Research Article

Received: 21 July 2017 Revised: 23 November 2017 Accepted article published: 28 February 2018 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.4883

Does mating disruption of *Planococcus ficus and Lobesia botrana* affect the diversity, abundance and composition of natural enemies in Israeli vineyards?

Idan Shapira,[a] Tamar Keasar,[a*] Ally R Harari,[b] Efrat Gavish-Regev,[c] Miriam Kishinevsky,[d] Hadass Steinitz,[b] Carmit Sofer-Arad,[e] Maor Tomer,[e] Almog Avraham[e] and Rakefet Sharon[e]

Abstract

BACKGROUND: Mating disruption (MD) employs high doses of a pest's synthetic sex pheromone in agricultural plots, to interfere with its reproduction. MD is assumed to have few behavioral effects on non-target arthropods, because sex pheromones are highly species-specific and non-toxic. Nevertheless, some natural enemies use their host's sex pheromones as foraging cues, and thus may be attracted to MD plots. To investigate this hypothesis, we compared parasitoid and spider assemblages in paired plots in five Israeli vineyards during 2015. One plot was MD-treated against two key pests, *Lobesia botrana* (Denis & Schiffermüller) and *Planococcus ficus* (Signoret). Both plots were insecticide-treated as needed. Natural enemies were suction-sampled and collected from pheromone-baited monitoring traps.

RESULTS: The total abundance, species diversity and species composition of most natural enemies were unaffected by MD. An important exception involved *P. ficus*' main parasitoid, *Anagyrus* sp. nr. *pseudococci* (Girault). *Anagyrus* sp. nr. *pseudococci* females were mainly captured in control plots, while male captures were low and not influenced by MD. Parasitized *P. ficus* occurred only in MD plots.

CONCLUSION: Non-target effects of MD involved mostly *A.* sp. nr. *pseudococci* females and hardly affected other natural enemies. These findings support the use of MD as an environmentally friendly pest management strategy.

Supporting information may be found in the online version of this article.

Keywords: *Anagyrus* sp. nr. *pseudococci*; arrestment; parasitoid; spider; sex pheromone

1 INTRODUCTION

Mating disruption (MD) is an environmentally friendly approach to pest control, based on flooding of agricultural plots with the synthetic sex pheromone of a crop pest. This impairs the ability of the males to find their mating partners, and interferes with pheromone emission, fecundity and longevity in the females.[1] Sex pheromones comprise blends of volatiles that are highly species-specific, and thus MD is generally assumed to have limited behavioral effects on non-target organisms.[2] Nevertheless, some insects are known to respond to sex pheromones of other species to which they are closely related.[3,4] Others are arrested by or attracted to the sex pheromone of their prey, which they use as a foraging cue.[5,6] One well-studied example of this phenomenon involves several parasitoids of aphids, which are attracted to their hosts' sex pheromones.[6,7] Another example involves predatory insects [*Elatophilus spp*. (anthocorid bugs), *Hemerobius* lacewings and *Aplocnemus* spp. (beetles)] that were attracted to traps baited with sex pheromones of their prey (pine bast scales).[8,9] Similarly, monitoring traps that were baited with the pheromone of the lepidopteran pest *Helicoverpa armigera* (Hübner) attracted (among other bycatch) lady beetles, which are potential predators on immature stages of this moth.[10]

The attraction of natural enemies to MD pheromones may interfere with agricultural pest control in some scenarios, and

* *Correspondence to: T Keasar, Department of Biology and Environment, University of Haifa – Oranim, Tivon 36006, Israel. E-mail: tkeasar@research.haifa.ac.il*

a *Biology and Environment, University of Haifa – Oranim, Tivon, Israel*

b *Entomology, Volcani Center, Bet Dagan, Israel*

c *The National Natural History Collections, The Hebrew University, Jerusalem, Israel*

d *Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel*

e *Northern R&D, MIGAL-Galilee Technology Center, Kiryat Shmona, Israel*

enhance it in other cases. MD might impede biological control by attracting natural enemies to the pheromone dispensers, and consequently reducing their densities on the crop plant foliage.[11] Alternatively, the pheromone cue may arrest natural enemies, inhibit their dispersal, and enhance their foraging in the vicinity of the pheromone source, and thus potentially contribute to pest control efficiency. Evidence for the latter hypothesis has accumulated concerning the encyrtid parasitoid *Anagyrus pseudococci sensu lato* [*Anagyrus pseudococci* (Girault) and the closely related *Anagyrus* sp. nr. *pseudococci*]. This wasp is an important natural enemy of mealybugs. The synthetic sex pheromone of the vine mealybug *Planococcus ficus* (Hemiptera: Pseudococcidae) attracted the parasitoid when pheromone dispensers were placed in agricultural plots with no MD,[5,12] and in olfactometer trials.[5] Sentinel hosts that were placed next to a single pheromone source, in agricultural fields that were not MD-treated, were parasitized by *A. pseudococci* more often than controls that were placed away from pheromone dispensers.[13] A study that compared the rates of parasitism on *P. ficus* between insecticide-treated plots and MD-treated plots found no effect of MD.[14] Another similar study, in contrast, found that parasitism rates were higher in MD plots than in control plots in the second of two experimental seasons.[15] A third study yielded the qualitative finding that parasitism levels of *P. ficus* were consistently higher in MD vineyards compared with controls.[16] These findings fueled the suggestion that the sex pheromone of *P. ficus* arrests the dispersal of *A. pseudococci* wasps, causing them to forage more intensively in pheromone-saturated areas and consequently to parasitize more hosts.[13]

It is still largely unknown whether the behavior of other natural enemies is affected by the synthetic pheromones used to disrupt the mating of mealybugs, or, in fact, of any other agricultural pest. Here, we applied this question to assemblages of arthropod natural enemies in wine-producing vineyards. A few previous studies conducted in vineyards compared the composition of arthropods in plots that were treated either with MD or with conventional insecticides. Gallardo *et al.* found that vineyards treated with MD against *L. botrana* received fewer insecticide applications than vineyards without the MD treatment.[17] This, in turn, led to a resurgence of secondary vineyard pests [the coleopteran *Altica ampelophaga* (Guérin-Méneville) and the hemipterans *Planococcus citri* (Risso), *Jacobiasca lybica* (Bergevin and Zanon) and *Aphis gossypii* (Glover)]. Aslan compared parasitism rates of *L. botrana* in vineyards treated with MD and *Bacillus thuringiensis* (Berliner) versus insecticide-treated control vineyards over 2 years.[18] The two types of vineyard were dominated by the same parasitoid species, but parasitism was more frequent in the MD plots than in the control plots in the second year. Parasitism rates on the north American grape berry moth *Endopiza viteana* (Clemens) were also higher in MD vineyards than in vineyards with intensive insecticide applications.[19] Similar findings were reported from orchards of pears, apples and peaches, where plots treated with MD had higher levels of pest parasitism and predation than insecticide-treated plots.[20–22] However, as a consequence of the design of the above studies, it is not possible to separate the effects of MD on parasitoids and predators from those of reduced insecticide use. Thus, the effects of MD *per se* on communities of natural enemies remain unclear.

In the current study, we focused on MD applied against two major vineyard pests, and tested its community-level effects on parasitic and predatory arthropods, while using the same insecticide schedule in both MD-treated and no-MD control plots. One of the pests, the vine mealybug *Planococcus ficus* (Hemiptera: Pseudococcidae), damages grapes by secreting honeydew that attracts molds, and by feeding on leaves and fruit. It also acts as a vector of viral diseases of grapevines.[16,23] The second pest, the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae), causes direct economic damage by feeding on the plant, and indirect damage by increasing the susceptibility of the berries to gray mold, *Botrytis cinerea* (Pers).[24,25] Grape cluster loss from *L. botrana* may reach 25% and 60% in table and wine grapes, respectively. Both pests are polyphagous and exhibit an aggregated spatial distribution.[23,26] They are effectively controlled using MD, which delays mating and reduces reproductive performance in the females.[27,28] We tested whether the MD treatment to control these pests influences the abundance, diversity and composition of parasitoid wasps and spiders in wine-producing vineyards located in the eastern Mediterranean basin. We studied multi-species assemblages of parasitoids and spiders, which included specialist natural enemies of vineyard pests and of non-pest arthropods, as well as species that are generalist foragers.

2 MATERIALS AND METHODS

2.1 Study sites

The study was conducted during the 2015 crop season in five wine-producing vineyards, planted on brown grumosol soil and nested in an agro-ecological matrix of rural northern Israel. The area is characterized by a Mediterranean climate, with cool winters and hot, dry summers. Annual rainfall is ca. 600 mm, mostly occurring between October and April. The vineyards were composed of Cabernet Sauvignon vines planted on at least 6 ha of land. Insecticides and herbicide were applied to all vineyards, according to the instructions of an extension specialist, as detailed in Supporting Information Table S1. Three adjacent 2-ha plots were marked in each vineyard: a treatment sampling plot that received MD against *L. botrana* and *P. ficus*; a buffer plot with no insect sampling; and a control sampling plot without MD. All three plots received similar insecticide applications.

2.2 MD dispensers

Dispensers with the synthetic female sex pheromones of the two pests were hung in the MD plots in the first week of May, 2 weeks before the first sampling of arthropods. The *L. botrana* pheromone components are the E7,Z9-12:Ac isomer with 15–20% of the E7,E9-12:Ac isomer and trace amounts of the other isomers in a polyethylene tube dispenser. The dispensers and pheromone were manufactured by Shin-Etsu (Tokyo, Japan). Each tube contained an average of 220 mg of active ingredient. Shin-Etsu's tubes (500 tubes per hectare) were placed 6 m apart in every row, and the distance between rows was 3 m. This provided a pheromone application rate of 86 g ha^{-1}, as recommended by the manufacturer. The pheromone release rate was 1 mg/day. The dispensers for *P. ficus* MD were loaded with 150 mg of racemic (+)/(−)-lavandulyl senecioate. Both the dispensers (CheckMate VBM-XL) and the pheromone were produced by Suterra (Bend, OR, USA). They were placed at a density of 620 ha^{-1}, at canopy height. The pheromone release rate was ca. 0.8 mg/day, depending on local weather conditions. The dispensers were effective for 4–5 months and did not require replacing during the crop season.[29]

2.3 Monitoring traps

To test whether MD effectively reduced the pests' flight towards pheromone sources, 12 monitoring funnel traps (six for each pest)

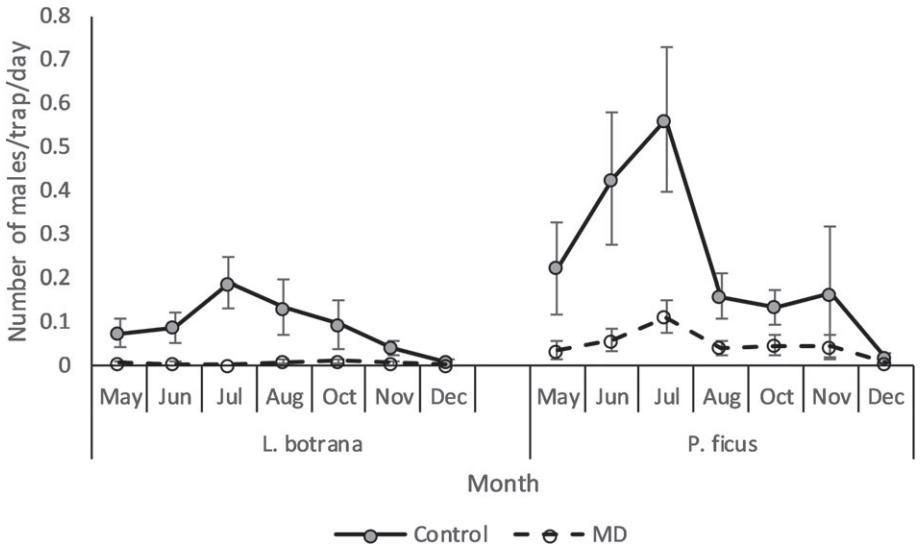

Figure 1. Mean daily captures of *L. botrana* (left) and *P. ficus* (right) males in the pheromone-baited monitoring traps, in MD versus control plots. Error bars are 1 SD.

were baited with the sex pheromones of *P. ficus* and *L. botrana* and placed in each vineyard. Three traps were evenly distributed in the MD plot, and three in the control plot (Fig. S1). The chemical composition of the pheromone lures was similar to that used for MD, and the traps were baited with 100 mg of pheromone. The lures were replaced every 6 weeks (*P. ficus*) or every 4 weeks (*L. botrana*). Traps were emptied at ca. monthly intervals during May–November, and the trapped male *P. ficus* and *L. botrana* were counted.

2.4 Sampling of pests on the vines

To monitor mealybugs on the grapevines, we searched the stems, branches and fruit of six grapevines along each of eight vine rows per vineyard. Each vine was searched for 5 min. The abundance of adult mealybugs and crawlers was scored using a discrete scale (0, no mealybugs; 1, 1–10 individuals; 2, 11–30 individuals; 3, >30 individuals). Mealybug egg sacs were counted. Sampling was conducted in May, July and October.

To estimate infestation by *L. botrana*, we inspected 100 grape bunches per vineyard for damaged berries in May and again in July. The bunches were sampled randomly from different areas in the vineyards.

2.5 Sampling and identification of parasitoids and spiders

Vegetation-dwelling arthropods were suction-sampled in May, July and September in the MD and control plots, using a Vortis Insect Suction Sampler (Burkard Manufacturing Co. Ltd, Rickmansworth, UK). We collected three samples from the vine foliage and three samples from herbaceous vegetation in each plot (Fig. S1). Sampling was conducted in the margins of the vineyards that bordered natural habitats (Fig. S1) because preliminary studies indicated that these areas contain the highest abundance and diversity of parasitoids within the plots (I. Shapira). We collected a total of 180 samples (3 samples × 2 habitats × 2 plots × 5 vineyards × 3 sampling dates). Samples were collected on clear days between 08:00 and 13:00 h. The ambient temperatures during the sampling sessions ranged from 20 to 25 °C in May, and from 25 to 35 °C in July and September. Suction duration was 15 s per sample, and the area covered per sample was about 1 × 3 m. Samples were stored in 75% ethanol and refrigerated until sorting. The parasitoids from the samples were classified to families and sorted to morpho-species. The abundant parasitoid species (>20 individuals in all samples combined) were also determined to genus level. Classification was based on several taxonomic keys.[30–42] Spiders were identified to family level, as only 1.5% of the collected individuals were adults that could be identified to species level.

Anagyus sp. nr. *pseudococci* wasps were sampled from the pheromone-based monitoring traps baited with the *P. ficus* synthetic sex pheromone. The trapped parasitoids were sexed and counted at approximately monthly intervals.

The frequency of parasitism on *P. ficus* was estimated by inspecting the leaves, branches and stems of 24 vines in each MD and control plot of all vineyards. Each vine was inspected for 5 min, in May, August and October 2015. Six vines, at different locations along four planting rows, were selected for inspections. Parasitized *P. ficus* were identified based on the grayish color of the mummy or the parasitoid's exit hole. We recorded the numbers of vines that carried at least one parasitized individual. Based on our previous studies of *P. ficus* in this area, most of the parasitism is attributable to *A.* sp. nr. *pseudococci*.

2.6 Data analysis

2.6.1 Suction samples

Parasitoid and spider abundance counts from the suction samples were averaged across the three replicates collected from each combination of vineyard, habitat, date and MD treatment, to prevent pseudo-replication. The total abundances of all parasitoids and spiders were calculated for each of the averaged suction samples. Shannon's species-level diversity for parasitoids and family-level diversity for spiders were also calculated for each averaged sample. We rounded the total mean abundances to the nearest whole number, and used an exponential transformation on the mean species diversity values, to obtain data sets that were Poisson- and gamma-distributed, respectively. General linear mixed models (GLMMs) were used to test the effects of MD (yes or no), habitat (vine or herbs) and sampling month (May, July or September) on the abundance and diversity of parasitoids and spiders. The GLMM for abundance included a Poisson link function,

and the GLMM for diversity included a gamma link function. MD, habitat and month were defined as fixed effects, while site (the repeated measures factor) was considered a random-intercept effect. Using likelihood ratio tests, we compared each full GLMM with three reduced models, in which we consecutively excluded effects of MD, habitat and month as explanatory variables. This allowed us to test the effect of each variable separately on the total abundance of parasitoids and spiders, and on their diversity scores.

Eight parasitoid species (to which we refer as "common species") were represented by >20 individuals in all samples combined. Individuals from most of these species were sampled from several combinations of site, month and habitat. We used the data set of these common species to test whether the frequency of capturing an individual in an MD plot varied among parasitoid species, habitats and months. For this we calculated a GLMM for binomial data with a logit link function, where presence in an MD plot was defined as a binary response variable (either yes or no) for each individual of the common species. As in the previous GLMMs, species, habitat and month were treated as fixed effects, and site was a random-intercept effect. We compared the full model with reduced ones to test for the effects of species, month and habitat on the abundance of the common species in the MD plots, as described above.

We used "Adonis" [permutational multivariate analysis of variance (PERMANOVA)] analyses to examine the effects of MD, sampling month and habitat on the composition of the parasitoid and spider assemblages. The data were stratified according to site to account for the paired sampling design.

2.6.2 Anagyrus *sp. nr.* pseudococci *wasps from pheromone traps*

The daily capture rates of male and female *A.* sp. nr. *pseudococci* wasps were averaged over the three replicate traps for each combination of site, date and MD/control plot. Following an exponential transformation, we used a GLMM for gamma-distributed data with an inverse link function to analyze the effects of MD, month (fixed factors) and site (a random-intercept factor) on *A.* sp. nr. *pseudococci* abundances. Separate models were run for male and female wasps.

All statistical analyses were conducted in R version 3.1.2.[43] The packages "lme4" and "vegan" were used for the GLMMs and the Adonis tests, respectively.[44,45]

3 RESULTS

3.1 Captures of pests in monitoring traps

Planococcus ficus captures per vineyard were 28.00 ± 13.49 (mean ± standard error) males in MD plots and 152.40 ± 85.41 males in control plots. In MD and control plots, 7.20 ± 2.03 and 60.80 ± 28.20 adult *L. botrana* males, respectively, were captured per vineyard. Accordingly, the mean daily capture rates of both pests were much lower in MD plots than in control plots throughout the study (Fig. 1).

3.2 Monitoring of pests on the vines

The mean (± standard error) score for adult mealybugs per vine (on a scale of 0–4) was 0.21 ± 0.03 in MD plots and 0.27 ± 0.03 in control plots. Crawler scores were 0.22 ± 0.03 and 0.26 ± 0.04, while egg sac counts were 0.49 ± 0.15 and 0.68 ± 0.19 in MD and control plots, respectively. Grape infestation levels for *L. botrana* larvae were negligible, and therefore larval densities of *L. botrana* could not be compared between the two types of plot.

Table 1. Mean (± SD) per-vineyard abundances and diversity scores of parasitoids and spiders in MD and control plots. Shannon's species-level diversity for parasitoids and family-level diversity for spiders are reported

	Parasitoids		Spiders	
	MD	Control	MD	Control
Per-vineyard abundance	24.13 ± 6.17	28.60 ± 6.70	22.00 ± 14.88	14.4 ± 11.37
Per-sample diversity score	1.04 ± 0.18	1.27 ± 0.09	0.57 ± 0.14	0.44 ± 0.15

3.3 Arthropods from suction samples

3.3.1 Overall arthropod abundance

A total of 8991 arthropod individuals were collected in all suction samples combined. They were dominated by aphids (37% of all sampled individuals), whiteflies (16%), thrips (16%) and leafhoppers (4%). Parasitoid wasps (8% of the arthropods) and spiders (2%) were the most common natural enemies.

3.3.2 Parasitoids

Totals of 283, 186 and 300 parasitoids were present in the suction samples obtained during May, July and September, respectively, with 114 morpho-species represented. The per-vineyard abundance and diversity of parasitoids in MD and control plots are reported in Table 1. Parasitoid abundance was significantly affected by sampling habitat (vines versus herbs within the vineyard) and sampling month, but not by the MD treatment (GLMM: $\chi^2 = 11.27$; df = 2; $P = 0.004$ for month; $\chi^2 = 105.51$; df = 1; $P < 0.0001$ for habitat; $\chi^2 = 1.53$; df = 1; $P = 0.22$ for MD). Shannon's index for parasitoid species diversity was also influenced by month and habitat, but unaffected by MD (GLMM: $\chi^2 = 15.85$; df = 2; $P = 0.0004$ for month; $\chi^2 = 13.64$; df = 1; $P = 0.0002$ for habitat; $\chi^2 = 1.73$; df = 1; $P = 0.19$ for MD).

Details of the common species (represented by >20 individuals over all suction samples combined), including information about their putative hosts, are provided in Table 2. Note that several of the common species are not potential natural enemies of *P. ficus* and *L. botrana*. The abundance of the common parasitoids in the MD plots varied significantly among species (logistic regression: $\chi^2 = 53.10$; df = 7; $P < 0.0001$) and months ($\chi^2 = 24.24$; df = 2; $P < 0.0001$), but not among habitats ($\chi^2 = 1.15$; df = 1; $P = 0.28$). However, when we considered the composition of all 114 parasitoid species we found no significant difference between MD and control plots. Both sampling month and habitat, but not MD, explained significant proportions of the variation in parasitoid composition among samples (Table 3).

3.3.3 Spiders

A total of 182 spiders from 12 families were found in the suction samples. Ninety-two individuals were sampled in May, 62 in July and 28 in September. Table 1 lists the per-vineyard abundances and family-level diversity scores of the spiders in MD and control plots. As with the parasitoids, the abundance and diversity of spiders in the samples were significantly influenced by month and habitat, but not by MD (GLMM for abundance: $\chi^2 = 15.55$; df = 2; $P = 0.0004$ for month; $\chi^2 = 22.27$; df = 1; $P < 0.0001$ for habitat;

Table 2. Common parasitoid species (represented by >20 individuals) in the suction samples. The number of sampling sites and dates denote the number of vineyards (out of five) and months (out of three) in which each species was found

Species	Family	Putative hosts	No. sampled		No. sampling sites	No. sampling dates
			MD plots	Control plots		
Anagrus sp.	Mymaridae	Leafhoppers	88	11	2	1
Encarsia lutea (Masi)	Aphelinidae	Whiteflies	13	33	3	1
Eretmocerus sp.	Aphelinidae	Whiteflies	36	35	4	2
Diglyphus isaea (Walker)	Eulophidae	Leafminer flies	14	16	4	2
Ceranisus sp	Eulophidae	Thrips	14	35	4	2
Oligosita sp.	Trichogrammatidae	Leafhoppers	18	19	5	3
Telenomus sp.	Scelionidae	Lepidoptera	8	24	5	3
Alloxysta sp.	Figitidae	Hymenoptera (a hyperparasitoid)	0	21	2[a]	1

[a] 20 out of the 21 individuals were sampled from the same vineyard.

Table 3. PERMANOVA analysis of the factors affecting the assemblage composition of parasitoids in the suction samples

Explanatoryvariable	df	Sum of squares	Mean squares	*F*	R^2	*P*
MD	1	0.32	0.32	0.74	0.01	0.858
Habitat	1	0.89	0.89	2.08	0.04	0.001
Month	2	2.36	1.18	2.75	0.09	0.001
Residuals	51	21.88	0.43		0.86	
Total	55	25.45	25.45		1.00	

Table 4. PERMANOVA analysis of the factors affecting the assemblage composition of spiders in the suction samples

Explanatory variable	df	Sum of squares	Mean squares	*F*	R^2	*P*
MD	2	0.39	0.19	1.31	0.04	0.204
Habitat	1	0.44	0.44	2.93	0.04	0.020
Month	2	1.89	0.94	6.34	0.18	0.001
Residuals	49	7.29	0.15		0.72	
Total	54	10.01			1.00	

$\chi^2 = 2.34$; df = 1; $P = 0.13$ for MD; GLMM for diversity: $\chi^2 = 11.97$; df = 2; $P = 0.003$ for month; $\chi^2 = 19.39$; df = 1; $P < 0.0001$ for habitat; $\chi^2 = 1.32$; df = 1; $P = 0.25$ for MD). MD did not explain the variation in spider family composition among samples, whereas sampling month and habitat had significant effects (Table 4).

3.4 *Anagyrus* sp. nr. *pseudococci* from pheromone traps

Significantly more female *A.* sp. nr. *pseudococci* were trapped in control plots (per-vineyard mean ± SE: 44.80 ± 10.04 individuals) than in MD plots (13.00 ± 2.77 individuals; Fig. 2). The number of females sampled also varied among months (GLMM: $\chi^2 = 20.30$; df = 1; $P < 0.0001$ for MD; $\chi^2 = 34.39$; df = 6; $P < 0.0001$ for month). The numbers of trapped males were much lower (11.80 ± 6.91 and 15.40 ± 12.68 individuals in control and MD plots, respectively). Sampling month, but not MD, affected the abundance of males collected in the monitoring traps (GLMM: $\chi^2 = 0.59$; df = 1; $P = 0.44$ for MD; $\chi^2 = 12.07$; df = 5; $P = 0.03$ for month).

3.5 Parasitism on *P. ficus*

A total of 15.6% of the sampled vines in the MD plots and 19.2% of the vines sampled in the control plots were infested by *P. ficus*. Parasitized mealybugs were found on 2.8% of the 360 inspected vines (24 vines/plot × 5 vineyards × 3 sampling dates) in the MD plots. None of the vines in the control plots contained parasitized mealybugs.

4 DISCUSSION AND CONCLUSION

The considerable decrease in *P. ficus* captures and the almost complete shutdown of the *L. botrana* traps (Fig. 1) indicate that the MD treatment effectively interfered with the pests' orientation to pheromone sources. Yet, MD of *P. ficus* and *L. botrana* did not affect the total abundance, diversity and community composition of most parasitoids and spiders in the experimental vineyards. These results are in line with the common view that the sex pheromones used in agricultural MD are highly species-specific, and therefore have few non-target effects.[2] Many of the common species in our samples are not potential natural enemies of *P. ficus* and *L. botrana*, but rather parasitoids of leafhoppers, whiteflies, leafmining diptera and thrips. This may explain why the influence of MD on the assemblage of natural enemies was minor. The availability of non-crop plants and of arthropod prey in the vineyards, together with lethal and non-lethal effects of insecticides applied to the crop, probably had more influence on the community of natural enemies than the MD treatment.[46] Overall arthropod densities in the study vineyards were low because of repeated insecticide applications (Table S1). Therefore, the generality of our findings should be confirmed in sites with higher populations of pests and natural enemies.

Although MD did not affect the natural enemies' community-level metrics, we did sample some of the common parasitoid species mainly in MD plots, while others were mostly found in control plots. However, detailed inspection of the common parasitoids (Table 2) reveals that many of them are patchily distributed in time and space. This could generate, by chance alone, unequal abundances of some species in the MD versus control plots. For example, *Anagrus* sp. (Mymaridae) wasps, parasitoids of leafhoppers that were frequent in our samples, were collected in only two vineyards in a single month. Although they were seven times more abundant in MD plots than in control plots, this may merely reflect a tendency to aggregate; that is, individual

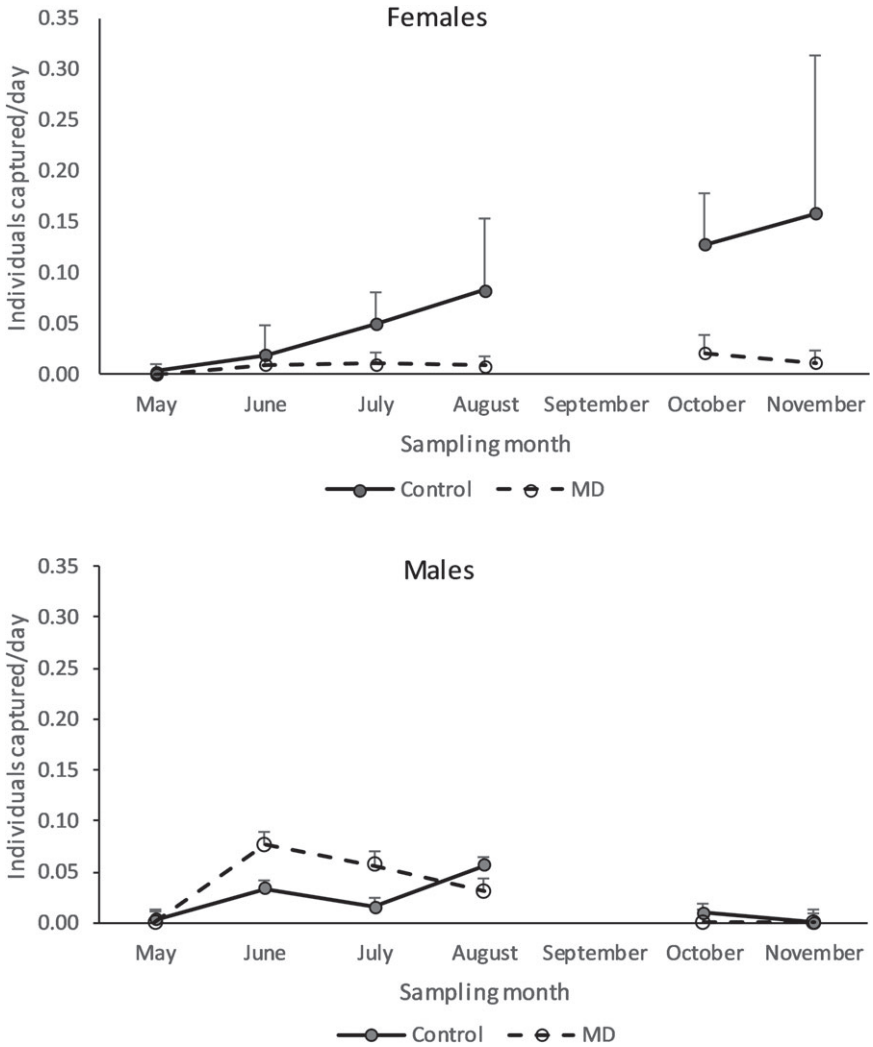

Figure 2. Mean daily captures of *Anagyrus* sp. nr. *pseudococci* females (top) and males (bottom) in pheromone-baited monitoring traps, in MD versus control plots. Error bars are 1 SD.

captures may not be independent of one another, leading to the occurrence of many individuals in a small number of samples. In support of this interpretation, ca. 80% of all sampled *Anagrus* sp. originated from just three samples which contained 37, 26 and 15 individuals, respectively. Similarly, 21 individuals of *Alloxysta* sp. (Figitidae) were found exclusively in control plots, but 20 of them were concentrated in two suction samples (17 wasps in one sample and three in the other) from a single vineyard. *Telenomus* sp. (Platygastridae), which was more abundant in control plots than in MD plots, provides a better-replicated example, as it was found in all five vineyards and three sampling months. The species from the genus *Telenomus* mainly parasitize eggs of Lepidoptera, potentially including the moth *L. botrana* that was targeted by MD. The possibility that *Telenomus* sp. preferred the control plots to the MD plots, as they contained higher densities of *L. botrana*, warrants further testing. However, experimental evaluation of this possibility is complicated by the presence of numerous additional host plants of the pest in and near the vineyards.[26,47]

Anagyrus sp. nr *pseudococci* females, which are known to use the sex pheromone of their *P. ficus* host as a foraging cue, showed two clear responses to MD. First, their captures in pheromone-baited traps were significantly higher in control plots than in MD plots (Fig. 2). Second, parasitism on *P. ficus* (presumably by *A.* sp. nr. *pseudococci*), although low overall, was restricted to plots treated with MD. These findings suggest that the wasps foraged actively for hosts in the MD plots, but that they were not attracted to the monitoring traps.

In agreement with our results, earlier studies found enhanced levels of parasitism by *A.* sp. nr. *pseudococci* in MD-treated vineyards.[15,16] Possibly, flooding of the plots with *P. ficus*' sex pheromone interfered with the parasitoids' orientation towards the pheromone-baited monitoring traps and reduced their captures. In previous experiments, the presence of a single pheromone source in non-MD plots attracted *A.* sp. nr. *pseudococci* females, and increased their parasitism rates on nearby hosts.[5,13] In our study, in contrast, the environment was saturated with the host pheromone, which originated from several MD dispensers in the vineyards. This could have interfered with the parasitoids' navigation to the monitoring traps. We propose that a similar "shutdown" of captures of *A.* sp. nr. *pseudococci* females in the monitoring traps occurred in our MD plots, but not in the control plots. Consequently, pheromone-baited traps may not be suitable for monitoring of *A.* sp. nr. *pseudococci* parasitoids in agricultural areas with MD. Unfortunately, suction sampling was

also ineffective for monitoring of *A*. sp. nr. *pseudococci*, as only one individual was collected in all suction samples combined.

The captures of *A*. sp. nr. *pseudococci* males in the monitoring traps were not affected by MD, and were much lower than those of females. This could indicate that male wasps orient less strongly to *P. ficus*' sex pheromone than do females. As males seek mates, rather than hosts, they may be more strongly attracted to cues associated with *A*. sp. nr. *pseudococci* females, or to semiochemicals emitted by host plants, than to kairomones associated with *P. ficus*. Nevertheless, in the parasitoid *Lariophagus distinguendus* (Förster), both males and females respond to kairomones emitted by their weevil hosts. This was proposed to improve the males' chances of encountering female aggregations near hosts, and thereby enhance their mating prospects. Possibly, *A*. sp. nr. *pseudococci* males are also somewhat attracted to host-produced volatiles. The odor preferences of male and female *A*. sp. nr. *pseudococci* should be compared in olfactometer assays to test this possibility.

In summary, the MD protocol used in our study most clearly influenced a single species (*A*. sp. nr. *pseudococci*) of the diverse community of parasitoids and spiders in the vineyards, and the effect was positive from a pest control point of view. Environmentally friendly pest management aims to promote plant protection strategies with minimal adverse effects on non-target beneficial organisms. Our findings support the use of MD to achieve this aim.

ACKNOWLEDGEMENTS

We thank Ben Dvir and Einat Bar-Ziv for fieldwork assistance. This study was part of a Monitoring Biodiversity in Agro-Ecosystems Project funded by the Chief Scientist at the Israel Ministry of Agriculture and HaMaarag – Israel's National Nature Assessment Program at the Steinhardt Museum of Natural History, Tel Aviv University.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Harari AR, Zahavi T, Gordon D, Anshelevich L, Harel M, Ovadia S and Dunkelblum E, Pest management programs in vineyards using male mating disruption. *Pest manag Sci* **63**:769–775 (2007).
2 Witzgall P, Kirsch P and Cork A, Sex pheromones and their impact on pest management. *J Chem Ecol* **36**:80–100 (2010).
3 Keathley CP, Stelinski LL and Lapointe SL, Attraction of a native Florida leafminer, *Phyllocnistis insignis* (Lepidoptera: Gracillariidae), to pheromone of an invasive citrus leafminer, *P. citrella*: evidence for mating disruption of a native non-target species. *Fla Entomol* **96**:877–886 (2013).
4 Jakubíková K, Komínková J, Šefrová H and Laštůvka Z, Target and non-target moth species captured by pheromone traps for some fruit Tortricid moths (Lepidoptera). *Acta Universit. Agricult. Silvicult. Mendelianae Brunensis* **64**:1561–1568 (2016).
5 Franco JC, Silva EB, Cortegano E, Campos L, Branco M, Zada A and Mendel Z, Kairomonal response of the parasitoid *Anagyrus spec. nov. near pseudococci* to the sex pheromone of the vine mealybug. *Entomol Exp Appl* **126**:122–130 (2008).
6 Zappalà L, Campolo O, Grande SB, Saraceno F, Biondi A, Siscaro G and Palmeri V, Dispersal of *Aphytis melinus* (Hymenoptera: Aphelinidae) after augmentative releases in citrus orchards. *Eur J Entomol*, **109**:561–568 (2012).
7 Powell W and Pickett JA, Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest Manag Sci* **59**:149–155 (2003).
8 Branco M, Lettere M, Franco JC, Binazzi A and Jactel H, Kairomonal response of predators to three pine bast scale sex pheromones. *J Chem Ecol* **32**:1577–1586 (2006).
9 Branco M, van Halder I, Franco JC, Constantin R and Jactel H, Prey sex pheromone as kairomone for a new group of predators (Coleoptera: Dasytidae, *Aplocnemus spp.*) of pine bast scales. *Bull Entomol Res* **101**:667–674 (2011).
10 Spears LR, Looney C, Ikerd H, Koch JB, Griswold T, Strange JP and Ramirez RA, Pheromone lure and trap color affects bycatch in agricultural landscapes of Utah. *Environ Entomol* **45**:1009–1016 (2016).
11 Pekas A, Navarro-Llopis V, Garcia-Marí F, Primo J and Vacas S, Effect of the California red scale *Aonidiella aurantii* sex pheromone on the natural parasitism by *Aphytis* spp. in Mediterranean citrus. *Biol Cont* **90**:61–66 (2015).
12 Millar JG, Daane KM, Mcelfresh JS, Moreira JA, Malakar-Kuenen R, Guillén M and Bentley WJ, Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California vineyards. *J Econ Entomol* **95**:706–714 (2002).
13 Franco JC, Da Silva EB, Fortuna T, Cortegano E, Branco M, Suma P, La Torre I, Russo A, Elyahu M, Protasov A, Levi-Zada A and Mendel Z, Vine mealybug sex pheromone increases citrus mealybug parasitism by *Anagyrus sp. near pseudococci* (Girault). *Biol Cont* **58**:230–238 (2011).
14 Walton VM, Daane KM, Bentley WJ, Millar JG, Larsen TE and Malakar-Kuenen R, Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. *J Econ Entomol* **99**:1280–1290 (2006).
15 Cocco A, Lentini A and Serra G, Mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in vineyards using reservoir pheromone dispensers. *J Ins Sci* **14**:1–8 (2014).
16 Daane K, Bentley W, Walton V, Malakar-Kuenen R, Millar J, Ingels C, Weber E, and Gispert C, New controls investigated for vine mealybug. *California Agric* **60**:31–38 (2006).
17 Gallardo AM, Lopez A, Lara M, Maistrello L, Molejon A and Ocete R, Resurgence of secondary pests following the implementation of mating disruption in sherry vineyards (Spain). *Vitis* **55**:37–43 (2016).
18 Aslan MM, A comparison of the parasitoids of grapevine moths *Lobesia botrana* (Denis et Schiffermuller) in the vineyards where conventional and mating disruption techniques are applied. *Agricult J* **10**:1–6 (2015).
19 Williamson JR and Johnson DT, Effects of grape berry moth management practices and landscape on arthropod diversity in grape vineyards in the southern United States. *HortTechnology* **15**:232–238 (2005).
20 Westigard PH and Moffitt HR, Natural control of the pear psylla (Homoptera: Psyllidae): impact of mating disruption with the sex pheromone for control of the codling moth (Lepidoptera: Tortricidae). *J Econ Entomol* **77**:1520–1523 (1984).
21 Biddinger DJ, Felland CM and Hull LA, Parasitism of tufted apple bud moth (Lepidoptera: Tortricidae) in conventional insecticide and pheromone-treated Pennsylvania apple orchards. *Environ Entomol* **23**:1568–1579 (1994).
22 Atanassov A, Shearer PW and Hamilton GC, Peach pest management programs impact beneficial fauna abundance and *Grapholita molesta* (Lepidoptera: Tortricidae) egg parasitism and predation. *Environ Entomol* **32**:780–788 (2003).
23 Sokolsky T, Cohen Y, Zahavi T, Sapir G and Sharon R, Potential efficiency of grapevine leafroll disease management strategies using simulation and real spatio-temporal disease infection data. *Austr J Grape Wine Res* **19**:431–438 (2013).
24 Fermaud M and Le Menn R, Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae. *Phytopathology* **82**:1393–1398 (1992).
25 Ioriatti C, Anfora G, Tasin M, Cristofaro A, Witzgall P and Lucchi A. Chemical ecology and management of Lobesia botrana (Lepidoptera: Tortricidae). *J Econ Entomol* **104**:1125–1137 (2011).
26 Sciarretta A, Zinni A, Mazzocchetti A, Trematerra P, Spatial analysis of *Lobesia botrana* (Denis and Schiffermüller) male population in a Mediterranean agricultural landscape in central Italy. - *Environ Entomol* **37**:382–390 (2008).
27 Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS *et al.* A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protect* **97**:70–84 (2017).

28 Lentini A, Mura A, Muscas E, Nuvoli MT, and Cocco A, Effects of delayed mating on the reproductive biology of the vine mealybug, *Planococcus ficus* (Hemiptera: Pseudococcidae). *Bull Entomol Res* 1–8, https://doi:10.1017/S000748531700075X (2017).

29 Sharon R, Zahavi T, Sokolsky T, Sofer-Arad C, Tomer M, Kedoshim R and Harari AR, Mating disruption method against the vine mealybug, *Planococcus ficus*: effect of sequential treatment on infested vines. *Entomol Exp Appl* **161**:65–69 (2016).

30 Masner L, Revisionary notes and keys to world genera of Scelionidae (hymenoptera: Proctotrupoidea). *Memoirs Entomol Soc Canada* **108**:1–87 (1976).

31 Masner L, Key to genera of Scelionidae of the Holarctic region, with descriptions of new genera and species (Hymenoptera: Proctotrupoidea). *Memoirs Entomol Soc Canada* **112**:1–54 (1980).

32 Hayat M, The genera of Aphelinidae (Hymenoptera) of the world. *Syst Entomol* **8**:63–102 (1983).

33 Shaw M and Huddleston T, *Classification and biology of Braconid wasps. Handbooks for the Identification of British Insects* Vol. **7**, Part 11 (1991). British Museum of Natural History, London

34 Goulet H and Huber JT, *Hymenoptera of the World: An Identification Guide to Families*. Research Branch, Agriculture Canada (1993).

35 Schauff ME, LaSalle J and Coote LD, *Eulophidae. Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera)*. National Research Council Research Press. Ottawa, Ontario, Canada, 327–429 (1997).

36 Grissell E, Schauff M, Gibson G, Huber J and Woolley J, *Annotated keys to the genera of neartic Chalcidoidea (Hymenoptera)*. National Research Council Research Press. Ottawa, Ontario, Canada, pp. 709–725 (1997).

37 Woolley JB, Aphelinidae. Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, *Canada*, **794**:134–150 (1997).

38 Noyes JS, Universal Chalcidoidea Database (2003). [Online]. Available: http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/ [2015 and 2016].

39 Pinto JD, A review of the new world genera of Trichogrammatidae (Hymenoptera). *J Hymenopt Res* **15**:38–163 (2006).

40 Ulrich W, Body weight distributions of European Hymenoptera. *Oikos* **114**:518–528 (2006)

41 Huber JT, Viggiani G and Jesu R, Order Hymenoptera, family Mymaridae. *Arthr Fauna UAE* **2**:270–297 (2009).

42 Pricop E, Identification key to European genera of the Mymaridae (Hymenoptera: Chalcidoidea), with additional notes. *ELBA Bioflux* **5**:69–81 (2013).

43 R Core Team, R: *A language and environment for statistical computing*, R Foundation for Statistical Computing, Vienna, Austria (2013).

44 Bates D, Maechler M, Bolker B and Walker S, lme4: Linear mixed-effects models using eigen and S4. *R Package Version*, 1 (2014).

45 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D *et al.* Community Ecology Package. [Online]. R package version 2.4-3 (2017). Available: https://CRAN.R-project.org/package=vegan [November 2017].

46 Biondi A, Zappalà L, Stark JD and Desneux N, Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? *PLoS One* **8**:e76548 (2013).

47 Sciarretta A, Zinni A and Trematerra P, Development of site-specific IPM against European grapevine moth *Lobesia botrana* (D. & S.) in vineyards. *Crop Prot* **30**:1469–1477 (2011).

48 Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U and Weber E, Growth Stages of the Grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. Vinifera) - Codes and descriptions according to the extended BBCH scale. *Austr J Grape Wine Res* **1**:100–103 (1995).