



# Managing the vine mealybug, *Planococcus ficus*, through pheromone-mediated mating disruption

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## Abstract

The vine mealybug (VMB), *Planococcus ficus* (Hemiptera: Pseudococcidae), is a key insect pest of vineyards. While pheromone-based mating disruption (MD) has been successfully tested against a wide range of insect pests, knowledge about its efficacy against key mealybug species, such as *P. ficus*, is scarce. In this study, a novel MD product, Isonet® PF, was evaluated by testing 300, 400, and 500 dispensers/ha at four study sites located in Northern (Veneto) and Southern (Sicily) Italy. Experiments were carried out over 2 years by monitoring the mealybug populations in wine grape and table grape vineyards managed with and without the application of MD. Pheromone dispensers were periodically collected during the grapevine-growing season, extracted, and analyzed by GC-MS, to determine their pheromone content and the release in mg/ha/day. The results showed that use of the MD dispenser Isonet® PF reduced the percentage of VMB-infested bunches and the number of VMB specimens per bunch compared with the untreated controls. This was recorded over 2 years at all experimental sites. Differences in the incidence of infested bunches among the three tested rates of Isonet® PF were not detected. Overall, the results presented here contribute to optimizing the sex pheromone dosage used in MD control programs against VMB allowing a reduction of broad-spectrum insecticides currently employed to manage this important pest.

**Keywords** Biological control · Chemical ecology · Integrated pest management · Sustainable pest control · Sex pheromones

## Introduction

*Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), commonly known as vine mealybug (VMB), is an important insect pest of vineyards in various regions worldwide (Daane et al. 2012), including several European countries, with special reference to Italy, Southern France, and Spain, as well as

the USA (CA), Mexico (Baja California and Sonora), Argentina, Brazil, and South Africa (da Silva et al. 2016; Le Vieux and Malan 2016; Walton and Pringle 2017; Daane et al. 2018a). *Planococcus ficus* infestations lead to severe economic losses, both on wine and table grape: VMB populations produce abundant honeydew that can cover vine plants, leading to sooty mold development on foliage and bunches (Chiotta et al. 2010). In addition, *P. ficus* can transmit various grape pathogens, including the grapevine leafroll-associated virus (GLRaV) (Douglas and Krüger 2008; Tsai et al. 2008, 2010) and the corky-bark disease (Tanne et al. 1989). Notably, it has been reported that heavy infestations by VMB can lead to the destruction of whole vineyards (Walton et al. 2006; Sharon et al. 2016).

The economic importance of this pest highlights the need to develop suitable control strategies for *P. ficus*. The effective and environmentally sustainable control of this pest is extremely important for ensuring the quality of wine and table grape production, as well as to prevent the further spread of GLRaV (Walton et al. 2004; Franco et al. 2009; Almeida et al. 2013; Sokolsky et al. 2013). Finding adequate pest

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management tactics against the VMB is a challenging issue. The use of insecticides against VMB populations—including systemic ones (e.g., spirotetramat)—may not show consistent results, since a relevant percentage of the population feeds on roots or is concealed under the bark (Gutierrez et al. 2008; Mansour et al. 2010). Various control strategies have been attempted, ranging from hot water treatments for preparing nursery stock (Haviland et al. 2005) to the use of VMB biological control agents (Fallahzadeh et al. 2011), in particular parasitoids (Güleç et al. 2007; Suma et al. 2012; Mansour et al. 2017a; Mgocheki and Addison 2009; Romano et al. 2018). Within the field of eco-friendly control tools that can be used against insect pests, a prominent role is played by sex pheromone-based mating disruption (MD) (Millar 2007; Miller and Gut 2015). This strategy is species-specific, is safe for human health, and does not lead to acute or chronic toxicity on non-target organisms (Welter et al. 2005; Witzgall et al. 2010). While MD has been successfully tested against a wide range of insect pests (Gut et al. 2004; Lance et al. 2016; Ioriatti et al. 2008), in particular certain moth species (e.g., codling moth, *Cydia pomonella* (L.)), European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller), and European grape berry moth, *Eupoecilia ambiguella* (Hübner) (Miller and Gut 2015; Ioriatti and Lucchi 2016; Lucchi et al. 2018a,b), knowledge about its efficacy against important mealybugs species, such as *P. ficus*, is limited. Indeed, little research is available on this topic (Walton et al. 2006; Cocco et al. 2014, 2018; Sharon et al. 2016; Mansour et al. 2017b). The studies conducted by da Silva et al. (2010) suggest that biparental mealybug species, including the *Planococcus* genus, do not rely on facultative parthenogenesis for reproduction (see also Lentini et al. 2018). Furthermore, Waterworth et al. (2011) reported that VMB must mate to produce viable offspring, outlining the potential of MD to control their populations.

The sex pheromone of *P. ficus* was first identified by Hinkens et al. (2001) as lavandulyl senecioate. Later, Millar et al. (2002) better clarified the VMB sexual communication, showing that the VMB-synthesized (S)-enantiomer was equally attractive for males, if compared to the racemic enantiomer mixture (see also Zada et al. 2003, 2008). Subsequently, various researchers developed monitoring methods based on the use of synthetic VMB sex pheromone (Millar et al. 2002; Walton et al. 2006).

Furthermore, the control of *P. ficus* through MD has been investigated by testing the VMB sex pheromone in sprayable microencapsulated formulations (Daane et al. 2006; Walton et al. 2006), as well as in reservoir dispensers (Cocco et al. 2014; Sharon et al. 2016). Results obtained with hand-applied dispensers were promising. In addition, they are easy to use for farmers compared to sprayable microencapsulations. However, the appeal of this eco-friendly control tool is limited by the high density of dispensers per hectare; indeed, studies

currently available tested MD products at a density higher than 600 units per hectare (Cocco et al. 2014; Sharon et al. 2016). Costs of MD programs for farmers need to be reduced by decreasing either the amount of pheromone per hectare (i.e., costs for material) or the number of dispensers per hectare (i.e., labor costs for deployment) or both.

In this research, a novel hand-applied reservoir dispenser (Isonet PF®, Shin-Etsu Chemical Co. Ltd., Japan) loaded with 180 mg of racemic lavandulyl senecioate was evaluated: three different dispenser densities (i.e., 300, 400, and 500 dispensers/ha) were tested at four study sites located in Northern (Veneto region) and Southern (Sicily region) Italy. Experiments were carried out over 2 years by monitoring the VMB populations in untreated wine and table grape vineyards as well as in vineyards managed with MD. Lastly, Isonet® PF dispensers were periodically collected during the whole grape-growing season, extracted, and analyzed by GC-MS, to monitor content and release of VMB sex pheromone in mg/ha/day.

## Materials and methods

### Experimental sites

Trials were conducted in 2016 and 2017 in areas typical for Italian grape cultivation, on different wine and table grape varieties. In 2016, three trials were carried out in the area of Acate, Ragusa province, Sicily region, Southern Italy (Table 1). Two trials were performed on early-ripening wine grape varieties, one on Chardonnay and one on Pinot Grigio (expected harvest time: beginning of August), and another trial was carried out on the later-ripening wine grape variety Nero d'Avola (expected harvest time: end of August–beginning of September) (Table 1).

In 2017, a total of four trials were conducted. Again, three trials were performed in the province of Ragusa (Sicily region, Southern Italy): one on the early-ripening wine grape variety Chardonnay, one on the later-ripening wine grape variety Nero d'Avola, and one on the table grape variety Italia. A fourth trial was carried out in Colognola ai Colli, Verona province (Veneto region, Northern Italy), on the wine grape variety Garganega. Details on vineyard location and pest history are found in Table 1. The study sites 1 and 4 are the same vineyard block tested over different years; this also applies to sites 3 and 5. A detailed description of each cultivar is given in Table 2.

Reservoir dispensers (Isonet® PF, Shin-Etsu Chemical Co. Ltd., Japan) were tested at three different application rates: 300, 400, and 500 dispensers/ha. Isonet® PF consists of two parallel polyethylene tubes, one of which contains an aluminum wire that enables their placement on supports and the other is filled with 180 mg of racemic lavandulyl senecioate TGAI. The VMB racemic pheromone amount per hectare tested in our MD trials was 54, 72, and 90 g, corresponding

**Table 1** Location of trial site vineyards, indicating pest history (low, medium, or high crop damage and pest population in previous years) and year of mating disruption trials with Isonet® PF dispensers. Low ≤ 15% infested bunches; medium 16–40% infested bunches; high > 40% infested bunches, where infested means one or more *Planococcus ficus* per bunch

Trial	Site	Province	Region	Longitude	Latitude	Pest history	Year
1	Acate	Ragusa	Sicily	14,4051 E	37,0211 N	Medium	2016
2	Acate	Ragusa	Sicily	14,4039 E	37,0176 N	Medium	2016
3	Acate	Ragusa	Sicily	14,4135 E	37,0013 N	Medium	2016
4	Acate	Ragusa	Sicily	14,4051 E	37,0211 N	Medium	2017
5	Acate	Ragusa	Sicily	14,4135 E	37,0013 N	Medium	2017
6	Chiaramonte Gulfi	Ragusa	Sicily	14,6218 E	37,0719 N	Low	2017
7	Colognola ai Colli	Verona	Veneto	11,1871 E	45,4258 N	High	2017

to a dispenser density of 300, 400, and 500 Isonet® PF dispensers/ha, respectively.

## Experimental design

A randomized block design is not applicable to the large plots required for studies on MD products (European and Mediterranean Plant Protection Organization 2016), so each treatment (i.e., Isonet® PF at 300, 400, and 500 dispensers/ha, respectively, and untreated control) was applied to a large plot. There were six sampling units per plot, which were distributed in a grid pattern. Each sampling unit was sufficiently large for there to be at least 100 bunches per assessment. Isonet® PF-treated plots were at least 2 ha in size (range 2.01–4.75 ha). Untreated control plots were not as large (range 0.12–0.64 ha), but had a comparable pest history and allowed for assessments on the same number of bunches per sampling unit. In all Isonet® PF-treated plots, the dispensers were deployed before the beginning of the migration of VMB nymphs in spring (mid-April in Sicily, end of April in Veneto).

## Male captures

Following the method by Walton et al. (2006), *P. ficus* males were monitored during both 2016 and 2017 using red Pherocon Delta IIID sticky traps (Trécé Inc., Adair, OK). Traps were baited with 100 µg of racemic synthetic lavandulyl senecioate from Pherobank B.V. (Wijk bij Duurstede, The Netherlands) in rubber septa lures (Suterra Europe). One trap was used per large plot. Trap sticky plates and sex

pheromone lures were replaced monthly, and trapped males were counted by direct observation using a stereoscopic microscope (Leica, Germany). Data were analyzed as the total number of males caught before harvest.

## Infestation and population density evaluation

The MD efficacy of Isonet® PF was evaluated on 100 bunches per sampling unit by determining the percentage of bunches infested by *P. ficus* and the number of VMB/bunch at harvest (BBCH 89). Within each of six sampling units, the number of bunches infested by VMB was counted on 100 randomly selected bunches, giving a total of 600 bunches per plot. Then, the percentage of *P. ficus*-infested bunches was calculated. In addition, the number of VMB per bunch was noted to provide an indication of the damage severity.

## Sex pheromone release

In both study years, Isonet® PF dispensers were periodically collected in Sicily during the whole grapevine-growing season, extracted, and analyzed by GC-MS, according to the method by Lucchi et al. (2018c). Isonet® PF residual content in racemic lavandulyl senecioate was estimated based on internal (SEC) standard GC-MS analysis. The latter was done using 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 × ID 0.25 mm × 0.25-µm film thickness, J & W Scientific, Folsom, CA, USA)

**Table 2** Crop details for vineyards where Isonet® PF dispensers were tested to manage *Planococcus ficus* populations

Trial	Crop	Variety	Rootstock	Training system	Row spacing (m)	Spacing within rows (m)	Plant age (years)
1	Wine grape	Chardonnay	1103 P	Low cordon	2.2	0.9	12
2	Wine grape	Pinot Grigio	110 R and 1103 P	Low cordon	2.2	0.9	10–12
3	Wine grape	Nero d'Avola	1103 P	Low cordon	2.2	0.9	9–11
4	Wine grape	Chardonnay	1103 P	Low cordon	2.2	0.9	13
5	Wine grape	Nero d'Avola	1103 P	Low cordon	2.2	0.9	10–12
6	Table grape	Italia	140 Ruggeri	Tendone	2.5	2.5	17
7	Wine grape	Garganega	SO4	Pergola veronese	3.5	0.65	10

with helium 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 set at 150 °C. The GC-MS estimate of the dispenser residual content allowed the calculation of the pheromone release for the duration of the field deployment of the dispenser. Each value was a mean  $\pm$  SD of four replicates, each obtained extracting and analyzing a single dispenser, except for the value presented on April 22, 2016, obtained as a mean  $\pm$  SD of 10 analyzed dispensers.

### Statistical analysis

Mealybug populations in vineyards are typically clumped in their spatial distribution (Geiger and Daane 2001); in our study, the incidence of *P. ficus*-infested bunches and number of VMB per bunch were not normally distributed. Data transformation, including  $\ln(x + 1)$ , was not able to normalize the distribution and homogenize the variance (Shapiro-Wilk test, goodness of fit  $P < 0.001$ ). Thus, non-parametric statistics were used. Differences in male catches, incidence of bunches infested by *P. ficus*, and the number of VMB per bunch among treatments (i.e., the tested sex pheromone dispenser Isonet® PF at three different densities per hectare and the untreated control), years, and study site were assessed using the Kruskal–Wallis test followed by the Steel–Dwass multiple comparison; a  $P$  value of 0.05 was selected as threshold to assess significant differences.

## Results

### Male captures

VMB male captures in pheromone-baited traps, on wine grape in 2017, were significantly lower in MD plots at all Isonet® PF-tested rates (300, 400, and 500 dispensers/ha) compared with the untreated plots ( $\chi^2 = 37.381$ ,  $d.f. = 3$ ,  $P < 0.0001$ ). This suggests that Isonet® PF, irrespective of the tested dispenser rate, can be effective in preventing VMB males from finding females (Fig. 1a).

Furthermore, a significant difference in the abundance of trapped VMB males was noted between the trial sites ( $\chi^2 = 13.325$ ,  $d.f. = 4$ ,  $P = 0.001$ ), with higher catches in Colognola ai Colli over other vineyards (Fig. 1b).

VMB total catches per trap also showed a significant difference between 2016 and 2017 ( $\chi^2 = 3.725$ ,  $d.f. = 1$ ,  $P = 0.05$ ) (Fig. 1c). In the trials carried out on table grape in 2017, as well as in all experiments conducted in 2016, negligible trap catches were recorded in all treatments. The total number of VMB male catches per trap up to harvest never exceeded 15 individuals in all treatments (Fig. 1). Due to the low number of *P. ficus* individuals caught during the entire

study period, no conclusions can be drawn on the efficacy of Isonet® PF, based on pest monitoring in table grapes.

### Incidence of bunches infested by *Planococcus ficus*

Bunch infestation by *P. ficus* was statistically different among treatments ( $\chi^2 = 65.131$ ,  $d.f. = 3$ ,  $P < 0.0001$ ) (Fig. 2a), with significantly higher infestation levels in the untreated control compared with vineyards where Isonet® PF dispensers were deployed at 300 ( $Z = 6.792$ ,  $P < 0.0001$ ), 400 ( $Z = 6.649$ ,  $P < 0.0001$ ), and 500 units ( $Z = 5.990$ ,  $P < 0.0001$ ) per ha, respectively. No significant differences were noted in the incidence of bunches infested by *P. ficus* among the three tested densities of Isonet® PF dispensers (300 vs. 400 dispensers  $\text{ha}^{-1}$ :  $Z = -0.031$ ,  $P = 1.000$ ; 400 vs. 500 dispensers  $\text{ha}^{-1}$ :  $Z = 1.200$ ,  $P = 0.626$ ; 300 vs. 500 dispensers  $\text{ha}^{-1}$ :  $Z = 1.093$ ,  $P = 0.693$ ) (Fig. 2a).

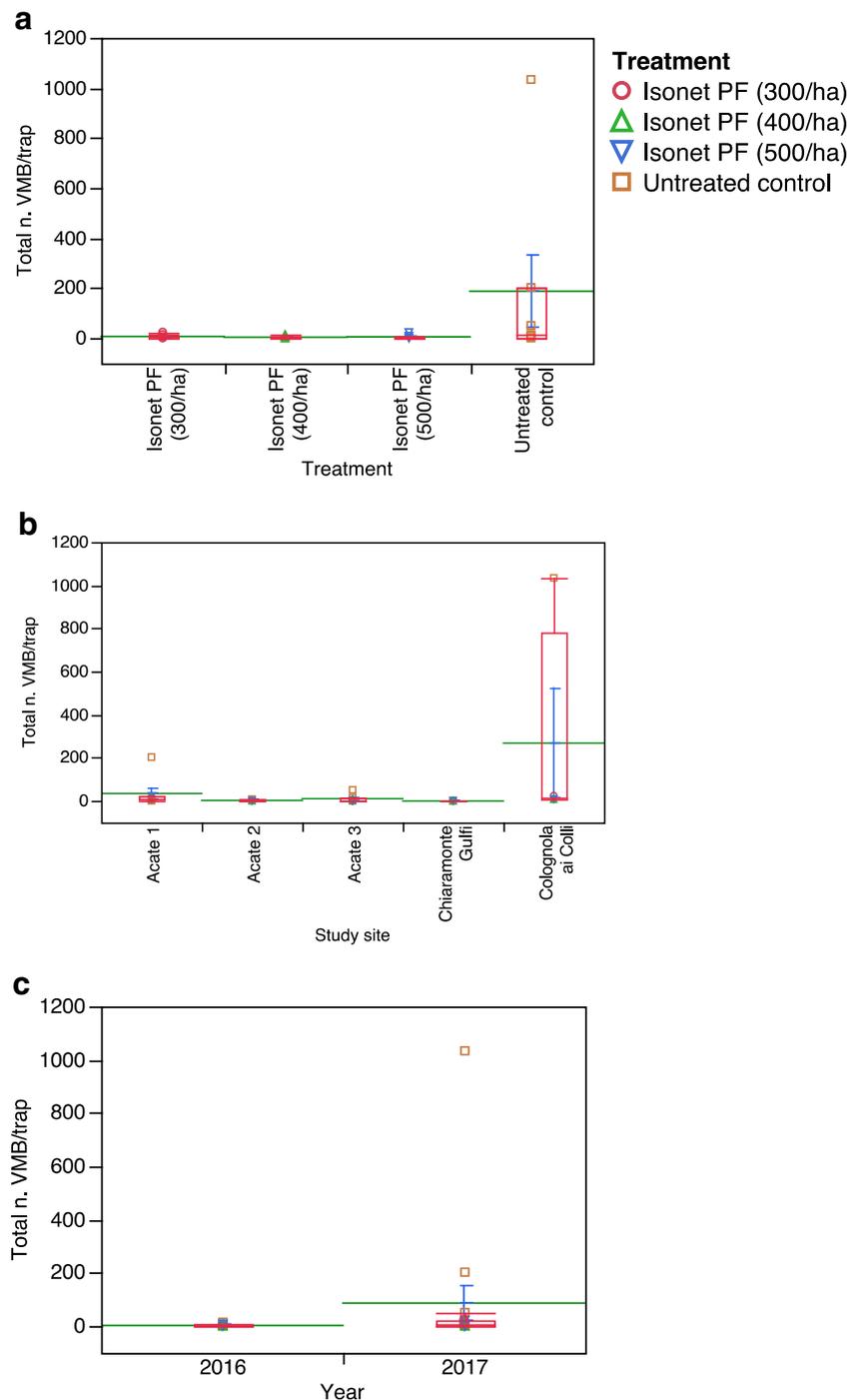
Furthermore, significant differences in the incidence of VMB infestation were detected among the trial sites ( $\chi^2 = 29.962$ ,  $d.f. = 4$ ,  $P < 0.0001$ ), where Chiaramonte Gulfi showed lower infestation incidence when compared to Colognola ai Colli ( $Z = -4.023$ ,  $P = 0.0005$ ), Acate 1 ( $Z = -4.544$ ,  $P < 0.0001$ ), Acate 2 ( $Z = -3.981$ ,  $P = 0.0007$ ), and Acate 3 ( $Z = -4.684$ ,  $P < 0.0001$ ) (Fig. 2b). Also, the incidence of *P. ficus* infestation on bunches varied between the two experimental years ( $\chi^2 = 43.318$ ,  $d.f. = 1$ ,  $P < 0.0001$ ) (Fig. 2c).

### Number of vine mealybugs per bunch

The number of VMB per bunch significantly differed among treatments ( $\chi^2 = 55.406$ ,  $d.f. = 3$ ,  $P < 0.0001$ ) (Fig. 3a), with significantly higher values in the untreated control than in the vineyards managed using Isonet® PF dispensers at 300 ( $Z = 5.989$ ,  $P < 0.0001$ ), 400 ( $Z = 6.640$ ,  $P < 0.0001$ ), and 500 units ( $Z = 5.639$ ,  $P < 0.0001$ ) per ha, respectively. No significant differences were noted in the number of VMB per bunch among the three tested densities of Isonet® PF dispensers (300 vs. 400 dispensers  $\text{ha}^{-1}$ :  $Z = -0.428$ ,  $P = 0.973$ ; 400 vs. 500 dispensers  $\text{ha}^{-1}$ :  $Z = 0.576$ ,  $P = 0.939$ ; 300 vs. 500 dispensers  $\text{ha}^{-1}$ :  $Z = 0.071$ ,  $P = 0.973$ ) (Fig. 3a).

Significant differences were observed in the number of VMB per bunch across the study sites ( $\chi^2 = 25.213$ ,  $d.f. = 4$ ,  $P < 0.0001$ ), with Chiaramonte Gulfi showing a lower number of VMB/bunch than Colognola ai Colli ( $Z = -4.211$ ,  $P = 0.0002$ ), Acate 1 ( $Z = -4.226$ ,  $P = 0.0002$ ), Acate 2 ( $Z = -3.609$ ,  $P = 0.0029$ ), and Acate 3 ( $Z = -4.199$ ,  $P = 0.0003$ ) (Fig. 3b). Lastly, significant differences in the number of VMB per bunch were found between the two study years ( $\chi^2 = 12.801$ ,  $d.f. = 1$ ,  $P = 0.0003$ ) (Fig. 3c).

**Fig. 1** Box plots showing differences in *Planococcus ficus* (VMB) male total catches among **a** mating disruption treatments, **b** study site, and **c** year. Red box plots indicate the median (line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median capture parameter. Green lines and blue T-bars indicate mean values and standard errors, respectively



### Sex pheromone release

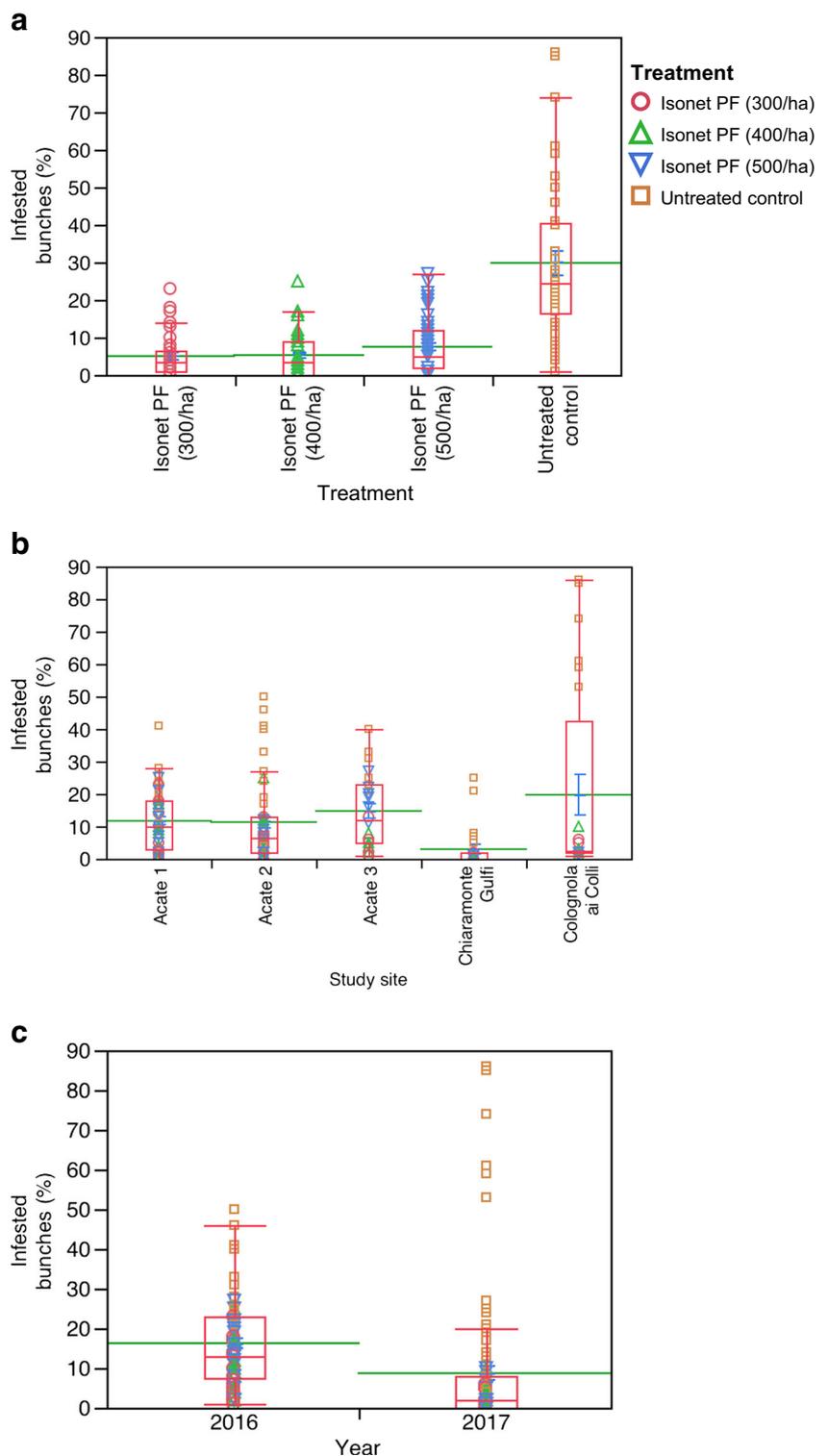
Figure 4 shows the load of racemic lavandulyl senecioate per dispenser in Sicilian study vineyards during the growing season 2016 (Fig. 4a) and 2017 (Fig. 4b). Results highlighted a rather homogenous release rate of the active ingredient during the whole grapevine-growing season.

In 2016, GC-MS analyses outlined a halving of the dispenser load in late July (i.e., from  $173.1 \pm 1.7$  on April 22 to

$97.5 \pm 1.8$  mg/dispenser), declining to  $59.9 \pm 4.4$  mg/dispenser in mid-late September (Fig. 4a).

Furthermore, the 2017 data show a comparable release trend, with a halving of the racemic lavandulyl senecioate content in late July (i.e., from  $169.3 \pm 2.2$  on April 11 to  $85.1 \pm 6.5$  mg/dispenser). Later, the active ingredient content dropped to  $41.6 \pm 6.3$  mg/dispenser on September 21; the residual content was  $29.0 \pm 2.3$  mg/dispenser on November 27 (Fig. 4b).

**Fig. 2** Box plots showing the differences in the incidence of *Planococcus ficus* (VMB)-infested bunches (%) among **a** different mating disruption treatments, **b** study site, and **c** year. Red box plots indicate the median (line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median infestation parameter. Green lines and blue T-bars indicate mean values and standard errors, respectively

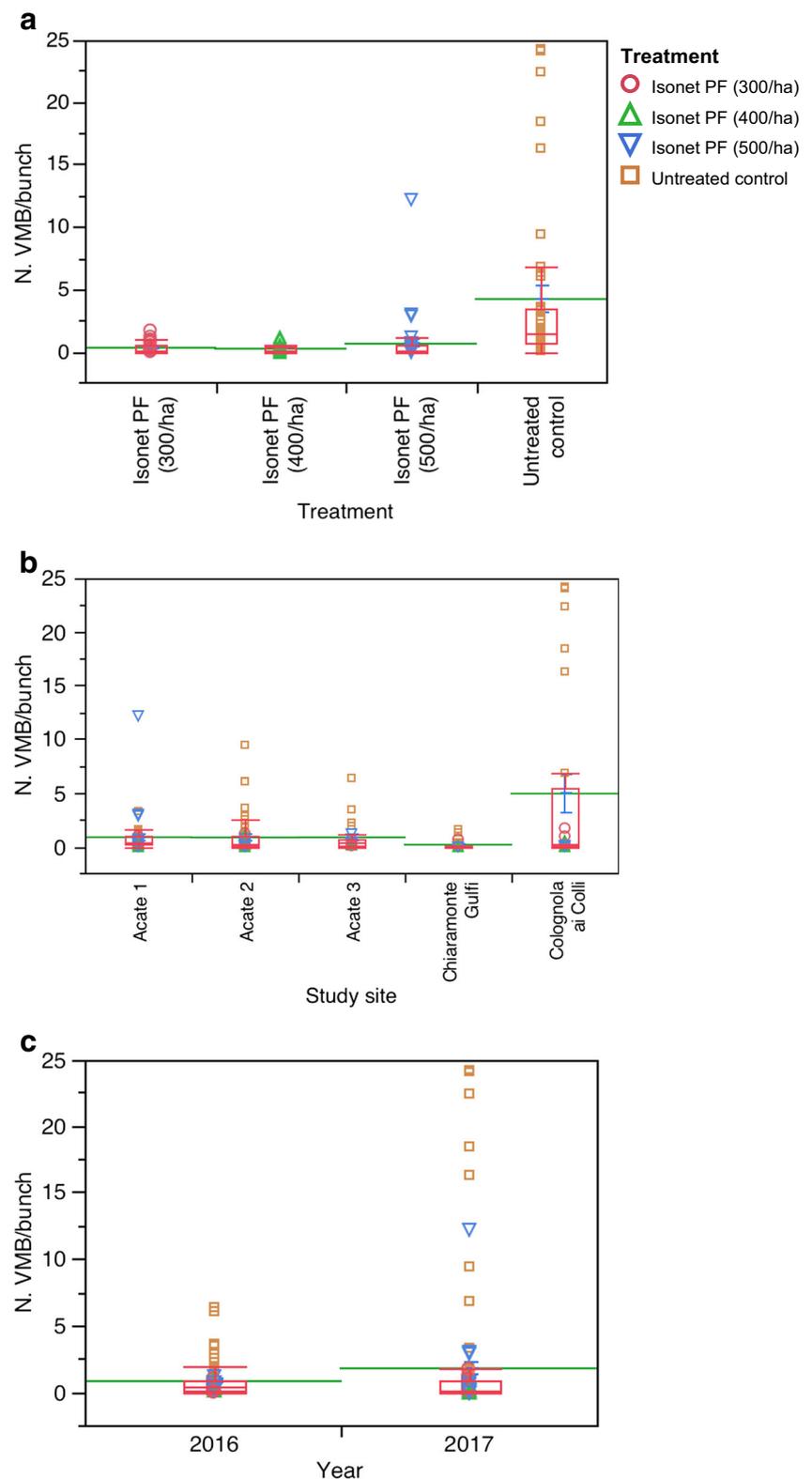


**Discussion**

The results show the successful use of MD dispenser Isonet® PF, which led to a significant reduction of VMB-infested bunches (%) and number of VMB per bunch, compared with the untreated vineyards, in both wine and table grape varieties.

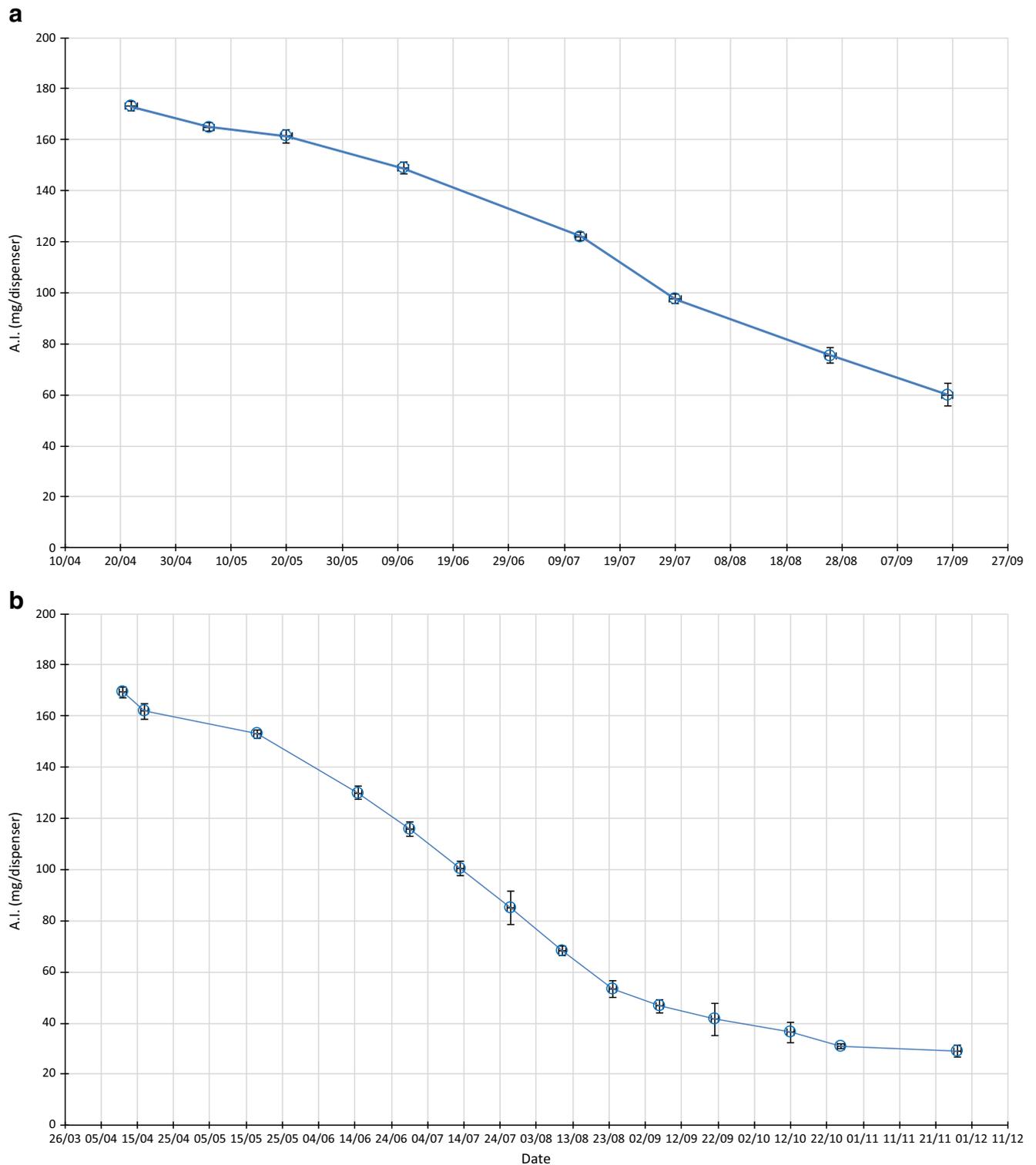
On the other hand, the strong variation in the abundance of male catches between 2016 and 2017 highlighted the poor reliability of male trapping for evaluating MD efficacy compared to VMB direct sampling on grapes. The history of MD against *P. ficus* is relatively recent. Walton et al. (2006) firstly tested MD efficacy against VMB. They conducted field

**Fig. 3** Box plots showing the differences in number of *Planococcus ficus* (VMB) per bunch among **a** different mating disruption treatments, **b** study site, and **c** year. Red box plots indicate the median (line) within each box and the range of dispersion (lower and upper quartiles and outliers) of the median infestation parameter. Green lines and blue T-bars indicate mean values and standard errors, respectively



assays in California, using a sprayable microencapsulated formulation of racemic lavandulyl senecioate. After three to four applications per year, the authors observed that VMB trap catches were lower, if compared to the control without MD.

In addition, as shown in our study, VMB density and crop damage significantly decreased. Moreover, *P. ficus* females exposed to MD treatments produced a limited number of eggs, if compared to control ones (Walton et al. 2006). Research



**Fig. 4** Load (mg/dispenser) of racemic lavandulyl senecioate in Sicilian vineyards during the growing season 2016 **(a)** and 2017 **(b)**. a.i. active ingredient

conducted in California vineyards showed that VMB can be successfully controlled with a pheromone dispenser density of 437 dispensers per hectare (93.75 g of a.i. per hectare) (Langone et al. 2014). Cocco et al. (2014) attempted MD

against VMB in Sardinian vineyards. The authors observed no significant differences among treatments in grape damage at harvest due to very low damage rates throughout all treatments in both field trials. However, they observed that hand-

applied reservoir dispensers (625 units/ha) releasing multiple plumes of the VMB racemic sex pheromone influenced pest density and modified age structure of the population. In addition, the effect was dose-dependent: MD at a pheromone dose of 62.5 g/ha did not affect VMB populations, while racemic lavandulyl senecioate at 93.8 g/ha reduced *P. ficus* density and also affected the population's age structure, showing a lower abundance of ovipositing females (Cocco et al. 2014). Later, Mansour et al. (2017b) showed that deploying 625 Checkmate® pheromone dispensers per hectare (150 mg of a.i.) led to the effective management of VMB populations in Tunisian table grape vineyards. Very recently, Cocco et al. (2018) pointed out that the MD dispenser tested led to a large reduction (i.e., minimum reduction 18.8%; maximum reduction 66.2%, depending on the year) in ovipositing females in MD vineyards, over a 3-year study period. In addition, this study highlighted that MD led to a longer pre-oviposition period by up to 12.5 days (Cocco et al. 2018).

It can be argued that MD on wide areas may affect behavioral traits of non-target arthropods. The use of MD as an eco-friendly IPM strategy has been widely promoted as insect pheromones are usually species-specific and non-toxic (Witzgall et al. 2010; Lucchi and Benelli 2018). However, Shapira et al. (2018) recently showed that managing VMB with MD may affect the abundance of *Anagyrus vladimiri* Triapitsyn (ex *Anagyrus* sp. near *pseudococci* (Girault)), the main parasitoid of *P. ficus*, outlining the urgent need of further research on this issue.

Interestingly, in the MD experiments reported in this study, all three tested pheromone doses (54, 72, and 90 g/ha, corresponding to 300, 400, and 500 Isonet® PF dispenser/ha, respectively) resulted in a significant reduction of VMB infestation in comparison to the untreated control. No significant differences in the incidence of bunches infested by *P. ficus* and number of VMB/bunch among the three tested pheromone doses were detected. In addition, a lower amount of VMB racemic pheromone was used, if compared to earlier research, where 93 g/ha (Sharon et al. 2016) or 62.5 and 93.8 g/ha (Cocco et al. 2014) of VMB racemic lavandulyl senecioate had been tested. It is well known that the efficacy of MD products depends on several factors (Neumann 1996; Ioriatti and Lucchi 2016). Among them, a key role is played by the size of the area where MD products are applied, the curve of pheromone release over time, and the pheromone concentration per area, which is to a great extent determined by the type of dispenser. In these MD experiments, the VMB sex pheromone release was rather constant during the grapevine-growing season (Fig. 4). In addition, the residual content of racemic lavandulyl senecioate detected in late November 2017 (Fig. 4b) ensures a good release during the mating period of overwintering

females. A constant and long-lasting pheromone release is extremely important for effective MD, especially for VMB, which in Mediterranean countries can develop four generations per year, with males searching for mates until late October (Cocco et al. 2014). It can be assumed that the constant and long-lasting release of pheromone over time by Isonet® dispensers and the large size of the study plots (at least 2 ha) enabled the authors to achieve a reliable MD efficacy during the whole growing season, including with a VMB pheromone dose of only 54 g/ha.

The findings in this research support earlier studies highlighting that MD can represent a reliable control method to significantly reduce *P. ficus* populations, particularly after one season characterized by low population density (Walton et al. 2006; Cocco et al. 2014; Mansour et al. 2017b). Sharing this theoretical background, Sharon et al. (2016) showed that in presence of high VMB populations, the MD effectiveness achieves a substantial increase only after 2 years of application. In other words, MD needs to be deployed over several seasons to result in acceptable population levels. However, in our field experiment conducted in Veneto region, the MD approach ensured an effective control of VMB, despite the high pest pressure characterizing the study site. In contrast to earlier observations (Lentini et al. 2008), the overwintering population in our study plots was mainly composed of young instars, with negligible presence of mated females (data not shown), which can contribute to VMB population build-up despite MD.

In this study, it was observed that there was a lower VMB infestation in the table grape variety Italia over the other varieties. This difference is probably due to the differences in historic pesticide strategies in the selected vineyards, since table grape vineyards have a lower tolerance threshold and are more subjected to insecticide treatments (Cabras and Angioni 2000; Ravelo-Pérez et al. 2009).

Overall, the results presented here outline a successful MD approach that should be taken into consideration within an IPM program to effectively manage VMB populations. Indeed, if it cannot be used alone as a fully efficient sustainable control option, especially in the case of high mealybug infestations, the use of MD in combination with an effective and safer insecticide could represent the appropriate way to ensure long-term field efficacy to control vine mealybugs in vineyards (Mansour et al. 2018). Contrary to earlier studies, this research provides evidence for successful MD programs for *P. ficus* control using low pheromone doses per hectare. This can help farmers to reduce costs in terms of both material to be purchased and labor for field application, as well as to adopt area-wide sustainable VMB control strategies for vineyards (Daane et al. 2018b).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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