

# Mating Disruption of *Paralobesia viteana* in Vineyards Using Pheromone Deployed in SPLAT-GBM™ Wax Droplets

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**Abstract** A paraffin wax formulation releasing pheromone for mating disruption of insects was tested during 2005 and 2006 in *Vitis labrusca* vineyards infested by grape berry moth, *Paralobesia viteana* (Lepidoptera: Tortricidae). In early May of each year, 1-ml droplets of SPLAT-GBM™ wax containing 3% sex pheromone of *P. viteana* were applied to every wooden post at a rate of 400/ha in replicated 1.3-ha plots. Moth captures in sex pheromone baited traps placed at the vineyard borders and interiors revealed significant disruption of male moth captures in treated plots, with activity of one application lasting over 10 weeks during both years. Treatment with SPLAT-GBM™ did not affect the proportion of clusters infested until the end of the second growing season, when infestation was 27% lower in the treated plots than in the control plots. Comparisons of moth captures in traps placed inside 15.2 × 16.5 m vine plots that were untreated or received varying densities of 0.2-ml wax drops or Isomate-GBM hand-tied dispensers at the recommended rate of 450/ha indicated that orientational disruption increased with droplet density. Similar numbers of moths were captured in plots that received 10 or 30 drops per vine as were trapped in plots with twist ties spaced at 0.4 per vine. Moth captures in monitoring traps baited with increasing sizes of wax droplets (0.2, 0.5, or 1-ml drops) or red septa containing *P. viteana* sex pheromone suggest decreasing ability of male moths to reach traps with increasing pheromone loading. This study indicates that wax-deployed pheromone can reduce crop infestation by *P. viteana* after 2 years of deployment, and that the increasing of pheromone release

by using application of greater droplet densities or by using larger droplets will improve the level of disruption achieved.

**Keywords** Grape berry moth · IPM · Vineyard · *Endopiza viteana* · Lepidoptera · Tortricidae

## Introduction

Vineyards in the eastern United States are at risk of infestation by the grape berry moth, *Paralobesia viteana* (Clemens), a tortricid moth that lays eggs directly onto clusters, and whose larvae then bore into the fruit (Hoffman and Dennehy 1989; Tobin et al. 2003). This insect is the most economically important direct pest of eastern US and Canadian vineyards, and protection of fruit is achieved typically by using broad-spectrum insecticides. Effective alternative control strategies are needed for the control of *P. viteana* to enhance adoption of IPM programs and to help minimize the risk of resistance to insecticides (Jenkins and Isaacs 2007a, b). The use of sex pheromones for mating disruption of *P. viteana* is one approach that has been tested in research trials but which has relatively limited commercial adoption.

The major component of the sex pheromone of *P. viteana* was identified by Roelofs et al. (1971) as (*Z*)-9 dodecenyl acetate with the minor component, (*Z*)-11 tetradecenyl acetate, being identified by Witzgall et al. (2000). These discoveries led to evaluations of mating disruption using hand-applied rope dispensers (subsequently referred to as twist ties) and sprayable formulations of this pheromone, that provided promising results in New York and Ontario vineyards (Dennehy et al. 1990; Trimble 1993; Trimble et al. 1991, 2003). Despite these findings, adoption

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of hand-applied rope dispensers for season-long control of this pest by grape growers has been low, and no companies currently produce sprayable pheromones for *P. viteana* control. Among the reasons for the low adoption of this technology is the 2- to 3-week duration of pheromone release by sprayable formulations (R. Isaacs, unpublished data) and the labor required to apply rope dispensers to the crop.

Recently, a paraffin wax emulsion has been developed, into which insect sex pheromones can be mixed at the required concentration (Atterholt 1996; Atterholt et al. 1998; de Lame 2003). This wax is a viscous homogenate that hardens once applied to crop foliage or branches and it can act as a long-lasting discrete source of pheromone emission. The wax can be applied into crop systems by using manual methods that are more rapid than manual application of twist ties (de Lame 2003; Stelinski et al. 2005; Epstein et al. 2006), or it can be applied by mechanical methods (Stelinski et al. 2005) that enable application to large orchards or vineyards from motorized vehicles (Stelinski et al. 2006).

The optimization of mating disruption requires that the density and size of droplets, as well as the pheromone release rate and duration, are appropriate for the biology of the targeted pest (Miller et al. 2006a, b). Because the size, density, and distribution of wax droplets can be easily manipulated, this delivery system also provides researchers with a flexible tool to aid investigations of how moths can be optimally disrupted. For example, Stelinski et al. (2005) documented increased mating disruption of male oriental fruit moth, *Grapholitha molesta*, in apple orchards as droplet density increased, thus leading to increased suppression of mating in virgin female moths. The authors compared mating disruption in plots treated with wax droplets or the standard twist-tie dispenser, and found superior performance when wax was used. This suggests that the typical deployment density of pheromone twist ties may not provide sufficient point sources of pheromone for optimal disruption of *G. molesta*. More recent studies that evaluated mating disruption of the codling moth, *Cydia pomonella*, in apple orchards treated with the codlemone sex pheromone in wax droplets provide further evidence that higher densities of pheromone release sources improve disruption of mating (Epstein et al. 2006). The success of these studies and others in cotton, pear, and walnut crops has led to the development of a commercial product called SPLAT™ (Specialized Pheromone and Lure Application Technology) that acts as the carrier for sex pheromones or other semiochemicals.

To ascertain the potential for wax-based formulations for mating disruption of *P. viteana*, we determined the duration and efficacy of pheromone disruption of *P. viteana* in Michigan vineyards over two growing seasons by using the GBM-SPLAT™ commercial formulation of the wax matrix.

We also tested the hypotheses that orientation of *P. viteana* to droplets would decrease with increasing pheromone release, and that mating disruption would be improved by increasing the density of droplets.

## Methods and Materials

**Study Sites** All studies were conducted in mature *Vitis labrusca* var. ‘Concord’ vineyards in Van Buren Co., Michigan. These vineyards received standard fungicide and insecticide programs applied by the growers, but low crop levels due to a late spring freeze in 2006 led to a reduced pesticide program and higher activity of *P. viteana* in the second year.

**Large-scale Mating Disruption Study** This study was conducted during 2005 and 2006 in four vineyards at the same farm, each of which was split into two equal-sized plots [ca. 1.3 ha (100×130 m)]. The plots were assigned to be treated with pheromone in wax, or not treated, in a randomized complete block design with four replicates. Plant spacing in these plots was 2.7 m between vines and 3 m between rows, with posts distributed every three vines, resulting in 1200 vines and 400 posts per hectare. In both years, 1-ml droplets of GBM-SPLAT™ (ISCA Technologies, Inc., Riverside, CA, USA) were deployed by hand on the north side of each wooden post in the treated plots with a 30-ml syringe. The applications were made on 9 May in 2005 and 1 May in 2006, with the same plots treated in both years with wax containing 3% (v/v) of *P. viteana* pheromone (12 ml AI/ha) at a 10:1 ratio of (Z)-9 dodecenyl acetate: (Z)-11 tetradecenyl acetate (Shin-Etsu Chemical Co., Tokyo, Japan). In 2005, the formulation also contained 3% (v/v) cypermethrin, but no moths were trapped near these droplets when placed on sticky traps, suggesting that *P. viteana* moths did not contact the droplets (R. Isaacs, unpublished data).

Adult male *P. viteana* were monitored in each plot with large plastic delta traps (Suterra LLC, Bend, OR, USA) baited with rubber septa that contained female sex pheromone (90:10 ratio of (Z)-9–12Ac and (Z)-11–14Ac) (Suterra LLC, Bend, OR). Two traps were suspended from the top trellis wire at a height of 1.5 m at both the border and the interior of each vineyard, with at least 33 m between traps. Those in the interior were 65 m from the border, in the same rows as the border traps. The number of male *P. viteana* captured was monitored weekly, and moths were removed or sticky inserts were replaced. Pheromone lures were replaced every 4 wk, with lures from the same lot throughout each season.

Infestation by *P. viteana* was quantified by visually examining 50 clusters (25 clusters at two sampling sites) at

the border and interior of each plot. Both the number of clusters, and the number of berries infested were recorded in the first generation of GBM on 29 June 2005 and 6 July 2006, and during the second generation on 15 August 2005 and 4 August 2006.

**Droplet Release Rates** To determine the release of pheromone from wax droplets, five 1-ml droplets of GBM-SPLAT™ were applied to each of five 30.5×2.9 cm untreated wood garden stakes (Dayton Garden Labels, Dayton, OH, USA) and deployed on 24 May 2006 at one corner of each of the four pheromone-treated plots described above. Garden stakes were scored every 3.8 cm with a utility knife to allow for ease of individual drop collection. Each stake was nailed to the north face of a post located farthest from monitoring traps. One drop was collected from a randomly selected stake in each of the four treated plots each week until the termination of the experiment. Each sample was placed into a 60-ml glass bottle (Qorpak, Bridgeville, PA, USA) and stored at -20°C until it was extracted using the procedure of Stelinski et al. (2005), which was modified from Meissner et al. (2000). Fifty milliliters of an internal standard solution of 232 ml/l methyl myristate (99%, Acros Organics, Geel, Belgium) in acetonitrile (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ, USA), were added to each sample. The bottles were placed into a water bath shaker (model 406015, AO Scientific Instruments, Keene, NH) at 70–75°C without shaking for 10 min, followed by 10 min of shaking, then briskly agitated by hand for 10 sec, before an additional 3 min of shaking in the water bath. Samples were then hand-agitated for 10 sec and frozen at -20°C to precipitate the wax. After 20 hr, samples were thawed, vortexed, and 1 ml of the solution was removed and filtered into a 2-ml GC vial (Agilent Technologies, Santa Clara, CA, USA) through a disposable glass Pasteur pipette fitted with a paper plug (Kimberly-Clark Corp., Roswell, GA, USA) at the tapered end. The pheromone in each sample was quantified by gas chromatography (GC) (HP-6890, Hewlett-Packard, Palo Alto, CA, USA) by using a 30-m HP DB-23 polar column (model 122-2332, J&W Scientific, Folsom, CA, USA) with the internal standard method (McNair and Miller 1998).

**Droplet Density Study** This study was conducted during 2006 within one large vineyard to determine the effect of droplet density on disruption of male *P. viteana* flight to pheromone traps. The experiment consisted of a randomized complete block design replicated six times. Treatment plots were 15.2×16.5 m with 80 m between plots and between blocks. Plots received either 0, 1, 3, 10, or 30 0.2 ml droplets of wax containing 3% (v/v) *P. viteana* pheromone per vine, applied by hand with a 1-ml syringe,

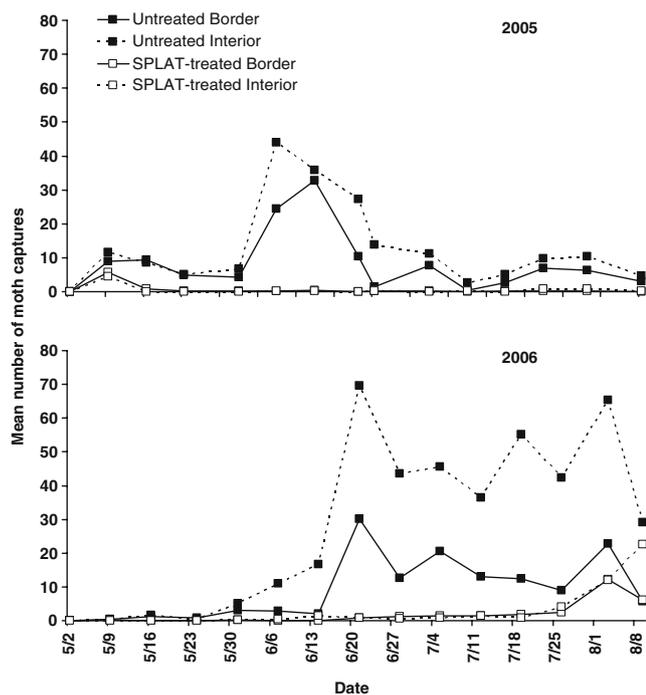
or Isomate GBM Plus rope dispensers (Shin-Etsu Chemical Co., Tokyo, Japan) at a density of 450/ha. Treatments were applied on 1 and 3 June 2006. A large plastic delta trap (Suterra LLC, Bend, OR, USA), baited with rubber septa that contained *P. viteana* sex pheromone (Suterra LLC, Bend, OR) was placed in the middle of each plot. Traps were monitored weekly until 24 August 2006 to record the number of male *P. viteana* captured, at which time the moths were removed or sticky inserts replaced. Pheromone lures were replaced every 4 wk with lures from the same lot.

**Droplet Size Study** This experiment used the same protocol as the droplet density study, but in this case the traps were baited with 0-, 0.2-, 0.5-, or 1.0-ml wax droplets (approximately 6, 15.9, and 30 mg *P. viteana* sex pheromone, respectively) applied to aluminum foil suspended inside the traps, or a rubber septum loaded with 0.1 mg *P. viteana* sex pheromone. All treatments were applied on 24 May 2006, and the traps were monitored weekly until 24 August 2006. To help minimize the effect of pest pressure within experimental blocks, treatments were rotated within each block every week, and droplets were changed every 4 wk.

**Statistical Analyses** In the mating disruption study, the significance of treatment effect on the number of moths captured for 13 and 15 wk after application in 2005 and 2006, respectively, was compared among treatments for both locations (plot interior and border) by using ANOVA (SAS/STAT 2003). For each sample date, the average number of moths per trap was compared among treatments within each vineyard position by using ANOVA (SAS/STAT 2003). All data were log-transformed ( $n+1$ ) for normality prior to analysis, and Tukey's HSD test was used to determine differences between means at  $\alpha=0.05$ . Percent disruption values were calculated as the proportional reduction in the number of moths caught in the treated plots compared to the untreated plots. Infestation data were arcsine squareroot transformed prior to analysis and compared between treatments at the border and at the interior positions by using ANOVA for each sampling date (SAS/STAT 2003).

To determine the relationship between time after application and the amount of pheromone released, residual concentration of pheromone droplets in the release rate study was analyzed by regression analysis (SAS/STAT 2003). These values also were compared to the percent disruption values calculated from moth captures, to determine a critical release rate for disruption of *P. viteana*.

The significance of treatment effect on total moths captured in the droplet density and droplet size study was compared among treatments by using analysis of variance (ANOVA) (SAS/STAT 2003). Data were log-transformed ( $n+1$ ) prior to analysis and Tukey's HSD test was used to determine differences between means at  $\alpha=0.05$ .



**Fig. 1** Average number of adult male *Paralobesia viteana* captured in pheromone traps at the vineyard border or vineyard interior of vineyard plots that were either untreated or treated with 1-ml droplets of SPLAT-GBM™ containing 3% pheromone

## Results

**Large-scale Mating Disruption Study** Before application of treatments in 2005, there was no significant difference in the number of moths trapped between treatments ( $F=3.67$ ;  $df=1, 2$ ;  $P=0.20$ ) or positions ( $F=0.05$ ;  $df=1, 8$ ;  $P=0.83$ ), indicating that pest pressure was similar across vineyards (Fig. 1). However, following the SPLAT-GBM™ treatments, the seasonal total number of moths per trap was lower in treated ( $2.6\pm 0.7$ ) than untreated ( $148.9\pm 28.1$ ) plots ( $F=96.76$ ;  $df=1, 3$ ;  $P=0.002$ ) (Fig. 1), a pattern

observed both within ( $F=126.6$ ;  $df=1, 3$ ;  $P=0.002$ ) and at the borders ( $F=37.55$ ;  $df=1, 3$ ;  $P=0.009$ ) of plots (Fig. 1).

In 2006, *P. viteana* were not trapped before treatment applications (moths had been trapped at nearby vineyards), but post-treatment the captures of *P. viteana* were significantly lower in the SPLAT-treated ( $36.5\pm 9.3$ ) than the untreated ( $279.6\pm 47.2$ ) vineyards ( $F=39.82$ ;  $df=1, 3$ ;  $P=0.008$ ). Most moth captures in the treated plots occurred in the last 2 wk of the trial as the disruption efficiency of the formulations declined (Fig. 1). In contrast to 2005, in 2006 more male *P. viteana* were captured at the interior compared to the border ( $F=35.4$ ;  $df=1, 12$ ;  $P<0.001$ ) within plots. However, similar to 2005, fewer moths were captured at the interior ( $F=33.97$ ;  $df=1, 3$ ;  $P=0.01$ ) and the border ( $F=21.22$ ;  $df=1, 3$ ;  $P=0.019$ ) of treated plots compared to untreated plots.

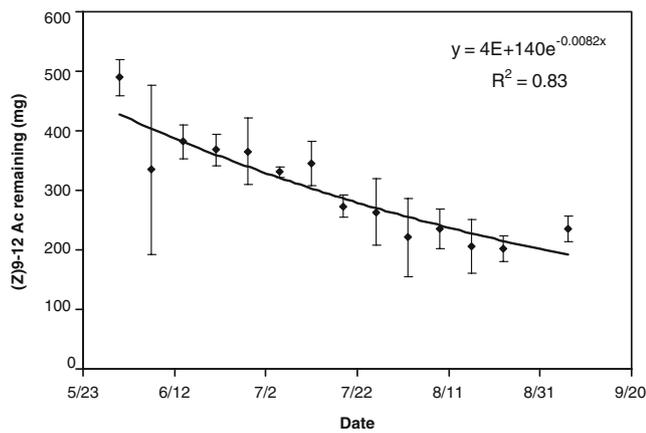
Percent disruption exceeded 90% at the vineyard interior for approximately 12 wk in 2005 and 11 weeks in 2006. In 2006, when there were no insecticide applications to the vineyards, the level of disruption had declined to 22.7% after 13 wk. In both years, disruption was greater at the interior traps than in traps placed at the borders.

In 2005, neither the total number of larvae found in each sample ( $F\leq 0.53$ ;  $df=1, 3$ ;  $P\geq 0.52$ ) nor the proportion of clusters infested with *P. viteana* larvae ( $F\leq 3.71$ ;  $df=1, 3$ ;  $P\geq 0.15$ ) varied significantly between treatments or vineyard positions for either sampling date (Table 1). During the first generation samples of 2006, there was no significant difference between treatments in the proportion of clusters infested or the number of larvae in cluster samples at the border ( $F=2.32$ ;  $df=1, 3$ ;  $P=0.23$ ) or interior ( $F=6.91$ ;  $df=1, 3$ ;  $P=0.078$ ) of the vineyard (Table 1). However, during the second generation, there were differences in these parameters between the SPLAT-treated and untreated borders ( $F=12.76$ ;  $df=1, 3$ ;  $P=0.038$ ).

**Droplet Release Rates** Droplets of SPLAT-GBM™ released pheromone as a first-order exponential decay function

**Table 1** Average number of *Paralobesia viteana* larval berry infestations and the percentage of clusters infested with *P. viteana* ( $\pm$ S.E.) at the borders and interiors of vineyard plots that were untreated or treated with SPLAT-GBM™ during 2005 and 2006

Date	Position	No. of infestations per 25 clusters			Percent of clusters infested		
		Untreated	SPLAT	<i>P</i> value	Untreated	SPLAT	<i>P</i> value
6/29/2005	Border	6.1 $\pm$ 1.3	7.4 $\pm$ 2.2	0.62	7.8 $\pm$ 1.0	10.0 $\pm$ 1.8	0.15
	Interior	1.9 $\pm$ 0.7	1.4 $\pm$ 0.9	0.52	2.8 $\pm$ 0.9	2.0 $\pm$ 1.1	0.48
8/15/2005	Border	—	—	—	20.0 $\pm$ 2.0	21.5 $\pm$ 3.2	0.64
	Interior	—	—	—	5.8 $\pm$ 3.4	3.5 $\pm$ 1.6	0.33
6/27/2006	Border	11.1 $\pm$ 2.5	7.5 $\pm$ 1.4	0.23	15.8 $\pm$ 4.5	9.5 $\pm$ 1.3	0.23
	Interior	6.8 $\pm$ 0.6	3.4 $\pm$ 1.1	0.078	9.3 $\pm$ 1.0	6.5 $\pm$ 2.2	0.27
8/4/2006	Border	22.5 $\pm$ 1.9	18.4 $\pm$ 1.5	0.038	35.5 $\pm$ 3.5	25.8 $\pm$ 2.4	0.004
	Interior	11.8 $\pm$ 3.9	8.6 $\pm$ 1.2	0.43	18.0 $\pm$ 5.9	15.3 $\pm$ 2.2	0.70

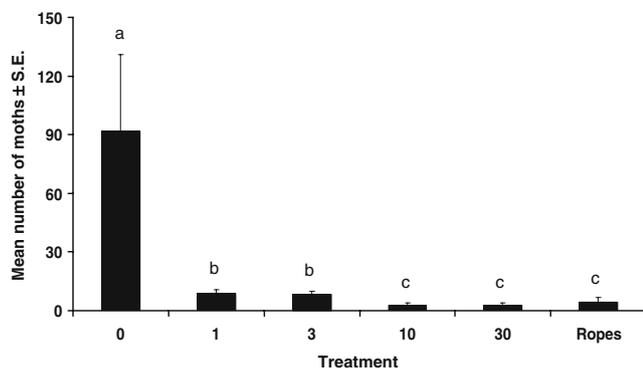


**Fig. 2** Release of pheromone from 1-ml droplets of SPLAT-GBM™ during 2006. The release profile best fits an exponential decay function, and the best-fit curve and equation are presented on the graph

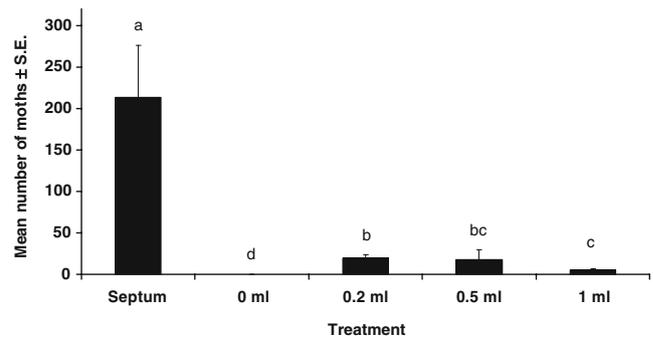
(Fig. 2), although the high variability among replicates (shown by the high standard error values), resulted in a relatively low predictive power of the exponential equation. The release function suggests that almost 50% of the pheromone in the wax droplets was still bound in the wax in late July when captures of *P. viteana* were first observed in the SPLAT-treated plots.

**Droplet Density Study** Significantly more moths were captured in the untreated control plots compared to all other treatments, but the one and three drops per vine treatments had higher moth captures than the 10 drops per vine, 30 drops per vine, and Isomate treatments ( $F=27.06$ ;  $df=5, 25$ ;  $P<0.001$ ) (Fig. 3).

**Droplet Size Study** The size of each droplet affected how many moths were trapped ( $F=66.1$ ;  $df=4, 20$ ;  $P<0.001$ ) (Fig. 4). The 0.2- and 0.5-ml droplets trapped low numbers



**Fig. 3** Average (+SE) number of adult male *Paralobesia viteana* captured in pheromone traps in vineyard plots treated with varying densities of 0.2-ml droplets of SPLAT-GBM™ wax that contained 3% sex pheromone, or pheromone ropes. Bars with the same letter are not significantly different at  $\alpha=0.05$



**Fig. 4** Average (+SE) number of adult male *Paralobesia viteana* captured in pheromone traps baited with different sizes of SPLAT-GBM™ wax droplets that contained 3% sex pheromone, or a commercial pheromone lure containing 0.1 mg of sex pheromone. Bars with the same letter are not significantly different at  $\alpha=0.05$

of moths, and increasing the droplet size to 1.0 ml caused a slight decrease in the number of moths trapped (Fig. 4). The greatest captures of moths were in traps baited with the lure that contained the standard 0.1 mg of sex pheromone, an amount much lower than that in any of the applied droplets (approximately 6 mg or greater). No moths were caught when the traps were unbaited.

**Discussion**

Application of 1-ml droplets of SPLAT-GBM™ that contained 3% pheromone to vineyards before the first generation flight of *P. viteana* resulted in high levels of disruption of male moth orientation to monitoring traps during two growing seasons, with reduced crop infestation in treated plots in the second growing season. This first report of using a wax formulation for pheromone deployment in vineyards provides evidence for the potential of this formulation to control *P. viteana*.

Wooden vineyard posts provided a practical target for application of the wax, and droplets applied on the north side of these structures in early May each year remained in place throughout the growing season. These droplets were easy to apply by hand with a 30-ml syringe, and their application was faster than that of Isomate twist ties at 450/ha (Isaacs, unpublished data). The large droplets used in this study released pheromone until late July. The longevity of these droplets is lower compared with those used for mating disruption of *G. molesta* (Stelinski et al. 2006, 2007).

Pheromone dispenser density affects the distribution of pheromone that permeates the crop habitat, thus influencing the degree of mating disruption achieved (Rothschild 1975; Flint and Merkle 1983; Lawson et al. 1996; Stelinski et al. 2005; Miller et al. 2006a, b). A similar pattern was found in this study, as trap catches in untreated plots were greater than one or three droplets per vine (0.2-ml volume) >10 and 30 droplets per vine. The higher doses gave similar results

as Isomate GBM ropes applied once every 0.4 vine. Overall, our results are similar to those observed for *G. molesta* (Stelinski et al. 2005), although they reported higher densities of droplets outperformed twist ties and completely prevented mating of tethered female moths. A profile analysis of variation in moth catch with dispenser density, similar to that conducted by Miller et al. (2006a), was not possible in our study because of an insufficient range of pheromone point-source densities.

A pheromone formulation lasting >20 wk, as achieved with Isomate-GBM twist ties, is necessary to cover the full activity period of *P. viteana* in the Great Lakes region of the US and Canada. Our results suggest that this cannot be currently achieved by using SPLAT-GBM™, so one approach to would be to re-treat vineyards in late July. Alternatively, one could apply an appropriate Lepidoptera-specific insecticide for control of first-generation *P. viteana*, followed by application of SPLAT-GBM™ in early July to provide protection against this pest until harvest. Another promising line of research would be the development of a wax formulation that provides a pheromone release system lasting >20 wk.

Development of formulations that slowly release the pheromone is an important goal for mating disruption, not only to provide a long period of activity but also to ensure that expensive pheromone is not wasted by remaining bound in the wax matrix (de Lame 2003). Residual analysis of wax revealed that approximately 50% of the *P. viteana* pheromone was still bound in the drops when disruption performance declined in late July. The 1-ml droplets used in this study are larger than those deployed in many recent studies (Stelinski et al. 2005, 2006, 2007), so the use of more, smaller-sized droplets should result in a greater proportion of the pheromone being released (Stelinski et al. 2005). This is currently under investigation, especially as the amount of pheromone released per hectare in the 10-drops-per-vine treatment (58.1 mg/Ha) was lower than the Isomate treatment (99.8 mg/Ha).

Evaluations of sex pheromone formulations for control of *P. viteana* have shown that mating disruption can help control this pest (Dennehy et al. 1990; Trimble 1993), yet there has been relatively low adoption of this approach in vineyard IPM programs in eastern North America where vineyards are infested by *P. viteana*. Primary impediments to adoption are the perception that pheromone formulations have lower efficacy, and the cost of pheromone products and their application is greater when compared with insecticides. An additional challenge to controlling infestation of *P. viteana* is that this insect typically causes higher levels of crop infestation at the vineyard borders than in the interiors (Hoffman and Dennehy 1989; Botero-Garcés and Isaacs 2003). Our results showed that while application of SPLAT-GBM™ caused an immediate shutdown of traps, a

significant reduction in cluster infestation at the vineyard borders was observed only in the second year of treatment, suggesting that multiyear application of pheromone may be required for mating disruption of GBM.

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