Effect of a Pheromone Antagonist-based Disruption Blend on Dogwood Borer (Lepidoptera: Sesiidae) Mate-finding and Infestation in a Commercial Apple Orchard[1]

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Abstract The effect of a pheromone antagonist-based disruption blend on disruption of dogwood borer, *Synanthedon scitula* (Harris), mate-finding behavior and incidence of infestation was evaluated in a commercial apple orchard from 2006 - 2008. Although the pheromone antagonist-based disruption blend treatment significantly disrupted male mate-finding behavior during each year of the study, there was a significant increase in the percentage of infested trees from the start to the conclusion of the experiment, likely due to the immigration of mated females from surrounding untreated orchard blocks. In 2007, pheromone-baited traps deployed in an east-west transect through the study area showed that capture of male moths increased as the distance from the disruption plot increased. The results of geostatistical analysis indicated that there were high degrees of aggregation in dogwood borer infestations in the study area, with ranges of spatial autocorrelation from 10.2 - 22.5 m. For each year, the spatial distribution of larval infestation was best described by the exponential semiovariogram model. Interpolated surface maps revealed areas of high infestation levels, which were more prevalent within the control 2 plot in 2006 and 2007. Infestation sites increased in size and intensity each year of the study in the disruption and control 1 plot.

Key Words *Synanthedon scitula*, mating disruption, pheromone antagonist, geostatistics

The dogwood borer, *Synanthedon scitula* (Harris), has been recognized as an economically-important pest for over 100 years (Herrick 1904). Given that it has been recorded from 19 species of trees and woody shrubs belonging to 10 families, it is considered to have the broadest host range among the North American Sesiidae (Eichlin and Duckworth 1988). Larvae are the damaging stage, feeding predominately on the cambium and phloem tissues of host plants (Underhill 1935, Wallace 1945). Consecutive years of larval feeding can result in the destruction of vascular plant tissue and girdling of trunks and/or branches (Underhill 1935, Wallace 1945), which can result in the death of young trees (Weires 1986, Howitt 1993).

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Dogwood borer was first considered primarily a major pest of dogwood, *Cornus florida* L., in nurseries and urban landscapes (Underhill 1935, Wallace 1945, Potter and Timmons 1981). Since the mid1980s, it has become an increasingly important pest of apple trees, *Malus domestica* Borkh, in eastern North America (Riedl et al. 1985, Bergh and Leskey 2003). Bergh et al. (2009) showed that captures in apple orchards exceeded those in urban and woodland habitats by about 13 and 206 times, respectively. The increasing abundance of dogwood borer in apple orchards appears to have coincided with the adoption of size-controlling rootstocks (Riedl et al. 1985, Kain and Straub 2001, Bergh and Leskey 2003). These rootstocks have enabled growers to increase tree densities whereas reducing the time, labor, and cost associated with their management. Many apple varieties propagated on size-controlling rootstocks have a propensity to produce burr knots, which are adventitious root initials that form primarily below the graft union and, in some varieties, on the scion (Rom 1970, 1973, Marini et al. 2003). Burr knot tissue appears to be an important resource for dogwood borer; in newly-established apple orchards the quantity of available burr knot tissue has the greatest influence on the initiation, persistence, and extent of infestations (Leskey and Bergh 2005, Frank et al. 2011).

The most effective and consistent control of dogwood borer in apple hosts has been provided by the organophosphate insecticide, chlorpyrifos (Riedl et al. 1985, Kain and Straub 2001, Kain et al. 2002, 2004). The integration of additional tactics that target multiple aspects of dogwood borer biology and life stages would provide a more sustainable management approach for this pest. Behavioral manipulation of pest insects is an environmentally benign tactic that has received increasing attention for pest control and that can be readily aligned with other management strategies (Foster and Harris 1997). Pheromone-based mating disruption has been used to control several important sesiid pests (Pfeiffer et al. 1991, Agnello and Kain 2002, Kittelson 2006). An initial, 3-year attempt at dogwood borer mating disruption using a commercial formulation of the peachtree borer sex pheromone resulted in the elimination of male captures in traps but not reductions of infestations (Pfeiffer and Killian 1999), likely due to suboptimally attractive disruption dispensers and pheromone lures for monitoring treatment effects (Leskey et al. 2009). The compound, (*E,Z*)-3,13-octdecadienyl acetate (ODDA), acts as a strong behavioral antagonist to male dogwood borer response to the main component of its sex pheromone, (*Z,Z*)-3,13-ODDA (Karandinos et al. 1977, Greenfield and Karandinos 1979), and to its trinary pheromone blend (Zhang et al. 2005). Isomate-LPTB mating disruption dispensers for the congeneric species, lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson), contain primarily (*E,Z*)-3,13-ODDA, but also (*Z,Z*)-3,13-ODDA. Leskey et al. (2009) showed that Isomate-LPTB dispensers effectively disrupted captures of male dogwood borer in pheromone traps baited with lures containing the trinary pheromone blend, although effects on infestations were not evaluated. Kain and Agnello (2011) also demonstrated disruption of mate-finding activity by male dogwood borer in orchard plots treated with Isomate-LPTB dispensers and that larval infestation of burr knots decreased over the course of 3 consecutive seasons.

Here we describe a 3-yr study evaluating the effects of a pheromone antagonist-based disruption blend on disruption of dogwood borer mate finding and occurrence of infestation in a commercial apple orchard. In addition, we used geostatistics to help provide insights on the distribution and extent of dogwood borer infestation within the study area.

Materials and Methods

Orchard site. Studies were conducted in a commercial apple orchard located in Berkeley Co., WV from 2006 - 2008 (Fig. 1). A 5.1-ha block of newly-planted 'Honeycrisp' and 'Gala' apple trees on M.26 rootstock was divided into 2 plots; 3.5 ha in the eastern part of the block was selected as the disruption plot containing pheromone dispensers whereas the remaining 1.6 ha on the west side was used as an untreated control (control 1). An additional 2.4 ha block of 2-yr-old 'Ginger Gold' apple trees on M.26 rootstock on the west side of the control 1 plot was used as an additional untreated control (control 2), so that the disruption and control plot would be roughly equal in size. The disruption and control 1 plot consisted of ∼120 trees in 20 and 10 rows, respectively, with 6.1 m between rows and 2.4 m between trees. The control 2 plot consisted of ∼45 trees in 12 rows with 7.9 m between rows and 6.1 m between

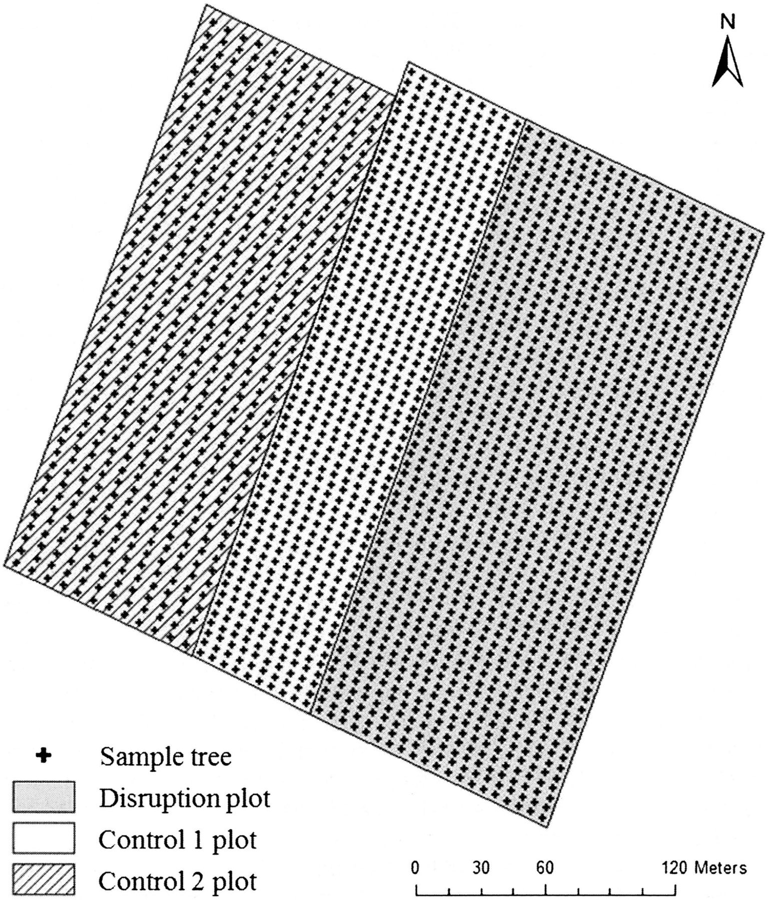

Fig. 1. Layout of the study area in a commercial apple orchard located in Berkeley County, WV, showing treatment plots and sample trees.

trees. Although the orchard was under an active management program for arthropod pests and diseases, none of the insecticide products applied during the study specifically targeted dogwood borer except during the 2008 season. In May of 2008 an application of Lorsban^{TM} (Dow AgroSciences LLC, INpolis, IN) was applied to the base of trees in the control 2 plot to control damage caused by larval infestation.

Mating disruption dispensers. In the disruption plot, Isomate-LPTB (lesser peachtree borer) disruption dispensers (CBC America, Commack, NY) were uniformly deployed within tree canopies at a height of ~2 m above the ground at a rate of 250/ha (Leskey et al. 2009) in late April to early May of each year. Each 50-mg dispenser contained 60.5% (*E,Z*)-3,13-ODDA (the behavioral antagonist of dogwood borer), 22.7% (*Z,Z*)-3,13-ODDA (the main pheromone component of dogwood borer pheromone), and 16.8% other ODDA isomers.

Mating disruption assessment and infestation surveys. Disruption of male dogwood borer mate finding was assessed with delta-style sticky traps baited with rubber septa lures formulated with a 1 mg load of the complete dogwood borer sex pheromone blend (Zhang et al. 2005). Three traps were deployed in both the disruption and collective control plot (control plots 1 and 2). Traps were evenly spaced and placed in tree canopies at a height of 1.5 - 1.8 m above the ground. In 2007, seven additional pheromone-baited traps were deployed in an east-west transect spaced 3 rows apart across both the disruption and collective control plot. Because the lures used for these studies remain effective for the duration of a field season (Bergh et al. 2006), they were not replaced within each season of the study. All traps were checked at approximately weekly intervals throughout the adult flight period, and the number of male moths captured were recorded and removed.

Infestation of burr knots by dogwood borer larvae was evaluated twice annually (Aug. and Nov. 2006, May and Nov. 2007, May and Dec. 2008) with the exception of the control 2 plot, which was not evaluated on the last sample date in 2008 because an insecticide application targeting dogwood borer was applied after the start of the season. Every other tree within each row of the disruption and control 1 plot (~60 trees in 20 and 10 rows, respectively), and every tree within each row of the control 2 plot (~45 trees in 12 rows) was evaluated. Trees were recorded as infested (1) or not infested (0) based on the presence of fresh frass below the graft union at the base of trees (Leskey and Bergh 2005).

Data analysis. To meet the assumptions of normality and homogeneity of variances, numbers of male moths in traps were log (x + 1) transformed prior to analysis. A two-sample *t*-test (SAS Institute 2008) was performed to determine if there was a significant difference between the mean number of moths captured per pheromone-baited trap in the disruption and collective control plot in 2006 - 2008. For larval infestation surveys, trees within each row represented a sample unit. The disruption, control 1, and control 2 plot contained 20, 10, and 12 sample units, respectively. The number of infested trees recorded from each sample unit was converted to percentages and transformed using arcsine square-root to stabilize the variance. A two sample *t*-test (SAS Institute 2008) was performed to determine if there was a significant difference between the mean percentage of infested trees within each plot at the start of the experiment (Aug. 2006) and at the conclusion (Dec. 2008 for the disruption and control 1 plots and May 2008 for the control 2 plot). All statistical comparisons were considered significantly different at $P < 0.05$.

Geostatistical analysis. The small-scale spatial structure in the larval infestation data was investigated using semivariogram analysis (Isaaks and Srivastava 1989, Rossi et al. 1992). The semivariogram provides a measure of the spatial dependence (autocorrelation) between sample values with distance, direction, or both within the sampling space and is typically estimated using the mathematical function,

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2,$$

where $\hat{\gamma}(h)$ is the estimated semivariance for lag distance h, $N(h)$ is the number of pairs of samples separated by h, and $z(x_i)$ is the observed value at location x_i (Rossi et al. 1992). When the semivariance is computed for all pairs of locations and plotted against lag distance, a variogram model (e.g., linear, spherical, exponential, or Gaussian) is fit to the data. The shape of the fitted model is described by 3 parameters, the nugget (C_o), sill ($C_o + C$), and range (A). The nugget (C_o) is the value of the semivariance at which the lag distance equals zero (i.e., where the model intercepts the y-axis of the semivariogram graph); the sill ($C_o + C$) is the value of the semivariance at which the model levels off and is equivalent to the variance of the data (Farias et al. 2004); and the range (A) is the average lag distance at which the model levels off and spatial dependence is no longer detected. The nugget-to-sill ratio, $C_0/(C_0 + C)$, also can be used to provide a measure of the degree of spatial dependence in the sample data with values of the ratio < 0.25, 0.25 - 0.75, and > 0.75 indicating strong, moderate, and weak spatial dependence, respectively (Cambardella et al. 1994).

Because infestation data exhibited a nonnormal and skewed distribution due to the absence of larval infestation in many trees, indicator semivariograms were used to describe the spatial dependence of infestation in the study area as suggested by Journel (1983) and Rossi et al. (1992). Briefly, a binary indicator value (1 or 0) at each sampled location was determined by redefining the probability of infestation for each tree during the season based on a cutoff or threshold value of 0. That is, if the probability that the tree at a sampled location was infested < 0, the location was assigned a value of 1; otherwise the location was assigned a value of 0. Indicator semivariograms were calculated by substituting the indicator value $i(x_i)$ for the z-data $z(x_i)$ in the above function. Because no evidence of directionality was detected at 0, 45, 90, or 135°, only omnidirectional semivariograms were evaluated. Among the variogram models examined, the best fit model was determined by the coefficient of determination (r^2). After selecting the best variogram model for each spatial pattern, the parameters of the model were used in a block kriging process to create an interpolated surface map depicting infestation of dogwood borer larvae throughout the study area. All geostatistical analyses were performed using GS$^+$ version 9.0 (Gamma Design Software, Plainwell, MI).

Results

Mating disruption assessment and infestation surveys. In the disruption plot, moth captures in pheromone-baited traps were virtually eliminated throughout the entire season in 2006 - 2008 (Fig. 2). Significantly more males were captured in traps within the collective control plot compared with the disruption plot for each year of the study (Table 1). In 2007, the capture of male moths in traps deployed in an east-west transect through the study area increased as the distance from the disruption plot increased (Fig. 3). Infestation surveys revealed a significant increase in the mean (± SE) percentage of infested trees between 2006 (0.0 ± 0.0) and 2008 (22.0 ± 2.4) in the disruption plot (t = -16.85, df = 38, $P \leq 0.0001$). Similarly, there was a significant increase in the mean percentage of infested trees between 2006 (0.0 ± 0.0) and 2008

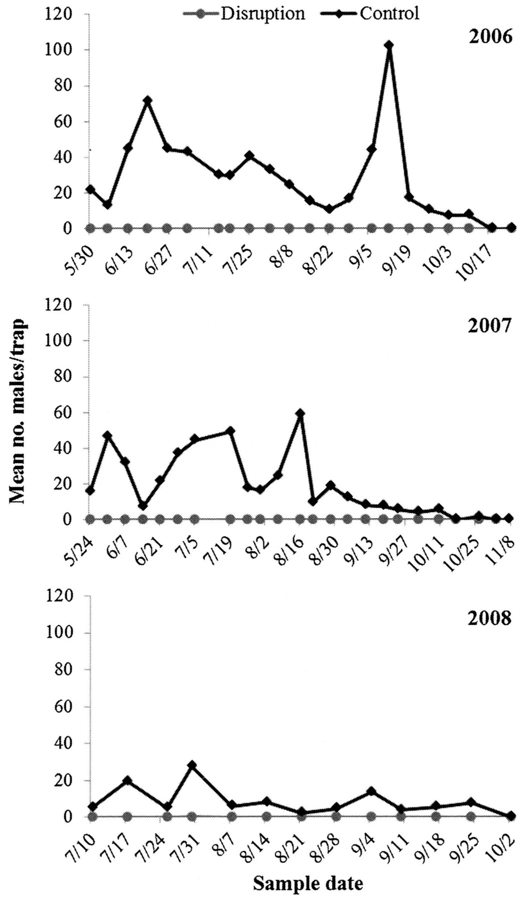

Fig. 2. Mean number of male dogwood borer captured throughout the season in pheromone-baited traps deployed in plots treated with a pheromone antagonist-based disruption blend or untreated control in 2006 - 2008.

(13.7 ± 1.5) in control 1 plot (t = -19.42, df = 18, $P \leq 0.0001$). However, in the control 2 plot the percentage change of infested trees between 2006 (12.0 ± 4.4) and 2008 (7.3 ± 1.3) was not significant (t = -1.70, df = 22, P = 0.1026).

Geostatistical analysis. An exponential model provided the best fit to the semivariance for each year (Table 2), which suggested that dogwood borer infestations showed an aggregated distribution. The nugget-to-sill ratio, $C_0/(C_0 + C)$, which provides a measure of spatial dependence, ranged from 0.00 - 0.12 (Table 2), also indicating strong spatial aggregation in infestations among trees at the distances and directions tested. The range of semivariograms, or the distance at which spatial dependence was no longer detected, varied from 10.2 - 22.5 m (Table 2). Interpolated surface maps revealed the extent of dogwood borer infestation within the study area for each year. Areas of high infestation levels were more prevalent near the eastern

Table 1. Total and mean (± SE) number of male dogwood borer captured in pheromone-baited traps deployed in plots treated with a pheromone antagonist-based disruption blend or untreated control in 2006 - 2008.

Year	Treatment	Total no. of males captured in traps	Mean (± SE) no. of males/trap
2006	Disruption	0	0.0 ± 0.0
	Control	1074	23.9 ± 3.7*
2007	Disruption	0	0.0 ± 0.0
	Control	1050	17.3 ± 10.8*
2008	Disruption	1	0.0 ± 0.0
	Control	329	8.4 ± 3.4*

* Significant difference between traps deployed in disrupted and control plot based on two-sample *t*-test ($P < 0.05$). For 2006, $t = 21.61$, df = 4, $P \leq 0.0001$; for 2007, $t = 4.57$, df = 4, $P = 0.0102$; for 2008, $t = 5.74$, df = 4, $P = 0.0046$.

border of the control 2 plot until an insecticide application targeting dogwood borer was applied in 2008 (Fig. 4). In the disruption and control 1 plot, infestation sites increased in size and intensity each year (Fig. 4).

Discussion

Mate-finding behavior by male dogwood borer was successfully disrupted by deployment of dispensers containing a pheromone antagonist-based disruption blend composed of a high percentage of (*E,Z*)-3,13-ODDA in combination with the main pheromone component, (*Z,Z*)-3,13-ODDA. These results were consistent with those of Leskey et al. (2009) and Kain and Agnello (2011). In 2007, deployment of additional

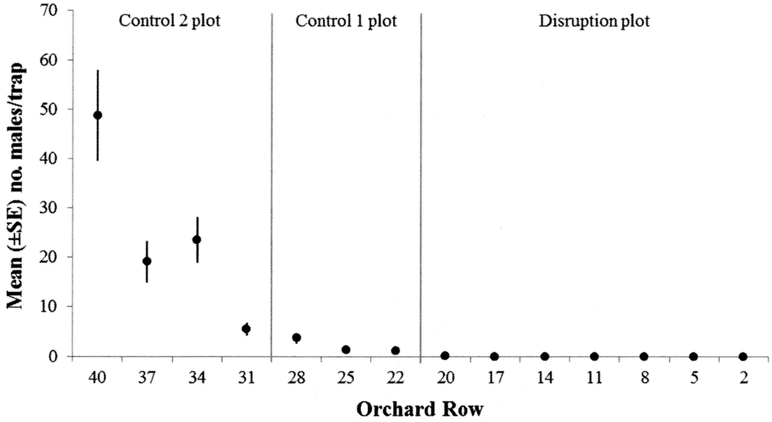

Fig. 3. Mean (±SE) number of male dogwood borer captured in pheromone-baited traps deployed in an east-west transect across plots treated with a pheromone antagonist-based disruption blend or untreated control in 2007.

Table 2. Semivariogram model parameters for larval dogwood borer infestation.

Year	Model	Nugget (C_0)	Sill ($C_0 + C$)	Range (m) (A)	r^2	Nugget/sill ratio C_0/($C_0 + C$)
2006	Exponential	0.000	0.035	22.5	0.50	0.00
2007	Exponential	0.000	0.112	11.1	0.86	0.00
2008	Exponential	0.024	0.198	10.2	0.86	0.12

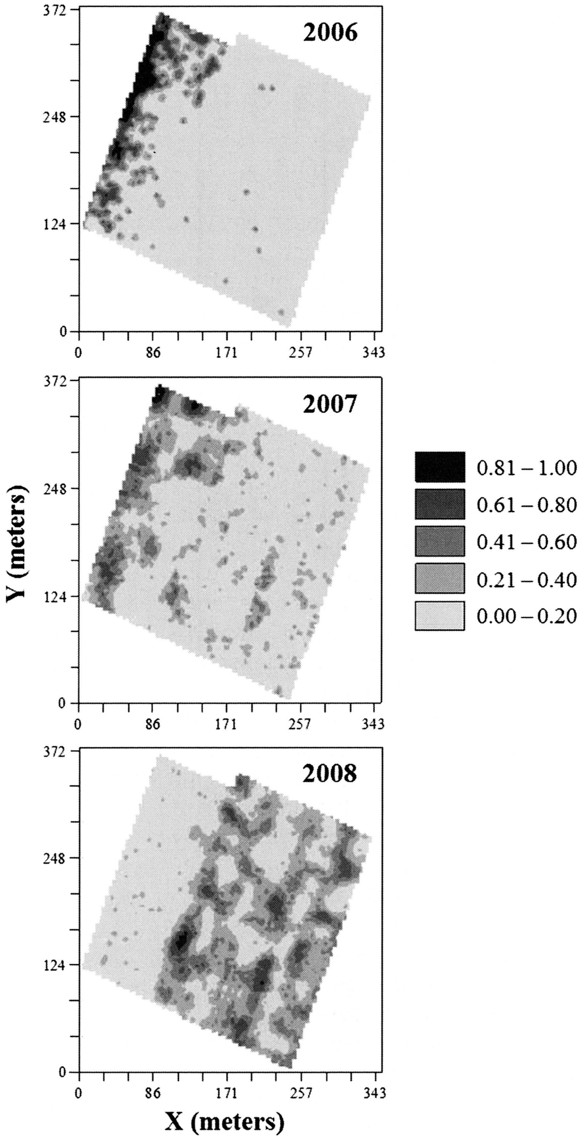

Fig. 4. Interpolated surface maps based on block kriging depicting the probability of trees in the study area becoming infested with dogwood borer in 2006 - 2008.

pheromone-baited traps in a transect through the study area to determine the distribution of moth catches showed that moth captures were almost completely eliminated throughout the disruption plot and that captures in the control plots increased as the distance from the disruption plot increased, likely due to the downwind location of control plots relative to the disruption plot.

Although virtually no dogwood borer males were captured in pheromone-baited traps deployed in the disruption plots in this study and in that reported by Kain and Agnello (2011), results at the conclusion of the 2 studies differed with respect to treatment effects on the percentage of burr knots infested by dogwood borer larvae. These differences appeared to be independent of dispenser type, deployment rate, study duration (which were identical), and experimental plot sizes, which were similar. In the present study, we recorded a significant increase in the percentage of infested trees from the start to the conclusion of the experiment; whereas, Kain and Agnello (2011) showed significant reductions in 2 of 3 plots and a numerical reduction in the third. However, it was unclear if the reduced infestations in disruption plots reported by Kain and Agnello (2011) were due to treatment effects or to other factors, because larval infestation of burr knots also decreased in the untreated control plots during the same period. In the present study, the failure of mating disruption to prevent infestation in newly-planted control and disruption blocks, or to reduce infestations in an adjacent 2-yr-old block is unclear, but may be explained by immigration of mated females into the plot from nearby orchard blocks. Pheromone-based management strategies are typically less effective at high population densities and often require large areas under treatment to combat the effects of immigrating females (Gut and Brunner 1998, Thomson et al. 1998, Hughes and Dorn 2002). Given that our study area was surrounded on all sides by older, established apple orchard blocks with detectable levels of dogwood borer infestation, and that female dogwood borer are active fliers when searching for potential oviposition sites (Frank et al. 2009), it is highly likely that they could have moved from other surrounding orchard blocks into the disruption plot. In the control 1 plot, the increase in the percentage of infested trees from the start to the conclusion of the experiment was not surprising, because Frank et al. (2011) showed that dogwood borer can attack newly-planted orchards during their first season of growth. The relative stability in the number of infestation sites from the start to the conclusion of the experiment in the control 2 plot was likely the result of these apple trees being older with established populations of dogwood borer already present.

Based on the nugget-to-sill ratios, there was a high degree of spatial dependence (< 0.25) in infestation within the study area. The range of spatial dependence or autocorrelation in infestation among the orchards was found to vary from 10.2 - 22.5 m. In newly-planted apple orchards, Frank et al. (2011) similarly reported that dogwood borer showed strong to moderate degrees of spatial aggregation in larval infestation with ranges of spatial dependence averaging ~10.2 m. Interpolated surface maps revealed areas of high infestation levels for each year of the study. Not unexpectedly, infestation sites were more prevalent within the control 2 plot during the first 2 years of the study until insecticide applications targeting dogwood borer larvae were initiated. Within the disruption plot infestation sites increased in size and intensity for each year of the study, suggesting that the spatial structure of larval infestation was not affected by the pheromone antagonist-based disruption blend.

In conclusion, although our results revealed that dispensers containing (*E,Z*)-3, 13-ODDA effectively disrupted mate-finding of male dogwood borer, they also suggest that successful management of dogwood borer may require deployment of dispensers across the whole orchard to reduce the impact of mated females immigrating from untreated blocks. Bergh et al. (2009) showed that dogwood borer captures in pheromone traps in urban landscapes and woodlands were very low compared with those in apple orchards, and thus woodlands adjacent to orchards would not be expected to add significantly to dogwood borer pressure. Given the recent registration of a mating disruption formulation specifically for dogwood borer, Isomate-DWB, our results may guide further research on its optimal use and impact, which may be best achieved via farm-scale demonstration trials.

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