Persistent effects of aerial applications of disparlure on gypsy moth: trap catch and mating success

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Abstract

In forest plots treated aerially with a plastic laminated flake formulation (Disrupt® II) of the gypsy moth sex pheromone disparlure to disrupt gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), mating was monitored the year of treatment and 1–2 years after treatment to determine the effects of the treatment on suppression of trap catch and mating success. In the year of treatment, there was a greater than 95% reduction in trap catch and a greater than 98% reduction in mating success compared to controls. One year after treatment at a dosage of 37.5 g active ingredient (a.i.) ha^{-1}, trap catch was reduced by 46–56% and mating success was reduced by 60–79%. Both trap catch and mating success were significantly reduced compared to controls in plots treated 1 year previously at 15 g a.i. ha^{-1}. Trap catch, but not mating success, was significantly reduced 2 years after treatment at 37.5 g a.i. ha^{-1}. The efficacy of mating disruption (MD) treatments in the Slow-the-Spread of the Gypsy Moth program was significantly reduced 2 years compared to 1 year after treatment. No such reduction was observed in plots treated with aerial applications of *Bacillus thuringiensis kurstaki*. The higher apparent efficacy of MD treatments 1 year after application may result to some extent from the suppression of moth capture in pheromone traps from the persistent effects of the previous year's treatment.

Introduction

Since its introduction into Medford, MA, USA, around 1869, the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), infestation has expanded to the west and south at a variable rate of 3–28 km year^{-1}, and the area generally infested with the gypsy moth currently extends northwest to Wisconsin and south to North Carolina (Tobin et al., 2007). The expansion of its current range is expected to continue until the gypsy moth eventually occupies all areas of the USA containing favorable habitat. It has been estimated that the ultimate range of this pest will be three times greater than its current range (Liebhold et al., 1997). To address the economic and environmental impacts caused by the expanding range of the gypsy moth infestation, a national strategy was developed to manage

gypsy moth populations along the leading edge of the infestation. The goal of this USDA Forest Service project, known as the 'Slow the Spread of the Gypsy Moth' (STS) program, is to intensively monitor populations along the leading edge and apply treatments such that the rate of expansion of the infested area is reduced by 50%. These goals are achieved through the use of a sophisticated Internet-based data management system and a decision algorithm to aid in decision making (Tobin et al., 2004). The STS program was pilot tested in 1993, became fully implemented in 2000, and currently includes 10 states and nearly 40 million ha (Sharov et al., 2002a). Since 2000, 82% of the nearly 1.2 million ha treated in STS used mating disruption (MD) (USDA, 2006). Mating disruption, which seeks to prevent reproduction by applying sex pheromone in sufficient quantities to prevent males from locating and mating with females, is a preferred tactic in STS, because it is target specific, relatively inexpensive (USDA, 2004), and effective in low-density, newly establishing populations (Sharov et al., 2002b).

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The ability of foliage to absorb pheromone and subsequently re-emit it in amounts sufficient to affect insect behavior has been reported for pea moth (Wall et al., 1981) and lightbrown apple moth (Karg et al., 1994; Suckling et al., 1996). Isomate C+ dispensers left in orchards continued to affect the behavior of male codling moths in the following season (Bäckman, 1997). Douglas-fir tussock moth pheromone applied by ground application to tree trunks suppressed trap capture by 80% 1 year after application (Hulme & Gray, 1997), but no such reduction in trap catch was detected following aerial applications. While contamination of the environment with the gypsy moth pheromone, disparlure, has not been previously documented, human contamination and re-emission after contact with disparlure is well documented (Cameron, 1983), although the mechanisms of absorption and re-emission in human tissues are not known. Cameron (1995) reported that he was still attractive to male gypsy moths 16 years after his last contact with disparlure.

Leonhardt et al. (1996) recovered dispensers from the forest floor that had been applied the year before to disrupt gypsy moth mating, and found that they contained 1.8% of their initial load of disparlure. However, it is not known how much, if any, of this disparlure continued to be released into the environment one or more years following initial treatment. This study was conducted to determine experimentally the extent to which the effects of gypsy moth MD treatments persist beyond the year of treatment, and to analyze historical data from STS treatment blocks to determine if there was a pattern of persistence in MD when compared to *Bacillus thuringiensis kurstaki* (Btk) treatment blocks.

Materials and methods

The study was conducted in the Appomattox-Buckingham (Appomattox and Buckingham Counties) and Cumberland (Cumberland County) State Forests, VA, USA, 78.2°N, 37.6°W to 78.7°N, 37.4°W. In 2003, as part of a field evaluation of experimental gypsy moth MD formulations, one 25-ha plot in each state forest was treated with Disrupt® II plastic laminated flakes (Hercon® Environmental, Emigsville, PA, USA) at 37.5 g active ingredient (a.i.) ha^{-1}. The treatment was applied by fixed-wing aircraft (Air Tractor, Olney, TX, USA) using specialized pods designed for that purpose and utilizing a differentially corrected global positioning satellite (DGPS) system for navigation and tracking. The flakes ($1 \times 3 \times 0.5$ mm; 17.9% a.i.) are composed of polyvinyl chloride outer layers and a polymer inner layer containing racemic disparlure [(Z)-7,8-epoxy-2-methyloctadecane]. At the 37.5 g a.i. ha^{-1} dosage, 209 g

of flakes and 140 g of sticker (Gelva 2333®; Solutia Inc, Springfield, MA, USA) were applied per ha. 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 this 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). An untreated control plot was established in each state forest. In both 2004 and 2005, two additional 25-ha plots were established in each state forest and treated with Disrupt® II flakes at 15 and 37.5 g a.i. ha^{-1}. At the 15 g a.i. ha^{-1} dosage, 84 g of flakes and 56 g of sticker were applied per ha.

In 2004, the following treatments were evaluated: disparlure applied at 15 and 37.5 g a.i. ha^{-1} the year of evaluation, disparlure applied at 37.5 g a.i. ha^{-1} the year prior to evaluation, and untreated control. In 2005, the treatments that were evaluated were: disparlure applied at 15 and 37.5 g a.i. ha^{-1} the year of evaluation, disparlure applied at 15 and 37.5 g a.i. ha^{-1} the year prior to the evaluation, disparlure applied at 37.5 g a.i. ha^{-1} 2 years prior to the year of evaluation, and untreated control.

Mating disruption was evaluated by the recapture of released laboratory-reared males in USDA milk-carton traps baited with 500 µg of (+)-disparlure in twine dispensers (Leonhardt et al., 1992) and the mating success of laboratory-reared females. Laboratory-reared rather than naturally occurring populations were used to ensure equal male moth density among plots and to extend the time period during which data could be collected. Male gypsy moths were shipped as pupae from the USDA–APHIS–PPQ–PSDEL (Animal and Plant Health Inspection Service–Plant Protection and Quarantine–Pest Survey Detection and Exclusion Laboratory), Otis ANGB, MA, USA. Pupae were kept in laminated paper cups with plastic lids and emerged adults were released in the field. The insects were reared on artificial diet containing a red dye that was visible in released adult males so that they could be distinguished from resident males. Twice each week, the same number of males (ca. 50) was released at each release point. All trap catch data were from released males only.

Each study plot had three male moth release points, one at the center of each plot and two at 150 m from the plot center in opposite directions. Female mating success was determined from 15 tethered females placed in a circle around the release point at the center of the plot. Females <24 h old were deployed on trees at a height of 1.5 m from the ground. A band of duct tape with a thin band of polybutene (Tanglefoot Bird Repellent®; The Tanglefoot Company, Grand Rapids, MI, USA) was placed at a height of 2 m and females were deployed below the band. After

24 h, the females and any egg masses they produced were collected into paper bags, held for 30 days, and then all eggs were checked for embryonation, which indicates that they were fertilized. The other two release points in each plot were each surrounded by four pheromone-baited traps placed 25 m to the North, South, East, and West from the release point. At the time of each release, all males were removed from the traps and later checked for the presence of dye, which indicated that they originated from the previous release.

Male trap catch for each release and female mating success for each group of 15 deployed females was calculated. Because gypsy moth MD 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 ≥5% fertile eggs. This definition of mating success is considered to be more biologically relevant, as females producing <5% fertile eggs contribute little to the next generation. Proportion data were transformed using the square root–arcsine transformation (arcsine $\sqrt{N}$). Data were analyzed by analysis of variance (ANOVA) (SAS Institute, 2000) as a randomized block design, with state forest serving as the blocking factor. When treatment effects were significant ($\alpha = 0.05$), treatment means were separated using the least significant difference (LSD) option at a comparison-wise error rate of 0.05. Values shown in figures are untransformed means and standard errors.

To supplement our experimental studies, historical data from blocks treated under STS were evaluated to determine if there was evidence of suppression of moth capture resulting from the environmental persistence of disparlure. Blocks that were treated with aerial applications of disparlure for MD or the biopesticide Btk from 1997 to 2002 were evaluated. Blocks treated with Btk were used for comparative purposes as Btk applications do not interfere with pheromone traps. Data from 182 Btk and 236 MD blocks were analyzed, and these blocks were located within the area managed in STS, which extends from Wisconsin to North Carolina (Tobin et al., 2004). As part of STS, the efficacy of treatments is evaluated using an index that measures the change in male moth population density in the treated area before and after treatment while adjusting for the corresponding changes in density in nearby, untreated areas that serve as a control. Gypsy moth population density was estimated through the use of pheromone-baited traps, which attract adult male moths, that are deployed 0.5–1.0 km apart in the treatment block as well as in the nearby control area.

Under standard operating procedures in STS, blocks that are treated with Btk, which targets larval populations, use trap catch data in the year of treatment as a post-treatment measurement of density. Because MD treatments target adults, data from traps in the following year are used as a post-treatment measurement of density. These data are used to measure treatment efficacy according to

$$T = 1 - \left(\frac{N_{\text{post-treatment}}}{N_{\text{pre-treatment}}} \times \frac{N_{\text{pre-treatment}}}{C_{\text{post-treatment}}} \right), \qquad (1)$$

where N is the average moth count in the treatment block (pre- or post-treatment), and C is the average moth count in the untreated control block (pre- or post-treatment) (Sharov et al., 2002b). Under this index, values → 1 indicate the highest level of treatment efficacy (i.e., T → 1 when $N_{\text{post-treatment}} \to 0$), and blocks with T>0.67 indicate a successful treatment application (Sharov et al., 2002b). For Btk treatment blocks, we calculated T using standard operating procedures (T0), and for those blocks in which T0>0.67, T was also calculated using trap catch data collected 1 year (T1) and 2 years (T2) post-treatment. For MD blocks, we calculated T using standard operating procedures (T1), and for those blocks in which T1>0.67, T was calculated also when using trap catch data collected 2 years (T2) post-treatment. Because of the potential for moth suppression following MD treatments, areas that were within 1.5 km from an MD treatment block from 1 and 2 years prior were excluded when calculating T1 and T2.

Blocks were scored with a binary response variable so that blocks with a reduction in treatment efficacy from T1 to T2 were assigned a 0, or 1 otherwise. This concept of a reduced treatment efficacy was used as one interpretation is that the suppression of moth catch due to environmental persistence of disparlure could result in an underestimate of the moth density subsequent to treatment application (i.e., an artificially low measurement of $N_{\text{post-treatment}}$ and hence a higher value of T1), but that this suppression effect would be reduced 2 years following treatment (i.e., a higher measurement of $N_{\text{post-treatment}}$ and hence a lower value for T2). However, as this is only one interpretation, the analyses were further restricted to comparisons between MD and Btk blocks. Stepwise logistic regression (SAS Institute, 2000) was used to test the interaction between treatment block (MD or Btk) and each of the following effects: (i) the pre-treatment density in the treatment block [$N_{\text{pre-treatment}}$ in Equation (1)]; (ii) treatment dose [6, 15, or 30 g acre^{-1} for MD; and 24, 36, 48 (two applications of 24), or 60 (two applications of 30) billion international units (BIU) acre^{-1} for Btk]; (iii) the minimum distance (km) between the treatment block and the gypsy moth generally infested area; (iv) the area (km^2) of the treatment block; and (v) the location of the treatment block, using the latitude coordinate of the treatment block center. Hosmer & Lemeshow (2001) model selection
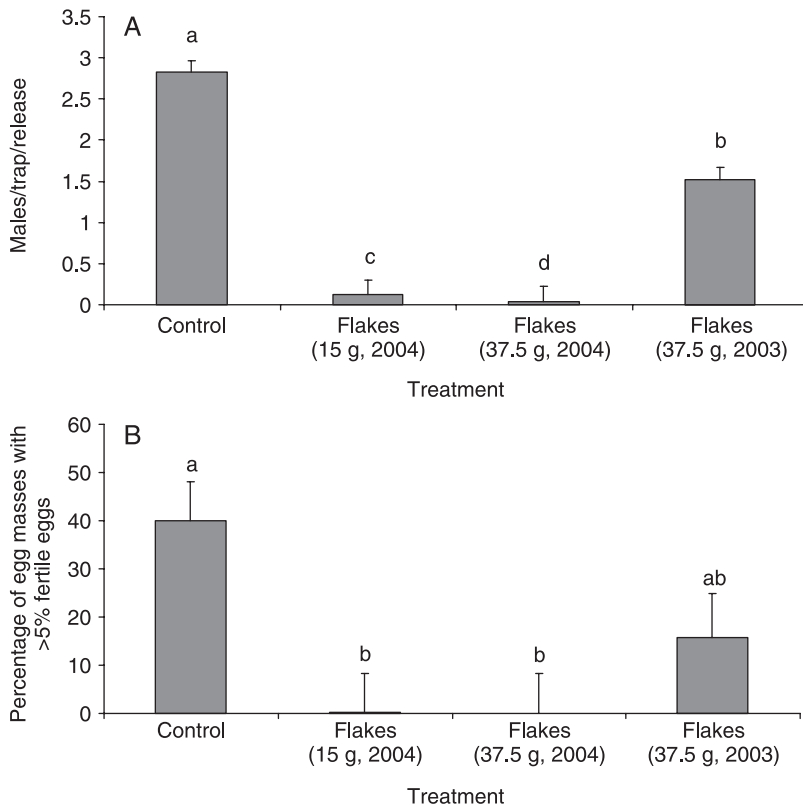
Figure 1 Suppression of (A) gypsy moth trap catch and (B) mating success following treatment with Disrupt® II flakes in 2003 at a rate of 37.5 g a.i. ha^{-1} and in 2004 at rates of 15 and 37.5 g a.i. ha^{-1}. All plots were monitored in 2004.

methods were used to determine the appropriate logistic regression model, and significance of effects were based on the Wald χ^2 (SAS Institute, 2000).

Results

Male trap catch was significantly reduced compared to the controls in plots treated and monitored in 2004 ($F_{3,3} = 71.2$, P = 0.003) (Figure 1A). Trap catch was reduced by 95.4 and 98.6% compared to controls in plots treated at 15 and 37.5 g a.i. ha^{-1}, respectively. Trap catch in the plots treated in 2003 at 37.5 g a.i. ha^{-1} and monitored in 2004 were reduced by 46.3% compared to controls. Trap catch in these plots was significantly higher than that in the plots treated in 2004, but was reduced significantly compared to the controls. Male trap catch in plots treated and monitored in 2005 was reduced significantly compared to controls ($F_{5,5} = 6.4$, P = 0.03) (Figure 1B). Trap catch was reduced by 97.1 and 99.8% compared to controls in plots treated at 15 and 37.5 g a.i. ha^{-1}, respectively. Trap catch was significantly lower compared to controls in all plots treated in 2004 and monitored in 2005. Reductions compared to controls were 56.0 and 39.6% in plots treated at 37.5 g a.i. ha^{-1} in 2004 and 2003, respectively, and 50.1% in plots treated at 15 g a.i. ha^{-1} in 2004.

Female mating success was significantly reduced compared to the controls in plots treated and monitored in 2004 ($F_{3,3} = 8.8$, P = 0.05) (Figure 2A). Mating success was reduced compared to controls by 99.2 and 99.7% in plots treated at 15 and 37.5 g a.i. ha^{-1}, respectively. The reduction in mating success in the plots treated at 37.5 g a.i. ha^{-1} in 2003 and monitored in 2004 (60.7%) was not significantly different from the controls or from the plots treated in 2004. Mating success in plots treated and monitored in 2005 was reduced significantly compared to controls ($F_{5,5} = 14.8$, P = 0.005) (Figure 2B). Mating success was reduced by 98.7 and 99.4% compared to controls in plots treated at 15 and 37.5 g a.i. ha^{-1}, respectively. Mating success was significantly reduced in all plots treated in 2004 and monitored in 2005 (79.4 and 91.4% reductions compared to controls in plots treated in 2004 at 15 and 37.5 g a.i. ha^{-1}, respectively). The reduction in mating success compared to controls in plots treated in 2003 at 37.5 g a.i. ha^{-1} and monitored in 2005 (33.6%) was not significant.

The results of the analysis of treatment efficacy data from the STS program indicated that there was a significant difference in the reduction of treatment efficacy 2 years following treatment due to an interaction between (i) the treatment block (MD vs. Btk) and the pre-treatment
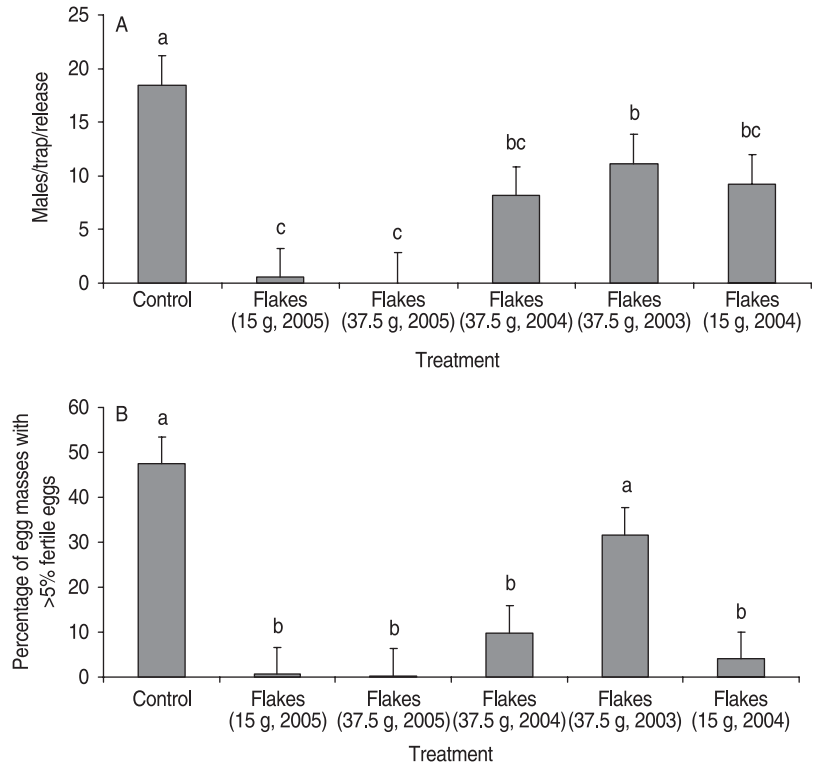
Figure 2 Suppression of (A) gypsy moth trap catch and (B) mating success following treatment with Disrupt® II flakes in 2003 at a rate of 37.5 g a.i ha^{-1} and in 2004 and 2005 at rates of 15 and 37.5 g a.i. ha^{-1}. All plots were monitored in 2005.

moth density in the block ($\chi^2 = 4.6$, P = 0.03), and (ii) the treatment block (MD vs. Btk) and the treatment dose ($\chi^2 = 5.4$, P = 0.02). None of the other interaction effects were significant. For the former interaction effect in Btk blocks, there was an overall reduction in treatment efficacy 1 and 2 years following treatment (P<0.01), but the reduction did not differ between 1 and 2 years (P = 0.75), nor did the effect differ over increasing levels of pre-treatment moth density in the treatment block (P = 0.56, Figure 3A). In contrast, for MD blocks, there was a significant effect in the pre-treatment moth density such that higher population densities resulted in a greater reduction in treatment efficacy 2 years following treatment (P<0.01, Figure 3B).

The dose of the treatment block also affected the probability of a reduction in treatment efficacy 2 years following treatment (Figure 4). This relationship was more intuitive in Btk blocks, in which a higher Btk dose significantly resulted in a decreased rate of a reduction in treatment efficacy. In other words, the higher the Btk dose, the more likely the treated population is eradicated. However, the opposite relationship was observed in MD blocks, in which there was a significant tendency for a reduction in treatment efficacy in blocks treated at a higher dose (Figure 4).

Discussion

The capture of gypsy moth males in pheromone traps was reduced 46 and 50% and female mating success was reduced 61 and 79% (2003 and 2004 applications, respectively) in the year following the aerial application of Disrupt II® flakes at 37.5 g a.i. ha^{-1}. Suppression of trap catch was also evident in plots treated the previous year at 15 g a.i. ha^{-1} and in plots treated 2 years previously at 37.5 g a.i. ha^{-1}, and female mating success was reduced in plots treated the previous year at 15 g a.i. ha^{-1}. The source of the residual pheromone in previously treated plots is unknown at present.

One possible explanation for the observed effects is that, in years subsequent to the application of the flakes, enough residual disparlure remains to release amounts sufficient to affect moth behavior. Leonhardt et al. (1996) recovered Disrupt II® flakes from the forest floor the year following treatment and found that they contained 1.8% of their initial load of disparlure. At an application rate of 37.5 g a.i. ha^{-1}, this would be 0.7 g a.i. ha^{-1} the year following flake application. In a dose–response experiment, Tcheslavskaia et al. (2005) found that trap catch was reduced by 67% at an application rate of 0.15 g a.i. ha^{-1}. This suggests the possibility that enough disparlure remains in year-old flakes
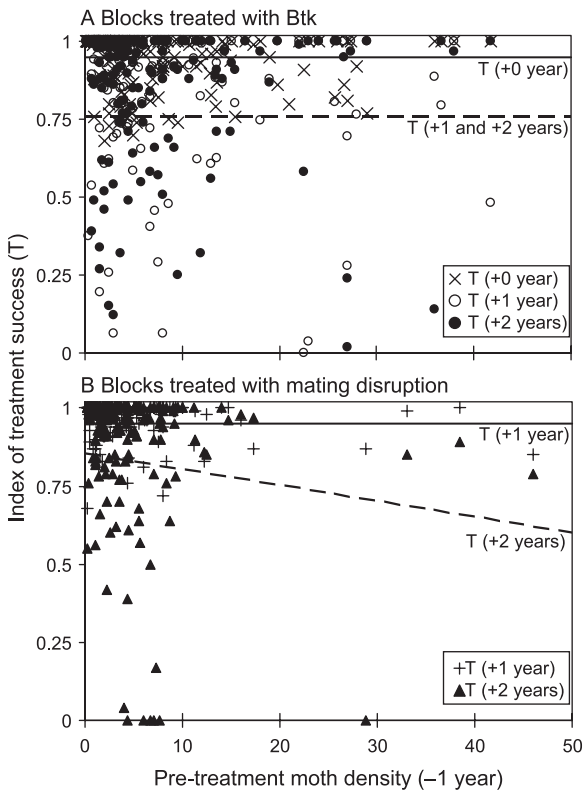
Figure 3 The index of treatment success [Equation (1)] in blocks treated with various dosages of (A) *Bacillus thuringiensis kurstaki* (Btk) and (B) mating disruption (MD) calculated using gypsy moth trap data collected in the year of treatment (e.g., +0 year), or 1 or 2 years following treatment (+1 or +2 years) relative to the pre-treatment moth density in the treatment block.

to cause the observed reductions in trap capture and mating success. However, the rate of release of disparlure from 1-year-old flakes compared to newly applied flakes is unknown.

Another possible explanation is that the environment within the treated plots becomes contaminated with disparlure and in subsequent years releases enough pheromone to reduce trap catch. Short-term contamination of environmental surfaces has been shown with other insect and pheromone systems (Karg et al., 1994; Suckling et al., 1996), and long-term absorption and re-emission of disparlure from human tissues has been documented (Cameron, 1983, 1995). Additional work will be needed to determine which, if either, of these possibilities is plausible.

An index of the success of MD treatments in STS (T) was significantly higher the year after compared to 2 years after treatment. There was no reduction in T from Year 1 to 2 in blocks treated with Btk. This pattern would be predicted if the suppression of trap catch in blocks treated with MD

inflated T-values in the year after treatment, but the effect was reduced or eliminated the following year. As Btk treatments do not interfere with trap catch, a similar effect would not be expected to occur in blocks treated with Btk. The lower value of T for MD treatments at higher pre-treatment moth densities is not surprising, because it is known that the effectiveness of MD is inversely related to moth density (Webb et al., 1988).

As expected, the probability of a reduction in T calculated 1 and 2 years after treatment decreased with increasing dose of Btk. However, in blocks receiving an MD treatment, the decrease in T from Year 1 to 2 was greatest at the highest dose. One possible explanation is that the suppressive effect of the treatment on trap catch the year after treatment increased with dosage, resulting in a greater reduction in T from the year after treatment to 2 years after treatment.

In the STS program, male moth capture in areas that previously received MD treatments is used to evaluate treatment effectiveness, delineate residual gypsy moth populations, and determine if additional treatments are needed (Tobin et al., 2004). In our study plots, female mating success in plots treated the previous year was
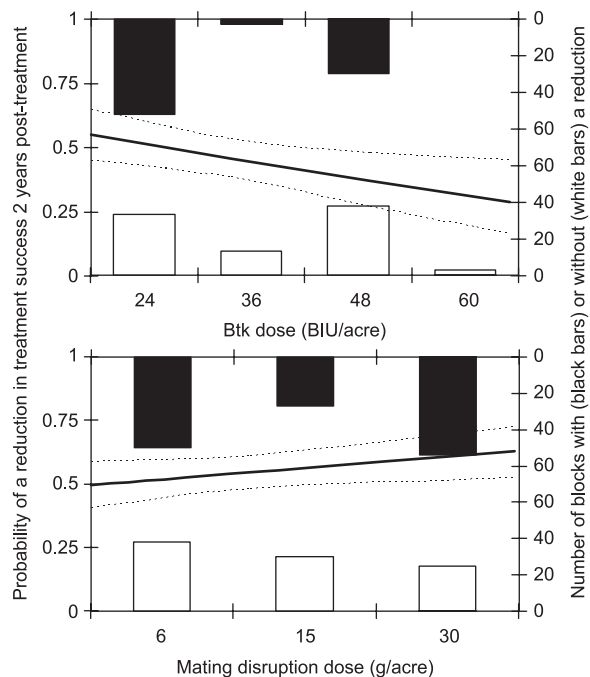

Figure 4 Effect of treatment dose on the probability (thick line, with 95% confidence intervals shown as dashed lines) of a reduction in treatment success 2 years following treatment in blocks treated with *Bacillus thuringiensis kurstaki* (Btk) (top) and mating disruption (MD) (bottom). The 48 and 60 BIU acre^{-1} in the top panel denote two separate applications of Btk at a 24 or 30 BUI acre^{-1} dose, respectively.

reduced 61% in 2004 and 79% in 2005. While this level of mating suppression is well below what would be considered as a successful treatment in STS, the persistence of treatment effects into subsequent year(s) could thus represent an additional benefit of the treatment. However, the continued suppression of trap capture in years following MD treatments could also be a concern in STS, because it may impair the evaluation of treatment effectiveness. Furthermore, this effect may lead to an underestimation of moth density in plots treated in previous years, which could result in a decision not to treat a population that should be treated.

Acknowledgements

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