#### RESEARCH ARTICLE



# Eco-friendly pheromone dispensers—a green route to manage the European grapevine moth?

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### Abstract

The development of environmentally sustainable control strategies to fight insect pests is a key challenge nowadays. Pheromonemediated mating disruption (MD) is based on the release of synthetic sex attractants into a crop, interfering with mate finding of a given pest species. However, a limited number of research items have been published on the optimization of MD strategies against the European grapevine moth, *Lobesia botrana*, as well as on the use of biodegradable dispensers to reduce waste production in vineyards, despite the high economic importance of this pest. Therefore, the present study evaluated the efficacy of the MD products Isonet® L TT and the biodegradable Isonet® L TT BIO, applied at various densities, in reducing L. botrana damage on grapevine in comparison to an untreated control and the reference MD product Isonet® L. Experiments were conducted in three different areas of grapevine cultivation, located in Central and Northern Italy, over three different years. Our MD approach allowed a reliable control of the three generations of L. botrana during the whole grape growing season, leading to a significant reduction in the infested flower clusters and bunches, as well as in the number of nests per flower cluster and bunch, if compared to the untreated control. The performances of Isonet® L TT BIO, Isonet® LTT, and Isonet® L did not differ in terms of infested flower clusters/bunches, as well as nests per flower cluster/bunch. This was confirmed in all experimental sites over 3 years of field experiments. Overall, the present research provides useful information for the optimization of MD programs against L. botrana, highlighting the interesting potential of biodegradable pheromone dispensers that can be easily applied at low densities in vineyards, reducing the use of chemical pesticides to control moth pests.

Keywords Chemical ecology  $\cdot$  Integrated pest management  $\cdot$  *Lobesia botrana*  $\cdot$  Mating disruption  $\cdot$  Pesticide-free farming  $\cdot$  Sex pheromones

# Introduction

Currently, about 1.8 billion people are involved in agricultural activities worldwide, and most of them rely on pesticides to protect crops and livestock (Aktar et al. [2009;](#page-13-0) Alavanja [2009](#page-13-0)). Nowadays, the European Commission Directives are

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directed towards a significant reduction in pesticide use in the short to medium term (Hillocks [2012](#page-14-0)), to produce residue-free foods and reduce the toxicological impact of pesticides on human health and the environment (Hicks et al. [2017;](#page-14-0) Silver et al. [2017](#page-15-0)). Therefore, growing research attention is devoted to the development of environmentally friendly and sustainable strategies to control insect pests of agricultural importance (Todd et al. [2015](#page-15-0); González-Chang et al. [2016](#page-14-0); Holland et al. [2016;](#page-14-0) Benelli et al. [2017;](#page-14-0) Benelli [2018;](#page-14-0) Athanassiou et al. [2018\)](#page-14-0). Besides classical biological control programs, the manipulation of insect chemical ecology has also been considered to develop novel, effective, and eco-friendly control tools (Witzgall et al. [2010](#page-16-0); Kaplan [2012;](#page-15-0) Pérez-Staples et al. [2013\)](#page-15-0).

In this scenario, a prominent role is played by pheromonemediated mating disruption, which is based on the release of synthetic sex attractants into a crop, thus interfering with mate finding of a given pest species (Cardé [1990;](#page-14-0) Cardé and Minks

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[1995;](#page-14-0) Suckling [2000](#page-15-0); Miller et al. [2006\)](#page-15-0). In Lepidoptera, mate finding is generally routed by female sex pheromones, which mediate scramble competition among males for access to females (Tcheslavskaia et al. [2005;](#page-15-0) Witzgall et al. [2008;](#page-16-0) Lance et al. [2016](#page-15-0)). Moth females release small amounts of their sex pheromone and the males detect these plumes relying on their highly sensitive neurosensory structures (Cardé and Haynes [2004;](#page-14-0) Cardé and Willis [2008\)](#page-14-0). Since moths strongly rely on sex pheromones to find their mates, dispensers releasing synthetic sexual pheromones can be efficaciously exploited in mating disruption programs to suppress pest reproduction in selected areas. This can be achieved by both non-competitive and competitive mechanisms, the first covering camouflage, desensitization, and sensory imbalance, the latter mainly due to false-plume following (Miller et al. [2006;](#page-15-0) Miller and Gut [2015\)](#page-15-0). Notably, up to now, no negative effects on non-target organisms have been observed, making this method compatible with modern integrated pest management strategies (Welter et al. [2005;](#page-16-0) Miller et al. [2006;](#page-15-0) Witzgall et al. [2010](#page-16-0); Ioriatti et al. [2012;](#page-15-0) Ioriatti and Lucchi [2016](#page-15-0)).

Concerning insect pests of vineyards, pheromone mating disruption was proven to be a reliable and effective tool for the control of the European grapevine moth, Lobesia botrana (Denis & Schiffermüller) (Lepidoptera: Tortricidae) (Ioriatti et al. [2008,](#page-15-0) [2011;](#page-15-0) Cooper et al. [2014](#page-14-0)). In mating disruption programs, a major issue to deal with—to allow large-scale use—is the optimization of the dispensers' performances, their comparative assessment of efficacy, and their cost-effectiveness, which is linked to the time required for field application. In particular, a reduced number of pheromone dispensers in the field allows a strong reduction in the time required for their deployment, thus in labor costs (Gut et al. [2004;](#page-14-0) De Lame et al. [2010\)](#page-14-0). Moreover, the development of biodegradable pheromone dispensers will also allow to reduce operational costs in the field (potentially no removal and plastic disposal at the end of the season required), as well as environmental pollution (Guerrini et al. [2017\)](#page-14-0).

However, while the optimization of the abovementioned features has been considered in researches on other insect pest species (e.g., Meissner et al. [2000](#page-15-0); Funes et al. [2016;](#page-14-0) McGhee et al. [2016;](#page-15-0) Sharon et al. [2016,](#page-15-0) Vacas et al. [2016](#page-16-0)), limited research has been done on L. botrana (Hummel [2017](#page-15-0)), despite the high economic importance of this pest. Most importantly, to the best of our knowledge, the use of biodegradable dispensers for L. botrana mating disruption programs has not yet been considered, with the unique exception of Ecodian (Isagro) dispensers—composed by Mater Bi® (Novamont, Novara) and cellulose—that have been tested with partial success (Anfora et al. [2008](#page-13-0)), without achieving substantial commercial interest.

On this basis, Shin-Etsu Chemical Co. (Japan) and CBC (Europe) S.r.l, (Italy) developed the two new pheromone dispensers for the mating disruption of L. botrana, namely

Isonet® LTT and Isonet® L TT BIO. Both products consist of two parallel capillary tubes filled with the main component [i.e.,  $(7E, 9Z)$ -7,9-dodecadien-1-yl acetate] of L. botrana sexual pheromone blend, joined, and sealed at the ends. The gap in the middle allows each dispenser to form a loop that can be easily and quickly deployed by placing the dispenser over the end of spurs or by looping it around cordons, instead of twisting it around cordons as required for the commercially available reference product Isonet® L. Furthermore, both products can be applied at a lower rate than the conventional reference product Isonet® L (200–250 vs. 500 dispensers/ha, respectively). Notably, Isonet® LTT and Isonet® L TT BIO differ in the material of which the dispensers are made, which is polyethylene for Isonet® L TT and biodegradable polymers for Isonet® LTT BIO.

The research herein reported is aimed at evaluating the efficacy of the mating disruption products Isonet® L TT and the biodegradable Isonet® L TT BIO in reducing European grapevine moth (L. botrana) damage on grape in comparison to an untreated control and the reference mating disruption product Isonet® L. The trials were conducted in three different areas of grapevine cultivation, one located in Tuscany (Central Italy) and two in Emilia Romagna (Northern Italy) over three different years (2014, 2015, and 2016). Each year, the impact of the mating disruption products on the three generations of L. botrana was evaluated by determining the percentage of infested bunches and the number of nests per bunch. Furthermore, the tested dispensers were periodically collected during the grapevine growing season, extracted and analyzed by GC-MS. By evaluating their residual content of (7E,9Z)-7,9 dodecadien-1-yl acetate, we estimated the pheromone release in mg/ha/day during the whole grapevine growing season.

# Materials and methods

#### Experimental sites

All experiments were conducted in areas representative for grapevine cultivation in Italy. Three trials were carried out in the area of Bolgheri, Livorno province, Tuscany region, Central Italy, an area representative for high-value grapevine cultivation in Italy, while additional two trials were conducted in Emilia-Romagna region, Northern Italy, respectively, one in Ravenna province (Campiano) and one in Forlì-Cesena province (Villafranca di Forlì). Details on the location of the study vineyards can be found in Table [1](#page-2-0), and a detailed description of the characteristics of the crop in Table [2.](#page-2-0)

## Experimental design of mating disruption trials

Since a randomized block design does not apply to large plots required for studies on mating disruption products (European <span id="page-2-0"></span>Table 1 Location of study vineyards and year of mating disruption trials



and Mediterranean Plant Protection Organization [2016\)](#page-14-0), each treatment was applied to 1 large plot, and 10 subplots, big enough to allow for assessments on at least 100 flower clusters/bunches per subplot (32–40 plants), were selected within each large plot (=treatment). All mating disruption products, both test and reference items, were deployed before the beginning of the first flight of the target pest in spring. Details on the size of the plots and the date of application of the MD products in the different trials can be found in Table [3](#page-3-0). The reference product Isonet® L, applied at a rate of 500 dispensers per ha, was included in 4 out of 5 trials. Both Isonet® L TT and Isonet® L TT BIO were tested at 200 dispensers per ha in 2014 and at 250 dispensers per ha in 2015 and 2016.

# Crop damage and L. botrana population density evaluation

In all trials, crop damage caused by L. botrana was assessed at the end of the 1st generation (=G1, BBCH 69–71), at the end of the 2nd generation (=G2, BBCH 79–81), and at harvest (=G3, BBCH 89). To assess the method effectiveness, we considered the following variables: (i) number of male captures per trap (Trap Test Isagro®, 1 trap per sampling site) per week, (ii) rate of infested flower clusters (G1) or bunches (G2 and G3), (iii) number of nests per flower cluster (G1) or bunch (G2 and G3).

Within each subplot and at each damage assessment, the number of flower clusters (G1) or bunches (G2 and G3) damaged by L. botrana was counted on 100 flower clusters/ bunches per subplot at G1 and G2, respectively, and on 50 bunches per subplot at G3. The percentage of L. botrana-damaged flower clusters or bunches at each assessment was then calculated. Furthermore, at each assessment, the number of L. botrana nests per flower cluster (G1) or bunch (G2 and G3) was noted.

In detail, G1 infestation was measured through on-site surveys on non-destructively sampled inflorescences. As to the two carpophagous generations (G2 and G3), an estimate of the infested bunches was made on samples collected in the vineyards and carefully dissected. This is necessary above all for the compact-bunch varieties, such as Sangiovese, Pinot, and Chardonnay, for which a mere field inspection would often lead to a marked underestimation of the infestation level.

## Pheromone release of the tested dispensers

For all tested dispensers, by evaluating the residual content of (7E,9Z)-7,9-dodecadien-1-yl acetate, we estimated the pheromone release in mg/ha/day during the whole grapevine growing season. Groups of Isonet® L, Isonet® LTT, and Isonet® L TT BIO dispensers  $(n = 5$  per group) were periodically collected during the grapevine growing season and stored at − 30 °C until chemical analysis. The dispenser residual content in (7E,9Z)-7,9-dodecadien-1-yl acetate was measured based on internal (SEC) standard GC-MS analysis. The analysis was achieved on an Agilent 6890 N gas chromatograph equipped with a 5973 N mass spectrometer (MS). MS settings were as follows: EI mode, 70 eV, mass to charge ratio  $(m/z)$ scan between 35 and 400. HP-5 MS capillary column (30 m x

Table 2 Crop description of vineyards where mating disruption dispensers were tested



Trial	Plot size (ha) $(N$ dispensers/ha)				Date of dispenser deployment
	Untreated control	Isonet <sup>®</sup> L TT	Isonet <sup>®</sup> L TT BIO	Isonet <sup>®</sup> L	
	0.05	2.10(200)	2.17(200)	1.48(500)	1 April 2014
2	0.65	2.98(200)	2.98(200)	2.38(500)	1 April 2014
3	7.50	5.00(200)	5.00(200)		27 March 2014
$\overline{4}$	1.50	8.50(250)	7.8(250)	7.20(250)	18 March 2015
5.	4.40	8.01 (250)	8.40 (250)	8.40 (500)	29 March 2016

<span id="page-3-0"></span>Table 3 Size of study plots, number of dispensers applied, and date of application of dispensers in the different mating disruption trials

ID 0.25 mm  $\times$  0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) with He gas flow (1.0 ml/min) was used for separation. GC temperature program was as follows: initial 50 °C for 5 min, then increasing with 20 °C/min to 300 °C. The injector temperature was 150 °C. The GC-MS estimate of the dispenser residual content, allowed us to calculate the pheromone release during the field exposure of the dispenser, as mg/ha/day. Each value was a mean of 5 replicates.

#### Statistical analysis

Differences in the incidence of infested flower clusters or bunches  $(\%)$  and nests per flower cluster or bunch  $(n)$  among treatments (i.e., tested pheromone dispensers and untreated control), years, and study site were assessed using nonparametric tests (Kruskal–Wallis test followed by Steel– Dwass multiple comparison) at the 5% significance level, since data did not show homogeneity of variance (Shapiro-Wilk test,  $P < 0.05$ ). All statistical analysis was performed using JMP® 9 (SAS Institute).

## Results

#### First-generation trials

Figure [1](#page-4-0) summarizes the field efficacy of mating disruption against the first generation of L. botrana. Isonet® L TT BIO, Isonet® L TT, and Isonet® L led to a significant reduction in the percentage of infested flower clusters if compared to the untreated control  $(Z = 5.756, P < 0.0001; Z = 5.156,$  $P < 0.0001$ ;  $Z = 4.811$ ,  $P < 0.0001$ , respectively), while no significant differences were noted among the efficacy of the three tested dispensers (Fig. [1a](#page-4-0)). Furthermore, also the number of nests per flower cluster was significantly lower in Isonet® L TT BIO, Isonet® L TT, and Isonet® L than in the untreated control  $(Z = 5.681, P < 0.0001; Z = 5.238, P < 0.0001; Z =$ 4.792, P < 0.0001, respectively), while no significant differences were noted among the efficacy of the three tested dispensers (Fig. [1a](#page-4-0)).

Both the percentage of infested flower clusters and number of nests per flower cluster varied significantly among the years (Fig. [1b](#page-4-0)). Concerning infested flower clusters (%), EGVM incidence was higher in 2014 and 2016 than in 2015 ( $Z =$ 4.534,  $P < 0.0001$ ;  $Z = -2.728$ ,  $P = 0.018$ ), while no significant differences were noted between 2014 and 2016. The number of nests per flower cluster followed the same trend  $(Z = 4.561, P < 0.0001; Z = -2.574, P = 0.027)$  $(Z = 4.561, P < 0.0001; Z = -2.574, P = 0.027)$  $(Z = 4.561, P < 0.0001; Z = -2.574, P = 0.027)$  (Fig. 1b).

The experimental site also played a significant role, showing varying L. botrana infestation levels (Fig. [1c](#page-4-0)). Concerning infested flower clusters (%), EGVM incidence was highest in Campiano (RA, Emilia Romagna), followed by Bolgheri (LI, Tuscany) and Villafranca di Forlì (FC, Emilia Romagna), with significant differences among them  $(Z = 7.398, P < 0.0001;$  $Z = -4.669, P < 0.0001; Z = -7.711, P < 0.001$ , respectively). A comparable trend was observed concerning the number of nests per flower cluster ( $Z = 7.141$ ,  $P < 0.0001$ ;  $Z = -4.899$ ,  $P < 0.0001$  $P < 0.0001$ ;  $Z = -7741$ ,  $P < 0.0001$ , respectively) (Fig. 1c).

#### Second generation trials

Mating disruption achieved significant results also in controlling the second generation of L. botrana, as shown in Fig. [2](#page-7-0). In this generation as well, Isonet® L TT BIO, Isonet® L TT, and Isonet® L significantly reduced the percentage of infested bunches compared to the untreated control (Z = 6.608,  $P < 0.0001$ ; Z = 6.236,  $P < 0.0001$ ; Z = 5.597,  $P < 0.0001$ , respectively), with no significant differences among the three tested dispensers (Fig. [2](#page-7-0)a). Also, the number of nests per bunch was significantly lower in the Isonet® LTT BIO-, Isonet® LTT-, and Isonet® Ltreated plots than in the untreated control  $(Z = 6.189)$ ,  $P < 0.0001$ ;  $Z = 5.936$ ,  $P < 0.0001$ ;  $Z = 6.012$ ,  $P < 0.0001$ , respectively), and no significant differences were observed among the three dispensers (Fig. [2](#page-7-0)a).

Infested bunches  $(\%)$  and nests per bunch  $(n)$  varied significantly among the years (Fig. [2b](#page-7-0)). L. botrana infested bunches were significantly more abundant in 2014 over 2015 and 2016 ( $Z = -4.126$ ,  $P = 0.0001$ ;  $Z =$  $-4.993$ ,  $P = 0.018$ ), while no significant differences were

<span id="page-4-0"></span>



Fig. 1 Field efficacy of mating disruption against the first generation of the European grapevine moth (EGVM) Lobesia botrana. Experiments were carried out over three different years and geographical sites. Box plots of infested flower clusters  $(\%)$  and nests per flower cluster  $(n)$  of

EGVM showing the effect of (a) the tested dispenser used for mating disruption, (b) the year, and (c) the geographical site. Box plots indicate the median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median infestation parameter

noted between 2015 and 2016. The number of nests per bunch followed the same trend  $(Z = -4.722, P < 0.0001;$  $Z = -5.554, P < 0.0001$ ) (Fig. [2b](#page-7-0)).

Significantly different infestation levels of L. botrana were found in mating disruption tests carried out in the three geographical sites (Fig. [2](#page-7-0)c). The percentage of EGVM infested bunches was significantly higher in

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Campiano (RA) than in Bolgheri (LI) and Villafranca di Forlì (FC) ( $Z = 6.956$ ,  $P < 0.0001$ ;  $Z = -7.588$ ,  $P < 0.0001$ , respectively), while no significant differences were found between the latter two sites. A comparable trend was observed concerning the number of nests per bunch  $(Z =$ 5.958,  $P < 0.0001$ ;  $Z = -7.650$ ,  $P < 0.0001$ , respectively) (Fig. [2](#page-7-0)c).



Fig. 1 (continued)

## Third generation trials

The third generation of EGVM was effectively controlled by the application of mating disruption dispensers, irre-spective of the type of dispenser tested (Fig. [3](#page-10-0)). Isonet<sup>®</sup> L TT BIO, Isonet® L TT, and Isonet® L resulted in a significant reduction in the percentage of infested bunches in comparison to the untreated control  $(Z = 4.783)$ ,  $P < 0.0001$ ;  $Z = 4.271$ ,  $P < 0.0001$ ;  $Z = 3.470$ ,  $P = 0.029$ , respectively), and no significant differences emerged among the three tested dispensers (Fig. [3a](#page-10-0)). The same trend was observed for the number of nests per bunch: significantly lower values were recorded in plots treated with Isonet® L TT BIO, Isonet® L TT, and Isonet® L than in untreated control plots  $(Z = 5.014, P < 0.0001;$  $Z = 4.379$ ,  $P < 0.0001$ ;  $Z = 3.612$ ,  $P = 0.0017$ , respectively), with differences among treated plots not being significant (Fig. [3](#page-10-0)a).



Fig. 1 (continued)

Infested bunches  $(\%)$  and nests per bunch  $(n)$  varied significantly among the years (Fig. [3b](#page-10-0)). The percentage of L. botrana infested bunches was significantly higher in 2014 than in 2015 and 2016 ( $Z = -5.554$ ,  $P < 0.0001$ ;  $Z = -4.608$ ,  $P < 0.0001$ ), while no significant differences were noted between 2015 and 2016. The number of nests per bunch followed the same trend  $(Z = -5.213, P < 0.0001; Z = -4.112,$  $P < 0.0001$ ) (Fig. [3](#page-10-0)b).

Also at harvest, significantly different EGVM infestation levels were observed in the mating disruption trials carried out in the three geographical sites (Fig. [3](#page-10-0)c). Percent EGVM infestation was significantly higher in Campiano (RA) than in Bolgheri (LI) and Villafranca di Forlì (FC)  $(Z = 9.356,$  $P < 0.0001$ ;  $Z = -7.671$ ,  $P < 0.0001$ , respectively), with the latter two sites differing from each other  $(Z = 4.959, P < 0.0001)$ . A comparable trend was observed concerning the number of

<span id="page-7-0"></span>

Fig. 2 Field efficacy of mating disruption against the second generation of the European grapevine moth (EGVM) Lobesia botrana. Experiments were carried out over three different years and geographical sites. Box plots of infested bunches  $(\%)$  and nests per bunch  $(n)$  of EGVM showing

nests per bunch (Z = 9.355,  $P < 0.0001$ ; Z = -7.639,  $P < 0.0001$ , and  $Z = 4.433$ ,  $P < 0.0001$ , respectively) (Fig. [3c](#page-10-0)).

In all mating disrupted vineyards, L. botrana males were not captured by Trap Test Isagro® during the whole grape growing seasons, providing a further evidence of proper (7E,9Z)-7,9-dodecadien-1-yl acetate dispersion within the tested fields. Lastly, Fig. [4](#page-13-0) shows the continuous release (mg/ha/day) of synthetic (7E,9Z)-7,9-dodecadien-1-yl acetate,

the effect of (a) the tested dispenser used for mating disruption, (b) the year, and (c) the geographical site. Box plots indicate the median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median infestation parameter

by the three mating disruption products Isonet® L, Isonet® L TT, and Isonet® L TT BIO.

# **Discussion**

The development of effective and environmentally sustainable control strategies against agricultural insect pests is a crucial



Fig. 2 (continued)

challenge nowadays, considering that more than two million tons of pesticides are employed each year in agricultural activities worldwide (De et al. [2014](#page-14-0)), of which more than 400,000 tons are currently used in European countries (Eurostat [2016](#page-14-0)). In this framework, the frequent overuse of insecticides rapidly led to the development of resistance in targeted insects (Bourguet et al. [2000;](#page-14-0) Franck et al. [2007](#page-14-0); Thomas and Read [2016](#page-15-0); Grégoire et al. [2017\)](#page-14-0), including moth pests (Reyes et al. [2007;](#page-15-0) Zhao et al. [2002,](#page-16-0) Zhao et al. [2006](#page-16-0)).

Furthermore, the third generation of the European grapevine moth, which is the most dangerous for late grapevine varieties, is difficult to control, since farmers are experiencing a lack of authorized reliable pesticides characterized by short pre-harvest interval, to avoid residues in grapes and wine. Insecticides commonly applied close to harvest against this target pest contain toxins from Bacillus thuringiensis subsp. kurstaki and aizawai, acting as microbial disruptors of insect midgut



Fig. 2 (continued)

membranes, or emamectin benzoate as active ingredient (Muccinelli [2017\)](#page-15-0).

main sex pheromone component of L. botrana females (Ioriatti and Lucchi [2016](#page-15-0); Lance et al. [2016\)](#page-15-0).

Therefore, developing eco-friendly and reliable control tools is crucial. Our results highlighted the high efficacy of the mating disruption programs carried out against L. botrana populations in Northern and Central Italian vineyards. The approach proposed enables to minimize the use of chemical pesticides, since it is based on the deployment of different dispensers releasing multiple plumes of (7E,9Z)-7,9-dodecadien-1-yl acetate — the

Notably, our mating disruption approach testing the efficacy of Isonet® L TT BIO, Isonet® L TT over the standard product Isonet® L allowed a reliable control of the three generations of this moth pest during the whole growing season. The field efficacy of the tested approach was validated in three different geographic sites over a study period of 3 years. As expected, we observed significant differences among

<span id="page-10-0"></span>

Fig. 3 Field efficacy of mating disruption against the third generation of the European grapevine moth (EGVM) Lobesia botrana. Experiments were carried out over three different years and geographical sites. Box plots of infested bunches (%) and nests per bunch (n) of EGVM showing

the effect of (a) the tested dispenser used for mating disruption, (b) the year, and (c) the geographical site. Box plots indicate the median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median infestation parameter

experimental sites, mostly due to different pest population sizes in early season in the tested vineyards. In particular, concerning the first generation, we detected a high incidence of L. botrana damage to grapes in Campiano (Emilia Romagna, Northern Italy), highlighting the presence of a larger pest population, if compared to the other sites. Thus, in this context, random encounters between mates may occur, leading to a decreasing efficacy of mating disruption (Millar [2007](#page-15-0)). In these scenarios, an effective strategy can be the integration of mating disruption with low-impact microbial insecticides (e.g., B. thuringiensis-based ones), since it is well recognized that mating disruption gives its best efficacy on starting pest populations characterized by medium-low densities (Ioriatti and Lucchi [2016\)](#page-15-0).



Fig. 3 (continued)

Regarding experiments conducted against the first generation of L. botrana, we noticed a significant reduction in the number of infested flower clusters, and number of nests per flower cluster as well, if compared to the untreated control. Besides, when mating disruption tests were conducted against the second and third generation of L. botrana, a strong reduction in the number of infested bunches and number of nests per bunch was achieved. Earlier, Anfora et al. [\(2008\)](#page-13-0) observed a significant field efficacy of mating disruption carried out against L. botrana using Ecodian® dispensers, showing a reduction in the overall attractiveness of traps lured with calling females and monitoring baits. However, the authors tested 1600 dispensers/ha (Anfora et al. [2008](#page-13-0)), while in the present study, the biodegradable dispenser was tested at 200–250 dispensers/ha, still allowing an adequate release of synthetic (7E,9Z)-7,9-dodecadien-1-yl acetate, and achieving a substantial reduction of the incidence of L. botrana damage on grapes.

As a general trend, the efficacy of Isonet® L TT BIO, Isonet® L TT, and Isonet® L was comparable. Indeed, the



Fig. 3 (continued)

performances of all the tested dispensers did not differ in terms of infested flower clusters/bunches and nests per flower cluster/ bunch. This was noted in all experimental sites over 3 years of field experiments. As indicated by the curves showing the release of (7E,9Z)-7,9-dodecadien-1-yl acetate over time (Fig. [4\)](#page-13-0), the three dispensers tested here can protect treated vineyards from L. botrana infestation during the whole growing season, ensuring a continuous release of sex pheromone plumes.

To our mind, there are three practical implications arising from these findings. First, the comparable field performances of Isonet® L TT BIO and Isonet® L TT vs. Isonet® L allow reducing the number of pheromone dispensers needed per hectare (200–250 vs. 500 dispensers/ha), thus direct costs for buying them.

Second, a lower number of sex pheromone dispensers has a direct impact on farmers' economy, reducing labor cost to apply the dispensers in the vineyards. Indeed, the time needed to apply sex pheromone dispensers is 3 h/ha for Isonet® L, while it drops to 1–1.5 h/ha using Isonet® L TT or Isonet® L TT BIO, due to the lower number of required dispensers per

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hectare. When designing this study, we considered that testing a lower number of dispensers per hectare can lead to reduced efficacy of mating disruption, as earlier outlined by several authors studying the effective rate of mating disruption dispensers per hectare in the fight against other moth pests of economic importance, such as Cydia pomonella (L.) (e.g., Epstein et al. [2006](#page-14-0); Stelinski et al. [2006b](#page-15-0); Patanita [2007](#page-15-0); Grieshop et al. [2010](#page-14-0)). However, the present results showed that this was not the case, since the tested numbers of dispensers allowed a reliable control of the three L. botrana generations in all the experimental sites.

Third, the comparable efficacy of the biodegradable dispenser Isonet® L TT BIO over the widely adopted nonbiodegradable Isonet® L ones contributes reducing waste disposal - which nowdays represents a serious environmental concern (Rochman et al. [2013;](#page-15-0) Vegter et al. [2014](#page-16-0); Jambeck et al. [2015\)](#page-15-0) - because they are made of more eco-friendly materials prepared from natural resources (Ashori [2008](#page-14-0); Boghossian and Wegner [2008](#page-14-0); Castellano et al. [2008](#page-14-0); Scarascia-Mugnozza et al. [2012](#page-15-0); Bledzki et al. [2015](#page-14-0)). Our results also support earlier findings by other authors, focusing on the use of biodegradable dispensers for mating disruption of insect pests of agricultural importance, including the grape berry moth, Paralobesia viteana (Clemens) (Teixeira et al. [2010;](#page-15-0) Jenkins and Isaacs [2008](#page-15-0)), the codling moth, C. pomonella (Angeli et al. 2007), the Oriental fruit moth, Grapholita molesta (Busck) (Frédérique et al. [2007](#page-14-0); Stelinski et al. [2005,](#page-15-0) [2006a](#page-15-0), [2007\)](#page-15-0), the light brown apple moth, Epiphyas postvittana (Walker) (Brockerhoff et al. [2012;](#page-14-0) Suckling et al. [2012](#page-15-0)), the Asiatic rice borer, Chilo suppressalis (Walker) (Vacas et al. [2009a\)](#page-15-0), the California red scale, Aonidiella aurantii Maskell (Vacas et al. [2009b,](#page-16-0) [2010](#page-16-0), [2012\)](#page-16-0), the grub beetle, Dasylepida ishigakiensis Niijima et Kinoshita (Arakaki et al. [2017](#page-14-0)), and the Oriental beetle, Anomala orientalis Waterhouse (Behle et al. [2008\)](#page-14-0).

Overall, the present research provides useful information for the optimization of eco-friendly mating disruption programs against L. botrana populations, highlighting the interesting potential of biodegradable pheromone dispensers that can be easily applied at low density in vineyards of high economic value, reducing the use of chemical pesticides to control moth pests.

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#### Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests. The mention of trade names or commercial products in this article does not imply recommendation or endorsement by the authors' institutions.

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