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# Disrupting mating of *Lobesia botrana* using sex pheromone aerosol devices

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## Abstract

Pheromone-mediated mating disruption (MD) is widely used as a control tool to manage the European grapevine moth (EGVM), *Lobesia botrana*. Most of the MD formulations are "passive" reservoir dispensers, which need to be used at a rather large number of units per hectare. A promising alternative is represented by automatic aerosol devices, releasing pheromone puffs at programmed time intervals. Herein, we investigated the effectiveness of MD aerosol product Isonet® L MisterX841 in reducing EGVM infestation on grape in comparison to the reference MD product Isonet® L and the grower's standard. Experiments were carried out over two years in two different study sites of Aragon region (Spain). EGVM male catches were monitored using traps baited with the female sex pheromone. The effectiveness of MD formulations against the three generations of EGVM was assessed by determining the percentage of infested bunches and the number of nests per bunch. As expected, a much greater amount of male catches in the grower's standard over Isonet® L MisterX841 and Isonet ® L was observed. No significant differences about EGVM male catches were found in vineyards where Isonet® L MisterX841 and Isonet® L were used. EGVM infested bunches, as well as number of nests per bunch, were higher in the grower's standard, if compared to vineyards where we tested Isonet® L MisterX841 and Isonet® L. However, the employ of the latter led to a lower EGVM bunch infestation, if compared to Isonet® L MisterX841. Overall, the MD approach proposed here is effective against EGVM. These aerosol devices require a lower number of units ha$^{-1}$ if compared to hand-applied dispensers, saving labor costs and contributing to reduce plastic disposal in agricultural settings.

**Keywords:** chemical ecology; European grapevine moth; insect pest; Integrated Pest Management; organic viticulture; pesticide-free agriculture; sexual pheromones; Tortricidae

## Introduction

The European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is a key pest of grape in most wine growing regions worldwide. It is responsible of direct damages leading to serious economic losses on table grape, as well as relevant indirect damages to wine grape, where larvae feeding on bunches lead to botrytis and sour rot development (Ioriatti et al. 2011). The recent spread of EGVM in the Americas, including regions of high economic importance for wine production, such as California, Argentina and Chile (Gilligan et al. 2011; Gutierrez et al. 2012; Lance et al. 2015), stressed the crucial importance to develop effective control tools to manage EGVM populations within the framework of organic viticulture and Integrated Pest Management (IPM), with special reference to Area-Wide Pest Management (AWPM) (Ioriatti and Lucchi 2016).

Besides, the reduction of the use of pesticides in agricultural settings is a key challenge for modern agriculture, to limit their serious detrimental effect on human health and the environment (Desneux et al. 2007; Guedes et al. 2016; Hoshi et al. 2016; Navarro-Roldán and Gemeno 2017; Benelli 2018). In this scenario, research and public attention focused on more eco-friendly control strategies to manage EGVM populations (Witzgall et al. 2010; Cooper et al. 2014), including pheromone-mediated mating disruption (MD) (Cardé and Minks 1995; Byers 2006). Indeed, MD is successfully used as an effective control tool to fight *L. botrana* populations in vineyards (Ioriatti et al. 2004, 2008; Hummel 2017; Lucchi et al. 2018). In 2017, over 249,000 ha of European vineyards have been managed using MD against *L. botrana*, with about 76,000, 60,000, 47,000 and 36,000 ha in Spain, Germany, France and Italy, respectively (Lucchi and

Benelli 2018). Notably, recent research failed to show any negative effect of MD on human health as well as against other non-target species, pointing out that it fully complies with IPM criteria (Welter et al. 2005; Millar 2006; Miller et al. 2006; Ting and Eya 2010; Ioriatti et al. 2012).

In this framework, a rather wide array of devices emitting the main component [i.e., (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate] of EGVM female sex pheromone, have been tested to improve the efficacy of MD operations, reduce the number of dispensers used per ha, thus labor cost (Anfora et al. 2008; Brockerhoff et al. 2012; Miller and Gut 2015; Lance et al. 2016), and replace plastic containers with biodegradable ones (Lucchi et al. 2018). However, most of the dispensers currently used for MD of EGVM are “passive” reservoir devices, which continuously release plumes of (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate (Ioriatti and Lucchi 2016). Even if their employ ensures an effective management of *L. botrana*, they need to be used at a rather large number of units per ha (in most of the cases, from 250 to 600 units per ha), with significant costs for the farmers to apply the dispensers in the field (Shorey and Gerber 1996; Gut et al. 2004; Hansen 2008).

To face this challenge, a promising alternative is represented by automatic aerosol devices, which release puffs of the sex pheromones at programmed time intervals. These devices have been successfully tested against several insect species of economic importance, with special reference to moth pests (Shorey and Gerber 1996; Burks and Brandl 2004; Knight 2004; Stelinski et al. 2007; Suckling et al. 2007; De Lame et al. 2010; McGhee et al., 2014, 2016). However, to the best of our knowledge, in the face of a series of efficacy tests necessarily conducted for the recent registration of the  CheckMate® Puffer® LB formulation in some European countries, no study on the effectiveness of pheromone aerosol devices for the control of EGVM has been published.

Therefore, in the present study, we compared several MD strategies to manage EGVM populations in Spanish vineyards. We attempted to address the following questions: (*i*) Can MD aerosol product Isonet® L MisterX841 strongly reduce EGVM male catches in pheromone-baited traps? (*ii*) Does the employ of this aerosol device significantly diminished EGVM damage on grapevine? (*iii*) Is this MD approach effective over various study years and sites? (*iv*) Is the overall efficacy of the MD aerosol product comparable to the reference MD product Isonet® L? (*v*) Should MD aerosol products be preferred over the grower's standard practices?

To tackle the arguments outlined above, herein we compared the effectiveness of EGVM control programs based on the use of the Isonet® L MisterX841 vs. the reference MD product Isonet® L and the grower's standard. Field experiments were carried out over two years (i.e., 2014 and 2015) in two different study sites located in the Aragon region (Spain). Each year, the effectiveness of MD products against the three generations of EGVM was assessed by determining the abundance of infested bunches and the number of nests per bunch. In addition, *L. botrana* male catches were monitored using traps baited with the EGVM synthetic sex pheromone.

## Materials and methods

### Field experimental sites and study period

The experiment was conducted in two commercially farmed vineyards belonging to Cariñena DO located in the north of Spain, Alfamén area, Aragon region (41º 29’ 49.8” N – 1º 16’ 29.5” W) (Table 1). Four efficacy trials were performed in two consecutive years, 2014 and 2015, on vineyards with homogenous conditions and varieties (Cabernet and Merlot). The vineyards selected for the trials registered medium-high *L. botrana* infestation in the previous years. Vineyards were scouted before harvest (at the end of the EGVM third generation) monitoring *L. botrana* infestation. More than 20 damage assessments were performed and the average infestation ranged from 20 to 40 % infested bunches. Study vineyards were located in a windy area. The average wind speed in the study area is 19 km/h. The 43 % of the days, the wind speed is >20 km/h, and in the 16% of the cases >30 km/h. Maximum wind speed is 80-100 km/h. The dominant wind is commonly named as “Cierzo” and blows from NW down the Ebro valley.

## Pheromone dispensers

The objective of this study was to test an aerosol formulation, named Isonet® L MisterX841 (Shin-Etsu Chemical Co., Tokyo, Japan), for mating disruption of EGVM. The aerosol dispenser consists in a pressurized aluminum can loaded with 52.1 g of (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate, solvent-diluted and mixed with a propellant. A programmable electronic device (Isomate® CM Mist), produced by Pacific Biocontrol Corp. (Vancouver, WA, USA), was used to release the formulation in the field over time.

All experiments were conducted using the same release program. Isonet® L MisterX841 devices were deployed at the density of 2 per ha, hanged on the top of the pole of the vineyard trellis system, and were daily releasing 297 mg of active ingredient (a pheromone puff every 20 min from 18:00 pm to 6:00 am).

## Experimental design and data collection

The design used for the experiment was large plots as recommended by EPPO guidelines and earlier studies (Baker et al. 1997; European and Mediterranean Plant Protection Organization 2016) for mating disruption products. Each treatment was applied on a large and homogeneous plot. Each plot was divided in 10 subplots composed by 100 vines minimum, homogeneously distributed in the internal part of the plot, 20 m away from the borders (Table 1).

EGVM flight was assessed during the season using three pheromone-baited delta traps (Trécé Inc., Adair, OK, USA) per plot. Traps were baited with septum lure for *L. botrana*, code 3104-25 EGVM (Trécé Inc., Adair, OK, USA) and hanged inside the vine canopy at 1.5 m from the ground. The traps were deployed before the beginning of the first flight of the pest and checked weekly. Data were analyzed as male catches at the end of each EGVM flight. The septum lure was changed every 30 days, as for manufacturer recommendation.

In our experiments, three treatments were tested, Isonet® L MisterX841 (tested MD product) at 2 units per ha, Isonet® L (Shin-Etsu Chemical Co) (standard MD reference) at 500 dispensers per ha, and the grower’s standard (control), where a conventional insecticide-based strategy was used (Table 2). All MD formulations were deployed before the beginning of the first flight of the target pest in both years (Table 2).

## Crop damage and *L. botrana* population density evaluation

In all trials, the crop damage caused by EGVM was assessed at the end of the 1st generation (=G1, BBCH 65), at the end of the 2nd generation (=G2, BBCH 79), and at the harvest time (=G3, BBCH 89). To assess the effectiveness of the three different strategies, we considered the following variables: (*i*) number of male captures per treatment per flight, (*ii*) rate of infested inflorescences or bunches, (*iii*) number of nests per inflorescence (G1) or number of larvae per bunch (G2 and G3).

Within each subplot and at each damage assessment, we examined 50 flower clusters per subplot at G1, 50 bunches per subplot at G2, and 30 bunches per subplot at G3, for a total of 15,000 (G1 and G2) and 9,000 (G3) examined samples. The percentage of EGVM-damaged flower clusters or bunches at each assessment was then calculated. Furthermore, at each assessment, the number of EGVM nests per flower cluster (G1) or bunch (G2 and G3) was noted. In detail, G1 and G2 infestation was measured through on-site surveys on non-destructively sampled inflorescences and bunches. As to G3, an estimate of the infested bunches was made on samples collected in the vineyards and carefully dissected as described by Lucchi et al. (2018).

## Statistical analysis

Data about male catches per EGVM flight, as well as the percentage of infested flower clusters (G1) and bunches (G2 and G3), and the number of EGVM nests per flower cluster/bunch, were not normally distributed. Data transformation reported by Stelinski et al. (2007) [i.e., *ln* ($x + 1$)] was not able to normalize the distribution and homogenize the variance of our data (Shapiro-Wilk test, goodness of fit post-transformation $P<0.001$). Therefore, non-parametric statistics was used. Differences in the abundance of EGVM catches, infested flower clusters (G1) and bunches (G2 and G3), and the number of nests per flower cluster/bunch among treatments (i.e., tested pheromone dispensers and the positive control, i.e., grower's standard), years, and study site were assessed using Kruskal–Wallis test followed by Steel–Dwass multiple comparison; $P=0.05$ was selected as threshold to assess significant differences.

## Results

### Male catches using pheromone-baited traps

The analysis of male catches per EGVM flight using traps baited with the main female sex pheromone component [i.e., (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate] showed significant differences among the three tested control strategies ($\chi^2_2=76.725$, $P<0.0001$), with higher abundance of male catches in the grower's standard, if compared to vineyards where we tested Isonet® L ($Z=-43.813$, $P<0.0001$) and Isonet® L MisterX841 ($Z=-41.021$, $P<0.0001$). No significant differences were detected about EGVM catches in vineyards where Isonet® L and Isonet® L MisterX841 ($Z=1.890$, $P=0.141$) were used (Figure 1).

In addition, male catches varied significantly among the three moth flights ($\chi^2_2$=29.928, *P*<0.0001). A significant difference emerged between the first and the second flight (*Z*=-3.163, *P*=0.009), as well as between the first and the third flight (*Z*=-3.796, *P*=0.0008). No differences in EGVM catches were observed between the third flight if compared to the second one (*Z*=4.236, *P*=0.957) (Figure 1).

Lastly, the effect of the study site was not significant ($\chi^2_1$= 1.592, P=0.207), while a significant difference was detected between the two years ($\chi^2_1$=6.625, *P*=0.010), showing higher EGVM male catches incidence in 2015 over 2014 (Figure 1).

### Infested flower clusters and bunches

The percentage of EGVM infested flower clusters and bunches varied significantly among the three tested control approaches ($\chi^2_2$=187.993, *P*<0.0001), with higher infestation rates in the grower's standard, if compared to vineyards where Isonet® L (*Z*=-12.592, *P*<0.0001) and Isonet® L MisterX841 (*Z*=-10.277, *P*<0.0001) were applied. In addition, a significant difference was detected between vineyards where Isonet® L and Isonet® L MisterX841 (*Z*=4.261, *P*=0.0001) were used, with higher percentage of infested flower cluster and bunches on Isonet® L MisterX841 over Isonet® L (Figure 2).

Furthermore, the percentage of EGVM infested flower cluster and bunches varied significantly among the species generations ($\chi^2_2$=24.924, *P*<0.0001). A significant difference in overall infestation was detected between the first and the second EGVM generation (*Z*=-4.460, *P*<0.0001). We observed higher infestation rates in the third generation if compared to the second one (*Z*=4.061, *P*<0.0001), while no significant differences were found in the percentage of EGVM infestation between the first and the third generation (*Z*=1.572, *P*=0.258) (Figure 2).

The effect of the study site was not significant ($\chi^2_1$=3.548, *P*=0.059), while a difference was detected between the two experimental years ($\chi^2_1$=4.246, *P*=0.039), showing higher infestation in 2015 over 2014 (Figure 2).

### Number of nests per flower cluster or bunch

The number of EGVM nests per one-hundred flower clusters and bunches varied significantly among the three pest management strategies ($\chi^2_2$=190.131, *P*<0.0001), with higher abundance of nests in the grower's standard, if compared to that of vineyards where we tested Isonet® L (*Z*=-12.601, *P*<0.0001) and Isonet® L MisterX841 (*Z*=-10.439, P<0.0001). A significant difference in the number of EGVM nests was detected between vineyards where Isonet® L and Isonet® L MisterX841 (*Z*=4.230, *P*=0.0001) were used, with higher value of this variable on Isonet® L MisterX841 over Isonet® L treatment (Figure 3).

Moreover, the abundance of EGVM nests per one-hundred flower clusters and bunches varied significantly among generations ($\chi^2_2$=27.297, *P*<0.0001). A difference was detected between the first and the second generation (*Z*=-4.622, *P*<0.0001). Higher abundance of nests was noted in the third generation if compared to the second one (*Z*=4.236, *P*<0.0001), while no significant differences were found about EGVM infestation values between the first and the third generation (*Z*=1.886, *P*=0.143) (Figure 3).

The effect of the study site was not significant ($\chi^2_1$=3.213, *P*=0.073), while a difference was detected between the two years ($\chi^2_1$=5.207, *P*=0.022), showing higher infestation levels in 2015 over 2014 (Figure 3).

## Discussion

The use of aerosol devices for MD of moth pests of fruits and nuts is still debated, since several authors pointed out that their employment as standing-alone control strategy is not enough to effectively manage pest populations (Isaacs et al. 1999; Stelinski et al. 2007; McGhee et al. 2016). However, the findings reported here about MD of EGVM are promising. Our results highlighted a higher abundance of male catches in pheromone traps located in the grower's standard vineyards over those placed in Isonet® L MisterX841 and Isonet® L vineyards. In addition, we showed that the percentage of EGVM infested flower clusters and bunches, as well as the number of nests per flower cluster or bunch, was significantly higher in the grower's standard, over vineyards where Isonet® L MisterX841 and Isonet L® were tested. Notably, no significant differences about EGVM male catches were found analyzing data from vineyards where Isonet® L MisterX841 and Isonet® L were used. However, the employ of the latter led to lower values of EGVM flower cluster and bunch infestation, if compared to the performances of Isonet® L MisterX841. As a general trend, the abundance of *L. botrana* populations was higher in the first and the third generation, if compared to the second one. The study site did not have a significant effect on the three variables used to monitor EGVM infestation, while the effect of the experimental year on infested clusters and bunches, number of nests per bunch and male catches was always significant.

The findings summarized above outlined the interesting potential of pheromone aerosol devices to control *L. botrana*. Even if most of the MD research conducted right now focused on the use of “passive” sex pheromone dispensers (see Ioriatti and Lucchi 2016 for a recent review), there are at least three advantages arising from the employ of pheromone aerosol devices against EGVM. First, aerosol devices require a lower number of units per ha (1-3 units ha$^{-1}$) if compared to hand-applied “passive” dispensers (200-600 units ha$^{-1}$). Second, the lower number of units per ha reduces labor cost, which is a key requirement for farmers (Gut et al. 2004; Stelinski et al. 2007). Third, the employ of pheromone aerosol devices contributes to lower plastic disposal in agricultural settings and close-related environments (Lucchi et al. 2018).

However, despite these promising features, no evidences have been published about the use of sex pheromone aerosol devices for MD of EGVM. On the other hand, several attempts have been done to evaluate similar aerosol devices against various moth pests (McGhee et al. 2014, 2016). Earlier, Stelinski et al. (2007) focused on the MD of *Cydia pomonella* (L.) and *Grapholita molesta* (Busck) testing Puffer® aerosol dispensers at 2.5 units ha$^{-1}$ (Suterra LLC, Bend, USA). The authors pointed out that the tested product was able to disrupt the male orientation towards pheromone-baited traps in trials conducted on both moth pests. However, the MD approach proposed by Stelinski et al. (2007) did not significantly affect the incidence of fruit infestation between Puffer®-treated fields and control ones. A similar result was obtained by Knight (2004), who tested the efficacy of MD against *C. pomonella* using a combination of sex pheromone dispensers (Isomate-C®) applied on the perimeter of apple orchards plus an internal grid of sex pheromone aerosol devices (1 unit $ha^{1}$) or dispenser clusters (4-8 units $ha^{-1}$). Again, the author did not find any impact of the proposed MD approach on fruit infestation levels (Knight 2004). Later on, McGhee et al. (2014) highlighted that aerosol devices (Isomate® CM MIST) to manage *C. pomonella* populations through MD, probably achieved their effect by inducing false-plume following, while their camouflage of traps and females is limited. More recently, McGhee et al. (2016) conducted an interesting attempt aimed to optimize the use of the above cited aerosol devices for *C. pomonella* MD, reducing the concentration of codlemone by 50 %. They outlined that the codling moth catches in MD-treated fields were about half of the untreated control, and none of the tested concentrations led to high (i.e. >95 %) reduction in male catches, at variance with earlier research discussed above (Knight 2004).

Furthermore, Suckling et al. (2007) studied MD of *Epiphyas postvittana* (Walker) in New Zealand apple orchards, testing the effectiveness of an electronically controlled aerosol system over pheromone polyethylene dispensers. As in our work, male moth catches were monitored in treated and control fields, showing that both MD products led to significantly lower male catches, with a 90 % reduction of male catches when both the sex pheromone aerosol devices (5 units $ha^{-1}$) as well as pheromone polyethylene dispensers (100 units $ha^{-1}$) were tested. Similar observations have been done by McGhee et al. (2014), pointing out the efficacy of 5 aerosol units (Isomate® CM MIST) per hectare in MD programs against *C. pomonella*. However, 5 aerosol devices per ha are a rather high number. Suckling et al. (2007) did not recommend the use of aerosol devices for *E. postvittana* MD programs, due to their high costs. This does not apply to the findings presented here, since we used only 2 devices per hectare.

In addition, our results highlighted that the efficacy of the sex pheromone aerosol device Isonet® L MisterX841 is higher if compared to the grower's standard practices, where the latter relied also to the use of insecticides to manage EGVM. In addition, the performances of the aerosol device tested here did not significantly differ from the commercial MD product Isonet® L, at least in term of male catches on pheromone-baited traps.

## Conclusions

The MD approach proposed here allowed an effective management of *L. botrana* populations, leading to a strong reduction in the number of pheromone dispensers in vineyards, thus labor cost. On the other hand, it should be noted that the standard hand-applied dispensers tested here achieved better results in term of crop damage reduction. Besides, the use of aerosol devices leads to several additional requirements. Indeed, a careful study of the agricultural settings where the MD approach is needed to locate the best sites to install these devices. In addition, the MD product tested here needs proper maintenance over time, and the cost per hectare to purchase is higher if compared to hand-applied dispensers. Further research to develop aerosol devices with reduced pheromone content and finely tunable release programs (see also McGhee et al. 2016) is ongoing, with the final aim to propose highly effective, cheap and easy-to-manage aerosol devices.

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## Conflict of Interest

The authors declare no competing interests. The mention of trade names or commercial products in this publication is only aimed to provide specific information; it does not imply recommendation or endorsement by the authors' affiliations.

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490

491

## Captions

**Table 1.** Location of the vineyards subjected to the tests of pheromone-based mating disruption using aerosol devices against the European grapevine moth, *Lobesia botrana*.

**Table 2.** Size of the vineyards subjected to the different treatments, and application dates of the two pheromone-based formulations.

**Figure 1.** Mating disruption against the European grapevine moth, *Lobesia botrana* (EGVM). Box plots showing the effect of **(a)** the treatment, **(b)** moth flight, **(c)** experimental site, and **(d)** year on EGVM male catches in pheromone-baited traps. Box plots indicate the median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the EGVM population parameter.

**Figure 2.** Mating disruption against the European grapevine moth, *Lobesia botrana* (EGVM). Box plots showing the effect of **(a)** the treatment, **(b)** moth generation, **(c)** experimental site, and **(d)** year on EGVM infested bunches (%). Box plots indicate the median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the EGVM population parameter.

**Figure 3.** Mating disruption against the European grapevine moth, *Lobesia botrana* (EGVM). Box plots showing the effect of **(a)** the treatment, **(b)** moth generation, **(c)** experimental site, and **(d)** year on EGVM nests/100 bunches. Box plots indicate the

median (solid line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the EGVM population parameter.

**Figure 1**

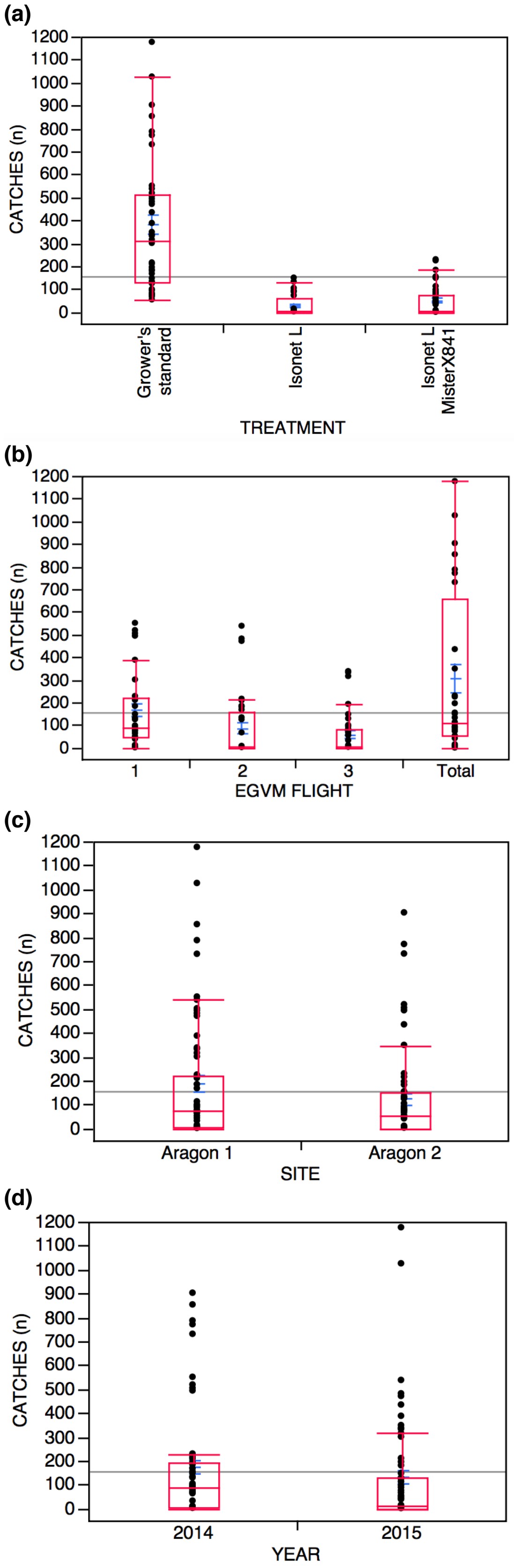
(a)
CATCHES (n)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Grower's standard
Isonet L
Isonet L MisterX841
TREATMENT
(b)
CATCHES (n)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
Total
EGVM FLIGHT
(c)
CATCHES (n)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Aragon 1
Aragon 2
SITE
(d)
CATCHES (n)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
2014
2015
YEAR

**Figure 2**

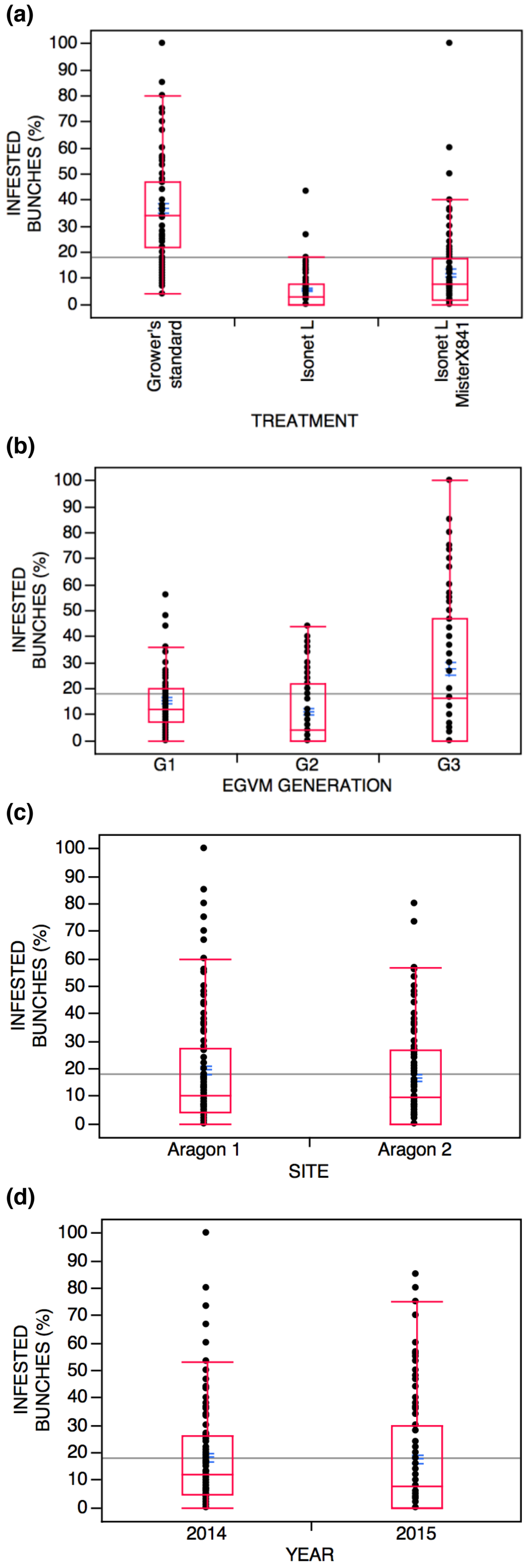
(a)
INFESTED BUNCHES (%)
100
90
80
70
60
50
40
30
20
10
0
Grower's standard
Isonet L
Isonet L MisterX841
TREATMENT
(b)
INFESTED BUNCHES (%)
100
90
80
70
60
50
40
30
20
10
0
G1
G2
G3
EGVM GENERATION
(c)
INFESTED BUNCHES (%)
100
90
80
70
60
50
40
30
20
10
0
Aragon 1
Aragon 2
SITE
(d)
INFESTED BUNCHES (%)
100
90
80
70
60
50
40
30
20
10
0
2014
2015
YEAR

**Figure 3**

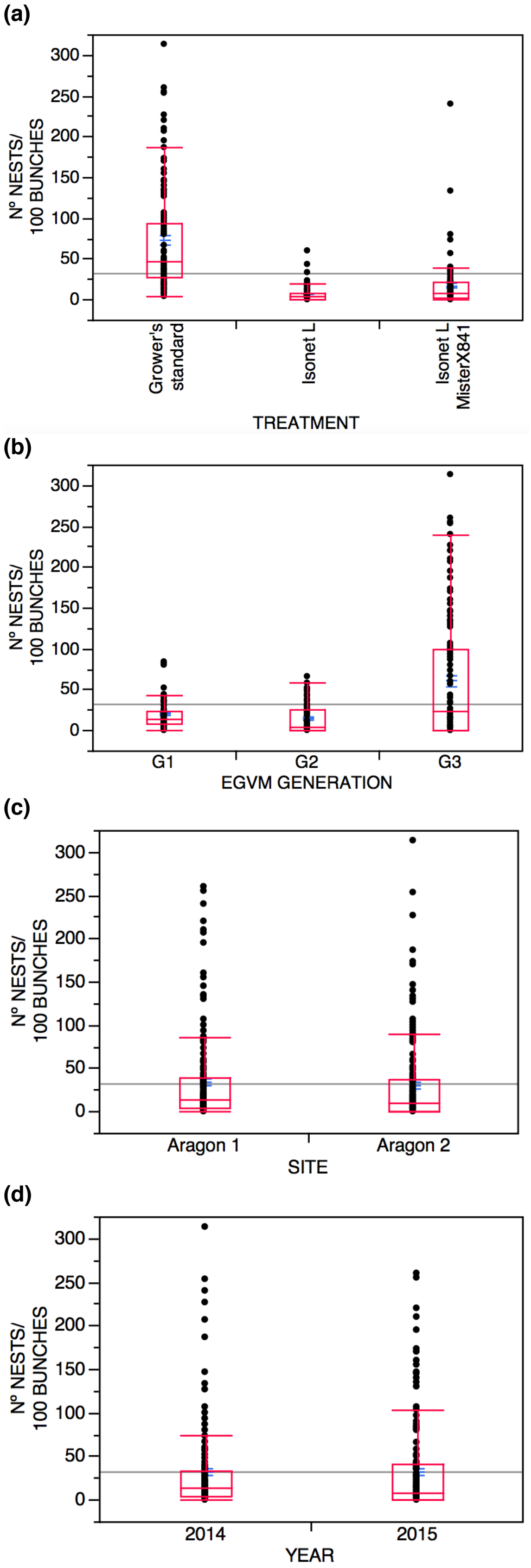
(a)
Nº NESTS/
100 BUNCHES
300
250
200
150
100
50
0
Grower's standard
Isonet L
Isonet L MisterX841
TREATMENT
(b)
Nº NESTS/
100 BUNCHES
300
250
200
150
100
50
0
G1
G2
G3
EGVM GENERATION
(c)
Nº NESTS/
100 BUNCHES
300
250
200
150
100
50
0
Aragon 1
Aragon 2
SITE
(d)
Nº NESTS/
100 BUNCHES
300
250
200
150
100
50
0
2014
2015
YEAR

**Table 1**

| Trial | Site | Province | Region | Longitude | Latitude | Year | Variety | Rootstock | Training system | Spacing between rows (m) | Spacing between vines (m) | Plant age (years) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Alfamén | Zaragoza | Aragon | 1º 16’ 29.5” W | 41º 29’ 49.8” N | 2014 | Cabernet | Richter 110 | Low cordon | 2.7 | 1 | 12 |
| 2 | Alfamén | Zaragoza | Aragon | 1º15’57.14” W | 41º29’51.04” N | 2014 | Merlot | SO4 | Low cordon | 2.7 | 1 | 11 |
| 3 | Alfamén | Zaragoza | Aragon | 1º 16’ 29.5” W | 41º 29’ 49.8” N | 2015 | Cabernet | Richter 110 | Low cordon | 2.7 | 1 | 13 |
| 4 | Alfamén | Zaragoza | Aragon | 1º15’57.14” W | 41º29’51.04” N | 2015 | Merlot | SO4 | Low cordon | 2.7 | 1 | 12 |

**Table 2**

| Trial | Vineyard size (ha) | | | Date of dispenser deployment |
|---|---|---|---|---|
| | Isonet$^{®}$ L Misterx841 | Isonet$^{®}$ L | Grower standard | |
| 1 | 9.2 | 9.2 | 2.2 | 2/4/2014 |
| 2 | 4.8 | 5.8 | 1.6 | 2/4/2014 |
| 3 | 4.8 | 4.5 | 4.2 | 1/4/2015 |
| 4 | 4.8 | 5.8 | 2.8 | 1/4/2015 |