

Gypsy moth mating disruption in open landscapes

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- Abstract**
- 1 Aerial applications of Disrupt II, a plastic laminated flake formulation containing a racemic form of the gypsy moth sex pheromone, disparlure, achieved > 99% reduction of mating among females on individual, isolated trees surrounded by an area cleared of trees.
 - 2 These results support the use of mating disruption to eradicate isolated gypsy moth populations in open landscapes, such as parks, residential areas and commercial settings.
 - 3 Mating success in both treated and untreated areas varied with the initial distance between males and females. When the initial distance between males and females was < 5 cm in an area receiving a dosage of 37.5 g of racemic disparlure per ha, mating success was reduced by 27% compared with a similar deployment in an untreated area. Mating was eliminated in areas treated at the same dosage when males and females were initially deployed 1 m apart but on separate trees.
 - 4 This suggests that mating disruption may not be an effective tactic for gypsy moth eradication in cases where the infestation is concentrated on a small number of trees and males and females are in close proximity in space and time.

Keywords Disparlure, gypsy moth, *Lymantria dispar*, mating disruption.

Introduction

Mating disruption is an effective tactic against low-density gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) populations. This has been demonstrated both experimentally (Leonhardt *et al.*, 1996) and operationally (Sharov *et al.*, 2002a). In the Slow the Spread of the Gypsy Moth Program (STS), a U.S. federally-funded effort involving ten states along the leading edge of the gypsy moth infestation (Sharov *et al.*, 2002b), population densities generally considered most appropriate for mating disruption treatments are those in which season-long captures of male moths in pheromone-baited traps do not exceed 30 (Thorpe *et al.*, 2006). The goal of the STS program is to reduce the rate of gypsy moth spread by treating and eliminating the numerous gypsy moth colonies that become established just ahead of the leading edge (Sharov *et al.*, 2002b). Subsequent to the implementation of STS in 2000, mating disruption has been used on 82% of the nearly 2.9 million ha treated (USDA, 2006) to slow the spread of gypsy moth, and the success rate was estimated to be 95% (Sharov *et al.*, 2002a).

The use of mating disruption to eradicate truly isolated gypsy moth populations that occur well beyond the leading population front has been limited primarily to areas considered too environmentally sensitive for the use of less target-specific tactics such as *Bacillus thuringiensis* or chemical insecticides. This results from a lack of confidence on the part of gypsy moth managers in the effectiveness of mating disruption for eradication. Specific concerns include the lack of research data on the efficacy of mating disruption for eradication, the lack of operational experience with the use of mating disruption for eradication, and the seriousness of the consequences of a failure (i.e. the potential establishment of an exotic invasive pest). Another important consideration is that the application of mating disruptants renders pheromone traps useless in the year of treatment, thus preventing their use to evaluate treatment effectiveness and to delineate any remaining residual populations until at least 1 year after treatment.

The source of isolated gypsy moth populations that are well beyond the leading edge and therefore candidates for eradication is usually inadvertent transport of immature life stages as a result of human activity (Liebhold *et al.*, 1992). This typically results in the introduction of small numbers of individuals. Successful colony establishment from such

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introductions is resisted by Allee effects, which refer to decreases in the growth rates of populations with a decrease in its density, and causes include the inability to locate mates, inbreeding depression and failure to satiate predators (Courchamp *et al.*, 1999). Allee effects are considered a major factor limiting the rate of gypsy moth spread (Tobin *et al.*, 2007).

Because long-range dispersal is primarily through anthropogenic movement of life stages, the site of introductions is often nonforest areas such as parks, residential areas and commercial settings. Such areas typically contain a much lower density of trees than occur in the forest, where most mating disruption applications have occurred. In the most extreme case, gypsy moth populations may become established on individual host trees surrounded by open areas. The effectiveness of gypsy moth mating disruption treatments, which target male searching behaviour, has not been tested outside of the forest environment.

Gypsy moth precopulatory sexual behavior has been well described (Cardé, 1981; Charlton & Cardé, 1990), but the mechanism(s) by which mating disruption treatments interrupt this process in gypsy moths (Cardé & Minks, 1995) are not well understood. Studies of the effects of pheromone treatments on gypsy moth behavior are complicated by the fact that racemic (\pm) disparlure, which contains (–) disparlure, a behavioral antagonist (Cardé & Hagaman, 1979), is used to disrupt mating. The (–)-enantiomer is not active as a pheromone, but is known to inhibit the response of gypsy moth males to (+)-disparlure (Yamada *et al.*, 1976, Cardé *et al.*, 1977, Miller *et al.*, 1977, Plimmer *et al.* 1977). However, the racemic disparlure is cheaper and was shown to be an effective mating disruptant (Kolodny-Hirsch & Schwalbe, 1990).

A further complication is that, under certain conditions, male gypsy moths shift from chemical to visual cues when searching for females (Richerson *et al.*, 1976a). This appears to occur in dense gypsy moth populations where males tend to orient to vertical silhouettes such as trees. Similar behaviour also occurs when males encounter areas treated with mating disruptants (Richerson *et al.*, 1976b). Based on numerous examples of successful operational and experimental mating disruption treatments on low-density gypsy moth populations in forested areas (Leonhardt *et al.*, 1996; Thorpe *et al.*, 1999, 2000; Sharov *et al.*, 2002a; Tcheslavskaja *et al.*, 2005a, b), it appears that a shift to visual searching in treated areas does not confer a sufficient advantage to males to overcome the effects of treatments. However, in situations where females are concentrated on isolated trees in open areas, a visual searching strategy could be more advantageous to searching males.

The present study aimed to determine the effectiveness of gypsy moth mating disruption treatments in situations involving individual, isolated host trees surrounded by an open landscape. In one experiment, we determined the mating success of females on isolated trees in an area treated with an aerial application of a plastic laminated flake formulation of pheromone. A second experiment examined the effect of the proximity of male and female gypsy moths at the time of eclosion on female mating success in the presence of different dosages of pheromone treatments.

Materials and methods

Open landscape experiment

The experiment was conducted in June to August of 2004 in the Goshen Wildlife Management Area, Rockbridge Co., Virginia [Universal Transverse Mercators (UTM): 637052E, 4223294N to 614250E, 4192715N, NAD 27, zone 17]. A 25-ha (500 × 500 m) plot that was half forested and half clear cut the previous year was treated for gypsy moth mating disruption at a rate of 37.5 g active ingredient (AI)/ha. The clear cut area contained a small number of isolated mature trees that escaped being cut. A similar plot was established 1 km away and used as an untreated control. Four trees in each of the four treatment combinations (clear cut versus forested; treated versus untreated) were used to evaluate mating success. Ten laboratory-reared females less than 24 h old were deployed directly on the trunk of each tree. A band of duct tape was applied to each trunk at a height of 2 m, and a thin bead of polybutene pest barrier (Tanglefoot Bird Repellent, The Tanglefoot Company, Grand Rapids, Michigan) was applied to the duct tape to prevent the females from ascending the trunk and to deter ant predation. Gypsy moths were reared at the USDA, APHIS, Pest Survey, Detection, and Exclusion Laboratory, Otis ANGB, Massachusetts, and shipped as pupae to the research site. Adult laboratory-reared males were released at a rate of 15 males per point per release from each of four points around each tree. Release points were 25 m from the tree. Females, along with any egg masses they produced, were recovered 24 h after they were deployed, placed in paper bags, and held for 30 days, after which the eggs were checked for embryonation, the presence of which indicates that the female was fertilized.

Male–female proximity experiment

The experiment was conducted in July and August of 2005 in the Appomattox-Buckingham State Forest, Appomattox and Buckingham Co., Virginia (UTM: 707562E, 4145752N to 707562E, 4146238N, NAD27, zone 17). Four 25-ha plots (500 × 500 m) were treated aerially for gypsy moth mating disruption at dosages of 0, 0.15, 15 and 37.5 g AI/ha. In each plot, 12 sites consisting of a pair of trees separated by a distance of approximately 1 m were established. A duct tape band with a thin ring of polybutene pest barrier was applied to one of each pair of trees at each site at a height of 2 m. Laboratory-reared females less than 24 h old were placed at a height of 1.5 m on each tree with a pest barrier. Laboratory-reared males that had emerged from their pupal cases within the previous 30 min and were not yet capable of flight were placed in one of three positions: within 5 cm of the female; on the same tree as the female but near the ground; or on the other tree of the pair of trees (approximately 1 m away). Females, and egg masses they produced, were recovered 24 h after they were deployed and placed in paper bags for 30 days, after which the eggs were checked for embryonation.

Treatments

A plastic laminated flake formulation (Disrupt II, Hercon Environmental, Emigsville, Pennsylvania) of racemic dispartlure [(Z)-7,8-epoxy-2-methyloctadecane] was applied by fixed-wing aircraft (Air Tractor, Olney, Texas) using specialized pods designed for that purpose and utilizing a differentially-corrected global positioning satellite system for navigation and tracking. The flakes ($1 \times 3 \times 0.5$ mm; 17.9% AI) are composed of polyvinyl chloride outer layers and a polymer inner layer containing the active ingredient. In the open landscape experiment, the mating disruption treatment was applied at a rate of 37.5 g AI/ha, which consisted of 209 g of flakes and 140 g of sticker (Gelva 2333, Solutia Inc, Springfield, Massachusetts) per ha. Gelva 2333 is a multipolymer emulsion used industrially primarily as a pressure-sensitive adhesive. In the male–female proximity experiment, the mating disruption treatment was applied at rates of 0.15, 15, and 37.5 g AI/ha. At the 37.5 g AI/ha rate, 209 g of flakes and 140 g of sticker were applied per ha. At the 15 g AI/ha rate, 84 g of flakes and 56 g of sticker were applied per ha. To achieve an application rate of 0.15 g a.i./ha, a sufficient number of blank flakes (with no dispartlure) was mixed with active flakes to achieve the target dosage when the flake mixture was applied at the same flow rate as used for the 15 g AI/ha rate. This use of blank flakes was necessary because the application equipment was not reliable at lower flow rates. The rate of release of dispartlure from the flakes was not determined in the present study but, in previous studies where Disrupt II flakes were applied under similar conditions, they released 30–50% of their dispartlure content over the 6-week period of male flight (Leonhardt *et al.*, 1996; Thorpe *et al.*, 1999).

Statistical analysis

In the open landscape experiment, percent mating success was calculated for each group of females deployed on a tree per day. In the male–female proximity experiment, percent mating success was calculated for all females deployed on a given day in each plot. Because gypsy moth mating disruption treatments sometimes fail to eliminate mating but do reduce the number of fertile eggs to a very low level (Thorpe *et al.*, 2000), mating is considered successful only if the female produces $\geq 5\%$ fertile eggs. This definition of mating success is considered to be more biologically relevant because females producing $< 5\%$ fertile eggs contribute little to the next generation. Data were analyzed by analysis of variance (SAS Institute, 2000; PROC MIXED). When treatment effects were significant, treatment means were separated using the latin square design option at a comparison-wise error rate of 0.05.

Results

Open landscape experiment

The effect of the mating disruption treatment on percent fertilization of deployed females (producing $> 5\%$ fertile eggs)

was significant ($F = 61$; d.f. = 1,8; $P < 0.001$) but there was not a significant difference between mating success on trees in an open landscape compared with trees in the forest ($F = 1.9$; d.f. = 1,8; $P = 0.2$) (Fig. 1). The mating disruption treatment \times landscape interaction effect was not significant ($F = 2.3$; d.f. = 1,8; $P = 0.2$). Mating success averaged 30.0% and 0.3% in untreated and treated areas, respectively, which is a 99% reduction due to the treatment.

Male–female proximity experiment

The effects of mating disruption treatment dosage ($F = 58.3$; d.f. = 3,123; $P < 0.001$) and male–female position ($F = 24.4$; d.f. = 2,123; $P < 0.001$) were significant, but the interaction of the two factors was not significant ($F = 1.2$; d.f. = 6,123; $P < 0.3$) (Fig. 2). Within each of the two factors, all means were significantly different at the 0.05% comparison-wise error rate. At all dosages, mating success was greatest when males and females were deployed within 5 cm of each other. When deployed this way, mating success ranged from 95% in the absence of a treatment to 27% at 37.5 g AI/ha. When males and females were deployed 1 m apart but on the same tree, mating success ranged from 84% in the absence of a treatment to 13% at 37.5 g AI/ha. When males and females were deployed 1 m apart but on separate trees, mating success ranged from 76% in the absence of a treatment to 0.2% at 37.5 g AI/ha.

Discussion

The open landscape experiment addressed two concerns over the use of aerial applications of mating disruptants to eradicate gypsy moth populations. First, most previous experimental and operational experience with gypsy moth mating disruption has been in forested areas where the dispensers are applied with a sticking agent and are distributed throughout the canopy. In an earlier experiment, gypsy moth mating was disrupted by the aerial application of flakes to a forest canopy without a sticking agent, even though the flakes fell to the forest floor (Thorpe *et al.*, 2000). In open

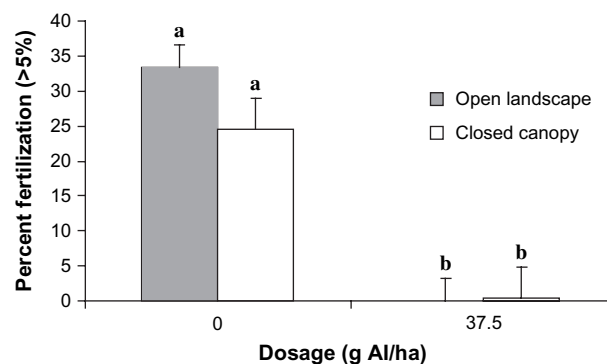


Figure 1 Gypsy moth mating success ($P \pm$ SEM) in open landscapes and closed canopies treated with Disrupt II flakes or untreated. Bars with the same letters are not significantly different.

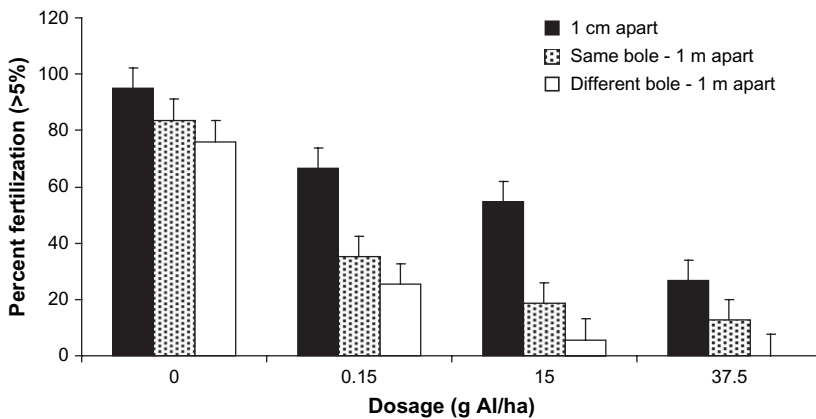


Figure 2 Gypsy moth mating success ($P \pm \text{SEM}$) in plots treated at pheromone dosages ranging from 0 to 37.5 g AI/ha when males were released at three different positions relative to females. All means are significantly different at the 0.05% comparison-wise error rate.

landscapes lacking a canopy, except for the isolated target trees, most of the dispensers fall to the ground, creating a much different distribution of dispensers than occurs in the forest. Furthermore, the canopy provides a barrier to the loss of pheromone and to the entry of air currents that can disperse the pheromone. However, the high level of efficacy achieved in this experiment is evidence that the lack of a canopy did not prevent the formation of appropriate atmospheric conditions for effective mating disruption. The second concern is that in the presence of high level of airborne pheromone males will switch to a visual searching behavior and the applied pheromone will be less effective. Again, the effectiveness of the treatment in this experiment indicates that this did not occur to a significant extent.

In the open landscape experiment, males were released 25 m from the females to determine the effect of the treatment on searching males. However, in an open landscape with few trees, gypsy moth life stages may be more spatially concentrated than with an infestation in a forest. The male–female proximity experiment was conducted to examine the effectiveness of mating disruption treatments when males and females emerge close together in space and time. In the absence of a treatment, mating success was high, regardless of the initial positions of males and females. In the presence of a mating disruption treatment at a dose of 37.5 g AI/ha, the highest dose currently used in the STS program, mating was greatly reduced but not eliminated when the initial positions of males and females was < 5 cm apart, and some mating occurred when males and females were released 1 m apart but on the same tree bole. Under this treatment, mating was essentially eliminated when males and females were initially positioned 1 m apart but on different trees.

The results of the open landscape experiment indicate that mating disruption should be an effective treatment for the eradication of isolated, low-density gypsy moth populations even in nonforest areas with a low density of trees provided the life stages are not concentrated on the same tree stems. In a situation where life stages are concentrated and males and females emerge close together in space and time, mating may not be completely disrupted. The frequency with which such a situation occurs is unknown but, given that long-range dispersal occurs primarily through anthropogenic movement of life stages, introductions to non-forest areas containing a low

density of trees may not be uncommon. The extent to which mating disruption treatments reduce mating among males and females initially positioned close to each other is dose-dependent, supporting the use of a high dose of 37.5 g AI/ha for eradication efforts.

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