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Pheromone Deployment Strategies for Mating Disruption of a Vineyard Mealybug

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Abstract

The mealybug, *Planococcus ficus* (Signoret), is a primary vineyard pest in California and other grape-growing regions throughout the World. Mating disruption programs are commercially available to manage *Pl. ficus*, but widespread adoption has been limited, in part, by high costs compared with insecticide programs. To improve mating disruption economic effectiveness, different deployment technologies (passive, aerosol, and microencapsulated formulations) were individually examined. Adult male *Pl. ficus* captures in pheromone traps and categorical ratings of vine infestation or crop damage suggest that all deployment strategies lowered mealybug densities or damage. Using passive dispensers, deployment rates of 310 and 465 per ha lowered *Pl. ficus* crop damage similar to 615 per ha, a rate commonly used in commercial operations; reduced rates would lower product and deployment costs. Meso dispensers, containing more a.i., deployed at 35 per ha did not have a treatment impact, but a microencapsulated formulation and aerosol canisters lowered male flight captures and/or crop damage. Male mealybug flight activity was greatest from 0500–1100 hr, which coincided with temperatures >16° and <32°C. These restricted times and temperatures suggest programable dispensers might allow pheromone deployment to coincide only with flight patterns. A large field trial using passive dispensers found greater treatment separation after 3 yr of pheromone deployment. Discrepancies in results among vineyards may be related to *Pl. ficus* density, but combined results from all trials suggest that different deployment technologies can be used to impact *Pl. ficus* densities and damage, even at reduced rates, especially with continued use over multiple seasons.

Key words: *Planococcus ficus*, semiochemical, sex pheromone, sustainable agriculture, vineyard pest

Grape production for wine, juice, raisin, or fresh table markets is a global, multi-billion-dollar industry that has, over the past two decades, seen an increase in production (currently about 77 million tons) and a decrease in area farmed (currently about 7.5 million ha) (OIV 2020). Concurrent with the adoption of more intensive management practices for increased production per ha has been an increased consumer demand for fruits and vegetables with perceived better health benefits (Wightman and Heuberger 2015) and more sustainable farming practices (Tilman et al. 2011, Chkanikova and Mont 2015). For vineyards, one of the constant pressures on sustainable systems is the invasion of arthropod pests (Daane et al. 2018a). Examples include phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera: Phylloxeridae), in Europe (Campbell 2004); European grapevine moth, *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae), in California (Cooper et al. 2014, Lucchi et al. 2014); spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera:

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Drosophilidae), in Europe and the Americas (Asplen et al. 2015) and more recently the spotted lantern fly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), in South Korea, Japan and the United States (Lee et al. 2019). A number of invasive mealybug species (Hemiptera: Pseudococcidae) have also become disruptive pests (Daane et al. 2012), in part because of their transmission of grape leafroll associated viruses (GLRaVs) (Almeida et al. 2013). *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is one of the more widespread and damaging vineyard mealybugs, being found in California, Mexico, South America, Europe, the Middle East, and South Africa (Daane et al. 2018b, Cocco et al. 2021).

Insecticides are the primary tool for vineyard mealybug control (Lo and Walker 2010, Mansour et al. 2018, Tacoli et al. 2018, O'Hearn and Walsh 2020). While there are insecticides approved for organic programs (e.g., Srinivas et al. 2007, Peschiutta et al. 2017), both conventional and organically-certified materials can impact beneficial arthropods (Mgocheki and Addison 2009, Mansour et al. 2018). Therefore, to meet consumer demands for more sustainable management practices, additional tools are needed for *Pl. ficus* and other vineyard pests (Wilson and Daane 2017, Daane et al. 2018a, Cocco et al. 2021). Mating disruption (MD) is a proven tool (Welter et al. 2005, Miller and Gut 2015), most commonly used for lepidopteran pest control or eradication (e.g., McLaughlin et al. 1994, Witzgall et al. 2008, Ioriatti et al. 2011, Lucchi et al. 2014). Rotundo and Trembly (1972) provided one of the initial descriptions of male mealybug response to a female-emitted sex pheromone for *Planococcus citri* (Risso), which was followed by the pheromone's identification and synthesis (Bierlleonhardt et al. 1981). Sex pheromones for other vineyard mealybug pests were later identified for *Pl. ficus* (Hinkens et al. 2001), *Pseudococcus viburni* (Millar et al. 2005), *Ps. maritimus* (Figadère et al. 2007), and *Ps. longispinus* (Zou and Millar 2009) and deployed to monitor mealybug densities and population dynamics in vineyards (Millar et al. 2002, Walton et al. 2004, Bahder et al. 2013, Cooper et al. 2018). The first report of a mealybug semiochemical used for control was Franco et al. (2003) with the mass-trapping of male *Pl. citri* in Portugal, Italy, and Israel. Semiochemicals have since been tested or commercially deployed for MD of a number of mealybug species, initially for *Pl. ficus* in California vineyards (Walton et al. 2006) and now for *Pl. kraunhiae* (Kuwana) in Japanese persimmons (Teshiba et al. 2009), citrophilous mealybug, and *Ps. calceolariae* in Australian citrus (Sullivan et al. 2019) and Chilean fruits (Ballesteros et al. 2021).

Mating disruption of *Pl. ficus* in vineyards has been one of the more successful and widely used semiochemical programs for a nonlepidopteran pest since it became available in 2009. A number of products for *Pl. ficus* MD have been tested worldwide, including in Europe, South America, Israel, and the United States (California) where various types of passive dispensers have been reported to lower *Pl. ficus* male flight activity and crop damage (Cocco et al. 2014, 2018; Sharon et al. 2016; Mansour et al. 2017; Lucchi et al. 2019; Daane et al. 2020; Hogg et al. 2021). Although MD programs have been successful, adoption has lagged behind that of other tools, particularly insecticidal products. Biological, social, and economic factors likely contribute to the lagging adoption (Lefebvre et al. 2015). For example, MD is often used in a similar manner to insecticide applications, this despite the difficulty of controlling moderate or large populations solely with MD and evidence that MD programs are increasingly efficient at lower pest densities (Witzgall et al. 2010, Hogg et al. 2021). Additionally, pest populations tend to respond more quickly to insecticides, whereas MD may require consecutive years of application (Sharon et al. 2016) and potential synergies with other practices, such as biological control (Daane et al. 2012). Moreover, growers concerned with mealybugs as vectors of GLRaVs should consider areawide MD programs to reduce vector densities lower than that achieved by insecticides alone. Growers integrating synergistic practices at the appropriate time and application interval may achieve long-term goals of reduced pest pressure and economic damage (Barzman et al. 2015). Economic considerations also factor into adoption of MD; the cost for full season and full label-rate deployment currently ranges from $150–300 (USD) per ha. Cost is based on dose per ha and the type of deployment device.

There are a number of different devices to release pheromone, as described in Benelli et al. (2019). Passive dispensers have been the most commonly used type and can be composed of plastic tubes, ampules or packets that release pheromone slowly over several weeks or months. For passive dispensers (e.g., CheckMate VMB-XL, Suttera LLC, Bend, OR and Isomate, Pacific BioControl, Vancouver, WA), dose is based largely on the deployment rate, load per dispenser, and release rate of the dispenser; within the category of passive dispensers are meso dispensers that are basically large passive dispensers that can hold and release more pheromone and are often deployed at lower rates per ha. In contrast, for flowable formulations that are applied using a spray rig similar to a foliar insecticide (e.g., CheckMate VMB-F, Suterra LLC) and aerosol technologies that release a metered aerosolized-volume of pheromone at controlled intervals (e.g., Semios VMB Eco DS, SemiosBio Technologies Inc., Vancouver, BC, Canada) the dose is based on the application rate and the number of applications per season. Most early studies used passive dispensers at deployment rates >600 dispensers per ha (Cocco et al. 2014, 2018; Sharon et al. 2016). A more recent study in Italy, however, reports a reduction in *Pl. ficus* cluster damage at deployment rates from 300 to 500 dispensers per ha (Lucchi et al. 2019), which could greatly reduce material and labor costs. The use of pheromone in pressurized cannisters might also reduce overall costs, as aerosol delivery systems can be cheaper to deploy because of lower densities (<5 per ha), and can release pheromones at selected time intervals when the target pest is active (Benelli et al. 2019). However, aerosol devices may reduce the number of point sources per ha, which could reduce overall MD effectiveness (de Lame et al. 2010), especially for vineyard mealybugs that tend to have clumped distributions (Geiger and Daane 2001). Here, we describe studies of *Pl. ficus* mating disruption that individually evaluated the impact of passive dispensers (both standard and meso), flowable formulations, and aerosolized cannisters. Our goal was to verify the performance of these deployment methodologies to develop best management parameters for lowered costs and greater adoption of this sustainable management tool.

Materials and Methods

Dispensers Deployment Rate

For passive dispensers, a standard deployment rate has been ~600 dispensers per ha, each with a load of ~150 mg of the pheromone active ingredient lavandulyl senecioate. *Planococcus ficus* response to different deployment rates was tested in wine grapes in San Luis Obispo County (Edna Valley American Viticultural Area (AVA)), from 2009 to 2011. Passive dispensers (CheckMate VMB-XL) loaded at 150 mg a.i. per dispenser were applied at four dispenser density rates: 125, 310, 465, and 615 dispensers per ha (18.7, 46.5, 69.7, and 92.2 g a.i. per ha). This was a large trial using 16 vineyard blocks, with each block ~4 ha in size and four replicates per treatment. All blocks were mature (>8 yr), trained to a T-trellis system and on drip irrigation, and managed for wine grapes (*Vitis vinifera* cultivars Chardonnay (10 blocks), Pinot noir (5 blocks), and a split

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block of Syrah/Riesling (1 block)). Three no-pheromone blocks were initially included in the design but were treated with imidacloprid (Admire, Bayer CropScience, St. Louis, MO) at the start of the trial and were excluded thereafter. Dispensers were deployed between 8–11 June 2009, 26 April–7 May 2010, and 2–13 May 2011; commonly deployed with help by the vineyard's field crew and hung on a trellis wire, just above the vine head and within the vine canopy (to reduce direct sunlight exposure). All sites had a history of *Pl. ficus* damage before treatment application, as well as Argentine ants, *Linepithema humile* (Mayr) tending the mealybugs (Daane et al. 2008).

Adult male *Pl. ficus* flights were monitored in 2009 and 2010 using three Pherocon Delta IIID sticky traps (Trécé Inc., Adair, OK) per plot, each baited with a rubber septum lure loaded with 100 µg a.i. of the pheromone. Traps were hung from trellis wires such that they were positioned above the cordon but within the vine canopy and were placed 15–50 vines from the edge. Traps were collected every 2 wk from April–May to November and lures were replaced every 4 wk. Trapped insects were counted in the laboratory using a dissecting microscope.

Mealybug densities on the vine can be difficult to accurately assess as vineyard mealybugs are typically clumped within each block and found in hidden locations on each vine (Geiger and Daane 2001, Daane et al. 2013) making detailed counts on individual vines a less accurate representation of the block density. For this reason, *Pl. ficus* densities were assessed each year during a 1-min per vine search using a 0–3 rating on 100 vines per plot at key seasonal periods: early (May–June) and late (late-July–August), where 0 = no mealybugs, 1 = 1–20 mealybugs, 2 = 21–50 mealybugs, and 3 = >50 mealybugs found. Rating 100s of vines rather than the more time-consuming counts of total mealybugs on individual vines allowed more vines per block to be assessed, thereby reducing variance resulting from fewer samples and a clumped mealybug distribution.

Crop damage was assessed each year near harvest time (August–October) using a 0–3 rating of sampled fruit, after Geiger et al. (2001), where 0 = no mealybug damage, 1 = a few mealybugs found or some honeydew present, 2 = fruit damage caused by mealybug and honeydew accumulation, and 3 = severe damage and an unmarketable cluster. Crop damage was rated on 100 clusters vines per plot. Vines were selected at random; although, clusters in contact with the upper trunk or cordon were preferentially selected as they typically have a higher likelihood of mealybug infestation, which makes the estimate of suppression more conservative.

Meso Dispensers

Meso dispensers, a larger version of the standard passive dispenser with more a.i., provide the opportunity to deploy fewer dispensers per ha but maintain a similar pheromone dose compared to standard passive dispensers. We compared standard and meso dispensers in a 231.3 ha wine grape operation in San Joaquin County (Lodi AVA) in 2012. Both dispenser types were hung on a trellis wire, as described previously. All blocks were mature, trained to a T-wire trellis system and on drip irrigation, and managed for wine grapes (*V. vinifera* cultivars were Sauvignon blanc (3 blocks), Merlot (2 blocks), and Cabernet Sauvignon (2 blocks)). Blocks ranged from 9–16 ha each, with 16 plots (~6 ha each) embedded in these blocks, with some larger blocks containing two plots. The farm was reportedly uniformly infested with *Pl. ficus* that had been suppressed by an annual insecticide application(s) (imidacloprid and/or buprofezin). Four treatments were applied: standard passive dispensers (CheckMate VMB-XL) at rates of 430 and 618 dispensers per ha (64.8 and 92.7 g a.i. per ha), experimental MESO emitters loaded at 2000 mg a.i. per dispenser at a rate of 89 emitters per ha (178 g a.i. per ha), and a grower-standard pesticide application of a systemic imidacloprid that was applied to all plots (the crop was too valuable for a no-spray control). As an alternative, a sub-plot (15 rows × the entire length of the respective block) in the middle of each of the treatment plots was not treated with insecticides; this nonspray area was used for all samplings. Treatments were replicated four times in a RCB design, with plots spread throughout the ranch to reduce near-neighbor impact of the sex pheromone volatiles. *Planococcus ficus* densities were assessed for a 1-min-per-vine search on 100 vines per plot at key seasonal periods: early (May), mid (late-July), and late (August); however, rather than a categorical rating of vine infestation, all 2nd instar to adult *Pl. ficus* were counted. At harvest time, one cluster per vine on 100 vines per plot was assessed for damage, using the 0–3 scale described previously.

Flowable Application

The first *Pl. ficus* MD trials were conducted with a microencapsulated flowable (sprayable) suspension (Walton et al. 2006) but this product only recently became available as CheckMate VMB-F (Suterra LLC, Bend, OR). This flowable material was tested with three treatments: 1) three sprays applied early (2 May), mid-(5 June), and late-season (11 July); 2) two sprays applied early- and mid-season; and 3) a no MD control. The flowable material was applied at 24.71 g a.i. per ha in 3.5 kl per ha water for each spray. The trial was conducted in Fresno County in two adjoining table grape blocks, one ~8 yr old (12.1 ha) and the other ~15 yr old (14.6 ha), both were Flame seedless *cv*., trained to an overhead trellis system and drip irrigated. The blocks were severely damaged by *Pl. ficus* in 2016 and received multiple, but similar, pesticide applications. Because of the previous year's damage, during the 2017 MD trial, both blocks received an April application of imidacloprid and a May application of Spirotetramat. The two MD treatments and control were applied in a randomized design, with twelve 2.1 ha plots; however working with the grower-collaborator there were five two-spray plots, four three-spray plots, and three control plots.

Adult male *Pl. ficus* flights were monitored using two Pherocon Delta IIID sticky traps per plot, as described previously. *Planococcus ficus* densities were assessed for 1-min-per-vine using the 0–3 rating, as described previously, on 100 vines per plot during (July–August) and after (September–October) the harvest period. Crop damage was assessed near harvest time (23–25 July) using the 0–3 rating, as described previously, on a single cluster from 100 vines per plot.

Aerosol Dispensers

Aerosolized cannisters were tested for trap suppression in Fresno County in 2011 and Napa County in 2012. In 2011, trials were done in two plots, both ~0.26 ha; the vineyards were mature raisin grape blocks (Thompson seedless *cv*.) trained to a single-wire trellis system and flood irrigated. The aerosol device (referred to as 'puffer') was placed in the center of one plot and the other plot was designated a control. Each puffer was hung from a metal hanger attached to an existing vineyard trellis post, which placed the device just above the vineyard canopy. Puffers were rotated between plots weekly, i.e., a plot with a puffer became a control plot the following week and vice versa after that. Puffers were set to release a pheromone 'puff' every 15 min from 0200 to 1400 hours; this time frame was selected to 'load' pheromone into the plot for several hours before and throughout the *Pl. ficus* adult male optimal flight period that occurs several hours after sunrise (see *Pl. ficus* Flight section and Zada et al. 2008). Two different

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rates were used, 5 mg a.i. per puff from August 4–22, and 1.5 mg a.i. per puff from September 7 to October 4. Each week constituted one replicate. Effect of puffers on male *Pl. ficus* capture rates were determined with an 8 × 8 grid of Pherocon Delta IIID traps centered around the aerosolized canister. Traps were hung from trellises and replaced the afternoon after the puffer was swapped. In 2012 at the Napa site, puffers were tested in two 0.14 ha plots from 6 July to 2 August. One plot was designated as a control and the other as a puffer for the duration of the trial. A different puffer rate was used each of the four weeks, 4.5 mg, 2.25 mg, 1.5 mg then 3 mg a.i. per puff. A 7 × 7 grid of Pherocon Delta IIID traps was used and replaced weekly.

Male *Pl. ficus* Flight

Daily male flight patterns can affect MD effectiveness and for that reason we monitored peak flight periods during the summer. Preliminary observations found no or few adult male *Pl. ficus* captured during the afternoon in California's San Joaquin Valley, where high summer temperatures can range from 35–42°C. To determine the effect of temperature, *Pl. ficus* colonies grown on butternut squash, as described in Daane et al. (2004), were placed inside two temperature cabinets, each set initially at 12°C. An unbaited pheromone trap was placed inside each cabinet for a 24 hr period to monitor adult male flight. This was repeated three times over the next 3–5 d, after which, the temperature in each cabinet was raised by 4°C and, after the new set temperatures were verified, the trial was repeated such that over the next 30 d temperatures of 12, 16, 20, 24, 28, 32 and 36°C were tested.

Two field studies were conducted to verify *Pl. ficus* daily flight activity. First, in a Fresno County vineyard (near Parlier, CA) managed for raisin grapes (Thompson Seedless *cv*.) with a moderate *Pl. ficus* infestation, six pheromone traps were deployed, as described previously, each separated by 5 rows and 20 vines. The traps were deployed in the evening (1800 hr) and then replaced hourly until 2100 hr, left overnight (2100–0500 hr), and then replaced hourly the next day until 1100. Second, concurrent with the vineyard study, colonies of *Pl. ficus* on butternut squash, which had been reared in an insectary room (25 ± 2°C, 16:8 LD), were taken to a peach orchard (e.g., no *Pl. ficus*) and four pheromone traps were deployed around the infested squash and monitored on the same schedule as in the vineyards. Both trials were repeated four times. Hourly temperatures were taken from a nearby California Irrigation Management Information System (CIMIS) station.

Commercial Application in Table Grapes

MD using passive dispensers (CheckMate VMB-XL) was demonstrated over a 3 yr period in six (2008) or five (2009–2010) large (~16–65 ha) table grape vineyards in Kern County (near Delano and MacFarland, CA). The vineyards included four Flame Seedless *cv*. (early-season harvest) and a Crimson *cv* (late-season harvest). All had some level of *Pl. ficus* damage in the previous years, were mature, drip irrigated, and trained to an overhead trellis system. A split plot design was used in each vineyard with 4–8 ha plots, widely separated at each site (20–50 rows). Each paired vineyard plot was treated similarly with pesticides, but pesticide use varied among sites, at the grower-cooperators' discretion to protect the valuable crops; materials used for *Pl. ficus* control over the three year period included chlorpyrifos (delayed dormant), imidacloprid, buprofezin, and clothianidin. In MD plots, Checkmate VMB-XL dispensers were deployed at a rate of ~600 dispensers per ha (~250 per acre) or (90 g a.i. per ha). The dispensers were hung on the fruiting wire and dispersed in May of each year (2008–2010). Essentially, this trial tested the additional suppressive effective of an MD program beyond the growers' insecticide program.

Each year, adult male *Pl. ficus* flights were monitored using three Pherocon Delta IIID sticky traps per plot, as described previously. Mealybug densities were assessed each year for 30-s-per-vine using the 0–3 rating on 200 vines per plot monthly (May–October), where 0 = no mealybugs, 1 = 1–2 mealybugs, 2 = 3–10 mealybugs, and 3 = >10 mealybugs found; this is a similar design as that described previously in the dispenser density trial but the rating was designed for fewer mealybugs given the lower threshold in table grapes at that time. Crop damage was assessed each year near harvest time (August–October) using the 0–3 rating of sampled fruit, as described previously, but adjusted for table grapes, where 0 = no mealybug damage, 1 = a few mealybugs found, or some honeydew present, 2 = fruit damage caused by mealybug and honeydew accumulation, and 3 = severe damage and an unmarketable cluster. Economic damage was measured by rating one cluster per vine on ~400 vines per plot, with clusters in contact with the trunk or cordon preferentially selected, as described previously.

Statistics

Results are presented as sample means ± SEM. Analyses were performed using Systat Software Inc. (version 13, San Jose, CA) and R version 3.4.1 (R Development Core Team 2017). For the pheromone trap counts and visual mealybug counts, we compared season-long treatment effects using the General Linear Model (GLM) function in Systat, with mealybug densities (trap counts, visual counts, percent infestation) as the dependent variable and treatment and date as the independent categorical variables, with a treatment × date interaction term. If more than two treatments were tested, Tukey Pairwise comparison was used to separate treatments. Data were transformed (log[x + 1] or square root [sqrt(x + 0.5)]) as needed to normalize the variance. For categorical ratings of mealybug density and cluster damage, treatment effects were compared in a 2 by 2 contingency table with treatments separated using Pearson's χ^2 test, with an experiment-wide error rate at $P < 0.008$ ($\alpha = 0.05/n$, where n is the number of possible pairwise comparisons). For mealybug density ratings, the mid- and late-rating periods were combined.

For the aerosol dispenser trial, the overall effect on trap catches were analyzed using a Generalized Linear Mixed Model with a negative binomial distribution. Due to low replication in these trials, data were pooled into a single puffer treatment for analysis each year, with each week being a replicate effectively comparing plots with and without the aerosol dispensers. Significance was determined using a Likelihood Ratio Test. Trap captures were averaged together within each replicate. Treatment and plot were independent variables and week a random variable. Distance from puffer was analyzed with transects along the grid's x- and y-axis. If the puffer was in between two trap rows, rows were averaged together. Statistical analysis was done in a similar manner as the total mean trap catches, with the additional variable of distance from puffer interacting with treatment. The grid of traps was also sectioned into center or border traps. Border traps were more than 18.3 m or 12.8 m away from the puffer in 2011 and 2012, respectively. Again, analysis was similar to total mean captures, with the addition of center/border grouping as an independent variable interacting with treatment. Analysis was conducted in R (R Core Team 2019) using the package 'glmmTMB' (Brooks et al. 2017).

Results

Dispensers Deployment Rate

There were higher season-long male mealybug trap captures in the 125 dispenser per ha treatment than the 310, 465, and 615 dispensers per ha rates, in both 2009 ($F = 10.732$, df = 3, $P < 0.001$;

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Fig. 1A) and 2010 (F = 7.498, df = 3, P < 0.001; Fig. 1B). In both years there was a significant effect of sample date (2009: F = 5.437, df = 9, P < 0.001; 2010: F = 7.158, df = 12, P < 0.001) as well as a treatment × date interaction (2009: F = 1.545, df = 27, P = 0.042; 2010: F = 3.519, df = 36, P < 0.001). In 2009, 14 of 16 plots were treated with Spirotetramat (Movento, Bayer CropScience) between 20–24 July due to high *Pl. ficus* pressure, as decided by the cooperating vineyard manager of these sites. For consistency, the two untreated blocks (treatments 465 and 615 dispensers per ha) were not included in the analyses. The low *Pl. ficus* male capture rates after July 2009 are likely a result of this pesticide treatment.

Planococcus ficus vine density ratings in the early-season 2009 (April), before deployment of pheromone dispensers and the application of Spirotetramat, indicate damaging *Pl. ficus* densities, ranging from 20.2–52.2% of the vines with measurable mealybug populations; there was a significant difference among treatment plots with more mealybugs in the 310 dispenser treatment (χ^2 = 130.549, df = 9, P < 0.001). By the late-season ratings (August), the 310 dispenser per ha plot still had more *Pl. ficus* (χ^2 = 108.023, df = 9, P < 0.001; Fig. 2A), but there was no treatment impact on crop damage, with 94–98.3% of the clusters clean and no clusters rated as unmarketable in any treatment (χ^2 = 5.316, df = 6, P = 0.504; Fig. 2D). In 2010, early-season vine ratings were low, with 98–100% clean vines and no treatment differences (χ^2 = 11.604, df = 6, P = 0.071); late-season ratings suggest a recovery of *Pl. ficus* populations and pheromone treatment differences, with lower densities in the 615 than the 125 dispenser per ha treatment (χ^2 = 21.920, df = 9, P = 0.009; Fig. 2B). In 2010, cluster damage was lower than 2009, ranging from 97.6–100% undamaged clusters; the previous trend of lower damage at 615 dispenser per ha was now significantly lower than the other treatments (χ^2 = 25.257, df = 9, P = 0.003; Fig. 2E). As *Pl. ficus* populations increased in 2011, treatment impacts became clearer, with lower mealybug densities in the three highest dispenser rates in early-(χ^2 = 74.103, df = 9, P < 0.001) and late-season counts (χ^2 = 146.72, df = 9, P < 0.001; Fig. 2C). Cluster damage was lower in the 310, 465 and 615 dispenser per ha treatments than the 125 dispensers per ha treatment (χ^2 = 52.739, df = 9, P < 0.001; Fig. 2F). Cluster damage, two years after the application of Spirotetramat, was increasing in all treatments and particularly in the 125 dispenser per ha density treatment, where 10% of the clusters had measurable damage.

Meso Dispensers

There was no early-, mid- or late-treatment difference on *Pl. ficus* densities (F = 2.021, df = 3,1596, P = 0.109; F = 1.750, df = 3,1596, P = 0.155; F = 2.306, df = 3,1596, P = 0.075; respectively; Fig. 3A). Similarly, there was no treatment difference in fruit infestation (χ^2 = 5.635, df = 9, P = 0.776; Fig. 3B).

Flowable Applications

There was a significant treatment impact of the flowable MD treatments (F = 52.464, df = 2, P < 0.001; Fig. 4A), with pairwise comparisons suggesting that an early-, mid-, and late-season application had lower *Pl. ficus* adult male season-long trap captures than the early- and mid-season application (P = 0.008) and control (P < 0.001) and the early- and mid-season application was lower than the control (P < 0.001). There was a significant impact of sample date (F = 15.093, df = 23, P < 0.001) and a treatment × date interaction (F = 5.335, df = 46, P < 0.001). *Planococcus ficus* vine density ratings during harvest time suggest lower infestation levels in the early-, mid- and late-season application than the other treatments (χ^2 = 22.012, df = 6, P = 0.001; Fig. 4B) but there was no postharvest treatment separation (χ^2 = 2.405, df = 6, P = 0.300; Fig. 4B). There was no treatment impact on crop damage ratings (χ^2 = 5.101, df = 2, P = 0.078), with only 5 of 1200 clusters with any measurable damage across all treatments.

Aerosol Dispensers

Mean male *Pl. ficus* per day captures were lower in plots with puffers in 2011 (puffer: 31.8 ± 9.1, control: 95.98 ± 22.7, LRT = 19.39, df = 1, P < 0.001) and 2012 (puffer: 5.99 ± 2.7, control: 13.17 ± 6.5, LRT = 146.54, df = 1, P = 0.004). *Planococcus ficus* trap captures along both axes decreased closer to plot centers in puffer treatments but not controls in 2011 (x-axis: LRT = 7.60, df = 1, P = 0.006; y-axis: LRT = 25.29, df = 1, P < 0.001) (Fig. 5A and B). In 2012, along the x-axis, trap counts increased from the plot center in both treatments (LRT = 8.32, df = 1, P = 0.004) (Fig. 5C), and *Pl. ficus* captures decreased closer to plot centers along the y-axis (LRT = 30.36, df = 1, P < 0.001), with puffer plots having overall lower captures (LRT: 65.66, df = 1, P < 0.001) (Fig. 5D). In 2011 trap counts in the center were lower around puffers (LRT = 15.27, df, P < 0.001); whereas, in 2012 trap location did not affect captures within treatments (LRT = 0.12, df = 1, P = 0.73) (Table 1).

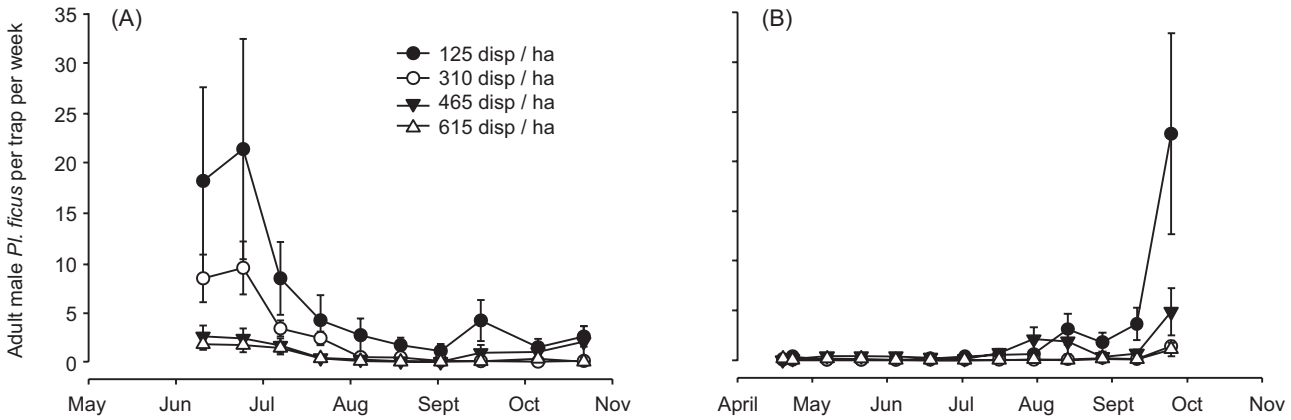


Fig. 1. Average *Pl. ficus* adult male (±SE) trap captures in treatment plots with different deployment rates of passive dispensers loaded at 150 mg a.i. per dispenser in (A) 2009 and (B) 2010; an application of Movento (Spirotetramat) in all plots between 20–24 July 2009 accounts for lowered mealybug densities until populations began to recover in August 2010. San Luis Obispo, CA.

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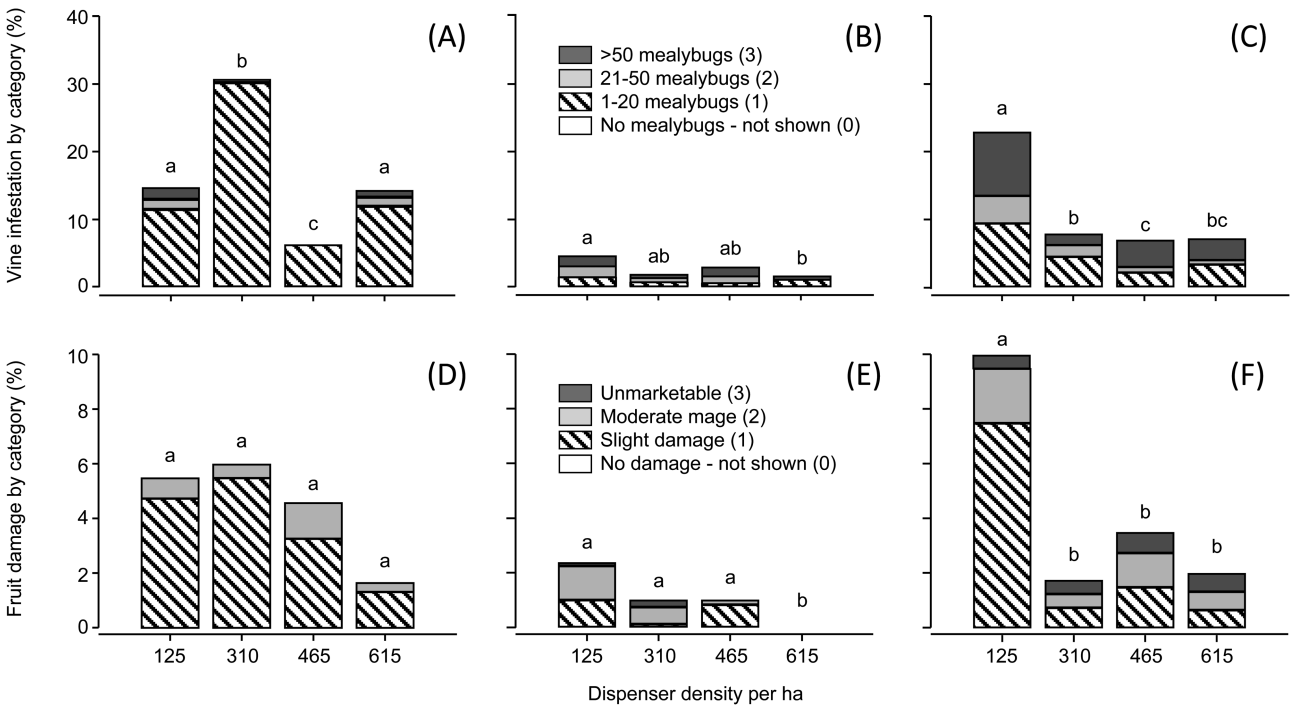


Fig. 2. The percentage for each categorical ranking of mealybug infestation levels during the late season period (July–August) for (A) 2009, (B) 2010, and (C) 2011 and for mealybug damage to fruit clusters at harvest time for (D) 2009, (E) 2010 and (F) 2011 (on each graph, different letters above each bar represent significant differences, pairwise comparisons using an experiment-wide error rate at $P < 0.008$). San Luis Obispo, CA.

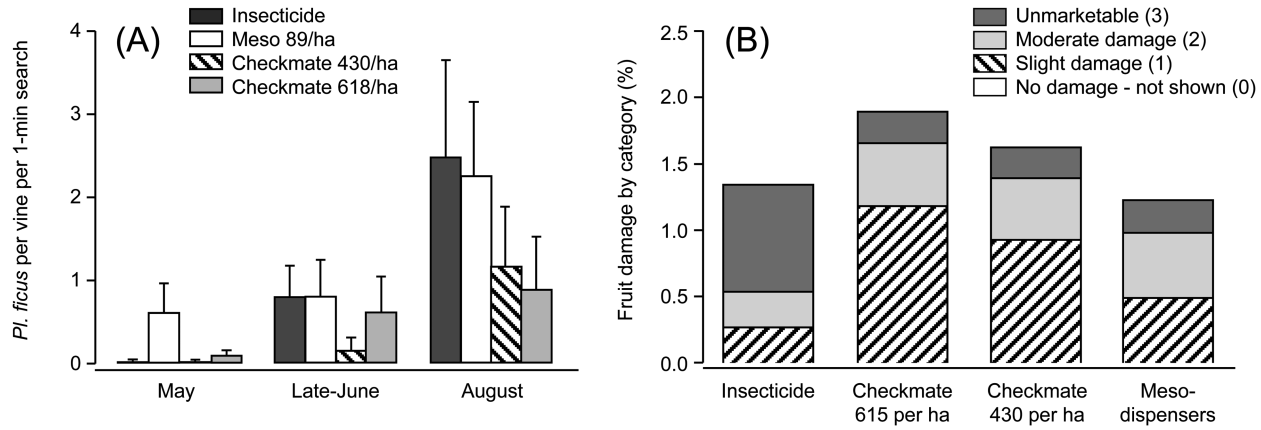


Fig. 3. Treatments compared delivery of *Pl. ficus* sex pheromone in meso dispensers (89 per ha, loaded at 2000 mg a.i. per dispenser) and standard dispensers (430 and 618 per ha, each loaded at 150 mg a.i. per dispenser) to a grower-standard insecticide treatment and suggest (A) no difference in average number of *Pl. ficus* 2nd instar to adults (±SE) found during a 1-min search of vines in early-, mid-, and late-season periods; (B) no treatment impact of the categorical ranking of mealybug damage to fruit clusters at harvest time. Lodi, CA.

Male *Pl. ficus* Flight

Male flight patterns in the temperature cabinet, based only on temperature, indicate that *Pl. ficus* flight occurred between 20 and 28°C, with no trap captures at 12, 16, 32, and 36°C (Fig. 6A). During the period of the trial (18–25 August; 1700–1300 h on the following day), ambient temperatures fluctuated from 18 to near 35°C (Fig. 6B). Pheromone trap captures from early evening, as temperatures cooled down, throughout the night (2100–0500 hr), and through the morning, as temperatures increased, show male flight activity was primarily in the early morning hours from 0500 to 1000 hr in both the vineyard trial (Fig. 6C) and manipulated *Pl. ficus* colony trial (Fig. 6D). During this period, morning temperatures ranged, generally, from 16–28°C, matching the temperature cabinet study. At these same temperatures in the evening, there was little *Pl. ficus* flight activity.

Commercial Application in Table Grapes

There were lower season-long male mealybug trap captures in the MD treatment in 2008, 2009, and 2010 (Table 2, Fig. 7); there was a significant effect of sample date and vineyard site, as well as the interaction between treatment and vineyard site (Table 2). *Planococcus ficus* vine density ratings in 2008 were largely similar between treatments across all sample dates, with vines rated as category 2 (3–10 mealybugs) or 3 (>10 mealybugs) at 6.7 and 7.2% in the MD and control treatments, respectively ($\chi^2 = 7.903$, df = 3, $P = 0.048$). The difference was largely based on the first sample date (May) with no

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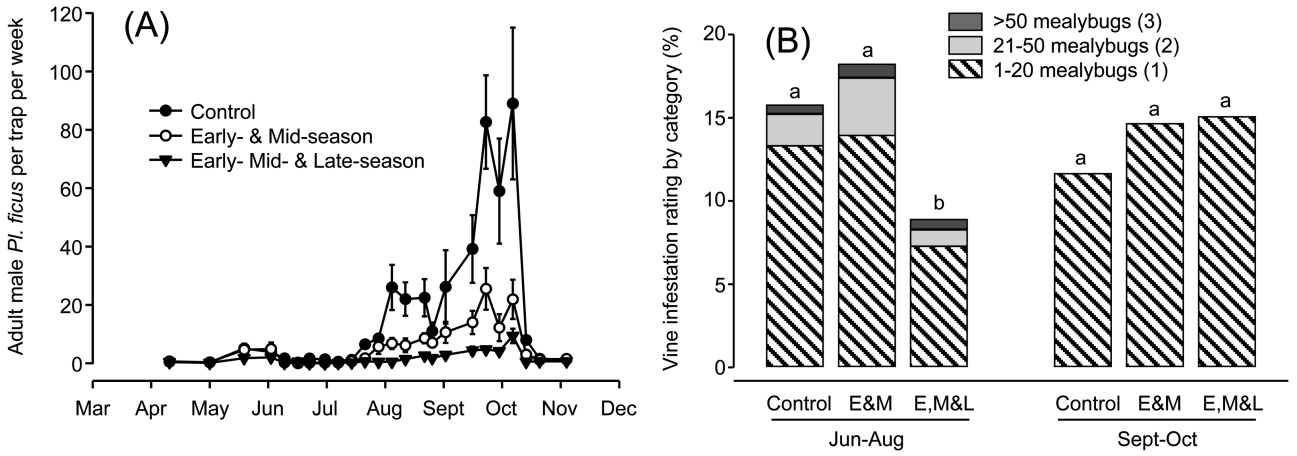


Fig. 4. Treatments compared a flowable application of *Pl. ficus* sex pheromone, either two or three times during the season (early – [E], mid – [M], and late – [L] season), to a grower-standard insecticide treatment (control). Adult male trap captures (A) were lower with three applications per season than two applications per season, and that two or three applications were lower than the control. Three applications per season resulted in lower *Pl. ficus* density ratings (B) during harvest time (June–August) but there was no postharvest (September–October) treatment separation. Different letters above each bar represent significant differences, pairwise comparisons using an experiment-wide error rate at $P < 0.008$. Lodi, CA.

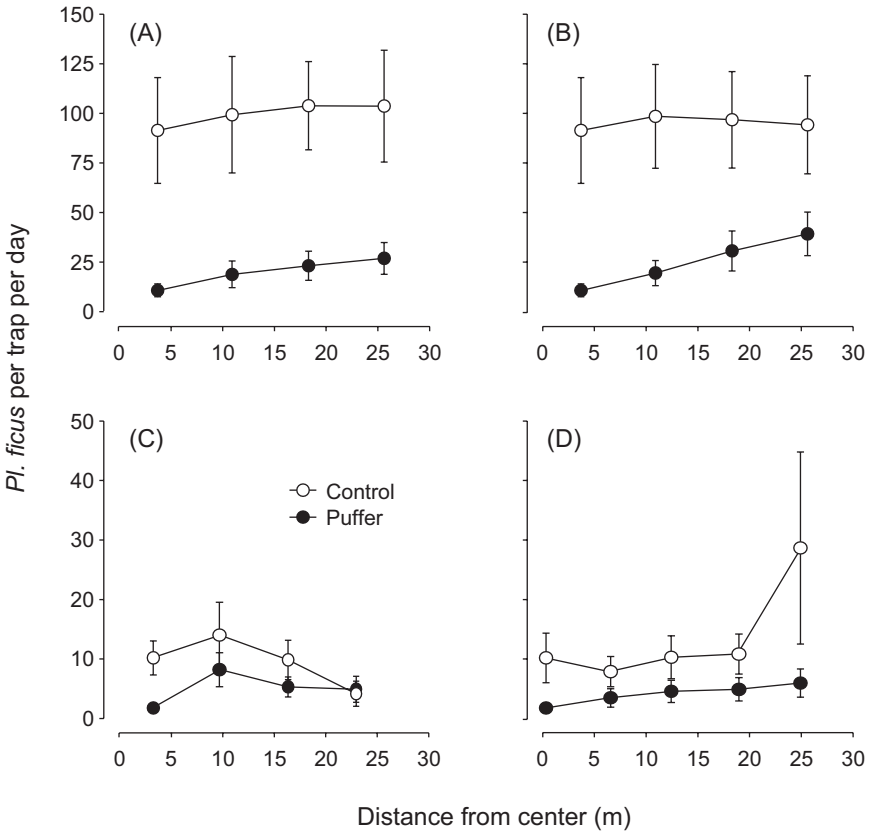


Fig. 5. Male *Pl. ficus* trap captures in pheromone traps placed in transects from the center of puffer (●) and control (○) plots reported as their distance (in meters) in 2011 along the x-axis (A) and y-axis (B) and in 2012 along the x-axis (C) and y-axis (D). Fowler (2011) and Napa (2012), CA.

significant differences on individual sample periods in June, July, August, or September samples. In 2009, across all sample dates there were lower category 2 and 3 ratings in the MD (3.6%) than the control (4.9%) treatment ($\chi^2 = 13.786$, df = 3, $P = 0.003$), however, the greatest treatment separation was later in the season near harvest-time in the August–September samples. By 2010, category 2 and 3 density ratings were lower in the MD (6.8%) than control (10.4%) treatment across all sample dates ($\chi^2 = 35.455$, df = 3, $P < 0.001$), with lower counts in June, July, August and September counts.

Crop damage was low in 2008, with only 0.8 and 1.3% of the clusters in the category 2 or 3 ratings, in the MD and control treatments, respectively ($\chi^2 = 5.730$, df = 3, $P = 0.125$). Similarly, in 2009 there was no treatment impact on cluster damage ratings (MD = 3.9, Control = 3.8%; $\chi^2 = 5.681$, df = 3, $P = 0.128$). In 2010, cluster

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damage (category 2 and 3) was marginally lower in the MD (3.1%) than control (5.1%) treatment ($\chi^2 = 7.368$, df = 3, $P = 0.061$). In this commercial-scale trial there was lower crop damage each successive year of MD application.

Discussion

Research reported herein suggests that different pheromone-release methodologies – passive dispensers, flowable suspensions, and aerosol technologies – can be used for *Pl. ficus* MD programs. Previous published trials focused on passive dispensers, typically loaded with 100–150 mg a.i. per dispenser and deployed at higher densities of ~600 per ha (e.g., Cocco et al. 2014, 2018; Sharon et al. 2016; Mansour et al. 2017; Lucchi et al. 2019; Hogg et al. 2021). Although successful MD programs are now used in commercial vineyards to suppress *Pl. ficus* populations, the research presented here provides information that can improve ongoing MD programs, highlights needed research directions, and provides supporting data on deployment strategies that could reduce MD costs and subsequently increase adoption. For example, deployment rates of 310 and 465 dispensers per ha, below the label rate near 618 dispensers per ha, reduced *Pl. ficus* densities and crop damage. Recently, Lucchi et al (2019) similarly reported a reduction in *Pl. ficus* cluster damage at deployment rates of 300, 400, and 500 dispensers per ha. We also demonstrated progressive impacts of MD on *Pl. ficus* populations over a 3-yr trial period. In the first year (2009) there was a treatment impact on male trap captures but not a clear difference in vine infestation levels or crop damage ratings. The second year there was a lower *Pl. ficus* incidence, due to the application of Spirotetramat across all plots; however, the highest deployment rate (615 dispensers per ha) had lower crop damage and there was a trend of lowered vine infestation and crop damage with increased dispenser deployment rates. By 2011, there was clear separation of the three highest density treatments (310, 465 and 615 dispensers per ha) from the

Table 1. Mean (±SE) adult male *Pl. ficus* trap captures per day from puffer trials between center and border traps

	2011		2012	
Treatment	Center traps	Border traps	Center Traps	Border traps
Control	94.0 ± 25.9 a	96.6 ± 22.2 a	10.4 ± 4.4 a	14.5 ± 17.4 a
Puffer	17.7 ± 5.6 b	35.9 ± 10.3 c	4.6 ± 2.5 b	6.6 ± 2.8 b

Within each year, means followed by different letters indicate a significant difference ($P < 0.05$) using Tukey's adjustment.

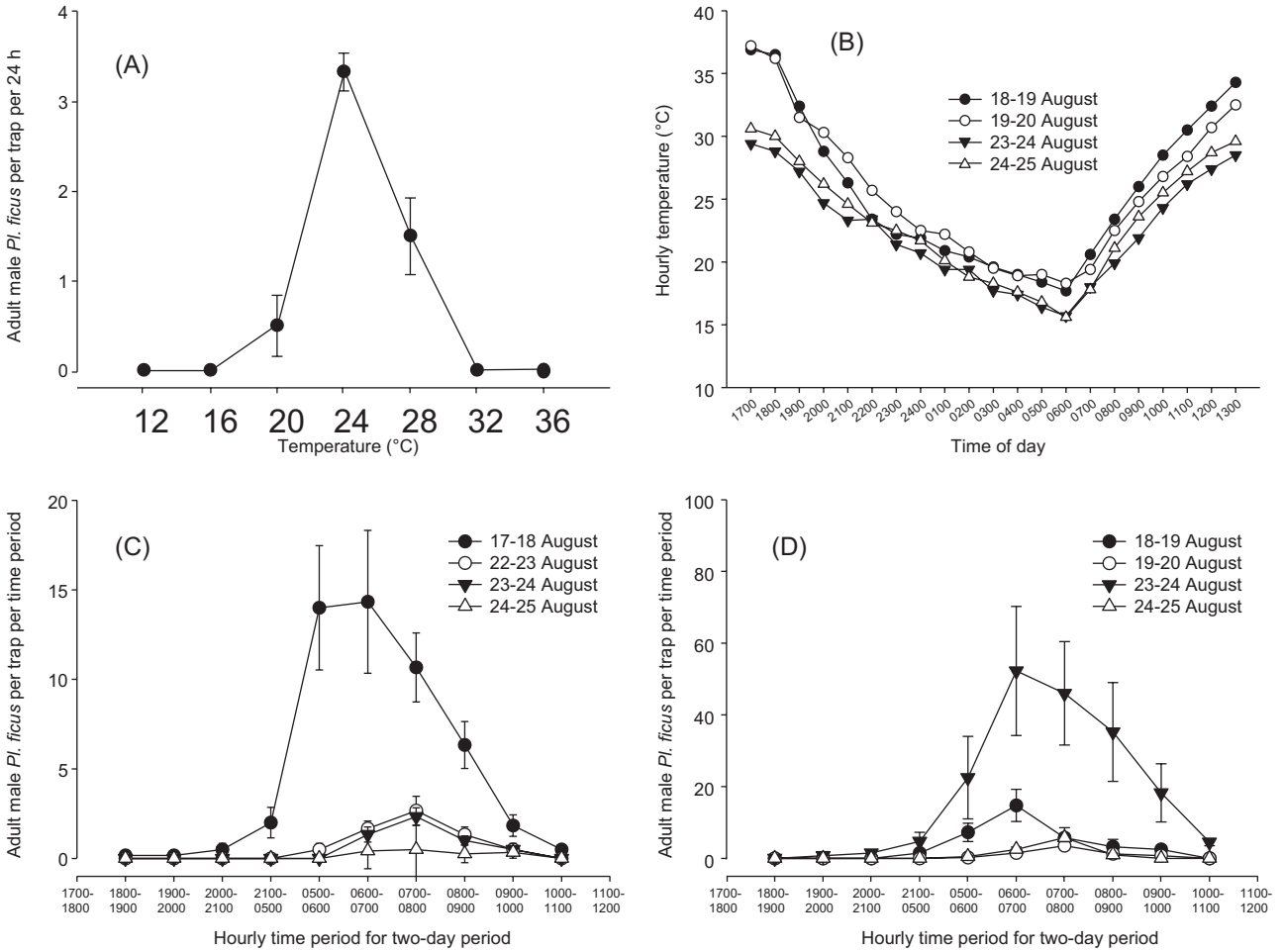


Fig. 6. Adult male *Pl. ficus* captures (mean ± SEM) in (A) a temperature cabinet study where temperatures ranged from 12 to 36°C; (B) ambient temperatures in Parlier, California during the evening and morning periods when *Pl. ficus* flight activity was monitored; *Pl. ficus* flight activity in (C) an infested vineyard where traps were changed every hour during the evening and following morning, and (D) a peach orchard where traps were placed around an insectary-manipulated *Pl. ficus* population contained on butternut squash. Parlier and Kingsburg, CA.

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Table 2. General linear model outputs for adult male *Pl. ficus* trap captures in mating disruption compared to control treatments in table grape plots from 2008 to 2010

Source	2008	2009	2010
Treatment (T)	$F = 72.52$, df = 1, $P < 0.001$	F = 15.90, df = 1, $P < 0.001$	F = 7.66, df = 1, $P = 0.006$
Sample date (SD)	F = 4.61, df = 16, $P < 0.001$	F = 5.80, df = 16, $P < 0.001$	F = 2.34, df = 12, $P = 0.007$
Vineyard site (VS)	F = 13.02, df = 5, $P < 0.001$	F = 45.52, df = 4, $P < 0.001$	F = 12.51, df = 4, $P < 0.001$
T × SD	F = 3.95, df = 16, $P < 0.001$	F = 1.31, df = 16, $P = 0.191$	F = 1.33, df = 12, $P = 0.200$
T × VS	F = 14.21, df = 5, $P < 0.001$	F = 6.32, df = 4, $P < 0.001$	$F = 5.19$, df = 4, $P < 0.001$

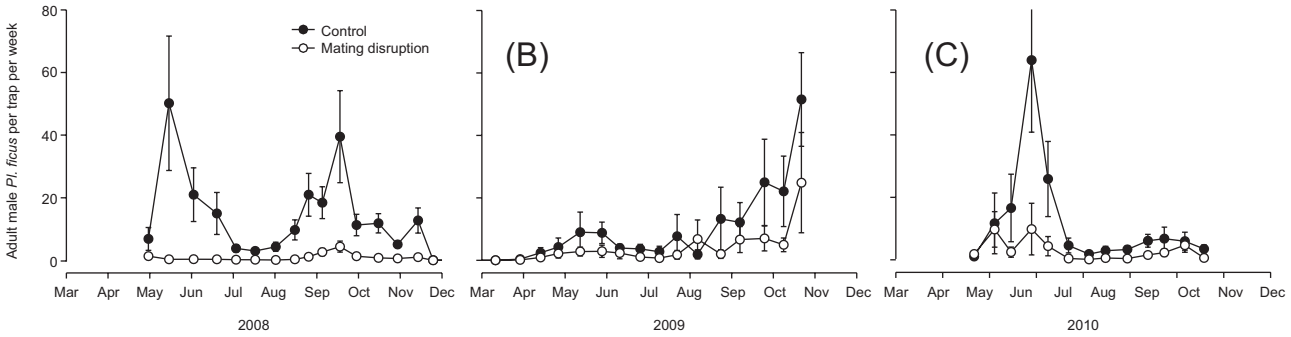


Fig. 7. Average *Pl. ficus* adult male (±SE) trap captures in large table grape plots with high density (618 dispensers per ha) deployment of standard *Pl. ficus* sex pheromone indicates lower trap counts than paired control plots in (A) 2008, (B) 2009, and (C) 2010 trials. MacFarland and Delano, CA.

lowest (125 dispensers per ha). Without a control it is difficult to draw conclusions on the effectiveness of 125 dispensers per ha and future studies will need to refine the impact of lower dispenser deployment rates in association with varying *Pl. ficus* densities. Our results also suggest repeated annual MD applications may be needed and we speculate that larger *Pl. ficus* densities or first year treatments may require a higher deployment rate, followed by lower rates in successive years of application if trap captures or harvest damage estimates support a reduction in populations. Approaches such as these that reduce the application rate of passive dispensers have the dual benefits of lowered materials and labor costs as well as reduced end-season disposal.

Meso dispensers have lower recommended application rates – about 90 per ha compared to about 600 per ha with standard dispensers – and are another means to reduce costs and address the additional challenge of labor availability to deploy dispensers across a large area at the appropriate time. In the meso dispenser trial, there was no treatment impact on *Pl. ficus* densities measured in May, late-June, or August or fruit damage at harvest time. However, mealybug densities in August samples were negatively associated with deployment rates ($y = 2.638 - 0.003x$, $R^2 = 0.941$, $P = 0.031$). We note that the meso treatment had the highest a.i. rate (178 g a.i. per ha), suggesting other explanations for the lack of treatment impact. For example, a greater number of point sources may be required when using passive dispensers, as has been demonstrated for moth pests (e.g., de Lame et al. 2010, Suckling et al. 2016). Additionally, multi-year MD deployment studies may identify cumulative impacts on *Pl. ficus* population densities, as discussed previously. A better understanding of biological factors such as male mealybug orientation to the pheromone plume and whether *Pl. ficus* MD is working via a competitive or noncompetitive disruption mechanism, may be important for determining the optimal deployment rate (Miller et al. 2006). For example, McGhee et al. (2014) reported that MD for codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) using aerosol dispensers at 5 units per ha disrupt male activity competitively by inducing false-plume following rather than by camouflaging females. Research is ongoing to investigate the deployment of lower rates of standard passive dispensers and meso dispensers for *Pl. ficus* control. This may particularly be relevant for mealybug MD programs as adult males are considered to be short-lived and weak fliers, which may be an important trait when considering point source reduction that may reduce pheromone plume. Moreover, female *Pl. ficus* tend to be hidden under the bark or within the vine canopy (Gutierrez et al. 2008) as compared to many moth pests successfully targeted in MD programs that have longer-lived males that are strong flyers and females in relatively open tree canopies.

We report that a microencapsulated (MEC) or flowable suspension reduced *Pl. ficus* adult male trap captures. Three sprays, covering most of the growing season, had lower captures than two sprays covering the early and mid-season period. Several studies have reported MEC sex pheromone formulations lowered pest densities of different moth species (e.g., Stelinski et al. 2007, Wins-Purdy et al. 2007)), as well as in the first MD trials against *Pl. ficus* (Walton et al. 2006). MEC formulations have a cost advantage over dispensers or aerosols because they can be tank mixed with other products (Il'ichev et al. 2006, Wins-Purdy et al. 2008)), or applied quickly by themselves at elevated drive speeds in relatively low volumes of water, to every other row. MEC products also solve the point source issue previously described by creating millions of point sources per hectare and placing them within the vine canopy in close proximity to the mealybugs. Producers also have flexibility in the amount of pheromone used per acre and can adapt the number of applications to pest density and harvest date. For example, a table grape producer might make two pheromone applications to vineyard with varieties harvested in early July, and three to four applications in a vineyard planted to a late-season variety harvested in the late fall. A disadvantage is that field longevity is often only 3–5 wk, thereby requiring repeated applications for season-long coverage (Walton et al. 2004, Knight and Larsen 2008). Currently, the CheckMate VMB-F MEC formulation is gaining popularity for *Pl. ficus* control because of its ease of application. Because MEC applications are closely tied to fungicide applications in western USA wine grape vineyards, further

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research is needed to determine the value of more common early season sprays, ending around veraison (mid- to late-July) even though the peak *Pl. ficus* flight period tends to be from August to October (Daane et al. 2020). Further areas of study include tank mixes to increase the field longevity of MEC formulations (Wins-Purdy et al. 2008) and whether ultra-low volume applications of MEC formulations produce a greater number of microcapsules per leaf area than a high-volume application (Knight and Larsen 2008, Reinke et al. 2014).

Aerosol technologies are another option for *Pl. ficus* MD programs. In our trials, adult male trap captures were lower in aerosol treatments and there was a negative association between dispenser location and trap captures in the aerosol plots. Benelli et al. (2019) review the history of aerosol dispensers, their mode of action, effectiveness, and deployment strategies. Compared with passive dispensers used at high densities, aerosol delivery systems can be applied at lower density, often 2–5 per ha, and programmed to release pheromones at selected time intervals when the target pest is active. We also found that *Pl. ficus* flight activity was concentrated in the morning hours, similar to Zada et al. (2008) when temperatures were between 17–28°C. Similarly, Burks and Thomson (2020) reported that only emission before midnight suppressed navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), communication when temperatures fell below 19°C. Baker (2016) further argued that emission rates from dispensers should be measured on a per-minute basis to better match knowledge of male flight patterns and contact with pheromone plume strands, thereby optimizing plume strand flux to improve dispenser field longevity and lower loading rates per dispenser. There also appears to be some fidelity to flight patterns, regardless of the deployment of pheromone emitters; for example, the peachtree borer, *Synanthedon exitiosa* Say (Lepidoptera: Sesiidae), did not change its diel rhythm response in the presence of MD (Teixeira et al. 2010). The primary disadvantage to aerosol emitters is the low number of point sources per hectare and potential for voids within the pheromone plume. This concern is elevated in table grapes where vines have very large canopies that completely envelop the trellis system such that there is minimal air circulation within the vine canopy where males are actively searching for females that are oftentimes under the bark. There was also difference in trap captures along the x- and y-axis and this may be related to the prevailing wind direction. For *Pl. ficus* MD programs, this might be a critical component for the use of aerosol dispenser use because the adult males are considered to be poor flyers and the population is often clumped. Moreover, it is not uncommon for wine grape vineyards to be rectangular along a hillside, where a prevailing wind might disperse much of the pheromone outside of the vineyard block. Clearly, work with aerosol dispensers presented herein represents an initial investigation of these devices, which have only recently become commercially available for *Pl. ficus*, and more detailed studies on their deployment rate, on–off timing with respect to *Pl. ficus* daily and seasonal flight patterns and seasonal temperatures are needed to optimize their use.

In the San Joaquin Valley table grape trial, results suggest that high density deployment of passive dispensers could reduce adult male trap captures in the first year of application as well as the following two seasons, which has been reported previously (Cocco et al. 2014, Lucchi et al. 2019, Daane et al. 2020). Perhaps the more intriguing information from this research was the difference among successive years and treatment plots. The trial was designed as a field demonstration using large plots and commercial management practices for table grapes, where there is little tolerance for mealybug infestation. In each year there was a significant difference among vineyard sites, as measured by pheromone trap captures, and this led to a significant treatment × vineyard site interaction. Of the five sites that remained in the MD program all five years, each a replicate, two sites had no measurable crop damage, two sites had significantly reduced crop damage in the MD plot (albeit this would be pseudo-replication) and one site had no treatment difference but very high *Pl. ficus* damage (>15%). This among-plot variation not only lowered the value of statistical comparisons but also highlights the question of when to use the high density deployment of passive dispenser or multiple applications of flowable materials. In this case, two of the sites could likely have received lower rates of dispensers without diminishing impact on *Pl. ficus* density or damage and the one site with extremely high *Pl. ficus* densities probably required insecticide applications rather than MD. As discussed by Walton et al. (2006) for *Pl. ficus* and by Witzgall et al. (2010) for MD programs in general, pheromones are increasingly efficient at lower pest population densities. It was also clear that for each successive year, there was a greater separation of MD from the control treatment even when including the high *Pl. ficus* density plot in the analysis.

Future research should also better integrate regional *Pl. ficus* population dynamics with pheromone deployment. As described in Daane et al. (2012) *Pl. ficus* populations overwinter primarily under the bark of the trunk and cordon, with some of the population found on the roots, especially when tended by ants. There is no diapause and, on warm days, development may occur during the winter months, with completion of the first generation almost entirely under the bark. From spring to summer, the *Pl. ficus* population follows the movement of plant resources from roots to shoots to leaves; four to seven generations per year typically occur in California's grape regions, although high summer temperatures, in excess of 40°C, may slow the population's growth and increase mortality. As berries ripen and sugars develop, mealybugs move into the berry clusters – first attacking those near the vine cordon. Adult male mealybugs have been found throughout the year, but trap captures are extremely low from December to April, perhaps because mating occurs under the bark, and flights are also suppressed during periods of high summer temperatures, perhaps because of high adult male mortality. Peak flights commonly occur from August to October, although there are regional differences. The rapid population increase in summer is followed by an equally rapid decline after harvest, resulting from biological controls and abiotic mortality associated with high temperatures and with vine senescence. Mating behaviors, seasonal adult male flight patterns, and seasonal sex ratio all should be further investigated to improve MD programs for *Pl. ficus*.

Overall, data presented herein support that passive dispensers, flowable formulations and aerosol devices delivering sex pheromone all can impact *Pl. ficus* densities. In a relatively short time, MD programs for *Pl. ficus* have gained commercial success in the Americas, Europe, the Middle East, and Africa (Cocco et al. 2014, 2018; Sharon et al. 2016; Mansour et al. 2017; Daane et al. 2020). Studies investigating reducing passive dispenser and flowable application rates, as reported herein and by Lucchi et al. (2019), provide support for lowered application costs that may, in turn, increase adoption of this sustainable tool, especially for use in areawide programs (Daane et al. 2018a, Hogg et al. 2021). There are still improvements to be made, particularly understanding the optimal rate of pheromone application for different *Pl. ficus* densities. Both flowable formulations and aerosol devices have the advantage of in-season adjustments to the application rate. For codling moth programs, McGhee et al. (2016) reduced pheromone concentration, operation time, and frequency of pheromone emission from aerosol dispensers while maintaining lowered pest densities. Whereas higher

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rates of deployment with passive dispensers typically provide better control, as shown herein and for some moth pests (Suckling et al. 2016), such high deployment rates may only be needed for high *Pl. ficus* densities or in the first years of MD program establishment. There is also a need to better understand the field longevity on grape leaves of pheromone delivered via flowable applications or aerosol devices (e.g., Knight and Larsen 2008, Gavara et al. 2020), the adult male mealybug flight patterns in relation to emitted plumes (e.g., Huang et al. 2013, McGhee et al. 2014, Baker et al. 2016) and the combined impact of MD and selected insecticides, especially delivered in a tank mix with a flowable pheromone suspension (e.g., Knight 2010, Suckling et al. 2016). A common goal of these research directions is to reduce overall costs of *Pl. ficus* MD programs to gain greater adoption of this sustainable tool (Daane et al. 2018a, Cocco et al. 2021).

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