Applications and Mechanisms of Wax-Based Semiochemical Dispenser Technology for Disruption of Grape Root Borer Mating

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ABSTRACT Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), is an important pest of cultivated grapes (*Vitis* spp.) in the eastern United States from Michigan to Florida. There are few registered insecticides for control of this pest, and their efficacy is limited. Pheromone-based mating disruption is a potential alternative to insecticides for management of *V. polistiformis*. Wax-based Specialized Pheromone & Lure Application Technology (SPLAT) was tested as a mating disruption method. Deployment densities of 150 dispensers per ha dosed with 5 mg of *V. polistiformis* pheromone were sufficient to achieve 95% mating disruption during a 7-wk trapping period. The disruption mechanism was determined to be competitive attraction. The release rate of pheromone from these dispensers was quantified to be approximately linear, 77.4 μg/g SPLAT/d. At this release rate, a minimum initial load of 5.4 mg of pheromone per dispenser would be needed to maintain disruption over a 9–10-wk *V. polistiformis* flight season, ≈19 August to 21 October in central Florida. It should be noted, however, that the main pheromone component alone, (*E*,*Z*)-2,13-octadecadienyl acetate (ODDA), was effective (presumably by a noncompetitive mechanism) at higher loads per area of crop. Due to the cost of synthesis of highly pure isomers of the *V. polistiformis* pheromone components, mating disruption of *V. polistiformis* may be more practical with higher doses of commercially produced *Zeuzera pyrina* L. blend [95% (*E*,*Z*)-2,13-ODDA:5% (*E*,*Z*)-3,13-octadecadien-1-ol] or with (*E*,*Z*)-2,13-ODDA alone than with the *V. polistiformis* blend at lower rates.

KEY WORDS *Vitacea polistiformis*, mating disruption, competitive attraction, pheromone dispenser

Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), was first reported attacking cultivated grapes (*Vitis* spp.) in 1854 (Kron 1854) and was formally described by Harris (1854a b). Since then, *V. polistiformis* has become one of the most destructive pests of grapes in North Carolina (Pearson and Schal 1999) and South Carolina (Pollet 1975). It is considered the key grape pest in Georgia (Dutcher and All 1979) and Florida (Liburd and Seferina 2004).

Grape root borer attacks European grapes, *Vitis vinifera* L.; muscadine grapes, *Vitis rotundifolia* Michx.; bunch grapes, *Vitis labrusca* L.; and hybrid bunch grapes (*Euvitis* spp. Planch). In addition, *V. polistiformis* are capable of completing their life cycle on wild grapes, which may serve as reservoirs of the pest, affecting adjacent commercial production (All et al. 1987, Olien et al. 1993). Grape root borer feed on and create gouge-like wounds in grape roots that can kill smaller roots and girdle large roots. These wounds cut off or restrict nutrient and water transportation from roots to the rest of the plant, reducing vine vigor (Clark and Ennis 1964) as well as average leaf area, fresh weight of berries, and pruning weight of canes per vine (Dutcher and All 1979). In addition, wounds on the roots render the plant more susceptible to freeze damage, drought, and pathogens (Pearson and Meyer 1996). A single larva is capable of causing 6% girdling of the vine trunk resulting in 47% yield reduction. Feeding by two to three larvae can kill a vine (Dutcher and All 1979). Grape root borer damage has resulted in loss of entire vineyards in Florida, and it is considered the cause of total cessation of grape production in South Carolina (Pollet 1975). According to Dutcher and All (1979), the economic injury level of *V. polistiformis* is extremely low (0.07 larvae per vine), indicating that use of insecticides should proceed immediately after detection.

Mating disruption is a potential alternative to insecticides for control of *V. polistiformis.* Use of pheromones has proven successful in vineyard agroecosystems for the moths *Eupoecilia guella* (Hübner) and *Lobesia botrana* (Denis & Schiffermüller) in Germany (Kast 2001, Louis and Schirra 2001). In >98% of vineyards implementing mating disruption in the Würtemberg region, attack damage did not exceed the economic threshold (Kast 2001, Louis and Schirra 2001). Mating disruption also has proven successful in controlling several sesiid pest relatives, including peachtree borer, *Synanthedon exitiosa* (Say)

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(McLaughlin et al. 1976, Alston et al. 2003); lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) (Pfeiffer et al. 1991); and currant borer, *Synanthedon tipuliformis* (Clerck) (Grassi et al. 2002). Catch of lesser peachtree borer in pheromone-baited traps was completely inhibited by pheromone treatment and populations were reduced by 19–97% compared with control plots as measured by pupal case counts (Pfeiffer et al. 1991). Furthermore, pheromone treatment outperformed pesticides recommended for lesser peachtree borer control (Pfeiffer et al. 1991).

Mating disruption with either of the two main components of the female sex pheromone blend also has been shown effective against *V. polistiformis* and population reductions have been documented up to a year after pheromone treatment (Johnson et al. 1991). In one investigation, disruption of *V. polistiformis* was more effective with the minor component (Z,Z)-3,13-octadecadienyl acetate (ODDA) than with the major component (*E*,Z)-2,13-octadecadien-1-ol acetate [(*E*,Z)-2,13-ODDA] (Johnson et al. 1991). Furthermore, Weihman and Liburd (2006) reported complete inhibition of orientation to pheromone traps by *V. polistiformis* with deployment of the sex pheromone of the leopard moth, *Zeuzera pyrina* (L.) [95% (*E*,Z)-2,13-ODDA:5% (*E*,Z)-3,13-octadecadien-1-ol] by using Shin Etsu (Tokyo, Japan) Isonet Z rope dispensers. Currently, the *V. polistiformis* two-component sex pheromone blend is prohibitively expensive to synthesize for commercial application, whereas the leopard moth blend is readily available; both of which share the major component (*E*,Z)-2,13-ODDA.

To develop cost-effective disruption of *V. polistiformis* by using the wax-based Specialized Pheromone & Lure Application Technology (SPLAT, ISCA Technologies, Riverside, CA), we conducted experiments to test the effects of pheromone blend, dispenser density per area of crop, dispenser aggregation within the crop, and pheromone loading per dispenser on effectiveness of *V. polistiformis* disruption. In addition, trapping studies and quantification of pheromone release from dispensers were conducted to gain insight into the possible mechanisms causing disruption of *V. polistiformis* in our experiments and to determine the duration over which different loads would be expected to achieve disruption.

Materials and Methods

Chemicals, Dispensers, and Traps. The semiochemicals used in the trapping and mating disruption experiments described below were the natural blend of *V. polistiformis* female sex pheromone [99% (*E*,Z)-2,13-ODDA:1% (Z,Z)-3,13-ODDA], the natural blend of *Z. pyrina* (leopard moth) pheromone [95% (*E*,Z)-2,13-ODDA:5% (*E*,Z)-3,13-octadecadien-1-ol], and (*E*,Z)-2,13-ODDA alone (major pheromone component of both species). Wax-based pheromone dispensers were made by mixing 99.0% pure pheromone purchased from PHEROBANK (Wageningen, The Netherlands) into 1.0-g deposits of the wax-based release matrix SPLAT from ISCA Technologies. Each dispenser was loaded with 5.0 mg of a given pheromone blend treatment unless specified otherwise. In 2008, dispensers were deployed with caulking guns calibrated to deliver 1.0 g of SPLAT, whereas in 2009 they were deployed using volumetric syringes to achieve the same amount of wax per dispenser.

In the various experiments below, male moth catch was monitored using Pherocon VI delta wing traps (Trécé Inc., Adair, OK). Except where stated, the traps were set at 1.5 m in height on the vine trellis posts, the male *V. polistiformis* were counted weekly, and the sticky cards were replaced weekly. Each trap was baited with a red rubber septum loaded with 1.0 mg of the *V. polistiformis* pheromone (Great Lakes IPM, Vesterburg, MI) or 1.0 or 10.0 mg of the *Z. pyrina* pheromone. The *Z. pyrina* lures were prepared as follows. Red rubber septa (West Pharmaceutical, Lionville, PA) were extracted for 24 h in hexane, 24 h in dichloromethane, and then air-dried for 48 h as per the methods of Knight (2002). Stock solutions of 1.0 and 10.0 mg of 95% (*E*,Z)-2,13-ODDA:5% (*E*,Z)-3,13-octadecadien-1-ol/100 μl were prepared in dichloromethane. Septa were loaded with 100 μl of the respective stock (1.0 or 10.0 mg) and then another 100 μl of dichloromethane was added to each reservoir. They were allowed to air dry until reservoirs were empty of liquid solvent. Control septa received 200 μl of dichloromethane.

Experimental Sites. Three different vineyards were used for experiments. A 0.5-ha, noncommercial vineyard in Citra, FL (29.4° N, 82.1° W) was divided into six 25- by 21-m plots. Plots were separated by aisles ≈5.3 m wide, and each plot contained a different grape cultivar ('Carlos bronze', 'Noble black', 'Triumph bronze', 'Alachua black', 'Blanc DuBois', or 'Conquistador'). The first four cultivars were planted using the bilateral cordon system, and the latter two were single cordon systems. In Lithia, FL (27.9° N, 82.2° W), a 4.1-ha commercial vineyard contained half 'Carlos black' and half Noble black grapes. Vines were spaced ≈3.7 m apart both within and between rows and all grapes were grown using the bilateral cordon system. A second 4.1-ha commercial vineyard in Bradenton, FL (27.5° N, 82.6° W) contained vines spaced 3.7 m within rows and 2.7 m between rows. 'Lake Emerald', 'Noble', Conquistador, and Blanc DuBois grapes were grown using the bilateral cordon system.

Relative Attractiveness of Pheromone-Baited Rubber Septa to Male *V. polistiformis*. An experiment was conducted (14–28 September 2007) in the Citra vineyard to compare attractiveness of the *V. polistiformis* and *Z. pyrina* pheromone blends to male *V. polistiformis*. The purpose of this comparison was to gain insight into the possible mechanism(s) of disruption by using the natural *V. polistiformis* pheromone blend versus unnatural blends containing the major component (*E*,Z)-2,13-ODDA. The treatments compared were lures containing 1.0 mg of *V. polistiformis* pheromone (described above), lures containing 1.0 or 10.0 mg of *Z. pyrina* pheromone, and a control. The experiment was arranged as a randomized complete

block replicated four times. Treatments were separated by 15–25 m.

Relative Attractiveness of Rubber Septum Lures and SPLAT Dispensers to Male *V. polistiformis*. The objective of this experiment was to compare the attractiveness of rubber septum lures used to monitor *V. polistiformis* in all pheromone disruption experiments with the 1.0-g SPLAT dispenser used to cause disruption. Our hypothesis was that SPLAT dispensers would attract male *V. polistiformis*, suggesting competitive attraction (false plume following) as an operative mechanism of disruption (Miller et al. 2006). This experiment was established on 24 August 2009 in the Citra vineyard described above, and data were recorded biweekly from 2 September to 12 October. This vineyard was otherwise not treated with pheromone disruption. The three treatments tested were replicated twice and were as follows: 1) one trap per plot baited with septum, 2) one trap per plot baited with SPLAT, and 3) two traps per plot; one trap baited with septum and the other trap baited with SPLAT. Plots were separated by 15–25 m. All septum and SPLAT treatments were loaded with the *V. polistiformis* blend. The two traps per plots in treatment three were placed in the central row, ≈8 m apart and at least 5 m from the plot edge. The traps of the remaining four plots, assigned one trap per plot, were hung 1.5 m above the ground on the central plant. All traps were checked biweekly; trapped *V. polistiformis* males were counted and the sticky cards were replaced.

Effect of Pheromone Blend on Disruption. The objective of this experiment was to compare disruption of *V. polistiformis* catch in monitoring traps using SPLAT dispensers releasing the "natural" *V. polistiformis* pheromone versus those releasing only the main pheromone component (*E,Z*)-2,13-ODDA. This experiment was established on 23 August, 2008 at Bradenton, and data were recorded weekly from 7 September to 26 October. The experiment was arranged as a randomized complete block with three treatments replicated four times. Wax dispensers were prepared as described previously with one treatment containing *V. polistiformis* pheromone, one treatment containing (*E,Z*)-2,13-ODDA alone, and the control receiving no pheromone. Dispensers were deployed within 0.02-ha plots of 25 grape vines, five plants per row in five rows, at a density of five per plant (125 per plot or ≈3,700 per ha). Dispensers were applied directly onto plants ≈30–45 cm from where the main trunk split and was trained along the trellis. Plots were separated by 15–25 m. Each plot contained one monitoring trap hung on the center-most plant 1.5 m above the crown.

Effect of Dispenser Density on Disruption. The objective of this experiment was to evaluate the effect of dispenser density per area of crop on disruption of male *V. polistiformis* orientation. This experiment was established on 22 August 2008 at Lithia, and data were recorded weekly from 7 September to 26 October. The experiment was arranged as a randomized complete block design with four treatments that were replicated four times. Each plot (0.03 ha) consisted of 25 grape plants in five rows (five plants per row). Treatments consisted of wax dispensers containing *V. polistiformis* pheromone, prepared as described above. All dispensers were deployed as described above at various densities: one dispenser per five plants (five total or 150 per ha), one dispenser per plant (25 total or 735 per ha), 10 dispensers per plant (250 total or 7,350 per ha), and a control. Plots were separated by 15–25 m. One monitoring trap was deployed per plot on the center-most plant 1.5 m above the crown.

Effect of Pheromone Dispenser Aggregation on Disruption. The objective of this experiment was to investigate the effect of pheromone dispenser aggregation on disruption of male *V. polistiformis*. We hypothesized that the treatment with the highest number of deployment sites per plot would result in the lowest moth catch if competitive attraction was the main operative mechanism of disruption. The experiment was established on 21 August 2009 in the Bradenton, FL, vineyard described above, and data were recorded weekly 23 from August to 24 October. The experiment was arranged as a randomized complete block with four treatments replicated four times. Wax dispensers, prepared using *V. polistiformis* pheromone, were deployed at a total density of 25–26 per plot; distribution of these dispensers was varied from a highly clumped distribution to one that was highly dispersed. Plots (0.02 ha) consisted of 25 grape vines, five plants per row in five rows. Treatments were: untreated control (without dispensers), one dispenser per plant (for a total of 25 dispensers per 25 vines), two dispensers on every other plant (for a total of 26 dispensers per 13 vines), and five dispensers on each corner plant and five on the most central plant (for a total of 25 dispensers per five vines). Plots were separated by 15–25 m. One monitoring trap was deployed on the center-most plant 1.5 m above the crown per plot. For the five dispenser location per plot treatment, the trap was not placed on the center-most plant because there were five dispensers deployed on it. In this case, the trap was placed one vine from the center-most location in an arbitrary direction.

Effect of Dispenser Dose on Disruption. The objective of this experiment was to determine the effect of pheromone loading rate on disruption of male *V. polistiformis*. We hypothesized that effective disruption could be maintained while reducing the 5.0-mg pheromone per dispenser (0.5% vol:vol) loading rate that was used in previous experiments. This experiment was established on 22 August 2009 in the Lithia, FL vineyard described above, and data were recorded weekly from 23 August to 24 October. The experiment was arranged as a randomized complete block with four treatments replicated four times. The treatments were 1.0-g SPLAT dispensers loaded with 0, 0.5, 2.5, or 5.0 mg of *V. polistiformis* pheromone. Each treatment was deployed at a density of one per plant (735 per ha) for 25 dispensers per plot in total. Each plot (0.03 ha) consisted of 25 grape plants in five rows, with five plants per row. Plots were separated by 15–25 m. One monitoring trap was deployed on the center-most plant 1.5 m above the crown.

Quantification of Pheromone Release From SPLAT Dispensers. The objective of this experiment was to quantify the release rate of pheromone from SPLAT dispensers used in mating disruption and trapping studies. The experiment was established on 21 August 2008. Individual dispensers containing the *V. polistiformis* pheromone blend were deployed as 1.0-g dollops (as described above) containing 5 mg of pheromone onto acetate strips (2.5 by 5 cm). Each dispenser was weighed and the acetate strip stapled to a numbered wooden board. Five blocks of 14 dispensers were prepared in this manner as well as 12 blank dispensers (negative control). Dispensers were deployed at Lithia in an area separated from the disruption experiments by ≈60 m for the duration of *V. polistiformis* flight (19 August–21 October).

During the first week of deployment, samples were collected daily to allow detection of a possible exponential decay in release rate near test onset. Thereafter, samples were collected weekly. One week's sampling consisted of one pheromone-loaded dispenser per block, for a total of five replicates, and one blank negative control dispenser from a randomly selected block. After removal, the dispensers were placed into separate glass vials and transported on ice to the laboratory where each vial received 5 ml of acetonitrile and internal standard, hexadecyl acetate (193.4 ng/μl, 99.0% purity, Sigma-Aldrich, St. Louis, MO). The samples were stored at −20°C until analysis.

Pheromone was extracted from SPLAT dispensers according to the protocol outlined in Stelinski et al. (2005). Pheromone within samples was quantified using a Varian 3800 gas chromatograph ([GC]; Varian Palo Alto, CA). The GC was equipped with a RTX WAX polar column (model 12423, Restek Corp., Bellefonte, PA) 30 m in length and with a 250 μm i.d. The initial GC temperature was held at 130°C for 2 min and then ramped at a rate of 2.5°C/min to 160°C, where it was held for 2 min. The program then ran at 40°C/min to a final temperature of 230°C. The carrier gas, He, entered the column at 20 psi. The pheromone content of the samples was calculated using the internal standard method (McNair and Miller 1998).

Statistical Analyses And Calculations. For all orientation disruption studies and the trapping study comparing attractiveness of various pheromone blends and doses, trap catch was not normally distributed and was therefore modeled by negative binomial regression. Differences among means were assessed using differences of least squares means ($P < 0.05$) (SAS/STAT version 9.2, SAS Institute 2009). To compare relative attractiveness of rubber septa and SPLAT dispensers, two-sample unpaired *t*-tests were used to determine whether catch values from traps in the double trap plots were different from single trap plots. Once trap catch was determined independent from number of traps per plot, trap catch data were pooled for the same lure treatments and trap catch means were analyzed using analysis of variance. Differences among means were determined by Tukey's means separation. Pheromone release rate from SPLAT dispensers was modeled with linear regression (SAS/STAT version 9.2, SAS Institute 2009). In addition, percentage of disruption was calculated as 1 − (mean moth catch per trap in the pheromone-treated plot/mean moth catch per trap in the control plot) × 100.

Table 1. Catch of male *V. polistiformis* in traps baited with *V. polistiformis* or *Z. pyrina* pheromone at various doses (14–28 September 2007)

Pheromone blend composition	Dose of pheromone per lure (mg)	Mean ± SD catch per trap over trapping period
No-pheromone control	0	0 ± 0.00
Z. pyrina	1.0	0 ± 0.00
Z. pyrina	10.0	0.94 ± 0.94a
V. polistiformis	1.0	7.0 ± 4.27b

Means followed by a different lowercase letter are significantly different ($P < 0.05$).

Results

Relative Attractiveness of Pheromone-Baited Rubber Septa to Male *V. polistiformis*. Both the unbaited negative control and 1.0 mg of *Z. pyrina* pheromone lure treatments yielded zero catch of male *V. polistiformis;* therefore, these data were excluded from the negative binomial analysis. Weekly mean male catch was significantly affected by treatment ($\chi^2 = 31.9$, df = 1, $P < 0.001$) but not by week ($\chi^2 = 2.7$, df = 1, $P = 0.26$) or block ($\chi^2 = 3.45$, df = 5, $P = 0.63$). Lures dosed with 1 mg of *V. polistiformis* pheromone attracted significantly ($\chi^2 = 47.3$, df = 1, $P < 0.001$) more *V. polistiformis* males per trap per week than lures dosed with 10 mg of *Z. pyrina* pheromone (Table 1).

Relative Attractiveness of Rubber Septum Lures and SPLAT Dispensers to Male *V. polistiformis*. Septum-baited traps deployed singly within plots caught a similar number (mean ± SD) of male *V. polistiformis* (5.5 ± 5.1 males per trap) compared with identical traps deployed two per plot (5.5 ± 2.9) ($t = -0.24$, df = 2, $P = 0.81$). Similarly, the difference between SPLAT-baited trap catch in single (1.8 ± 1.2 males per trap) and two-trap (2.3 ± 2.7 males per trap) plots was not statistically different ($t = -0.48$, df = 14, $P = 0.64$). Dispenser type ($F = 12.1$, df = 1, $P = 0.002$) and week ($F = 3.0$, df = 3, $P < 0.05$) were significant, whereas block ($F = 2.5$, df = 5, $P = 0.06$) was not. Overall, septum-baited traps caught significantly more moths (5.3 ± 3.9) than SPLAT-baited traps (2.0 ± 1.5) ($t = 1.9$, df = 22, $P < 0.05$).

Effect of Pheromone Blend on Disruption. Both pheromone blend treatments [5 mg of *V. polistiformis* pheromone or (*E*,*Z*)-2,13-ODDA alone] resulted in complete disruption of male *V. polistiformis* (zero catch) in traps baited with the *V. polistiformis* pheromone for the entire monitoring period. Mean ± SD catch of male *V. polistiformis* in control plots (7.0 ± 5.4) was significantly greater ($\chi^2 = 87.3$, df = 1, $P < 0.001$) than in plots receiving either of the two treatments.

Effect of Dispenser Density on Disruption. Mean weekly trap catch declined with increasing dispenser

Table 2. Catch of male *V. polistiformis* in traps baited with *V. polistiformis* pheromone as influenced by density of SPLAT dispenser point sources per plot (7 September–26 October)

Pheromone dispenser density per plot (per ha)	Mean ± SD catch per trap over trapping period	% disruption
0 (0)	7.1 ± 3.5a	
5 (150)	0.4 ± 0.8b	95.1
25 (735)	0.03 ± 0.06bc	99.2
250 (7,350)	0.0 ± 0.0c	100.0

Means followed by a different lowercase letter are significantly different ($P < 0.05$).

density and all treatments resulted in >95% disruption (Table 2). The effect of both week ($\chi^2 = 39.9$, df = 7, $P < 0.001$) and dispenser density ($\chi^2 = 123.1$, df = 3, $P < 0.0001$) on catch of male *V. polistiformis* was significantly different from zero. The effect of block was not significantly different from zero ($\chi^2 = 6.2$, df = 3, $P = 0.10$). Traps within the control plots caught significantly more moths than traps in plots of all other treatments (Table 2). Traps in plots containing five pheromone dispensers caught fewer moths than traps in control plots but more than in plots receiving 25 dispensers (Table 2). Traps in plots with 250 dispensers caught the fewest moths but not significantly fewer than traps in plots treated with 25 dispensers (Table 2).

Effect of Pheromone Dispenser Aggregation on Disruption. Trap captures of male *V. polistiformis* were significantly affected by dispenser aggregation ($\chi^2 = 39.7$, df = 3, $P < 0.001$), week ($\chi^2 = 39.7$, df = 8, $P = 0.003$), and block ($\chi^2 = 11.3$, df = 3, $P = 0.01$). Pairwise comparisons made using lsmeans with the Genmod procedure showed that traps in control plots caught significantly more moths than traps in all other treatment groups (Table 3). Traps in plots that received 25 dispenser release sites (one dispenser per vine) captured significantly fewer moths than any of the aggregation treatments (Table 3). The five dispenser site treatment resulted in 51.9% disruption, whereas the 13-dispenser site treatment resulted in 80.6% disruption; however, these treatments were not significantly different from one another (Table 3).

Effect of Pheromone Dose Per Dispenser on Disruption. Load rate ($\chi^2 = 28.9$, df = 3, $P < 0.00001$) and block ($\chi^2 = 30.9$, df = 3, $P < 0.001$) significantly affected catch of male *V. polistiformis* in the negative binomial regression model, whereas week did not

Table 3. Catch of male *V. polistiformis* in traps baited with *V. polistiformis* pheromone as influenced by aggregation of SPLAT dispenser point sources when all plots received a similar total number of dispensers (7 September–26 October)

No. of pheromone release stations per plot	Mean ± SD catch per trap over trapping period	% disruption
0	0.97 ± 1.10a	
5	0.47 ± 0.71b	51.9
13	0.28 ± 0.49b	80.6
25	0.03 ± 0.06c	97.2

Means followed by a different lowercase letter are significantly different ($P < 0.05$). In total, 25–26 dispensers were deployed in each plot (735 per ha).

Table 4. Catch of male *V. polistiformis* in traps baited with *V. polistiformis* pheromone as influenced by pheromone loading per 1.0 g SPLAT dispenser (23 August–24 October)

Milligrams of pheromone per dispenser	Mean ± SD catch of males per trap over trapping period	% disruption
0	1.13 ± 1.62a	
0.5	0.35 ± 0.52b	60.4
2.5	0.13 ± 0.25bc	90.0
5.0	0.08 ± 0.15c	91.5

Means followed by a different lowercase letter are significantly different ($P < 0.05$). In total, 25 dispensers were deployed in each plot (735 per ha).

($\chi^2 = 14.4$, df = 8, $P = 0.11$). Pairwise comparisons made using lsmeans with the Genmod procedure showed that catch of males in the control treatment was significantly higher than in all pheromone treatments (Table 4). Dispensers loaded with 0.5 mg of *V. polistiformis* pheromone caused an average of 60.4% disruption, which was significantly higher than the control but not significantly different from dispensers loaded with 2.5 mg. Dispensers loaded with 5.0 mg of *V. polistiformis* pheromone caused significantly higher disruption than the control and dispensers with the 0.5 mg loading rate, but this level of disruption was not significantly different from that obtained with dispensers containing the 2.5 mg load rate (Table 4).

Quantification of Pheromone Release From SPLAT Dispensers. Release rate of *V. polistiformis* pheromone from SPLAT dispensers fit a linear model ($F = 104.6$, df = 3, $P < 0.001$, adj. $r^2 = 0.859$) shown in Fig. 1. The release rate predicted by the model is ≈ 77.4 μg of pheromone per g of SPLAT per day. No pheromone was detected in the blank negative control.

Discussion

The current investigation sought to optimize pheromone-based disruption of male *V. polistiformis* by using SPLAT dispensers. A minimum density of ≈700 dispensers per ha was required to achieve nearly 100% disruption of male catch in traps when 1.0 g dispensers were loaded with 5.0 mg of *V. polistiformis* pheromone. Although this density seemed optimal, ≈95% disruption was obtained when density of such dis-

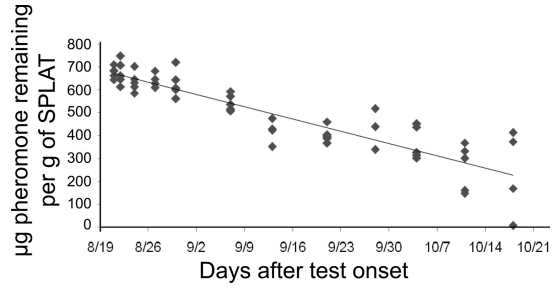

Fig. 1. Season-long release rate of *V. polistiformis* pheromone in the field from 1.0-g dispensers of SPLAT under Florida conditions.

pensers was reduced to only 150 per ha. When maintaining a total density of ≈700 dispensers per ha, aggregating release sites reduced efficacy. Thus placing one dispenser per vine seems optimal at this pheromone loading rate. Furthermore, efficacy was equivalent between a 2.5- and 5.0-mg pheromone dose per dispenser of SPLAT over the experiment duration.

Our results with dispensers releasing the natural *V. polistiformis* blend support the predictions outlined by Miller et al. (2006) for competitive attraction. First, traps baited with SPLAT dispensers attracted male *V. polistiformis.* Second, the initial impact of small increases of dispenser density was great but the return for a given increment in dispenser density progressively diminished as predicted for competitive attraction (Miller et al. 2006). If a noncompetitive mechanism such as camouflage were operative, the efficacy would have been expected to increase slowly as a function of dispenser density and then increase dramatically as plume coverage approached 100% (Miller et al. 2006).

One of our experiments also showed that *V. polistiformis* can be effectively disrupted with (*E*,Z)-2,13-ODDA alone (the major pheromone component of both *V. polistiformis* and *Z. pyrina*). When deployed at ≈3,700 dispensers per ha, disruption was equivalent between the 'natural' *V. polistiformis* blend and (*E*,Z)-2,13-ODDA alone, which is unattractive to male *V. polistiformis* as a single component. Our trapping study confirmed that the *Z. pyrina* blend is unattractive to male *V. polistiformis* males at a release rate that more closely approximates females and is only slightly attractive to males at a release rate that more closely approximates that of the SPLAT mating disruption dispensers. These results suggest that, in this case, disruption operated by a noncompetitive mechanism such as camouflage or some type of sensory desensitization. However, 3,700 dispensers per ha caused this effect, which is slightly >20 times the number of dispensers that resulted in effective disruption when the *V. polistiformis* blend was deployed. Given the 99:1 (*E*,Z)-2,13-ODDA:(Z,Z)-3,13-ODDA blend of the *V. polistiformis* pheromone, highly pure isomers of both components are needed to achieve competitive attraction, rendering the *V. polistiformis* blend ≈50 times more expensive than the *Z. pyrina* blend for practical application. Also, the *Z. pyrina* blend is already produced in large quantities by Shin Etsu for production of their Isonet Z polyethylene tube dispenser for leopard moth disruption. Therefore, it may be more economical to deploy more dispensers of the *Z. pyrina* blend per hectare than fewer of the *V. polistiformis* blend for disruption of grape root borer. Further work is needed to optimize the numbers of dispensers per hectare releasing the *Z. pyrina* blend for disruption of *V. polistiformis.*

Our attempt to optimize deployment of dispensers by investigating aggregation of release sites while maintaining an equivalent overall density of dispensers between treatments indicated that consolidating the number of release sites was not possible without a steep loss in disruption. Disruption with the most aggregated deployment was only 52%, whereas the most dispersed treatment was at 97%. Thus aggregating dispensers into larger dispenser release sites, when using the *V. polistiformis* blend, as a method of reducing hand application labor was not effective. Our results are congruent with those obtained for *Cydia pomonella* (L.) (Epstein et al. 2006) and *Grapholita molesta* (Busck) (de Lame et al. 2010).

The number of moths captured in control plots within experimental areas of the two vineyards where disruption trials were performed decreased from 2008 to 2009. The seasonal mean capture of male *V. polistiformis* within the experimental area of the Lithia vineyard was reduced from 7.9 ± 4.2 in 2008–2.8 ± 0.53 in 2009. Likewise, mean catch in control traps at Bradenton was reduced from 7.2 ± 4.3 in 2008–2.4 ± 0.6 in 2009. These were 64 and 67% reductions in average catch, respectively. However, monitoring traps in untreated areas placed 60 m away from the disruption experiment in Lithia caught 15.3 ± 6.7 and 15.2 ± 2.2 males per trap on average in 2008 and 2009, respectively. The weather conditions, grape cultivars, cultural practices, and proximity to nearby wooded areas were the same for both areas of the Lithia vineyard. These results suggest that pheromone treatment in the area of the mating disruption experiments reduced population densities of *V. polistiformis* during the following year. It is possible that the pheromone treatment reduced mating or delayed mating sufficiently to impact the number of eggs laid in the area of the disruption experiment in 2008 to reduce populations in the following year. Reduced or delayed mating negatively impacts both fecundity and fertility of *V. polistiformis* (Pritchard 2004). However, we cannot exclude the possibility that our pheromone treatments affected capture of male *V. polistiformis* in adjacent untreated plots given the 60-m spacing between these areas.

Our results indicate that mating disruption has promise as an alternative to insecticides for management of *V. polistiformis.* At a release rate of ≈77.4 μg of pheromone per day, control was observed when a sufficient number of dispensers were deployed per area of crop. To maintain that release rate for a season lasting 10 wk, a minimum initial load rate of 5.4 mg/g SPLAT would be required. However, an initial load rate between 2.5 and 0.5 mg/g release matrix was sufficient for effective disruption in these experiments. Our experiments also showed that as few as one dispenser every five grape vines (≈150 per ha) was sufficient to cause 95% disruption. This was a lower dispenser density than reported for effective disruption of *G. molesta* (Stelinski et al. 2005, Trimble et al. 2001) and *C. pomonella* (Epstein et al. 2006) in apple (*Malus* spp.) orchards and *V. polistiformis* in Arkansas vineyards (Johnson et al. 1991). The success of mating disruption; however, depends on pest population density when competitive attraction is operative and therefore dispenser deployment density may need to be tailored to the density of the target population. An advantage of the SPLAT formulation is that it is flowable and can be readily machine applied, which allows

precise tailoring of application rate to the population density of the target pest. Our results also show that aggregating dispensers into fewer release stations while maintaining equivalent numbers of dispenser per plot reduces disruption. However, *V. polistiformis* males can be effectively disrupted with (*E,Z*)-2,13-ODDA when dispensers are deployed at ≈3,700 per ha. Therefore, mating disruption of *V. polistiformis* with (*E,Z*)-2,13-ODDA alone or the less expensive *Z. pyrina* blend may be more economical when this pheromone is deployed at densities of dispensers that cause mating disruption by a noncompetitive mechanism. Given that the *Z. pyrina* pheromone blend is not currently registered for use in the United States, pursuing use of (*E,Z*)-2,13-ODDA alone for *V. polistiformis* mating disruption may result in a shorter term delivery of a registered product.

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