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RESEARCH

Mating Disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in Vineyards Using Reservoir Pheromone Dispensers

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ABSTRACT. Mating disruption field experiments to control the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), were carried out in 2008 and 2009 in two commercial vineyards in Sardinia (Italy). The effectiveness of mating disruption was evaluated by testing reservoir dispensers loaded with 100 mg (62.5 g/ha) and 150 mg (93.8 g/ha) of the sex pheromone in 2008 and 2009, respectively. The number of males captured in pheromone traps, the *P. ficus* population density and age structure, the parasitism rate, the percentage of ovipositing females, and the crop damage were compared between disrupted and untreated plots. In both field trials, the number of males captured in mating disruption plots was significantly reduced by 86% and 95%, respectively. Mating disruption at the initial dose of 62.5 g/ha of active ingredient gave inconclusive results, whereas the dose of 93.8 g/ha significantly lowered the mealybug density and modified the age structure, which showed a lower percentage of ovipositing females and a higher proportion of preovipositing females. Mating disruption did not affect negatively the parasitism rate, which was higher in the disrupted than in the control plots (>1.5-fold). Crop damage at harvest was very low in both field trials and did not differ between treatments. Mating disruption was effective in wide plots protected with dispensers loaded with 150 mg of the sex pheromone, showing its potential to be included in the overall integrated control programs in Mediterranean wine-growing regions.

Key Words: sex pheromone, age population structure, population density, sexual communication, pest control

The vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), has become a key pest in many grape-growing regions of the Mediterranean Basin, South Africa, and California (Ben-Dov 1994, Godfrey et al. 2003, Walton et al. 2004). Mealybugs affect crop quality and yield by excreting honeydew that promotes the growth of sooty mold fungi and reduces photosynthetic activity in leaves (de Lemos Filho and Sousa Paiva 2006). Severe infestations render table grapes unmarketable, lower the quality of wine grapes, and increase the risk of ochratoxin A contamination on grapes, affecting the wine production chain (Chiotta et al. 2010). Moreover, *P. ficus* can vector a number of viral diseases, such as grapevine leafroll-associated virus and corky bark disease (Rosciglione and Gugerli 1989, Tanne et al. 1989), and is therefore considered a primary pest even at low population densities. As mealybugs are phloem feeders, improvements in cultivation techniques (e.g., increased irrigation and nitrogen fertilization, diffusion of vigorous grapevine clones) have led to a buildup of *P. ficus* populations (Dalla Montà et al. 2001), requiring up to three applications with organophosphates (A.L., personal observations).

Vine mealybug control programs in the most important wine-growing countries rely on multiple in-season applications of chemical insecticides, mainly chlorpyrifos, imidacloprid, and spirotetramat (Walton et al. 2004, Mansour et al. 2010). However, insecticide applications are often of limited effectiveness because the majority of vine mealybugs are in concealed locations (e.g., under the bark, beneath the bud scale) for most of the growing season (Lentini et al. 2008). Repeated applications of pesticides have also a detrimental effect to beneficial insects (Walton and Pringle 1999), although some new insecticides were found to be selective (Mansour et al. 2011). The most common natural enemies in the Mediterranean region are the encyrtid wasps *Anagyrus* spp. and some coccinellid species (Berlinger 1977). For these reasons, an effective control strategy should rely on integrated pest management programs. Moreover, wine producers have become more conscious that the production of high-quality wines with no chemical residues can be achieved using selective and environmentally friendly tools, with a limited use of chemical insecticides. Restrictions in the use of pesticides have led to the development of alternative techniques for pest control, as promoted by the Directive 2009/128/EC (European Union 2012). Synthetic sex pheromones are widely used as nonpesticide insect control methods (mating disruption, mass trapping) by manipulating insect behavior and disrupting sexual communication (Rodriguez-Saona and Stelinski 2009).

Mating disruption is effectively used in viticulture as an area-wide control tool in Germany, Italy, Spain, France, Switzerland, and Austria on approximately 140,000 ha to control the European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller), and the European grape berry moth, *Eupoecilia ambiguella* (Hübner) (Kast 2001; Ioriatti et al. 2008, 2011). This control strategy significantly reduced the use of insecticides and improved the quality of life in the grape-growing areas (Ioriatti et al. 2011). The sex pheromone of *P. ficus* has been recently identified as (*S*)-(+)-lavandulyl senecioate and commercially produced in its racemic form (Hinkens et al. 2001). The synthetic pheromone has been used to develop monitoring protocols (Millar et al. 2002) and a mating disruption program in California using a sprayable microencapsulated formulation (Walton et al. 2006). This formulation, associated with an insecticide application, was effective in reducing vine mealybug density and crop damage at harvest. The downside is that the microencapsulated pheromone required several applications during the growing season to keep the pheromone density at an effective concentration. Reservoir dispensers that generate a season-long mating disruption of the vine mealybug by a slow release rate of sex pheromone have been developed and commercialized. These devices are widely used in commercial vineyards in California and showed promising results in Argentina (Miano et al. 2011). In this study, we aimed to evaluate the season-long efficacy of reservoir pheromone dispensers in disrupting the sexual communication of *P. ficus*. Our objective was to determine the influence of mating

Table 1. Vineyard and plot sizes and horticultural characteristics of experimental vineyards in 2008 and 2009

	Vineyard surface (ha)	Plot surface (ha)	Cultivar	Training system	Soil management system
2008					
Vineyard 1	0.4	0.2	Vermentino	Overhead	Soil tillage
2009					
		2.5	Nuragus	Cane-pruned	Soil tillage
Vineyard 2	23	1.2	Nasco	Cane-pruned	Soil tillage
		1.5	Cabernet	Spur-pruned	Soil tillage

disruption on pest population density and age structure in commercial vineyards in the Mediterranean climatic conditions.

Materials and Methods

Description of Sites and Treatment Applications. The study was carried out in two commercial vineyards moderately infested by *P. ficus* in northwestern and southern Sardinia (Italy) in 2008 and 2009, respectively. Vineyard and plot surfaces, cultivars, training, and soil management systems are described in Table 1. In vineyard 1, two adjacent plots were established as mating disruption and untreated control plots, while three plots per treatment were identified in vineyard 2. Reservoir dispensers tested (CheckMate, Suterra Inc., Bend, OR, USA www.suterra.com) were evenly distributed in the disrupted plots at the manufacturer's recommended rate (625 dispensers per hectare) before the first male flight (mid-May and late April in 2008 and 2009, respectively) by attaching them to trellis wires at bunch height. Dispensers were 4 by 9 cm in size with a solid matrix membrane loaded with 100 mg (62.5 g/ha) and 150 mg (93.8 g/ha) of racemic lavandulyl senecioate (purity 97.66%) in 2008 and 2009, respectively. The release kinetics of pheromone dispensers loaded with 150 mg of active ingredient was evaluated by the manufacturer in different vineyards under Mediterranean climatic conditions, exhibiting a constant release rate of 0.88 mg/d and an expected life span of 5 mo. To avoid wind drifting of pheromone cues from mating disruption to control plots that could interfere with the experiment, pheromone-treated plots were located downwind of control plots with respect of the predominant wind direction and separated by a buffer zone 40 m wide in 2008 and 100 m wide in 2009.

With the purpose to control *L. botrana* infestations, vineyard 1 was sprayed with chlorpyrifos (Dursban, Dow AgroSciences Milan, Italy, www.dowagro.com) on 4 July 2008, while vineyard 2 was treated with lambda-cyhalothrin (Karate, Syngenta Crop Protection Milan, Italy, www.syngentacropprotection.com) on 29 July 2009. Pesticides were applied with commercial air-blast sprayers at medium volume (400–500 liter/ha) at the recommended lowest label rates (Dursban: 110 ml/hl; Karate: 100 g/hl). Insecticide sprays were unavoidable because growers were concerned about the high potential crop loss. However, as both control and mating disruption plots received the same insecticide applications, differences in mealybug density, population structure, and crop damage were attributed to the pheromone dispenser application. None of the vineyards had previously been protected with the mating disruption control strategy.

Male Captures in Pheromone-Baited Traps. Suppression of trap captures (shutdown) in the disrupted plots is an indicator of the effectiveness of the mating disruption strategy because dispensers alter the ability of males to locate pheromone sources. Therefore, the efficacy of this control method was evaluated by monitoring the male flight activity with bottle traps baited with the commercial rubber septum lures (Isagro Italia, http://www.isagro.it) loaded with 0.01 and 0.25 mg of sex pheromone in 2008 and 2009, respectively. Bottle traps consisted of a Plexiglas container (8 cm tall, 6 cm in diameter) with the pheromone dispenser hung underneath the cap and four holes (1.5 cm in diameter) on the upper part of the container wall to allow male access (Ortu et al. 2006). Pheromone traps were filled with soapy water to kill the trapped males and hung to trellis wires inside the canopy of three vines per plot. Captured males were counted every week from the date of the exposure (mid-May and mid-April in 2008 and 2009, respectively) to late October, replacing the monitoring pheromone plugs every 4 wk and soapy water weekly. CheckMate pheromone dispensers were not replaced during the experiments; to assess their persistence in disrupting the sexual communication, the percentage of reduction ($\%_{RED}$) of males captured in pheromone traps between disrupted and control plots was calculated on peak flight dates as:

$$\%_{RED} = [1 - (M_{MD}/M_C)] \times 100,$$

where M_{MD} is the number of male catches in mating disruption plots and M_C is the number of trap captures in control plots.

Mealybug Population Density, Age Structure, and Parasitism Rate. To assess the density of *P. ficus* populations within plots before the experiments, 100 randomly chosen vines in the central part of each plot were inspected and classified as having none-low mealybug density (absence of ants or trunk discoloration) or medium-high pest density (ant activity by the Argentine ant, *Linepithema humile* (Mayr), or *Lasius* spp. and blackened trunk due to sooty mold infestation; Walton et al. 2006).

After the deployment of pheromone dispensers, 20 vines per plot were randomly selected and sampled in mid-July, August, September, and October 2008. To better evaluate the influence of mating disruption on population density and age structure, the experimental design was improved in 2009 using three plots for each treatment and increasing the sampling frequency. Ten vines per plot were randomly chosen and sampled weekly in June and July and biweekly in August, September, and October, for a total of 30 vines per treatment on each sampling date. In both control and disrupted plots, all mealybug stages (first, second, and third instars and adult females, further separated into "preovipositing" and "ovipositing" females) were counted for 5 min per vine (3 min on trunk and cordons, and 2 min on canes, leaves, and grape bunches) and recorded separately (Geiger and Daane 2001). First-, second-, and third-instar stages and preovipositing females were differentiated by body length, amount of waxy secretion, and length of waxy filaments. The preovipositing stage included females with no ovisac that could either be virgin or mated females before starting oviposition. Ovipositing females were discriminated by the presence of ovisacs (cottonlike masses containing eggs). Population density was expressed as mean number of mealybugs per plant, while the age structure was calculated as the percentage of preimaginal stages, preovipositing or ovipositing females over the mealybug population.

Parasitoid mummies observed during the mealybug counts were collected and stored individually in plastic containers until parasitoid emergence. The only parasitoid emerging from mealybug mummies was *Anagyrus sp. near pseudococci* (Girault) (see Results), which parasitized second and third instars and young (preovipositing) females of the cogeneric species citrus mealybug, *Planococcus citri* (Risso) (Chandler et al. 1980). Therefore, the parasitism rate ($\%_{PAR}$) was estimated as:

$$\%_{PAR} = \frac{M}{II + III + F + M},$$

where M is the number of mealybug mummies, II is the number of second instars, III is the number of third instars, and F is the number of preovipositing females.

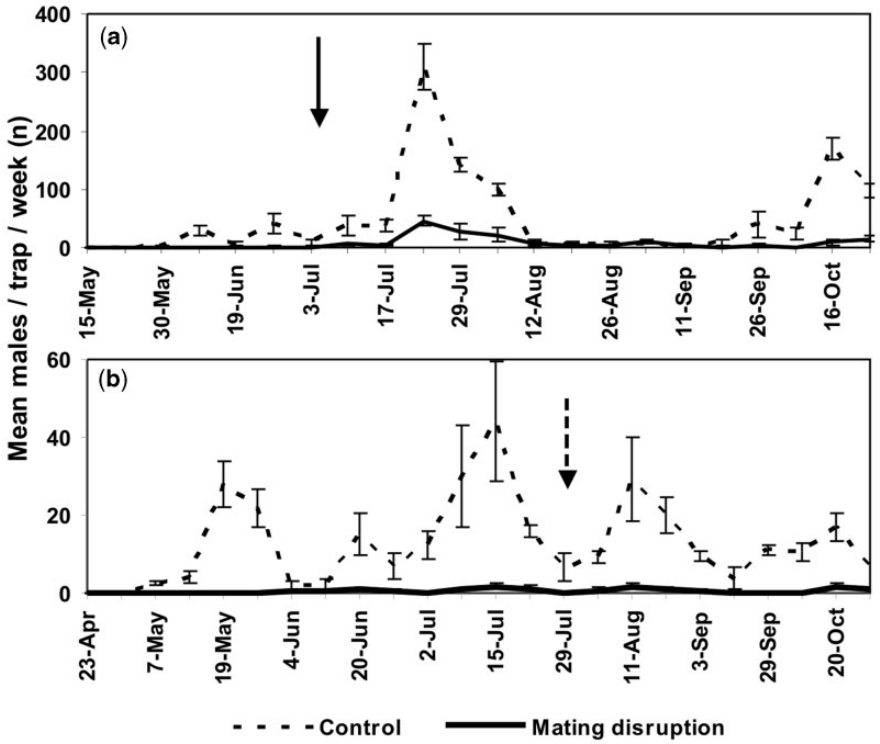

Fig. 1. Mean (±SE) number of *P. ficus* males captured in pheromone traps in control and mating disruption plots in 2008 (a) and 2009 (b). Black and dashed arrows indicate chlorpyrifos and lambda-cyhalothrin application dates, respectively. Treatments in each vineyard are significantly different by repeated measures analysis of variance ($P < 0.05$).

Effect of Mating Disruption on Female Mating. To determine the influence of mating disruption on vine mealybug mating, adult females with no ovisac (preovipositing) were randomly collected from both disrupted and control plots during the samplings. Females were placed individually on a sprouted potato inside Plexiglas containers (8 cm tall, 6 cm in diameter) secured with a double paper napkin and a rubber band to prevent male access. Mealybugs were kept in the laboratory under indoor conditions of temperature, relative humidity, and photoperiod until death, after which the percentage of females that produced ovisacs was determined.

Crop Damage. The damage on grapes was estimated at harvest by rating 40 bunches of each plot using the following categories: none (no mealybug damage), low (presence of honeydew, bunch salvageable), moderate (presence of honeydew and mealybugs, bunch partially salvageable), and severe (total bunch loss; Geiger and Daane 2001).

Statistical Analysis. In 2008, male captures in pheromone traps were compared throughout the season by repeated measure analysis of variance with treatments as fixed effects and traps as random effects ($P < 0.05$; PROC MIXED, SAS Institute 2008). The mealybug population density was evaluated date by date using analysis of variance throughout the experiment using vines as replicates ($P < 0.05$; PROC GLIMMIX, SAS Institute 2008). *P. ficus* monitoring and sampling was carried out from a single control and treated plot. Pseudoreplicates are sometimes unavoidable in mating disruption studies, due to the difficulties associated with multiple plots of adequate size in the same study area (Harari et al. 2007, Trona et al. 2009). The experimental design was modified in 2009, and differences between treatments were compared using plots as replicates. Therefore, the mean number of males captured per plot and the mean population density on vines per plot were compared throughout the season by repeated measure analysis of variance with treatments as fixed effects and plots as random effects (PROC MIXED, SAS Institute 2008). Prior to statistical analysis, numerical data were log transformed [log(x)] to meet the assumption of homogeneity of variance. In both trials, the population density of *P. ficus* before the application of pheromone dispensers, as categorized as none-low or medium-high pest densities, was compared between control and disrupted plots by G-test ($P < 0.05$; Sokal and Rohlf 1981). To evaluate differences in the overall age population structure, mealybug counts in both control and mating disruption plots were pooled by stage on each sampling date and compared by G-test ($P < 0.05$). When the age structure differed significantly, single G-tests were performed in each sampling date to determine which mealybug stage contributed to the significant difference. As the principle underlying mating disruption is to interfere with the pest mating, changes in the proportion of the ovipositing females over the total female population were investigated in both treatments by G-test ($P < 0.05$), with the aim to point out the influence of mating disruption on *P. ficus* reproduction. The percentage of field-collected ovipositing females, the parasitism rate, and the bunch damage at harvest were also compared by G-test ($P < 0.05$).

Results

Male Trap Captures. In the control plot of vineyard 1, male captures started in June and showed four peaks: 10 and 25 June, 23 July, and 16 October 2008 (Fig. 1a). In 2009, captures in control plots were lower than in vineyard 1, but with well-defined peaks on 19 May, 20 June, 15 July, 11 August, and 20 October (Fig. 1b). In disrupted plots of both trials, high reductions in trap captures were achieved, with $\%_{RED}$ in the peak dates ranging from 85 to 100%. The seasonal patterns of male captures were significantly lower in disrupted than in control plots in 2008 and 2009 (trial 1: $F_{84,1} = 203.42$, $P < 0.001$; trial 2: $F_{86,1} = 120.66$, $P < 0.001$), with a season-long reduction in male captures of 86 and 95% in vineyards 1 and 2, respectively.

Population Density. Before the deployment of pheromone dispensers, the percentages of vines with medium-high mealybug density were 28% and 33% in disrupted and control plots of vineyard 1. In 2009, the proportion of highly infested plants was 16% in plots

protected with mating disruption and 14% in untreated plots. The percentage of medium- or highly infested vines did not differ significantly between disrupted and control plots in both vineyards (*G*-test, vineyard 1: $P = 0.839$; vineyard 2: $P = 0.974$).

In vineyard 1, the pest population density in July was 19.2 and 29.3 mealybugs per vine in control and disrupted plots, respectively, with no significant difference between treatments ($F_{19,1} = 0.94$, $P = 0.346$; Fig. 2). The *P. ficus* density decreased to low levels in August and September and was not significantly different between treatments (August: $F_{19,1} = 0.02$, $P = 0.892$; September: $F_{19,1} = 0.11$, $P = 0.747$), but increased to significantly higher levels in the control plot compared with the disrupted plot in October ($F_{19,1} = 6.61$, $P = 0.019$; Fig. 2). In 2009, the population density of *P. ficus* throughout the season was significantly higher in control plots than in those protected with mating disruption dispensers ($F_{56,1} = 4.37$, $P = 0.041$; Fig. 3). The mean number (±SE) of mealybugs per vine across all the sampling dates was 16.0 ± 2.8 and 9.9 ± 1.2 in control and pheromone-treated plots, respectively, with a mean reduction in population density of 38%. The *P. ficus* density was higher in control than in disrupted plots in all sampling dates, except on 4 June, 15 July, and 9 October. The population density increased to a maximum on 25 June in both treatments, when 41 and 29 mealybugs per vine were observed in control and disrupted plots, respectively (Fig. 3). In July, the mean number of mealybugs per plant observed in 5-min counts ranged from 12 (mating disruption plots, 29 July) to 29 (control plots, 22 July).

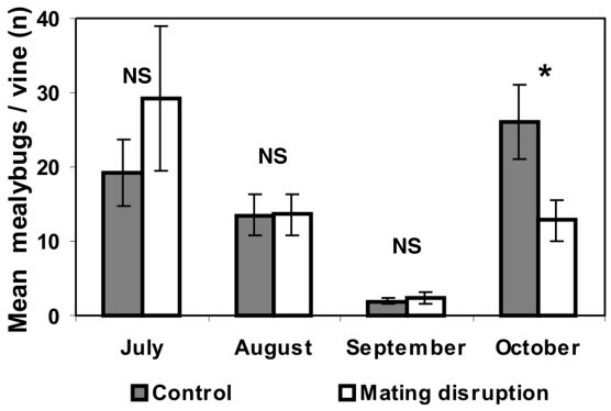

Fig. 2. Mean (±SE) number of vine mealybugs observed in control and disrupted plots during 5-min counts on 20 randomly selected vines in 2008. Treatment bars within each sampling date with an asterisk are significantly different by analysis of variance ($P < 0.05$); NS, not significant.

In August, September, and October, the *P. ficus* population density decreased to approximately 3 mealybugs per vine in both treatments. The progressive reduction of the population density might also have been affected by the application of lambda-cyhalothrin in late July to control *L. botrana* infestations. However, biotic and abiotic factors may have played a main role in reducing the pest population because 65% of mealybugs were observed beneath the bark in the sampling date before the insecticide application.

Age Structure. Preimaginal stages were the most numerous stage in both vineyards and treatments, whereas ovipositing females were the least represented population stage (Figs. 4 and 5). The wide differences in sizes among mealybug stages were due to the high fecundity of *P. ficus* females (up to 400 eggs per females) and the natural mortality caused by biotic and abiotic factors that reduced the more mature stages. In 2008, the age population structure differed significantly between untreated and pheromone-protected plots on all sampling dates. Except in September, a lower percentage of preimaginal stages and a higher proportion of preovipositing females were observed in disrupted plots (Fig. 4). In the disrupted plot, the percentage of ovipositing females over the total female population was significantly lower in July and higher in August, compared with the control plot (Fig. 6). In 2009, the age population structure of *P. ficus* in disrupted plots was significantly affected by the control strategy on all but two sampling dates, 29 September and 20 October. The percentage of preovipositing females was higher in disrupted than in control plots on all sampling dates except for 4 June, although significant differences were observed in 7 of the 14 samplings (Fig. 5). The percentage of ovipositing females over the total female population was significantly higher in control than in mating disruption plots in eight sampling dates, and lower only on 10 June, compared with pheromone-protected plots (Fig. 6).

Parasitism Rate. The parasitism rate observed during the 5-min sample in 2008 ranged from 0.9 to 3.0%, with no significant difference between treatments (data not shown). The mating disruption technique did not affect but rather enhanced the parasitoid activity in 2009, when a total of 96 and 74 mummies were observed in disrupted and control plots, respectively (Table 2). In the period July–October 2009, the mean parasitism rate was significantly lower in control (2.8%) than in disrupted plots (5.1%). The parasitism rate was significantly higher in pheromone-treated plots in July and August, whereas it did not differ significantly in September and October. All emerged parasitoids were identified as *A.* sp. near *pseudococci* (Triapitsyn et al. 2007).

Mated Females. In 2008, 74% of the 57 females collected in the disrupted plot produced offspring, and there were no significant differences with those collected from the control plot (81% of 80 specimens; $P = 0.294$). In contrast, the percentage of ovipositing females collected in mating disruption plots in 2009 (29% of 69 specimens) was significantly lower than that of control plots (85% of 67 females; $P < 0.001$).

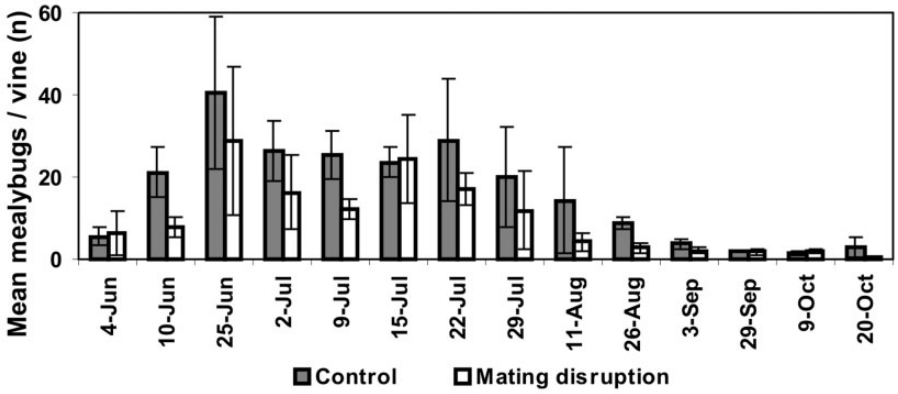

Fig. 3. Mean (±SE) number of vine mealybugs observed in control and disrupted plots during 5-min counts on 30 randomly selected vines per treatment in 2009. Treatments are significantly different by repeated measures analysis of variance ($P < 0.05$).

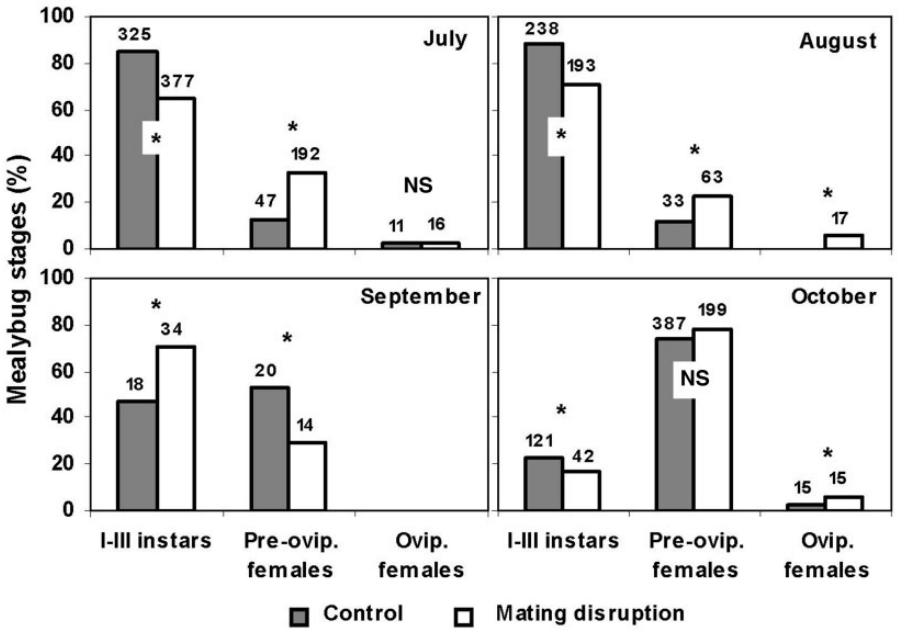

Fig. 4. Age population structure of *P. ficus* from 5-min counts of field populations in 2008. Numbers above bars indicate sample size for each mealybug stage. Asterisks between or above bars indicate significant differences in the percentage of mealybug stages between treatments (*G*-test, $P < 0.05$); NS, not significant.

Crop Damage. In 2008, the crop damage was very low, being the percentage of uninfested clusters of 75% in both plots. The proportion of low-damaged bunches was 20 and 25% and that of moderately infested clusters was 5 and 0% in control and disrupted plots, respectively. No significant difference in the cluster infestation between untreated or pheromone-protected plots was found ($P = 0.213$). In 2009, *P. ficus* infestation on clusters was lower than in 2008 and no damage was found at harvest in disrupted and control plots.

Discussion

The 2-yr mating disruption experiments provided valuable information on the effectiveness of CheckMate dispensers to control the vine mealybug at different pheromone doses and plot sizes in the Mediterranean climate. The disruptive effect on males, assessed with monitoring pheromone traps, was comparable with that obtained using three or four applications of sprayable microencapsulated formulation (Walton et al. 2006). Male captures in disrupted plots were reduced by 86–95% in vineyards 1 and 2, respectively. CheckMate dispensers, deployed in April–May before the first male flight, reduced consistently male captures in monitoring traps until September, showing a field lifetime of approximately 5 mo. However, a marked disrupting activity was observed in the last male peak flight in October ($\%_{RED} = 95\%$ and 85% in trials 1 and 2, respectively), before the overwintering period. As the vine mealybug overwinters mainly as mated female (Lentini et al. 2008), an effective mating disruption at the end of the vine growing season could lead most females to overwinter as virgin females rather than as mated females, with a delay of population buildup the following spring.

In mating disruption studies of some lepidopteran species, male trap shutdown was found to be positively correlated with the reduction of mated females (Stelinsky et al. 2007). However, male moth catch reduction did not always correspond to a decrease in crop damage (Michereff Filho et al. 2000, Atanassov et al. 2002). In our studies, the mating disruption was effective in 2008 in reducing the population density of *P. ficus* only before the pest overwintering (October). Conversely, mating disruption reduced significantly the pest density in 2009, when the mean number of mealybugs per vine observed throughout the season was 16.0 and 9.9 in control and pheromone-protected plots, respectively, with a mean density reduction of 38%. The better performance of mating disruption may be attributed to the higher pheromone concentration in the dispensers (150 mg per dispenser in 2009 and 100 mg per dispenser in 2008) and the wider size of disrupted plots. In fact, the reduction of pest population was unsatisfactory in the smallest plot (0.2 ha). Our results are in accordance with findings of Walton et al. (2006), who hypothesized that plot sizes affected treatment differences. In general, pest control mediated by pheromones is more effective in area-wide applications because the migration of mated females from surrounding untreated areas and the lower pheromone coverage along plot edges due to wind reduce the effectiveness of mating disruption in small plots. However, as vine mealybug females are apterous and unable to migrate, the reduced efficacy in small plots can be attributed to the inadequate pheromone density along the borders of disrupted blocks. Nonetheless, the effectiveness of the mating disruption technique in plots of 1.5 ha has been demonstrated for a similar pest, the California red scale, *Aonidiella aurantii* (Maskell) (Vacas et al. 2009), which the dispersal stage (first-instar nymphs) is apterous as well. In 2009, mating disruption significantly affected the age population structure, determining a lower season-long percentage of ovipositing females in disrupted plots. Rearing preovipositing females collected from the field under laboratory conditions gave similar results. In fact, the percentage of ovipositing females from pheromone-treated plots (29%) was significantly lower than that of control plots (85%). As virgin females live longer than mated ones (Waterworth et al. 2011), mating disruption could have modified the population structure by delaying the mating age, which caused the increase of the percentage of preovipositing females and reduced the proportion of ovipositing females.

The mating disruption technique is an effective control strategy even if the male mate-finding ability is not completely inhibited. In many lepidopteran species, aged females have a reduced reproductive output, caused by reduced fertility, fecundity, or both, or a decreased willingness to mate (Torres-Vila et al. 2002, and references therein). No studies have been carried out on the reproductive effects of delayed mating in *P. ficus* females. However, aged females of the citrus

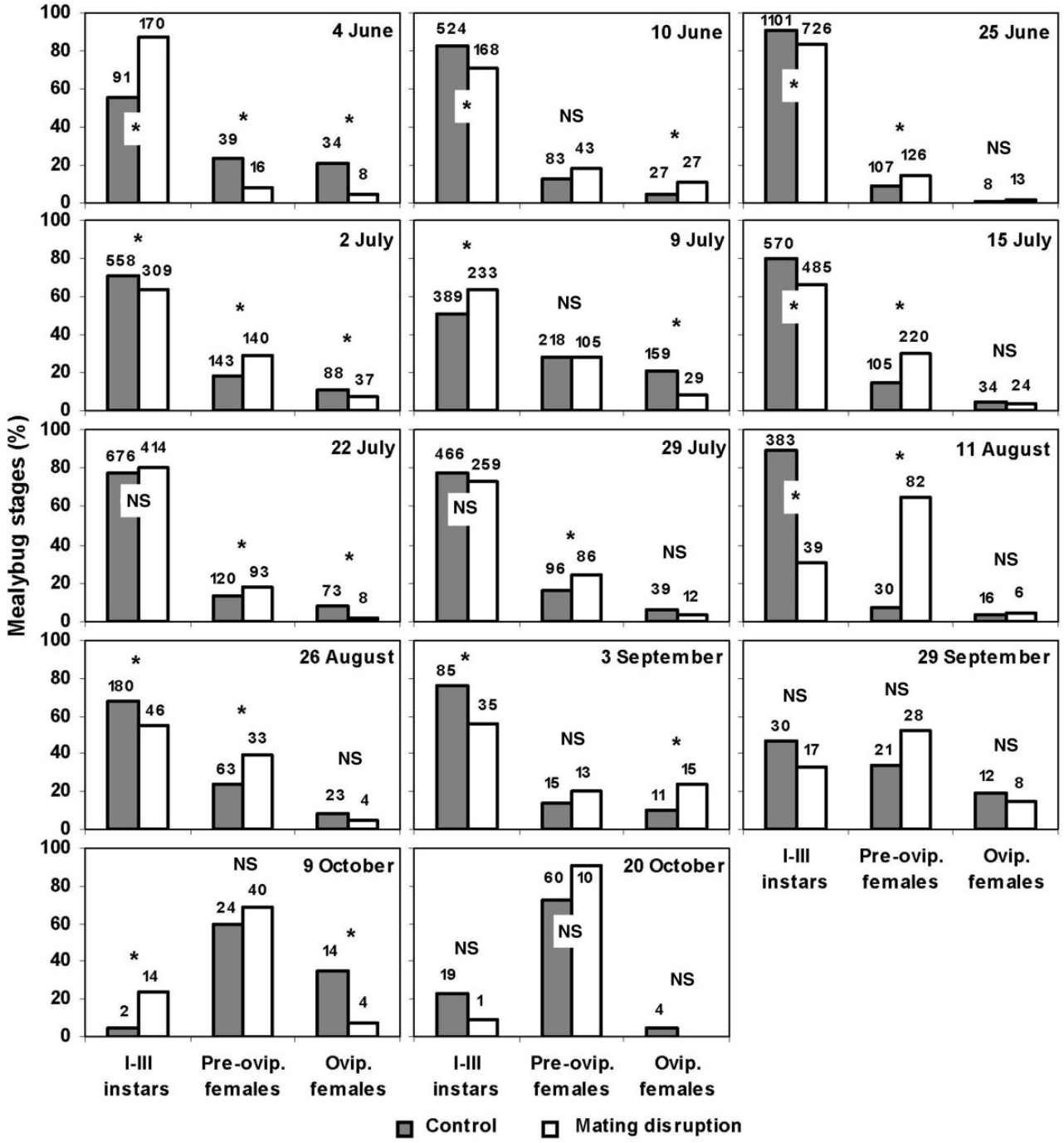

Fig. 5. Age population structure of *P. ficus* from 5-min counts of field populations in 2009. Numbers in and above bars indicate sample size for each mealybug stage. Asterisks between or above bars indicate significant differences in the percentage of mealybug stages between treatments (*G*-test, $P < 0.05$); NS, not significant.

mealybug produced fewer offspring than young females (Ross et al. 2011). The fecundity of females in disrupted and control plots was not investigated in our study, but Walton et al. (2006) observed that females in mating disruption plots produced fewer eggs. Delayed mating has a strong influence on the growth rate of multivoltine populations because the preoviposition period is extended and, consequently, the mating age is raised. For example, mating delay of 4–6 d caused a significant reduction of the population growth rate in the koa seedworm, *Cryptophlebia illepida* (Butler), although the percentage of mated females did not differ between control and delayed treatment (Jones and Aihara-Sasaki 2001).

The parasitism rate observed during the 2-yr field trials was very low, and *A.* sp. near *pseudococci* was the only parasitoid emerged from mealybug mummies. *A. pseudococci* has been reported to be attracted by *P. ficus* synthetic pheromone (Millar et al. 2002), and recent studies showed that the *P. ficus* sex pheromone has a kairomonal effect on *Anagyrus* sp., increasing the wasp parasitism rate (Franco et al. 2011). For these reasons, mating disruption could interfere on the host-finding process of *Anagyrus* sp. females, reducing the natural biological control. However, our results showed a higher parasitism rate (>1.5-fold) in mating disruption than in control plots, and no negative effects

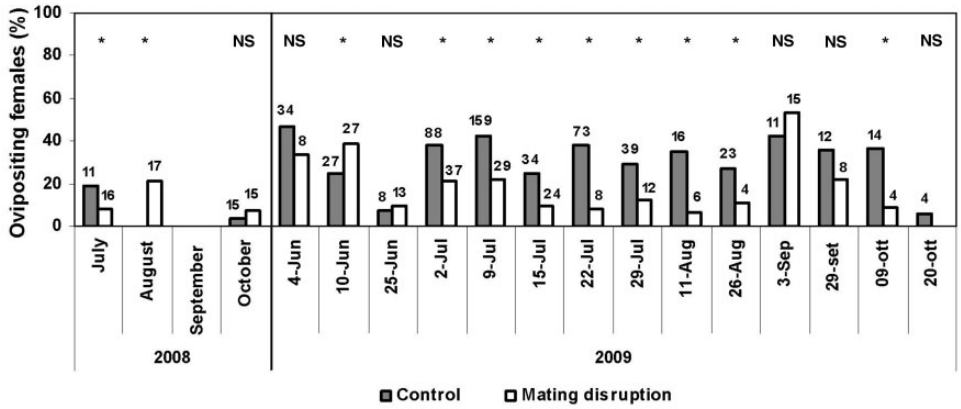

Fig. 6. Percentage of *P. ficus* ovipositing females over the total females in mealybug populations in control and disrupted plots in 2008 and 2009. Numbers and asterisks above bars indicate sample size for each mealybug stage and significant differences in the percentage of ovipositing females between treatments (*G*-test, $P < 0.05$), respectively; NS, not significant.

Table 2. Parasitism rate of vine mealybugs observed during 5-min counts on 30 randomly selected vines per treatment in 2009

Date	Treatment	Parasitizable mealybugs (*n*)[a]	Parasitized mealybugs (*n*)	Parasitism rate (%)	*P* value[b]
June	Control	2,008	0	0.0	
	Mating disruption	1,487	0	0.0	
July	Control	1,838	19	1.0	
	Mating disruption	1,432	28	1.9	0.032
Aug.	Control	468	15	3.1	
	Mating disruption	193	36	15.7	<0.001
Sept.	Control	145	19	11.6	
	Mating disruption	92	20	17.9	0.145
Oct.	Control	105	21	16.7	
	Mating disruption	65	12	15.6	0.839
July–Oct.	Control	2,556	74	2.8	
	Mating disruption	1,782	96	5.1	<0.001

[a]Second instars, third instars, preovipositing females.
[b]*P* values refer to significant differences between treatments by *G*-test.

of mating disruption on biological control of *P. ficus* were observed, in accordance with findings of Walton et al. (2006).

No significant crop loss due to *P. ficus* infestations was observed during our trials because of the low mealybug density at harvest observed in control and disrupted plots in both years. This was because the moderate *P. ficus* infestation at the beginning of the growing season was further lowered by insecticide applications against *L. botrana* in both years. However, control of the vine mealybug is crucial also at low population density because of its ability to vector the grapevine leafroll-associated virus, which causes severe crop loss (up to 40–60%) and reduces the quality of must and wine (Carstens 2011, and references therein).

CheckMate dispensers loaded with 150 mg of the sex pheromone at the application rate of 625 per hectare significantly reduced *P. ficus* infestations in the Mediterranean climate, characterized by high summer temperature and windiness. The nearly complete trap shutdown, the reduced population density, and percentage of ovipositing females observed in disrupted plots in 2009 show the potential of mating disruption as a promising tool to control the vine mealybug. Mating disruption control programs are more effective at low or moderate population densities (Cardé and Minks 1995), and might not be able to effectively control high vine mealybug infestations, requiring a complementary insecticide application. An integrated control strategy could be derived from a pest management tactic commonly adopted against *L. botrana*, in which the season-long mating disruption control is combined with an insecticide application against the first generation (Charmillot and Pasquier 2000, Louis and Schirra 2001), when the population density exceeds the threshold of 5–10% of infested clusters (Ioriatti et al. 2011). Similarly, a *P. ficus* population density threshold above which mating disruption should be integrated with an insecticide application could be determined. The pheromone-mediated control of *P. ficus* should be considered together with other grapevine pest management strategies rather than as a stand-alone treatment, and included in the overall integrated control programs in Mediterranean wine-growing regions. Moreover, mating disruption is at this moment the most suitable control strategy against *P. ficus* infestations in organic viticulture, especially when integrated with rational practices (i.e., pruning, nitrogen fertilization, and irrigation) that reduce the grapevine vigor and contribute to reduce pest populations. Further experiments will be needed to investigate the effects of mating disruption on *P. ficus* infestations over successive years and define the population threshold that would require a supplemental insecticide application to reduce the pest density and prevent grape damage.

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