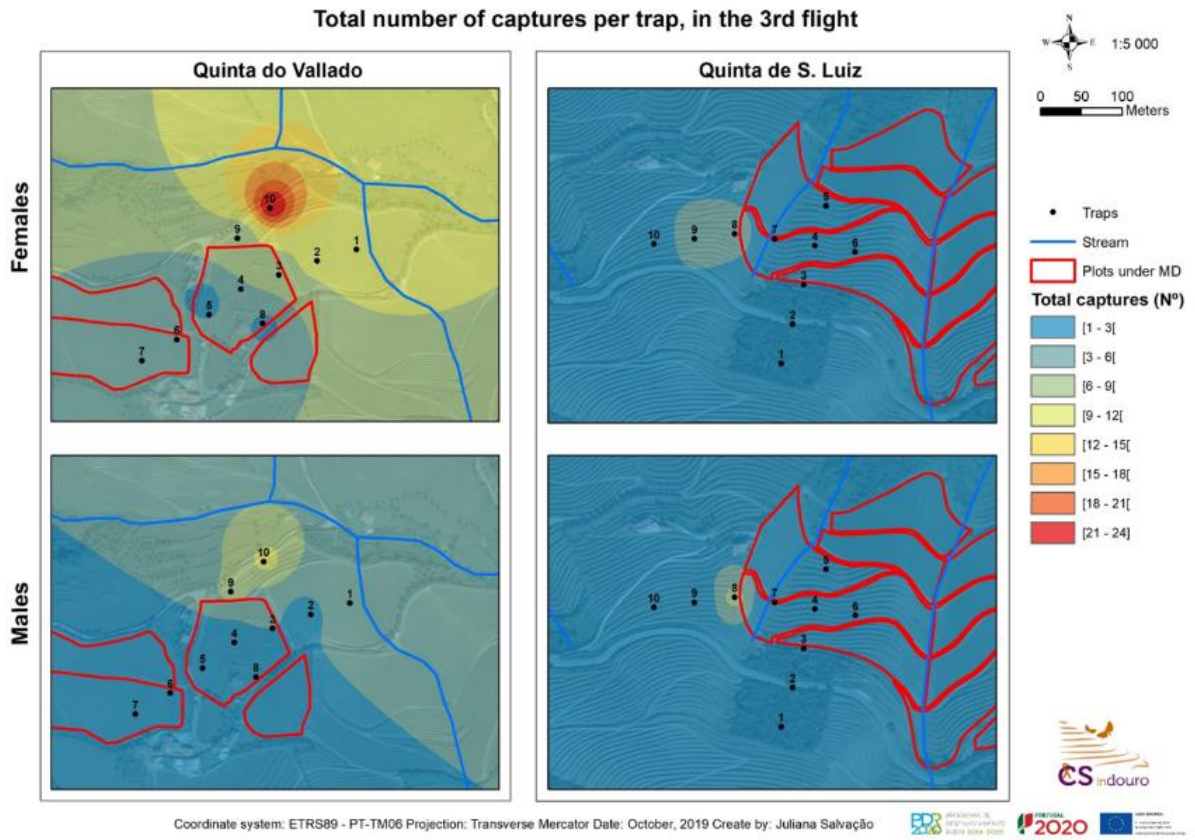

Figure 3. IDW results for the total number of captures of females and males in the 3rd flight of *Lobesia botrana*.

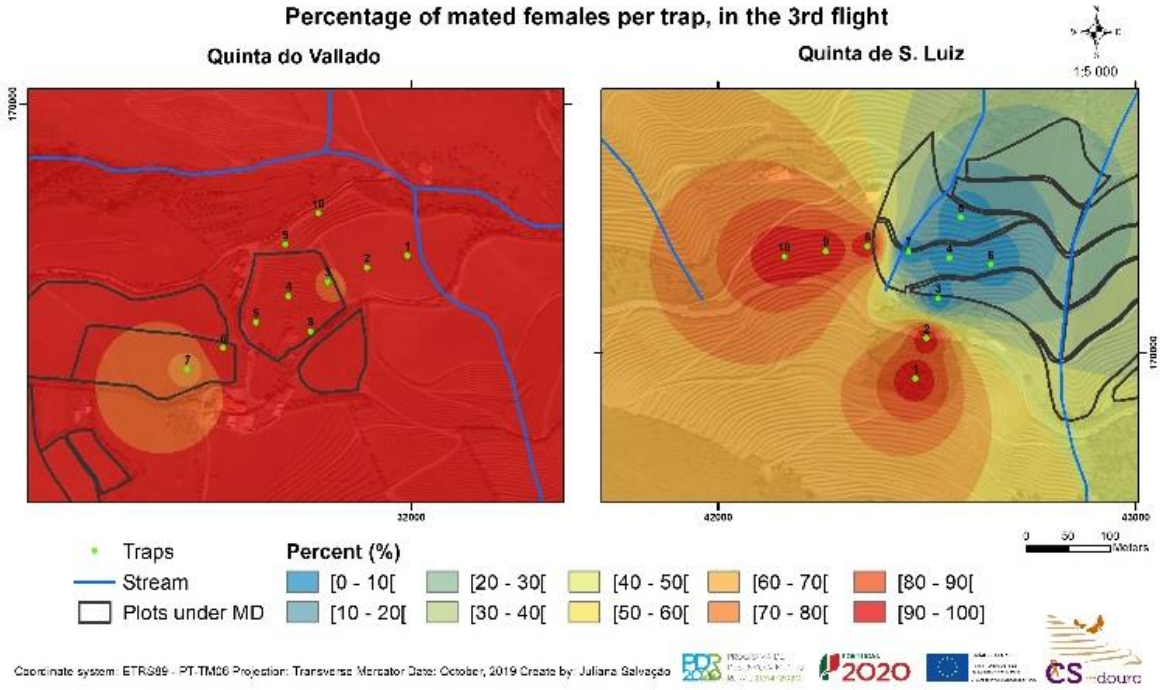

Figure 4. IDW results for percentage of mated females of *Lobesia botrana* in the 3rd flight.

Total captures were positively correlated with the proportion of olive groves (r = 0.610, p = 0.046) showing that vineyards under MD with larger proportions of olive groves were expected to have a higher density of adults. In this regard, Sciarretta et al. (2008) found that the the number of *L. botrana* males captured with pheromone traps was quite high inside olive groves, particularly during the first annual flight. The same authors hypothesized that in central Italy olive groves represent a primary overwintering host outside vineyards for the species, and part of the first generation larval population, develops on olive tree to then produce adults of the second seasonal flight that move toward vineyards.

Total and female captures were also positively correlated with the proportion of other crops (r_{ho} = 0.648, *p* = 0.031 for total captures and r_{ho} = 0.669, *p* = 0.024 for female captures). Negative correlations between both total and female captures and the proportion of woodlands and scrublands were also found (r_{ho} = -0.669, *p* = 0.024 for total captures and r_{ho} = -0.666, *p* = 0.025 for female captures). This negative correlation could be because in those woodlands and scrublands the larval and adult population of *L. botrana* is very small or absent due to the scarcity of suitable hosts. Hypothetically, also could be an indirect result of the positive impact that woodlands and scrublands have in the abundance and diversity of natural enemies (Gonçalves et al., 2018; Thomson and Hoffmann, 2013) and consequently in the natural control of *L. botrana*.

Further studies will be carried out in order to clarify some aspects related to the apparent low efficacy to capture adults in general and virgin females in particular, of the wine-baited trap tested by us, with respect to devices tested in other studies. In this regard, it will be useful to compare different types of traps, including the classic trap derived from the terra-cotta flower pot (truncated cone shape, height 20 cm and upper diameter 15 cm) also tested by Thiéry et al. (2006) and Bagnoli et al. (2013), and always appreciated for efficacy shown towards every category of adults of the species.

In conclusion, our results confirm that wine-baited traps are devices that, despite the lack of knowledge of their different attractiveness for males, virgin females and mated females of *L. botrana*, can be very useful for estimating and studying presence, structure and origin of the moth adult population, and in this sense to improve the knowledge on the ethology of the species and its adaptation to MD.

Acknowledgements

This research was developed under CSinDouro project, funded by the European Regional Development Fund (ERDF) and by the Portuguese Government through 1.1 «Grupos Operacionais», integrated on Measure 1. «Inovação» do PDR 2020 – Programa de Desenvolvimento Rural do Continente (PDR2020-101-031652 and PDR2020-101-031659), and supported by National Funds of Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019.

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