Effects of intentional gaps in spray coverage on the efficacy of gypsy moth mating disruption

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Abstract: The study was conducted during 2001 and 2002 in forested areas in Virginia, US to examine the effects of gaps in coverage of pheromone on gypsy moth, *Lymantria dispar* (L.) (Lep., Lymantriidae), mating disruption. Gypsy moth male moth catches in pheromone-baited traps were significantly reduced in plots treated with the gypsy moth sex pheromone, disparlure, at an overall application rate of 37.5 g of active ingredient (AI)/ha but with untreated gaps of 30 or 90 m between 30-m wide treated swaths. In one of the two plots with 90 m gaps, significantly more males were captured in traps in the untreated areas compared with the treated areas within the plot. However, in another plot, significant differences in trap catches between treated and untreated areas were not observed. No difference in male moth catches in the pheromone-baited traps was observed between treated and untreated areas within the plots treated with 30 m gaps. Female mating success did not differ significantly between treated and untreated areas within the one plot in which it was measured. These results suggest that it may be possible to lower costs associated with gypsy moth mating disruption applications by alternating treated and untreated swaths, which would reduce flight time and fuel costs, without a reduction in efficacy.

Key words: *Lymantria dispar*, disparlure, pheromone, plastic flakes, spray coverage, swath width

1 Introduction

The gypsy moth, *Lymantria dispar* (L) was accidentally introduced to Medford, MA in the late 1860s (Burgess and Baker, 1938; Liebhold et al., 1989) and since then has gradually expanded its range in North America. This insect is a defoliator of hardwood trees, primarily oaks (*Quercus* spp.), with over 300 tree species recorded as hosts (Leonard, 1981). Today, the gypsy moth is one of the most important forest pests in the eastern United States (Doane and McManus, 1981; USDA, 1995).

Gypsy moth females use a sex pheromone to attract males. The pheromone, disparlure, is the (+) enantiomer of cis-7,8-epoxy-2-methyloctadecane (Bierl et al., 1970; Iwaki et al., 1974). Shortly after a synthetic form of the pheromone became available, attempts were made to use it to disrupt mating in gypsy moth populations (Cameron, 1981). Field tests conducted in the 1990s demonstrated that aerial mating disruption applications could suppress low-density gypsy moth populations for several years following the treatment (Leonhardt et al., 1996; Thorpe et al., 1999). Mating disruption is currently used on over 200 000 ha/year (http://www.gmsts.org/reports/2002_accomp.htm) in the Slow-The-Spread of the Gypsy Moth Program (STS), a combined state and federal effort to slow the rate of gypsy moth population expansion in the United States (Sharov and Liebhold, 1998; Sharov et al., 2002b).

Currently, the only formulation used on a large scale for gypsy moth mating disruption is Disrupt® II (Hercon Environmental, Emigsville, PA). Disrupt® II is formulated as polymeric three-layer laminated flakes that contain 17.9% racemic disparlure. The emission rate of disparlure from laminated flakes depends on its concentration, the thickness of the outer layers of flake dispensers, and other factors such as temperature and air movement (Zedi et al., 1982). Because environmental parameters (e.g. temperature, humidity) affect the emission rate in a predictable manner, it is possible to provide a uniform concentration of pheromone in the forest atmosphere throughout the flight period of the gypsy moth (Bierl et al., 1976). Disrupt® II is applied aerially by aircraft using specialized equipment (Plimmer et al., 1982). Aerially applied flakes are deposited at all layers of the canopy, including the understory foliage, and only 16% of the flakes reach the ground (Leonard et al., 1992). However, little is known about the horizontal distribution of pheromone flakes and how the distribution relates to the efficacy of the pheromone.

The costs associated with the application of the pheromone flakes could be lowered by reducing the flight distance of the aircraft. Currently, the pheromone is applied in non-overlapping swaths in the same manner as are conventional pesticides. Although the spray aircraft is calibrated to deliver the correct amount of flakes in each swath, the flakes within a swath are not deposited uniformly, presumably because of lower rates of application under the fuselage and wing tips (Trent and Thistle, 1999). By intentionally skipping one or more spray swaths, reductions in costs associated with fuel and flight time could be realized. Sharov et al. (2002a) showed that gypsy moth mating is suppressed up to 600 m from the edges of treated plots. Therefore, uniform coverage of plots with mating disruptant dispensers may not be necessary to disrupt mating. The objective of the study reported here was to determine the effect of gaps in deposition of pheromone dispensers on the effectiveness of gypsy moth mating disruption.

2 Materials and Methods

2.1 Study sites

Experiments were conducted in the Appomattox-Buckingham State Forest (ABSF) [Buckingham and Appomattox Co., VA, (UTM 707562 E, 4145752 N to 707562 E, 4146238 N, NAD27, zone 17)], located in the Piedmont physiographic region (179–198 m elevation) and in the Goshen Wildlife Management Area (GWMA) [Rockbridge Co., VA, (UTM 632723 E, 4199588 N to 632432 E, 4200432 N, NAD27, zone 17)], located in the Appalachian Plateau physiographic region (600–800 m elevation). Plots delineated within these areas consisted of closed canopies that were predominately oak, with overstory trees reaching a height of 20–25 m. The ABSF was at the leading edge of the area generally infested by the gypsy moth. The GWMA had been infested by gypsy moth for over 10 years, but the plots have never experienced noticeable defoliation. Male moth catches in standard USDA milk-carton pheromone-baited traps in 2001 ranged from 0 to seven in the vicinity of the ABSF and from 82 to 700 in the vicinity of the GWMA. No trap data were available from within GWMA in 2002.

2.2 Plot layout and pheromone treatments

2.2.1 Experiments in 2001

At each of the two study sites, one 50 ha-plot (1 by 0.5 km) was treated on June 11 with racemic disparlure at an overall dosage of 37.5 g active ingredient (AI)/ha. Each plot was divided into 30 m wide strips corresponding to the swath width of the treatment aircraft. Every fourth strip was sprayed twice at a dosage of 75 g AI/ha, and the intervening three strips were left unsprayed (fig. 1). Thus, a 90 m gap was left between each pair of sprayed swaths.

To evaluate the efficacy of the pheromone treatments, a line of 32 gypsy moth male release points located 30 m apart from each other was placed across the plot in the ABSF perpendicular to the aircraft flight path. Two lines of pheromone-baited traps were located parallel to and 30 m to the east and west of the line of release points. Each line of pheromone-baited traps extended 150 m beyond the northern and southern edges of the plot. Male moths were released and traps were checked weekly during July and twice each week during August.

At GWMA, in addition to the two lines of pheromone-baited traps, the plots also contained four lines of female deployment points. No males were released in this plot. The lines of pheromone-baited traps were separated by 150 m, were perpendicular to the aircraft flight path, and consisted of traps spaced 30 m apart. Each line extended 150 m beyond northern and southern edges of the plot. The lines of female deployment points were also perpendicular to the aircraft flight path, but did not extend beyond the plot edges. The lines of females were separated by 50 m, and the points within each line were spaced 30 m apart. As a control, another line of female deployment points was set up 1 km from the treated plot. Females were deployed in this line on four dates (4, 5, 9, and 10 July).

2.2.2 Experiments in 2002

Two 25 ha-plots (500 × 500 m) were treated at the GWMA with racemic disparlure at an overall dosage of 37.5 g AI/ha. Each plot was divided into 30 m wide strips corresponding to the swath width of the treatment aircraft. Every other strip was sprayed twice at a rate of 37.5 g AI/ha to achieve a 37.5 g AI/ha application rate over the entire plot. Two plots of similar size to the treated plots were left untreated and used as controls.

Three lines of 35–37 pheromone-baited traps spaced 30 m apart were placed across each of the four plots (two treated and two control). The lines were separated by 100 m and extended 150 m beyond each edge of the plot. In the treated plots, the aircraft flight path was perpendicular to the trap lines. Traps were checked once each week for 3 weeks. Male moths were not released, so only native moths were used to evaluate treatments in 2002.

2.3 Pheromone formulation and application

Gypsy moth pheromone was aerially applied in a plastic laminated flake formulation (Disrupt® II). Each flake (1 mm wide × 3 mm long × 0.5 mm thick) consists of 3-mil polyvinyl chloride (PVC) outer layers and an inner polymer layer containing racemic disparlure (17.9%). The flakes were applied with 105 g/ha of sticker (Gelva 2333, Solutia Inc., Springfield, MA). Gelva 2333 is a multipolymer emulsion used industrially primarily as a pressure-sensitive adhesive.

The rate of pheromone release from the flake dispensers was not determined in this study. However, under similar conditions the flakes have been shown to release 30–50% of their disparlure content over the 6-week period of moth flight (Leonhardt et al., 1996; Thorpe et al., 1999). The pheromone flakes were applied from specialized pods (Schweitzer Aircraft Corp., Elmira, NY) mounted on fixed-wing aircraft (Air Tractor) flying 8–15 m above the canopy at a speed of 225 km/h and using a differential global positioning system (DGPS) for spray guidance. A ground-based DGPS system was used to determine the positions of the traps and deployed females relative to the treated swaths.

In 2001, in the ABSF, an examination of the trap capture data superimposed over the flight lines suggested that the treated swaths did not correspond exactly with observed suppressed trap captures, but appeared to be shifted to the left (fig. 2). Because it is possible that some off-target drift of the applied pheromone may have occurred, or there may have been a temporary loss of accuracy in the DGPS signal, the position of the traps relative to the treated swaths was recalculated after offsetting the position of the treated swaths 12 m to the right. A similar examination of trap catch data relative to the locations of the treated swaths in the other plots did not reveal evidence of a shift, so no other adjustments were made.

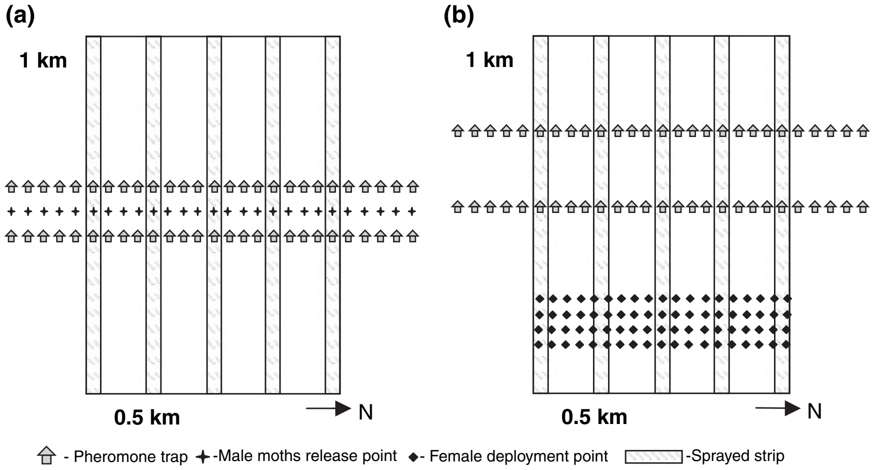

Fig. 1. *Layout of experimental plots. (a) Appomattox-Buckingham State Forest, VA, 2001; (b) Goshen Wildlife Management Area, VA, 2001*

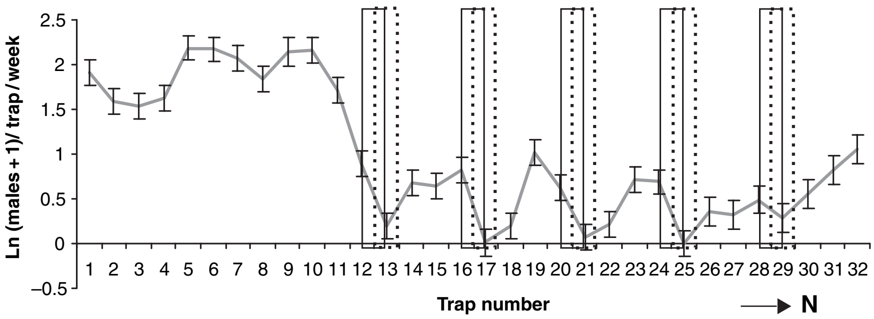

Fig. 2. *Male gypsy moth counts [ln(n + 1) ± SD] in pheromone-baited traps relative to the positions of 30-m wide swaths treated with disparlure, Appomattox-Buckingham State Forest, VA, 2001. Black solid bars represent flight lines according to GPS data. Dashed bars represent presumed area of treatment deposition*

2.4 Efficacy assessment

Treatment efficacy was assessed based on capture rates of male moths in pheromone-baited traps and mating success of deployed females. In the ABSF plot (2001), native populations of gypsy moth males were too low for adequate assessment, so laboratory-reared males were released in the plots. Male gypsy moths were shipped as pupae from USDA, APHIS, Pest Survey, Detection, and Exclusion Laboratory, MA. Pupae were kept in 500-ml paper cups with plastic lids and then transferred to cups that were modified to release moths in the field. The release cups had several openings at mid-height for moth escape and were stapled to the trunks of small trees. Tanglefoot (The Tanglefoot Co., Grand Rapids, MI) pest barrier was applied in circles around the tree trunk to reduce ant predation. A fluorescent powder dye was added to the cups so that released moths could be distinguished from native moths.

Approximately 200 male moths were released at each release point each week. Traps were checked each week, and all captured male moths were removed and stored in the freezer. Later captured individuals were illuminated with an ultraviolet light and examined under a microscope to distinguish between released and native moths.

Mating success was assessed by determining the fertilization status of deployed females after 24 h exposure to native or released males. Females were tethered around the base of a front wing using a 10–15 cm thread and attached to a tree by a pushpin (Sharov et al., 1995). To protect females from natural predators (e.g. ants), a tanglefoot pest barrier was applied in a 25 cm radius circle around each individual. After 24 h, females were retrieved and placed in paper bags. After 30 days, the eggs were examined to determine if they were embryonated. Females were considered to have successfully mated if they produced egg masses in which > 5% of the eggs were embryonated.

2.5 Statistical analysis

The general linear model analysis of variance (anova) procedure with Fisher's adjustment for multiple comparisons of mean values (SAS, 1988, Proc GLM) was used to test for differences in moth counts among groups of traps. In 2001, log-transformed weekly moth counts [ln(n + 1)] were modelled as a function of treatment, trap line, replication within line, and interaction of treatment and line. The treatments were: (i) inside the treated swath, (ii) between treated swaths, and (iii) outside the treated plot but within 300 m of the plot perimeter. In 2002, the log-transformed weekly moth counts [ln(n + 1)] were modelled as a function of block, treatment, trap line, replication within line, and interaction of treatment and line. A fourth treatment consisting of an untreated control was added to the three treatments included in 2001.

The general linear model anova procedure (SAS, 1988, Proc GLM) was used to test for differences in the proportions of fertilized females deployed inside treated swaths, between treated swaths, and in the control area on the 4 days that control females were deployed. A separate anova was used to compare the proportion of fertilized females inside and between treated swaths over the duration of the study. In both analyses, the interaction of treatment and date was used as the error term to test the significance of the treatment effect.

3 Results

In 2001, the experimental plots consisted of 30 m sprayed swaths separated by 90 m untreated gaps. Male gypsy moth recapture in pheromone-baited traps located within treated swaths in the ABSF plot was significantly lower than in traps located in untreated gaps. Moth capture in traps located outside the plot was significantly higher than in traps located within the plot ($F = 5.5$; $P < 0.03$; d.f. = 2, 8; fig. 3a).

In the GWMA plot, the difference between native male moth capture in pheromone-baited traps located within and between treated swaths was not significant (fig. 3b). Male capture was significantly higher in traps located outside the plot ($F = 5.1$; $P < 0.04$; d.f. = 2, 8). The proportion of fertilized females inside and between treated swaths from July 4 to July 10 did not differ significantly, but significantly more females were fertilized in the control area ($F = 56.2$; $P < 0.0001$; d.f. = 2, 6; fig. 4). Over the duration of the study, the proportion of fertilized females in treated swaths (0.015) and between treated swaths (0.021) was not significantly different ($F = 0.7$; $P = 0.4$; d.f. = 1, 17).

In 2002, the experimental plots consisted of 30 m sprayed swaths separated by 30 m gaps. Male moth capture in pheromone-baited traps within the treated plots was significantly lower than in traps outside the plots (fig. 3c). Moth capture in traps placed in the control area was significantly higher than in traps placed in or near the treated plots ($F = 54.7$; $P < 0.0001$; d.f. = 3, 12). However, there was no significant difference in trap capture between traps placed within and between the treated swaths.

4 Discussion

The study reported here was conducted to quantify the effect of pheromone treatments in adjacent untreated areas to determine if it might be possible to achieve effective mating disruption with non-uniform treatment patterns. These experiments showed that there was a significant reduction of male moth catches within treated plots that contained untreated areas from 30 to 90 m wide between 30 m wide treated swaths. Therefore, there was a clear indication that disparlure disperses from treated areas and can disrupt mating communication in areas not treated directly. These results agree with those of Sharov et al. (2002a), who found that trap capture was suppressed within 250 m of treated areas.

Milli et al. (1997) noted that leakage of pheromone from treated plots could lead to increased pest attack rates. They referred to the area where the leakage

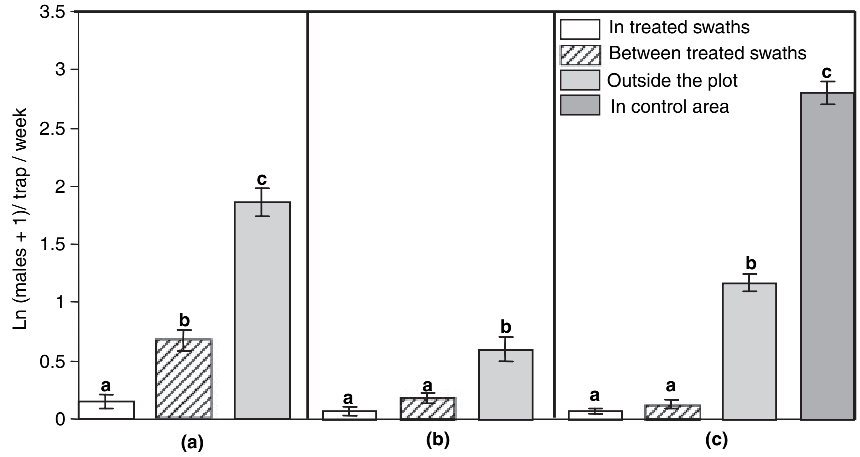

Fig. 3. Male gypsy moth counts [ln(n + 1) ± SD] within treated swaths, between treated swaths, outside plot perimeter but in the vicinity of the plot, and in separate untreated areas. (a) Appomattox-Buckingham State Forest, VA, 2001; (b) Goshen Wildlife Management Area, VA, 2001; (c) Goshen Wildlife Management Area, VA, 2002. Bars within each of the three groups with the same letters are not significantly different, Fisher's LSD (α = 0.05)

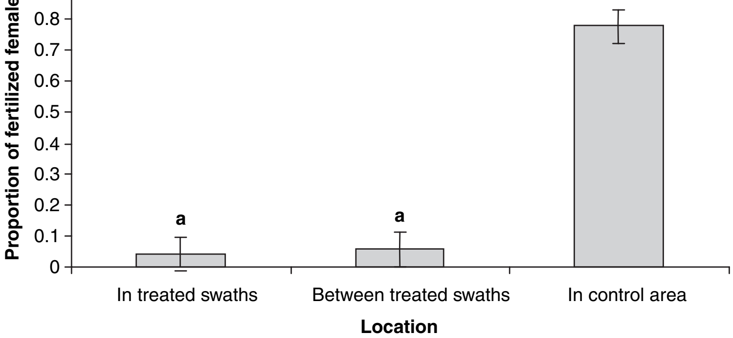

Fig. 4. Proportion of females fertilized within treated swaths, between treated swaths, and in a separate untreated area, Goshen Wildlife Management Area, VA, 2001. Bars with the same letter are not significantly different (α = 0.05)

occurs as a transition zone. A transition zone could occur because, as air flows across a treated plot as a result of wind currents, the pheromone cloud is likely to move further into the treated plot thus depleting the border. Wind blowing from treated to untreated areas pushes the pheromone cloud beyond the border of the treated plot thereby creating edge leakage. Therefore, a transition zone appears around the border of the plot where the pheromone concentration is unstable and is affected by wind (Milli et al., 1997). The pattern of male moth catches that was observed in pheromone-baited traps across the plots in this study (fig. 2) does not show evidence of a transition zone. In the experiments reported here, and in Sharov et al. (2002a), mating communication was disrupted at and beyond the boundaries of the treated area. This might have been due in part to the pheromone formulations used for mating disruption in these studies. The presence of sticking agent in the plastic flakes formulation allows flakes to stay attached to the foliage while constantly emitting pheromone, and therefore may mitigate the effects of edge depletion.

In 2001, each treated swath (30 m) was separated by an untreated area that was 90 m wide. Two plots were treated in this way, one in the ABSF which is located in the Piedmont geographic region of Virginia and one in the GWMA that is located in more mountainous terrain. In the former plot, significantly more moths were captured in traps in the untreated area compared with those in the treated swaths. However, in the latter plot, trap capture did not differ between treated and untreated areas in the plot. Sharov et al. (2002a) found that the effect of applied pheromone extended further along valleys in mountainous areas than in less mountainous areas. The differences in pheromone effects between the ABSF plot and GWMA plot with respect to skipped-swath applications of pheromone observed in this study in 2001 may be because of differences in the topographic relief at the two study sites. Wind and airflow patterns are influenced by the landscape, therefore the distance at which disparlure disperses may also be affected by characteristics of the landscape. In narrow valleys in mountainous terrain (such as GWMA), there is a venture (tunnelling) effect that increases turbulence and accelerates wind speeds along the valley (Tang and Peng, 1977; Kimmins, 1987). This has the effect of moving molecules (such as the gypsy moth pheromone) in the air further along narrow valleys in mountains, which may have led to greater spread of pheromone from treated areas in the GWMA plot in 2001.

Another possible explanation for the inconsistent results between the two plots in 2001 is that male moths were released in the ABSF plot but not in the GWMA plot. The distribution of native male gypsy moths is highly variable, which may have obscured the effect of the pheromone in the GWMA plot.

In 2002, with an untreated area of 30 m between treated swaths, suppression of trap capture did not differ between treated and untreated areas within the plots. Based on the results of this experiment and those of the previous year which provided some evidence of reduced suppression of trap capture within untreated areas that were 90 m wide, untreated gaps between treated swaths should not exceed 30 m. Further research into the effects of topography on pheromone movement and distribution may provide additional information that could be used to refine these recommendations.

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