Comparison of methods for deploying female gypsy moths to evaluate mating disruption treatments

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- **Abstract** 1 Mating disruption is the primary tactic used to reduce rates of gypsy moth population spread in the United States Department of Agriculture's Slow-the-Spread of the gypsy moth programme (STS). Because STS targets very low-density gypsy moth populations within which it is extremely difficult to collect females or egg masses, mating success in native populations cannot be determined. Therefore, the evaluation of mating disruption treatments in field experiments such as those designed to test new formulations and application methods requires deploying and recovering laboratory-reared female moths to determine mating success.
 - 2 Five methods of deploying females were evaluated for cost, rates of female and egg mass recovery, and female mating success. The deployment methods tested were: modified delta trap, square barrier, single and double trunk bands, and tethered females.
 - 3 Deployment of tethered females had the highest cost and mating success rate, but it did not yield the highest rates of female and egg mass recovery. Deployment of females in delta traps produced the lowest cost and mating success rate, but vielded the highest recovery rate. Neither of these deployment methods is recommended because of unacceptably high cost (tethered female) or low mating success (delta trap).
 - 4 There were no significant differences in cost or mating success among the other three deployment methods.
 - 5 The differences among the square barrier, single trunk band, and double trunk band methods in cost, female and egg mass recovery, and mating success are too small to recommend any one over the others.

Keywords Aerial application, disparlure, gypsy moth, laminate flakes, *Lymantria* dispar, mating disruption, mating success, pheromone.

Introduction

The gypsy moth Lymantria dispar (L.) (Lepidoptera: Lymantriidae) is a major pest of forests and shade trees in the north-eastern United States. Subsequent to its introduction from Europe in approximately 1868, it has defoliated more than 34 million ha, and more than 5 million ha have been treated with insecticides to suppress populations (Gypsy

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Moth Digest, 2005). Populations are currently established in 17 states, and the leading edge is continuing to spread to the south and west. The national Slow-the-Spread of the Gypsy Moth Project (STS) was initiated in 1999 in the nine states containing the advancing gypsy moth population front. STS is a coordinated effort by the United States Department of Agriculture (USDA) and affected states to decrease the rate of spread of the gypsy moth into uninfested areas (Sharov et al., 2002a; Tobin et al., 2004). In STS, an extensive grid of pheromone traps is used to detect and monitor gypsy moth populations, and treatments are applied as needed to slow the rate of population spread. Mating disruption is the primary

treatment tactic used in STS (Sharov *et al.*, 2002a). It is favoured because it is specific to the target pest, its cost is relatively low (Sharov *et al.*, 2002a), and it is effective (Sharov *et al.*, 2002b).

Starting in 1971, the management of gypsy moths using mating disruption has been the subject of considerable research effort (Doane & McManus, 1981; Reardon et al., 1998). Early research led to the development of suitable dispensers (Schwalbe et al., 1979; Plimmer et al., 1982) and the determination that aerial and ground application of mating disruptants could reduce gypsy moth mating success (Schwalbe et al., 1983; Webb et al., 1988, 1990). Later efforts demonstrated that mating disruption treatments targeting low-density populations could suppress population growth in subsequent years (Leonhardt et al., 1996). More recent work with Disrupt II plastic laminated flakes (Hercon Environmental, Emigsville, PA), currently the only commercial product available for operational use, quantified the effects of a sticking agent in the flake formulation (Thorpe et al., 2000), examined the effectiveness of non-uniform aerial application patterns (Tcheslavskaia et al., 2005a), and established a dose-response for the effect of pheromone application rate on suppression of male trap catch (Tcheslavskaia et al., 2005b).

Because of the importance of mating disruption in the STS programme, there is a continued need for research to address data gaps, develop new formulations, and improve the costeffectiveness of this tactic. Reliable methods are needed to evaluate the effectiveness of mating disruption treatments. Because mating disruption targets very low population densities, it is usually not possible to find life stages in the field other than males collected in pheromone traps. Therefore, the only methods available to measure treatment effectiveness in the year of treatment are the numbers of males collected in pheromone traps and mating success of females that have either been collected from areas with higher population density or reared in the laboratory and placed in the plots. Both methods have been used extensively in the past to measure mating disruption. Although reductions in trap catch correlate with reductions in mating success (Granett, 1974; Tcheslavskaia et al., 2005b), mating success is generally thought to be a more reliable indicator of the effectiveness of mating disruption treatments (Kolodny-Hirsch & Webb, 1993; Leonhardt et al., 1996), possibly because females are more attractive than traps because of chemical or visual cues.

A variety of methods has been used for the deployment of female gypsy moths in plots to measure the effectiveness of mating disruption treatments, including tethered females (Richerson *et al.*, 1976; Sharov *et al.*, 1995; Tcheslavskaia *et al.*, 2005a,b), delta trap-like structures (Webb *et al.*, 1988; Leonhardt *et al.*, 1996; Thorpe *et al.*, 1999), and the simple placement of females on tree boles (Granett, 1974; Schwalbe & Mastro, 1988). Tcheslavskaia *et al.* (2005b) compared the mating success of tethered females and females deployed in nonsticky delta traps. Mating success among tethered females was more than twice that among females deployed in delta traps (15.6% and 7.0%, respectively). Although the reasons for this difference are unknown, it could have resulted from a

reduction in the ability of males to find and mate with females in delta traps, or an increase in the ability of males to mate with tethered females. In either case, it is important to understand the effects of deployment method on mating success and recovery rates of deployed females and choose a method that minimizes inhibition of mating and maximizes cost-effectiveness.

The present study aimed to examine the cost, female recovery rate, and mating success of five methods of female deployment. The study was conducted in the absence of a mating disruption treatment and in plots treated with a low dose of mating disruptant to examine any effects of the treatment on mating success and female recovery rate.

Materials and methods

Study location

The study was conducted in 2004 in the Little North Mountain Wildlife Management Area, Augusta County, Virginia (79°20.4'W, 38°4.2'N to 79°21.9'W, 38°2.3'N). The overstory was primarily oak, Quercus spp., with hickory, Carya spp., black gum, Nyssa sylvatica Marshall, tulip tree, Liriodendron tulipifera L., white pine, Pinus strobus L. and Virginia pine, Pinus virginiana Miller. The understory was predominantly blueberry, Vaccinium spp., mountain laurel, Kalmia latifolia L. and flowering dogwood, Cornus florida L. In all plots, the canopy was approximately 30 m high. Gypsy moth population density was relatively low with no defoliating populations in the vicinity. The average seasonlong (early July to end of August) trap catch in standard USDA milk carton pheromone traps in the vicinity of the study area was 9.2 (range 1–22; n = 9). Female deployment methods were evaluated in an untreated area and in a 25-ha plot treated with disparlure at an extremely low dose [0.15 g active ingredient (a.i.)/ha]. In a previous study, this dose of Disrupt II flakes reduced trap capture by about 50% (Tcheslavskaia et al., 2005b), and we expected a treatment at this dose to cause a similar reduction in mating success. Our goal was to conduct the test in an area where moth behaviour could be affected by the disparlure treatment, but where mating would still occur so that the deployment methods could be compared.

Disparlure treatment

Disrupt II flakes (Hercon Environmental, Emigsville, Pennsylvania) were applied on 9 June 2004 with a fixed-wing aircraft (Air Tractor) using specialized pods designed for that purpose and utilizing a differentially corrected global positioning satellite system for navigation and tracking. To achieve an application rate of 0.15 g a.i./ha, a sufficient number of blank flakes (with no disparlure) was mixed with active flakes to achieve the target dosage when the flake mixture was applied at a flow rate of 85 g/ha. The flakes (1 \times 3 \times 0.5 mm) are composed of polyvinyl chloride outer layers and a polymer inner layer, which contain racemic disparlure in

the active flakes. Active flakes were composed of 17.9% a.i. The flakes were applied with 42 g/ha of sticker (Gelva 2333; Solutia Inc., Springfield, Massachusetts). Gelva 2333 is a multipolymer emulsion used industrially primarily as a pressure-sensitive adhesive. The rate of release of disparlure 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 disparlure content over the 6-week period of male flight (Leonhardt et al., 1996; Thorpe et al., 1999).

Female deployment methods

In all cases, females were deployed on large trees (> 30 cm diam.) at a height of 1.5 m from the ground. Five different methods of female deployment were tested: (i) modified delta trap; (ii) square barrier; (iii) single trunk band; (iv) double trunk band (above and below female); and (v) tethered female. The delta trap did not contain any sticky material. A small piece of brown paper was placed inside to provide a surface on which the female could cling. The delta trap was suspended from a coat hanger stapled to the side of the tree

bole (Fig. 1A). The square barrier consisted of a 30-cm wide square of duct tape applied to the surface of the tree bole and held in place with staples (Fig. 1B). A tray $(30 \times 6 \text{ cm})$ was fashioned from aluminium roof flashing and taped and stapled to the tree at the bottom of the duct tape square to catch falling moths. A thin band of polybutene (Tanglefoot Bird Repellent, The Tanglefoot Company, Grand Rapids, Michigan) was applied to the entire length of the duct tape square to restrict female movement and deter predators. The female was deployed in the centre of the square. The trunk bands (Fig. 1D) consisted of duct tape wrapped around the tree bole. Any gaps between the duct tape and the tree were filled with synthetic polyester fibre material. A thin band of polybutene was applied to the entire length of the duct tape. When a single band was used, the band was placed at a height of 2 m and the female was deployed below the band. When there were two bands, the upper band was placed at 2 m and the lower band was placed at 1 m. The female was deployed between the two bands. Females were tethered with a 10-cm length of thread tied around the base of a forewing (Fig. 1C). Tethered females were placed on the tree bole and the other end of the thread was pinned to the bole.

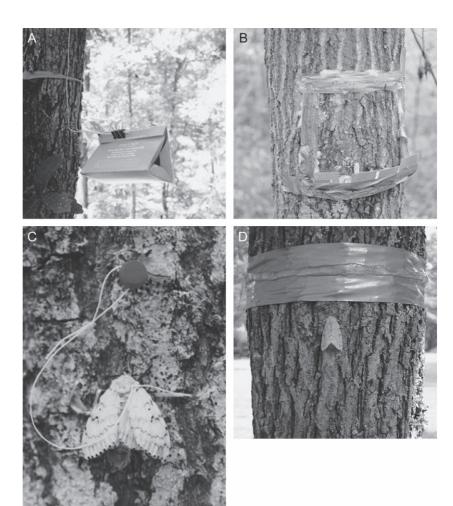


Figure 1 Methods used to deploy gypsy moth females. (A) modified delta trap; (B) square barrier; (C) tethered female; (D) single trunk band.

Evaluation of deployment methods

Six circles of 15 trees were established: three in the treated plot and 3 in the untreated area. Each circle was approximately 100 m in diameter. Each deployment method was applied to three trees in each circle. Females were obtained as pupae from the USDA, APHIS, Pest Survey, Detection, and Exclusion Laboratory, Massachusetts. The pupae were reared to adult and females were deployed within 24 h of eclosion. Females were collected, along with any egg masses, 24 h after they were deployed. After at least 30 days in the laboratory, all eggs were examined for embryonation. If any eggs in an egg mass were embryonated, the numbers of embryonated and unembryonated eggs were counted (Sharov et al., 1995). Because of the variability associated with the spatial distribution of low-density populations of wild moths, laboratory-reared males were released in the centres of the circles at an approximate rate of 50 males per plot per day. Male moths were also shipped as pupae from the USDA, APHIS facility.

Statistical analysis

Percent female and egg mass recovery, percent mating success, and yield (percent recovery × percent mating success) were calculated separately for each combination of type of deployment, day of deployment, circle of 15 females, and treated vs. untreated plot. The percent of egg masses with greater than 50 eggs was calculated separately for each combination of type of deployment, time period, and circle of 15 females. To eliminate missing values, several of the deployment days were combined into ten time periods prior to analysis. Percent fertile eggs produced by successfully mated females was calculated separately for each combination of type of deployment and time period (same grouping of days as before). The percent of egg masses with greater than 50 eggs and the percent fertilized eggs produced by successfully mated females were calculated for females deployed in untreated areas only. Because it has been found that 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), successful mating is defined as only those egg masses that contain $\geq 5\%$ 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 transformed using the square root-arcsine transformation and analysed by analysis of variance (SAS Institute, 2000; PROC GLM) as a randomized block design, with day or groupings of days serving as the block factor. When treatment effects were significant ($\alpha = 0.05$), treatment means were separated at a comparisonwise error rate of 0.05 using the Tukey adjustment option.

Results

The times required to prepare trees and moths using each of the five deployment methods, and to clean up at the end of the experiment, are shown in Table 1. All values were estimated

Table 1 Estimated tree and gypsy moth preparation times for five deployment methods

| Treatments | Tree preparation and clean-up time (minimum/tree) | Moth preparation time (minimum/moth) |
|-------------------|---|--------------------------------------|
| Tethered | 0 | 0.2–2 ^a |
| Square barrier | 20 | 0 |
| Delta trap | 5 | 0 |
| Single trunk band | 10 | 0 |
| Double trunk band | 15 | 0 |

^aDepending on worker skill.

based on discussions with workers with experience using each of the deployment methods. The time required to locate and mark each of the trees on which females were deployed, and to carry females into the plots and deploy them, was the same for each deployment method and therefore was not included in these estimates. Deploying tethered moths required no time to prepare the tree, but the tethering itself took 0.2–2 min per moth depending on the skill of the worker. Of the remaining methods, the delta trap method required the least preparation time. It took approximately 10 min to move to a tree and apply a duct tape and polybutene barrier trunk band. A second trunk band only added another 5 min because it did not require travel between trees. The square barrier required the most tree preparation. Because the duct tape could not be pulled tight against the tree, many staples were required to hold it in place. Also, additional time was required to fashion a tray out of flashing and attach it to the bottom of the square with duct tape.

Recovery of females is shown in Fig. 2. Significant differences occurred among the deployment methods both in the presence of disparlure (F=22.1; d.f. = 4,84; P<0.001) and in its absence (F=9.9; d.f. = 4,84; P<0.001). Recovery was greatest with the delta trap method and lowest with the single trunk band method, regardless of whether or not a disparlure treatment was applied. Female recovery rates were in the range 60–97%. The disparlure treatment had little effect on relative female recovery rates.

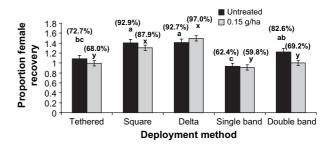


Figure 2 Recovery of female gypsy moths that were deployed using five methods in an untreated area and an area treated with a low dosage of disparlure. (Values are the mean \pm SE of square root-arcsine transformed proportions.) Means of untransformed data are given in parentheses. Treatments with the same letter are not significantly different at the 0.05 experiment-wise error rate.

In some cases, the female was not recovered (or she was dead), but an egg mass was recovered. A recovered egg mass provides useful information about whether or not the female mated. Total recovery (females and/or egg masses) is shown in Fig. 3. Significant differences occurred among the deployment methods both in the presence of disparlure (F = 13.8; d.f. = 4,84; P < 0.001) and in its absence (F = 10.4; d.f. = 4,84; P < 0.001). Recovery was greatest with the delta trap method and lowest with the single trunk band method. Total recovery rates were in the range 65.9-97.7%. Again, the disparlure treatment had little effect on relative total recovery rates.

Mating success, here defined as females that produced egg masses containing $\geq 5\%$ fertile eggs, is shown in Fig. 4. Significant differences occurred among the deployment methods both in the presence of disparlure (F = 3.2; d.f. = 4,84; P = 0.02) and in its absence (F = 6.6; d.f. = 4,84; P < 0.001). In the absence of disparlure, mating success was greatest among tethered females (48%) and lowest among females deployed in delta traps (14%). Mating success was reduced by approximately 50% in the presence of the disparlure treatment. Mating success in the treated plot varied from 3% among females deployed in delta traps to 21% among females deployed using the double trunk band method. There was no significant difference in mating success among tethered females and females deployed using the square barrier or trunk band methods, but mating success among females deployed using the delta trap method was significantly lower than that among tethered females (absence of disparlure only) and females deployed using the double trunk band method.

Total recovery (females plus egg masses) and percent mating success were combined into a single value called yield (Fig. 5). Higher yield values should be associated with more efficient deployment methods. There were significant differences among female deployment methods both in the presence (F = 2.7; d.f. = 4,84; P = 0.03) and absence (F = 5.5; d.f. = 4,84; P < 0.001) of disparlure. In the absence of disparlure, yield was greatest among tethered females (42%) and lowest among females deployed in delta traps (14%). In the presence of disparlure, yield values were much lower,

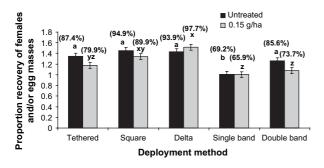


Figure 3 Recovery of female gypsy moths and/or their egg masses after females were deployed using five methods in an untreated area and an area treated with a low dosage of disparlure. (Values are the mean \pm SE of square root-arcsine transformed proportions.) Means of untransformed data are given in parentheses. Treatments with the same letter are not significantly different at the 0.05 experiment-wise error rate.

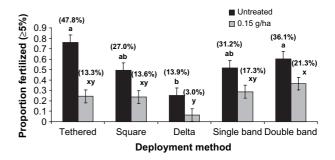


Figure 4 Mating success of gypsy moth females that were deployed using five methods in an untreated area and an area treated with a low dosage of disparlure. Females were considered mated if they produced egg masses containing \geq 5% fertile eggs. (Values are the mean \pm SE of square root-arcsine transformed proportions.) (Means of untransformed data are given in parentheses.) Treatments with the same letter are not significantly different at the 0.05 experiment-wise error rate.

and ranged from 14% among females deployed using double trunk bands to 3% among females deployed in delta traps.

The percent of egg masses with > 50 eggs and the percent of fertile eggs produced by successfully mated females averaged 84% and 55%, respectively, and did not differ among deployment methods (F = 0.8; d.f. = 4,36; P = 0.6 and F = 1.3; d.f. = 4,36; P = 0.3) (Table 2).

Discussion

Tethering yielded the highest mating success among females deployed in the absence of disparlure, and recovery was relatively high (73%). However, the process of tethering females is tedious and costly. In a typical mating disruption experiment, it is not unusual to use several hundred females per day for the evaluation of treatment efficacy. Tethering this many females requires several person-hours of work each day. Thus, the benefits of deploying tethered females would have to be great to justify the expense (and strain on worker morale). Although mating success was highest among tethered females in the absence of a disparlure treatment, it was

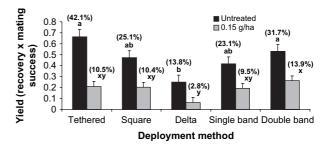


Figure 5 Yield (percent recovery of females and/or egg masses × percent mating success) of gypsy moth females deployed using five methods in an untreated area and an area treated with a low dosage of disparlure. Values are the mean \pm SE of square rootarcsine transformed proportions. Means of untransformed data are given in parentheses.

Table 2 Gypsy moth egg mass size and proportion fertilized eggs produced by successfully mated females for five deployment methods in the absence of a disparlure treatment

| Treatments | Proportion egg masses with > 50 eggs | Proportion fertilized eggs |
|--|--|--|
| Tethered Square barrier Delta trap Single trunk band Double trunk band | 1.32 ± 0.07 (87) 1.31 ± 0.07 (86) 1.16 ± 0.07 (77) 1.28 ± 0.07 (84) 1.31 ± 0.07 (86) | $0.86 \pm 0.08 (53)$ $0.84 \pm 0.10 (53)$ $0.67 \pm 0.13 (40)$ $0.94 \pm 0.12 (60)$ $0.68 \pm 0.09 (42)$ |

All data were square root-arcsine transformed prior to analysis. Values are mean \pm SE. Untransformed means are given in parentheses. Differences among treatments within a column are not significant ($\alpha=0.05$).

not significantly higher than mating success of females deployed using trunk bands or the square barrier methods. Furthermore, the tethered-female deployment method did not yield the highest recovery rates. Therefore, given the extremely high relative cost, tethering is not a recommended female deployment method.

Although recovery rate of females plus egg masses was highest among females deployed in delta traps, mating success was lowest with this deployment method, both in the presence and absence of disparlure. Mating success was extremely low (3%) for females deployed in delta traps in the presence of disparlure. Although the cost of female deployment in delta traps was lowest of all the deployment methods, the difference in cost is not great enough to justify this deployment method given the significantly reduced rates of mating success.

Mating success among the three remaining deployment methods did not differ, but total recovery was significantly lower with the single trunk band method in the absence of disparlure. However, yield, which combines these two values, did not differ significantly. Among these methods, the single trunk band method had the lowest application cost and the square barrier method had the highest, although the cost difference was probably too small to be a significant consideration. Given the small difference in cost and the lack of difference in yield among the three remaining methods, there is insufficient basis for recommending any one over the others. Individual preferences should dictate which method is used. The square barrier method results in somewhat higher recovery rates, but the barriers are more complicated to fabricate, install and remove than are the trunk bands. Given the small differences involved, and the low cost of installation, the single or double trunk band methods may be preferable in most situations.

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