# Optimization of pheromone dosage for gypsy moth mating disruption

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**Abstract**

The effect of aerial applications of the pheromone disparlure at varying dosages on mating disruption in low-density gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), populations was determined in field plots in Virginia, USA during 2000 and 2002. Six dosages [0.15, 0.75, 3, 15, 37.5, and 75 g active ingredient (AI)/ha] of disparlure were tested during the 2-year study. A strongly positive dose–response relationship was observed between pheromone dosages and mating disruption, as measured by the reduction in male moth capture in pheromone-baited traps and mating successes of females. Dosages of pheromone ≥15 g AI/ha (15, 37.5, and 75 g AI/ha) reduced the mating success of females by >99% and significantly reduced male moth catches in pheromone-baited traps compared to untreated plots. Pheromone dosages <15 g AI/ha also reduced trap catch, but to a lesser extent than dosages ≥15 g AI/ha. Furthermore, the effectiveness of the lower dosage treatments (0.15, 0.75, and 3 g AI/ha) declined over time, so that by the end of the study, male moth catches in traps were significantly lower in plots treated with pheromone dosages ≥15 g AI/ha. The dosage of 75 g AI/ha was initially replaced by a dosage of 37.5 g AI/ha in the USDA Forest Service Slow-the-Spread (STS) of the Gypsy Moth management program, but the program is currently making the transition to a dosage of 15 g AI/ha. These changes in applied dosages have resulted in a reduction in the cost of gypsy moth mating disruption treatments.

## Introduction

The management of populations of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), by mating disruption has been attempted since 1971 (Stevens & Beroza, 1972; Schwalbe et al., 1974; Granett & Doane, 1975). During the last 20 years extensive research has been conducted towards finding an appropriate formulation (e.g., Plimmer et al., 1982; Thorpe et al., 1999) and dosage (e.g., Webb et al., 1988) of the gypsy moth pheromone, disparlure, for use in mating disruption. Studies have shown that for mating disruption to be successful, the pheromone must be released slowly at a constant rate and be present in the air in sufficient quantities for the entire period during which the moths are sexually active (Cardé et al., 1975; Howse et al., 1998; Reardon et al., 1998). Thus far, the Hercon Disrupt® II plastic flake formulation of disparlure is a gypsy moth mating disruption product that satisfies the above criteria, and this formulation is therefore currently in use in the USDA Forest Service Slow-the-Spread (STS) of the Gypsy Moth program (Reardon et al., 1998; Sharov et al., 2002b).

In one of the earliest dose–response experiments to be conducted (Webb et al., 1988), Hercon Disrupt® II was applied aerially at dosages of 7.5, 30, and 75 g active ingredient (AI)/ha. A strong negative relationship was observed between pheromone dosage and male moth response to pheromone-baited traps and females. The large number of males that were trapped suggested that the study was conducted in an

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area with a high population density of gypsy moths. A number of other studies also showed decreasing mating success (i.e., proportion of mated females) and male moth captures with increasing dosages of dispalure, from 0 to 700 g AI/ha (Schwalbe & Mastro, 1988; Kolodny-Hirsch & Schwalbe, 1990; Webb et al., 1990; Kolodny-Hirsch & Webb, 1993).

Studies of the effects of pheromone dosage on gypsy moth mating disruption have usually been conducted employing high dosages against high-density populations (e.g., Schwalbe & Mastro, 1988; Webb et al., 1988, 1990); however, it now appears that mating disruption is most suitable for the suppression and eradication of isolated and semi-isolated low-density gypsy moth populations along the leading edge of an expanding front (Sharov et al., 2002a). The strategy of mating disruption in low-density populations was therefore implemented in 1998 by the STS program in eight US states, from Wisconsin in the north to North Carolina in the south (Sharov et al., 2002b). In isolated, low-density gypsy moth populations, mating disruption may also be an effective management strategy when the pheromone is applied at relatively low dosages. However, little information is available on the effects of low dosages of disparlure on low-density gypsy moth populations.

The objective of this study was to evaluate the relationship between the dosage of applied pheromone and mating disruption in low-density gypsy moth populations using a wider range of pheromone dosages than was applied in previous studies. Experiments were conducted in 2000 and 2002 using two and five dosages of pheromone, respectively, to evaluate the effects on mating disruption in gypsy moth populations, as measured by the mating success of females, and male moth catches in pheromone-baited traps.

## Materials and methods

### Dose–response experiment: 2000

Twelve plots, each 500 × 500 m and separated by at least 1 km, were selected in the George Washington National Forest near Millboro Springs (Bath Co), VA [UTM 637052 E, 4223294 N to 614250 E, 4192715 N, NAD 27, zone 17]. Four plots were used as controls and left untreated; the remaining eight plots were treated with racemic disparlure formulated as Disrupt® II (Hercon Environmental Corporation, Emigsville, PA, USA) plastic flakes at 37.5 and 75 g AI/ha. Each treatment was therefore replicated four times.

*Treatments.* The gypsy moth mating disruption formulation consisted of plastic flakes (3 mm long × 1 mm wide × 0.5 mm thick) composed of polyvinyl chloride (PVC) outer layers and an inner polymer layer containing 17.9% racemic disparlure ((Z)-7,8-epoxy-2-methyloctadecane). The flakes were mixed with diatomaceous earth (3% wt/wt) to reduce clogging and were applied from a fixed-wing aircraft (Air Tractors) using specialized application pods (Schweitzer Aircraft Corp., Elmira, NY, USA). Within the pods, the flakes were mixed with a multipolymer emulsion glue (Gelva 2333, Solutia Inc., Springfield, MA, USA) and dispensed through a spinner (Reardon et al., 1998). At the highest dosage of 75 g AI/ha, the pods were calibrated to deliver 85 g of flakes and 113 ml of glue per ha. Disparlure release rate from applied flakes was not determined in this study. However, in previous studies where plastic flakes were applied under similar conditions, the flakes released 30–50% of their disparlure content over the 6-week period of male moth flight (Leonhardt et al., 1996; Thorpe et al., 1999). A Global Positioning Satellite (GPS) navigation system was used to guide the spray applications.

An evaluation of treatment effects was done in a central 175 × 175 m core area (Figure 1). Gypsy moth mating success was evaluated using laboratory-reared virgin females. Each study plot had nine tethered females, nine females in mating stations, and four pheromone-baited traps (Figure 1). Each mating station was a cardboard delta trap containing a female with neither glue nor synthetic pheromone. Females were tethered around the base of a front wing

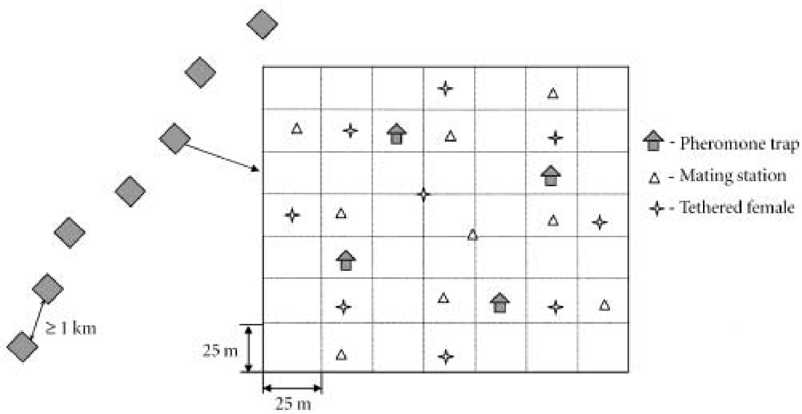


**Figure 1** Layout of pheromone-baited traps, mating stations, and tethered *Lymantria dispar* females in the 175 × 175 m core sampling area of an experimental forest plot in Millboro Springs, VA in 2000.

using a 10–15 cm thread and the thread was attached to a tree by a pushpin (Sharov et al., 1995). To protect the tethered females from predators, a Tanglefoot (The Tanglefoot Company, Grand Rapids, MI, USA) glue ring was applied at a radius of ca. 25 cm around each individual. Females were left on trees for 24 h, after which time they were removed and their fertilization status was determined via analyses of the spermatheca and by determining the embryonation of eggs (Stark et al., 1974; Sharov et al., 1995; Tcheslavskaia et al., 2002). Male moth capture was determined using standard USDA milk-carton pheromone traps baited with 500 μg of (+)-disparlure in twine dispensers (Hercon Environmental Corporation, Emigsville, PA, USA) (Schwalbe, 1981; Leonhardt et al., 1992).

*Data analysis.* The proportion of recovered females that were fertilized was determined for each treatment. All females with sperm present in the spermatheca, or that produced any fertile eggs, were considered fertilized. The General Linear Model analysis of variance (ANOVA) procedure with Tukey's adjustment for multiple comparisons of mean values (SAS, 1988, Proc GLM) was used to test the difference in moth counts between treatments. Log-transformed total moth counts per trap per week for each type of pheromone treatment, $\ln(N + 1)$, was modeled as a function of week and dosage without interactions of factors.

#### Dose–response experiment: 2002

A second study was conducted in the Appomattox–Buckingham (Appomattox and Buckingham Counties) and Cumberland (Cumberland County) State Forests, VA [UTM 746246 E, 4166292 N to 700180 E, 4136389 N, NAD 27, zone 17]. Eight experimental plots were established in each state forest. One plot in each state forest was used as a control, and the remainder of the plots were each treated aerially with Hercon Disrupt® II at 0.15, 0.75, 3, 15, or 37.5 g AI/ha. In each state forest, the low dosages (0.15, 0.75, and 3 g AI/ha) were replicated once, and the 15 and 37.5 g AI/ha dosages were each replicated twice. All treatments were randomly assigned to plots within each state forest.

*Treatments.* The mating disruption formulation consisted of Hercon Disrupt® II plastic flakes applied as described previously. At the dosage of 75 g AI/ha, pods were calibrated to deliver 85 g of flakes and 113 ml of glue per ha. Therefore, the application rates for the 37.5 and 15 g AI/ha dosages were controlled by proportionally reducing the flow rates of the flakes and glue. Because it was not practical to reduce flow rates further, lower-dosage applications were achieved by maintaining a flow rate equivalent to that used for the 15 g AI/ha dosage and diluting the flakes by mixing in an appropriate amount of blank (without disparlure) flakes.

Mating disruption was evaluated by recapture of released laboratory-reared males in USDA milk-carton traps baited with 500 μg of (+)-disparlure in twine dispensers (Schwalbe, 1981; Leonhardt et al., 1992). Laboratory-reared, rather than naturally occurring, moths were used to ensure equal male moth density among plots and to extend the time period during which data could be collected. In plots treated with 15 and 37.5 g AI/ha, the rate of fertilization of females was also used to evaluate the effect of applied pheromone. Each study plot had three male moth release points and eight pheromone-baited traps. The release points were established at the center of each plot and 150 m to the north and south of the plot center. The northern and southern release points were surrounded by four pheromone-baited traps, which were placed 25 m to the north, south, east, and west from the release point. Plots treated with pheromone at 15 and 37.5 g AI/ha had 15 tethered females placed in a 50-m radius circle around the release point at the center of the plot. Tethered females, protected from ant predation by a band of the Tanglefoot glue, were placed on tree boles for 1 day. Fertilization, as determined by the analysis of egg embryonation, was used as an indicator of mating.

Male gypsy moths were shipped as pupae from the USDA, APHIS, Pest Survey, Detection, and Exclusion Laboratory, MA, USA. Pupae were kept in paper cups with plastic lids and emerging adults were released in the field. Fluorescent powder dye was added to the cups with pupae to mark the emerging male moths. Each week, the same number of males (ca. 150) was released at each release point. Male moths were removed from the pheromone traps and stored in a freezer. They were subsequently examined under a microscope with UV light for the presence of fluorescent powder on wings, antennae or body in order to distinguish between released and natural moths.

*Data analysis.* The mating success of the females was analyzed using a General Linear Model ANOVA procedure (SAS, 1988, Proc GLM). The arcsine-transformed proportion of fertilized females ($\arcsin \sqrt{N}$) was modeled as a function of week and dosage with interaction of factors. Male moth catches in pheromone-baited traps were both analyzed using data from the entire period of the study, and separately using data from each of the three time intervals: 8–14, 15–49, and 50–56 days after pheromone application. To analyze the pooled data, a General Linear Model ANOVA with Tukey's adjustment for multiple comparisons of mean values (SAS, 1988, Proc GLM) was used to test the significance of differences in moth counts among dosages. The log-transformed total moth counts per trap per week for each type of pheromone treatment, $\ln(N + 1)$, was modeled as a function of week, dosage, and state forest without interactions of factors. For the first (8–14 day) and third (50–56 day) intervals, ln(N + 1) was modeled as a function of dosage and state forest without interactions of factors. For the second interval (15–49 day), ln(N + 1) was modeled as a function of week, dosage, and state forest without interactions of factors.

## Results

### Dose–response experiment: 2000

The mating success of laboratory-reared females in plots treated with disparlure was significantly reduced compared to females in control plots. In the control plots, 19.9% of 478 recovered females were fertilized, while in all treated plots 100% of 1460 females remained unmated. Moreover, in the control plots, the mean (± SEM) fertilization rate of tethered females (15.6 ± 3.0%) was more than twice as great as that of females deployed in mating stations (7 ± 2.4%) ($F_{1,51} = 4.2$, $P = 0.045$).

Trap catches of male moths were also suppressed by the pheromone treatments. Season-long male catches averaged 541, 7.33, and 8.25 males per plot at disparlure dosages of 0, 37.5, and 75 g AI/ha, respectively. Trap catches were significantly lower in treated plots compared to control plots ($F_{2,102} = 52.05$, $P<0.001$), but there were no significant differences in trap catches between plots treated at 75 and 37.5 g AI/ha (Figure 2).

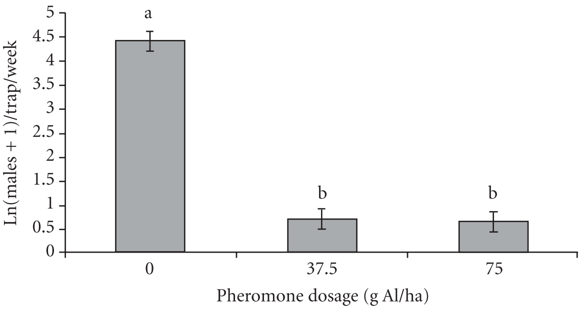


**Figure 2** Male gypsy moths (ln(N + 1) ± SEM) captured in plots treated with various dosages of pheromone in Millboro Springs, VA in 2000. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$).

### Dose–response experiment: 2002

No significant difference was found between the mating success of laboratory-reared females in plots treated with Hercon Disrupt® II at 15 g AI/ha (0.02 ± 0.0003%) and those in plots treated at 37.5 g (0.02 ± 0.0003%) ($F_{1,188} = 0.45$, $P>0.5$). Season-long male catches (from 25 June to 6 August) averaged 273.5, 91.5, 65.5, 23.5, 2.7, and 2.5 males per plot at disparlure dosages of 0, 0.15, 0.75, 3, 15, and 37.5 g AI/ha, respectively. Overall male moth recaptures were significantly lower in plots treated with Hercon Disrupt® II at 3, 15, and 37.5 g AI/ha compared to the plots treated with 0.15 and 0.75 g AI/ha ($F_{5,127} = 81.5$, $P<0.001$; Figure 3). Pheromone treatment significantly reduced trap captures of male moths in all treated plots compared to control plots.

Male moth recapture rates were compared over the periods of 8–14, 15–49, and 50–56 days after pheromone application (Figure 4). At 8–14 days, catches in the treated plots were significantly lower than in control plots ($F_{5,8} = 19.2$, $P<0.001$). Trap catches in the plots treated with Hercon Disrupt® II at 0.15 and 3 g AI/ha were greater than in plots treated with Hercon Disrupt® II at 15 and 37.5 g AI/ha, but the differences were not statistically significant. Trap catch was completely suppressed at dosages of 15 and 37.5 g AI/ha.

At 15–49 days after the pheromone application, trap catches of male moths decreased with increasing pheromone dosage ($F_{5,97} = 71.8$, $P<0.001$). The trap catches in the plots treated at 3, 15, and 37.5 g AI/ha were significantly lower compared to the catches in the rest of the plots. At 50–56 days after treatment, the trap catches in the plots treated with Hercon Disrupt® II at 15 and 37.5 g AI/ha

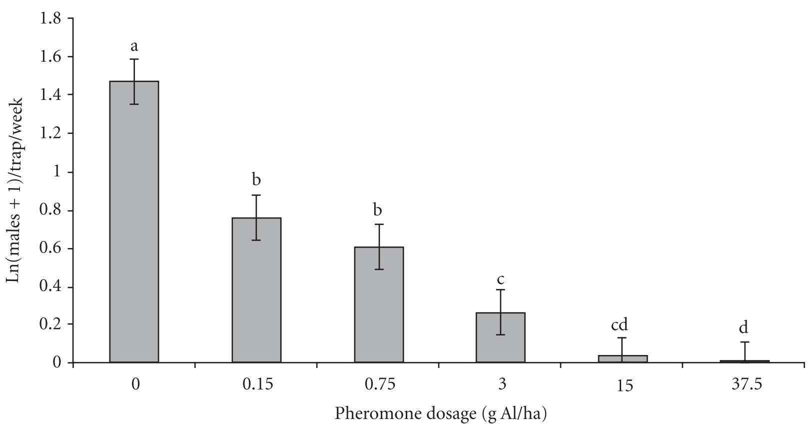


**Figure 3** Male gypsy moths (ln(N + 1) ± (SEM) recaptured in plots treated with various dosages of pheromone in Cumberland & Appomattox–Buckingham State Forests, VA in 2002. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$).

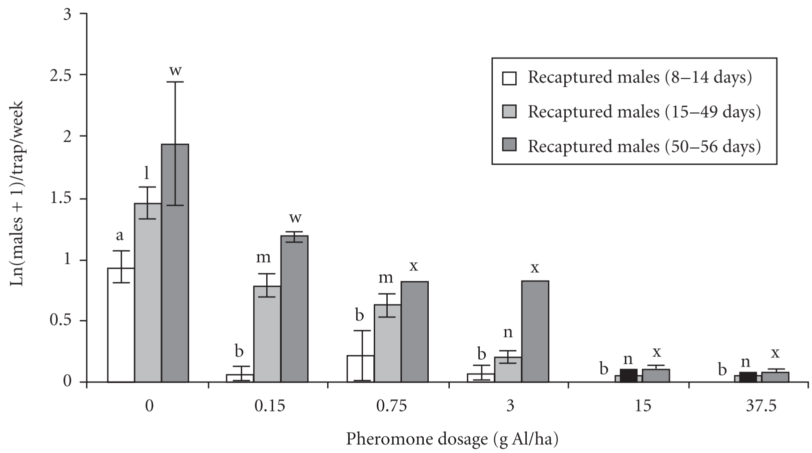


**Figure 4** Male gypsy moths (ln(N + 1) ± (SEM) recaptured at three time intervals in plots treated with various dosages of pheromone in Cumberland & Appomattox–Buckingham State Forests, VA in 2002. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$). Letters (a, b) (l–n), and (w, x) indicate significant differences between trap catches at 8–14, 15–49, and 50–56 days, respectively.

were significantly lower than in the remaining plots, including the plots treated at 3 g AI/ha ($F_{5,8} = 26.5$, $P<0.001$).

## Discussion

Previous studies have shown that there is a direct dose–response relationship in the disruption of mating communication in gypsy moth populations (Webb et al., 1988; Webb et al., 1990). In the present study a direct dose–response relationship was also observed between the dosage of applied pheromone and mating disruption. For example, the mating success of gypsy moth females and male moth catches in pheromone-baited traps were lower (i.e., mating disruption was high) in plots treated with relatively high dosages of disparlure (15, 37.5, and 75 g AI/ha) compared to untreated plots or plots treated with low dosages of disparlure (0.15, 0.75, and 3 g AI/ha). The absence of significant differences in mating disruption among the high dosages of pheromone suggests that an increase in pheromone concentration above a certain dosage (such as the 15 g AI/ha applied in this study) will result in a proportionally smaller increase in mating disruption. This phenomenon was also observed by Webb et al. (1990), but for much higher dosages of disparlure (>50 g AI/ha). The dfferences between the two studies could be due to the higher dosages of disparlure that were applied and the higher density of the gypsy moth population tested by Webb et al. (1990).

It has also been shown that there is a complete disruption of mating in gypsy moth populations in experimental plots treated with Hercon Disrupt® II at 75 g AI/ha (Leonhardt et al., 1996; Thorpe et al., 1999). For example, Thorpe et al. (1999) found that the mating success of females was reduced by > 99% after single aerial applications of Hercon Disrupt® II at 50 and 75 g AI/ha. In our experiments conducted in 2000, single aerial applications of Hercon Disrupt® II at 37.5 g AI/ha and 75 g AI/ha reduced the mating success of gypsy moth females and male moth catches in pheromone-baited traps by 100 and > 97%, respectively. Therefore, the disparlure dosage of 37.5 g AI/ha was as effective at decreasing the mating success of females and male moth catches in traps as the 75 g AI/ha dosage. As such, in 2000, the STS Program reduced its recommended dosage from 75 to 37.5 g AI/ha.

In the experiments conducted in 2002, aerial applications of Hercon Disrupt® II at 15 and 37.5 g AI/ha resulted in low (< 2.5%) mating success of females and a 99% reduction in male moth catches in pheromone-baited traps (Figure 3). While mating was eliminated in 2000 in plots treated at 37.5 g AI/ha, some mating did occur at the same dosage in 2002. This could be explained by the fact that in 2000, the experiments were conducted using naturally occurring males, but in 2002 laboratory-reared males were released and tested.

Although the results of the experiments conducted in 2002 confirmed the effectiveness of the 37.5 g AI/ha dosage, they also indicated that a disparlure dosage of 15 g AI/ha was as effective at decreasing the mating success of females and male moth catches in traps in low-density populations as were the dosages of 37.5 and 75 g AI/ha. As a result, in 2002, the 15 g AI/ha dosage was adopted for operational use in the STS program. The transition from a pheromone dosage of 37.5 to 15 g AI/ha resulted in a substantial reduction (ca. 50%) in the cost of mating disruption treatments in the STS program (http://www.gmsts.org/reports/2002_accomp.htm). In 2003, over 80% of the 222 585 ha treated with mating disruptant were treated at 15 g AI/ha (http://daento.vt.edu/stsdec.html).

For successful mating disruption, the artificial pheromone must be present in the air in sufficient quantities for the entire period of sexual activity of moths (Cardé et al., 1975; Howse et al., 1998; Reardon et al., 1998). The 2002 study of the time effect of the pheromone applied at different dosages showed that the dosage of 15 g AI/ha was also effective throughout the entire flight period of gypsy moths. Although the dosage of 3 g AI/ha reduced overall trap catches by 91%, the effect of pheromone applied at this dosage changed over the season. At the beginning of the season (8–49 days after pheromone application), there were no significant differences among trap catches in plots treated at 3, 15, and 37.5 g AI/ha. By the end of the season (50–56 days after pheromone application) trap catches in plots treated with 3 g AI/ha were significantly greater than in plots treated with 15 and 37.5 g AI/ha. In the STS program, it is required that the applied pheromone be effective for a period of 8 weeks to cover the entire period of gypsy moth flight (up to 6 weeks) and to provide a margin of safety in the event of early treatment application and late moth emergence. Thus, even though the dosage of 3 g AI/ha significantly reduced season-long trap catches, the effects of this dosage did not last long enough to satisfy the requirements for operational use in the STS program.

Mating success was found to be higher in females that were tethered than in those deployed in mating stations. It is likely that the delta traps used as mating stations make it more difficult for the males to find and mate with females. However, the deployment of tethered females is a much more time-consuming, costly, and tedious process than deploying females in delta traps. There is therefore a need to find alternative, lower-cost deployment methods that do not inhibit mating.

Finally, this study showed that male gypsy moth catches in pheromone-baited traps could be used as a single measure of the effectiveness of pheromone treatments against gypsy moth populations. In some earlier studies, the evaluation of mating disruption in pheromone-treated areas was based on a reduction in male trap catches (Plimmer et al., 1982). However, subsequent studies have shown that the mating success of females provided a more accurate measure of mating disruption (e.g., Schwalbe & Mastro, 1988; Kolodny-Hirsch & Webb, 1993). In the present study, male moth capture in pheromone-baited traps was significantly reduced at 15, 30, and 75 g AI/ha. At these dosages, mating success was either reduced to a very low level (0.02% mating at 15 and 37.5 g AI/ha in 2002) or completely eliminated (no mating at 37.5 and 75 g AI/ha in 2000). Therefore, the reduction in trap capture was a reliable indicator of a successful mating disruption treatment. A possible explanation for the capture of moths at dosages that nearly or completely prevented mating is that a female is a weaker source of pheromone compared with a pheromone-baited trap and is therefore more difficult for a gypsy moth male to locate. As such, treatments that significantly reduce male catch should also significantly reduce the mating success of females. Therefore, reductions in male moth catches in traps alone can provide a useful measure of the effectiveness of gypsy moth mating disruption treatments.

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