Effects of the 3M™ MEC Sprayable Pheromone® formulation on gypsy moth mating success

K. S. Onufrieva (Tcheslavskaia)[1], C. C. Brewster[1], K. W. Thorpe[2], A. A. Sharov[3], D. S. Leonard[4], R. C. Reardon[5], V. C. Mastro[6], P. Sellers[7] & E. A. Roberts[1]

1 Department of Entomology, Virginia Tech, Blacksburg, VA, USA
2 USDA, Agricultural Research Service, Beltsville, MD, USA
3 NIA/NIH, Pasadena, MD, USA
4 USDA, Forest Service, Asheville, NC, USA
5 USDA, Forest Service, Morgantown, WV, USA
6 USDA, APHIS, Otis ANGB, MA, USA
7 USDA, Forest Service, Harrisonburg, VA, USA

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Correspondence
Ksenia Onufrieva (corresponding author), Department of Entomology, 202 Price Hall, Virginia Tech, Blacksburg, VA 24061-0319, USA. E-mail: ktchesla@vt.edu

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Abstract

The study was conducted during 2000, 2001, 2003 and 2004 in forested areas in Virginia, USA to evaluate the 3M™ MEC-GM Sprayable Pheromone® formulation of the gypsy moth sex pheromone, disparlure, for its ability to disrupt mating in gypsy moth, *Lymantria dispar* (Lep.: Lymantriidae). Both mating success of gypsy moth females and male moth catches in pheromone-baited traps were significantly reduced in plots treated with the 3M™ MEC-GM formulation at dosages ranging from 15 to 75 g of active ingredient/ha. However, the 3M™ MEC-GM formulation reduced trap catch to a lesser extent than did the currently registered Hercon Disrupt® II plastic flakes used as a positive control and applied at similar or lower dosages. Furthermore, the effectiveness of the 3M™ sprayable formulation declined through time, so that by the end of the male flight season, male moth catches in traps were significantly higher than in plots treated with Hercon plastic flakes. Based on the reported results, 3M™ MEC-GM Sprayable Pheromone® formulation was never integrated into the operational treatment projects of USDA Forest Service Cooperative Slow-the-Spread of the Gypsy Moth management programme.

Introduction

Mating disruption is currently the most widely used method of gypsy moth, *Lymantria dispar* (L.) (Lep.: Lymantriidae), control. It is the primary treatment tactic used in the USDA Forest Service Cooperative Slow-the-Spread of the Gypsy Moth management programme (STS) initiated in 1993. The goal of the STS programme is to reduce the rate of expansion of gypsy moth populations in the USA by detection and suppression of low-density isolated colonies that are located just beyond the expanding population front of the infested area (Campbell 1981; McFadden and McManus 1991; Leonard and Sharov 1995).

Several hours after emergence, flightless gypsy moth females attract males with a sex pheromone disparlure (Leonard 1981) that is released by rhythmic protrusion and retraction of the last abdominal segments (Doane 1968). The pheromone was identified as the (+)-enantiomer of *cis*-7,8-epoxy-2-methyloctadecane (Bierl et al. 1970; Iwaki et al. 1974). In mating disruption, sources of artificial pheromone are introduced into the environment at a level that prevents males from locating calling females. For

successful disruption, the artificial pheromone must be present in the air in sufficient concentration throughout the mating period (Thorpe et al. 2006). This can be achieved by using controlled release dispensers that regulate the transfer of an active agent from a reservoir, such as a polymeric matrix, to a target surface to maintain a pre-determined atmospheric concentration for a specific period of time (Zedi et al. 1982).

Currently, the only product available for operational mating disruption treatments is Disrupt® II (Hercon Environmental, Emigsville, PA). Disrupt® II is a controlled-release formulation of polymeric 3-layer laminated flakes 1 mm × 3 mm. The flakes contain racemic disparlure at a concentration of 17.9% (Health-Chem Corporation, New York, NY). A sticker should be applied with Hercon Disrupt® II plastic flakes to achieve the maximum effect of the formulation (Thorpe et al. 2000). The application of Disrupt® II requires special equipment (Plimmer et al. 1982; Thorpe et al. 2006).

The 3M Corporation of Canada developed a liquid microencapsulated formulation of disparlure for mating disruption. The 3M^{TM} MEC-GM Sprayable Pheromone® contains 20.0% racemic disparlure (3M^{TM} MEC-GM Sprayable Pheromone® 2004). This product received EPA registration in 2003 (Thorpe et al. 2006) but was withdrawn from the market in 2004. This paper presents the results of the study conducted to evaluate the 3M^{TM} sprayable formulation for its ability to disrupt mating in gypsy moth population. Experiments were conducted in 2000, 2001, 2003 and 2004 using various dosages of pheromone to evaluate the effects on mating disruption as measured by mating success of females and male moth catches in pheromone-baited traps.

Materials and Methods

Study sites

In 2000, the two formulations of disparlure, Hercon Disrupt® II and 3M^{TM} Sprayable Pheromone® were evaluated in field plots in the Goshen Wildlife Management Area (GWMA) (Bath Co), VA (UTM 637052E, 4223294N to 614250E, 4192715N, NAD 27, zone 17). In 2001, 2003 and 2004 field evaluations of the same formulations were conducted at sites selected in the Appomattox-Buckingham (ABSF) (Appomattox and Buckingham Counties) and Cumberland (CSF) (Cumberland County) State Forests, VA (UTM 746246E, 4166292N to 700180E, 4136389N, NAD 27, zone 17).

Plot layout and pheromone treatments

Experiment 2000

Sixteen plots, each 500 by 500 m in size and separated by at least 1 km, were selected in GWMA for this experiment. The plots were grouped into four blocks with four plots per block. In each block, one plot was used as a control and left untreated, and the remaining three plots were treated with various dosages and formulations of pheromone, which was applied by airplane as follows: 3M^{TM} MEC-GM Sprayable Pheromone® (3M Canada Co., London, ON, Canada) at 75 g active ingredient (a.i.)/ha, Disrupt® II (Hercon Environmental) at 75 g a.i./ha, and Disrupt® II at 37.5 g a.i./ha. Thus, each treatment was replicated four times. Previous studies showed that the 75 g a.i./ha dosage of disparlure disrupts gypsy moth mating (Webb et al. 1990). Therefore, this dose was used to compare the two formulations.

The treatment effects were evaluated in a central 175 × 175 m core area of each plot using laboratory-reared virgin females. Nine tethered females, nine females in mating stations and four pheromone-baited traps were deployed in each plot (Tcheslavskaia et al. 2005). Mating stations consisted of cardboard delta traps containing a female but without glue or synthetic pheromone. Females were tethered around the base of a front wing using a 10–15 cm thread and the thread was attached to a tree by a pushpin (Sharov et al. 1995). A polybutene pest barrier (The Tanglefoot Company, Grand Rapids, MI) was applied in a radius of ca. 25 cm around each tethered female for protection from predators. Females were left on the trees for 24 h, after which they were removed for analysis and replaced with new females. This process was repeated for 5 days each week of the study for 4 weeks.

Experiment 2001

At both ABSF and CSF one block was selected and divided into three 500 by 500 m experimental plots separated by 1 km. One plot in each block was left untreated and used as a control and the two remaining plots were treated either with 3M^{TM} MEC-GM at 75 g a.i./ha or with Disrupt® II at 15 g a.i./ha.

Because the density of the resident population of gypsy moths was very low, mating disruption was evaluated by deploying laboratory-reared tethered females following the release of laboratory-reared males. Each study plot had two male moth release points. Fifteen tethered females were placed in a circle around a release point at the centre of the plot.

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Four pheromone-baited traps were placed around a second male release point 150 m to the north of the centre of the plot; the traps were positioned 25 m from the release point. One trap also was placed 150 m to the south, east and west of the central release point. Adult females were placed on tree boles for 1 day and protected from ant predation by a band of the Tanglefoot pest barrier. The plots were monitored for 9 weeks.

Experiment 2003

At both ABSF and CSF one block was selected and divided into three 500 by 500 m experimental plots separated by 1 km. Two plots in each block were treated either with 3M^{TM} MEC-GM or Disrupt® II at 37.5 g a.i./ha. The third plot in each block was used as a control and left untreated.

Each study plot had three male moth release points and eight pheromone-baited traps. The release points were established at the centre of each plot and 150 m to the north and south of the plot centre. Fifteen tethered females were placed in a 50-m radius circle around the release point at the centre of the plot. Tethered females, protected from ant predation by a band of the Tanglefoot pest barrier, were placed on tree boles for 1 day. 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 (fig. 1). The plots were monitored for 6 weeks.

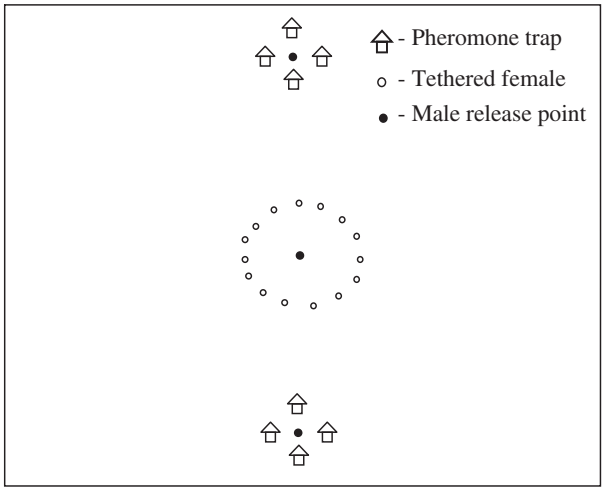

Fig. 1 Layout of pheromone-baited traps, male moth release points and tethered females in the 300 × 300 core sampling area of an experimental forest plot in Appomattox-Buckingham and Cumberland State Forests, VA in 2003.

Experiment 2004

Each of the two blocks selected in ABSF and CSF was divided into five 500 by 500 m experimental plots separated by 1 km. In each block, the plots were treated with 3M^{TM} MEC-GM at 15 g a.i./ha, 3M^{TM} MEC-GM at 37.5 g a.i./ha, Disrupt® II at 15 and Disrupt® II at 37.5 g a.i./ha. The untreated plot in each block was used as a control. The plot layout was similar to the one used in 2003 (fig. 1). Male moth catches in pheromone-baited traps alone were used to evaluate the treatment effects. The plots were monitored for 8 weeks.

Pheromone applications

Disrupt® II gypsy moth mating disruption formulation consisted of plastic flakes composed of polyvinyl chloride 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 aerially applied using a fixed-wing aircraft (Air Tractor) equipped with specialized application pods (Schweitzer Aircraft Corp., Elmira, NY). Within the pods, the flakes were mixed with a multipolymer emulsion glue (Gelva 2333; Solutia Inc., Springfield, MA) and dispensed through a spinner (Thorpe et al. 2006). At the highest dosage of 75 g a.i./ha the pods were calibrated to deliver 419 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).

The 3M^{TM} MEC-GM Sprayable Pheromone® (3M Canada) formulation consisted of small polymer capsules (5–100 μ in diameter). Disparlure is encapsulated in these microcapsules that are suspended in a thick liquid that preserves the formulation. Disparlure starts releasing through the capsule walls soon after the product is applied (Leonard 2004). The 3M^{TM} MEC-GM was applied using two CP® nozzles directed straight back (no deflection) at 40 psi. Lastick (2 oz/100 gallons) was added to the tank mix as a sticking agent. Disparlure release rate from applied microcapsules was not determined in this study. A Global Positioning Satellite (GPS) navigation system was used to guide the spray applications.

Treatment evaluation

Gypsy moth females were left on trees for 24 h, after which they were removed and their fertilization status was determined via analyses of the spermatheca (2000 only) 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) (Schwalbe 1981; Leonhardt et al. 1992).

Male and female gypsy moths were obtained as pupae from USDA APHIS Otis Methods Development Center, Massachusetts. Pupae were kept in laminated paper cups with plastic lids. In 2001–2004, 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 2001, male pupae were transferred to release cups, which were stapled to the trunks of trees in the field. The release cups were the same type of cups used for rearing males but with several openings cut at mid-height to allow emerging males to escape. Tanglefoot glue was applied in circles around the tree trunk. Fluorescent powder dye was added to the cups to mark emerging male moths. In 2003 and 2004, adult males were released in the plots to avoid wasp predation and a fluorescent dye was added to the caterpillars' diet at the rearing facility. Each week, the same number of males (~150) was released at each release point. Male moths captured in pheromone traps were removed and stored in the freezer. The moths were later examined under the microscope with a UV light for the presence of fluorescent powder on wings, antennae or body to distinguish between released and native moths. Only laboratory-reared recaptured moths were used for the data analysis.

Data analysis

For each treatment in the 2000 study, we determined the proportion of recovered females that were fertilized. In 2001 and 2003, mating success of females was analysed using the General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS Institute 2003, Proc GLM). The arcsine-transformed proportion of fertilized females (arcsin $\sqrt{}$N) was modelled as a function of week, dosage and block with interactions of factors. The interaction of dosage and block was used as an error term.

The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS Institute 2003, Proc GLM) was used to test for significance of differences in moth counts between groups of traps located in plots treated with various doses and formulations of pheromone for each of the four studies. The log-transformed total moth counts per trap per week for each type of pheromone treatment, ln (N + 1), was modelled as a function of week, dosage and block with interactions of factors. The interaction of dosage and block was used as an error term.

Results

In 2000, mating success of laboratory-reared females in plots treated with pheromone was reduced significantly compared with that of females in untreated control plots. In the control plots 19.9% of females were fertilized while in all treated plots 100% of females remained unmated (fig. 2).

Male trap catches were significantly suppressed by all applied pheromone formulations and dosages compared with control plots ($F = 83.2$, $P < 0.0001$, d.f. = 3, 9; fig. 3). Trap catches in plots treated with 3M^{TM} microcapsules at 75 g a.i./ha were lower than in plots treated with the same dose of Hercon plastic flakes, but the difference was not statistically significant.

In 2001, the female mating success was significantly reduced in the experimental pots compared with the control plots ($F = 34.5$, $P = 0.028$, d.f. = 2, 2; fig. 2).

Male moth catches in the pheromone-baited traps were also significantly reduced with both treatments ($F = 19.9$, $P = 0.05$, d.f. = 2, 2; fig. 3). Trap catches were significantly lower in traps treated with Hercon plastic flakes compared with plots treated with the 3M^{TM} microcapsules. The results of the ANOVA GLM also indicated a significant effect of time ($F = 14.7$, $P < 0.0001$, d.f. = 6, 15) and of time and treatment interaction ($F = 6.7$, $P = 0.0005$, d.f. = 12, 15) on the male moth catches in pheromone-baited traps. The analysis of male moth catches in pheromone-baited traps over time showed that during the first 62 days, there was no significant difference between the male moth catches in pheromone-baited traps in plots treated with plastic flakes and 3M^{TM} microcapsules (fig. 4). However, 64 days after the applications the trap catches in plots treated with the 3M^{TM} formulation increased significantly compared with plots treated with plastic flakes.

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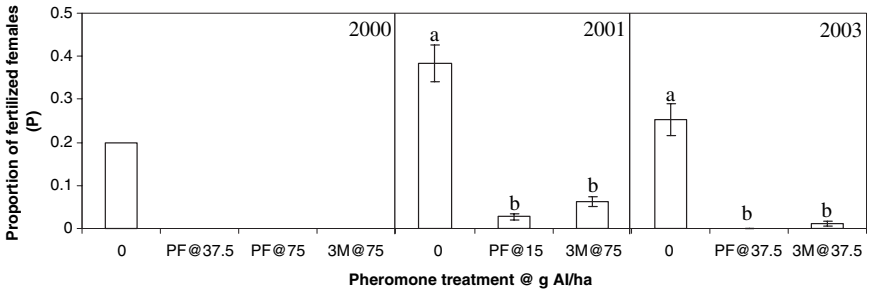

Fig. 2 Proportion of fertilized females (P ± SEM) in plots treated with various dosages of pheromone in Goshen Wildlife Management Area, VA in 2000 and in Appomattox-Buckingham and Cumberland State Forests, VA in 2001 and 2003. Bars with the same letter are not significantly different, Tukey's HSD (α = 0.05).

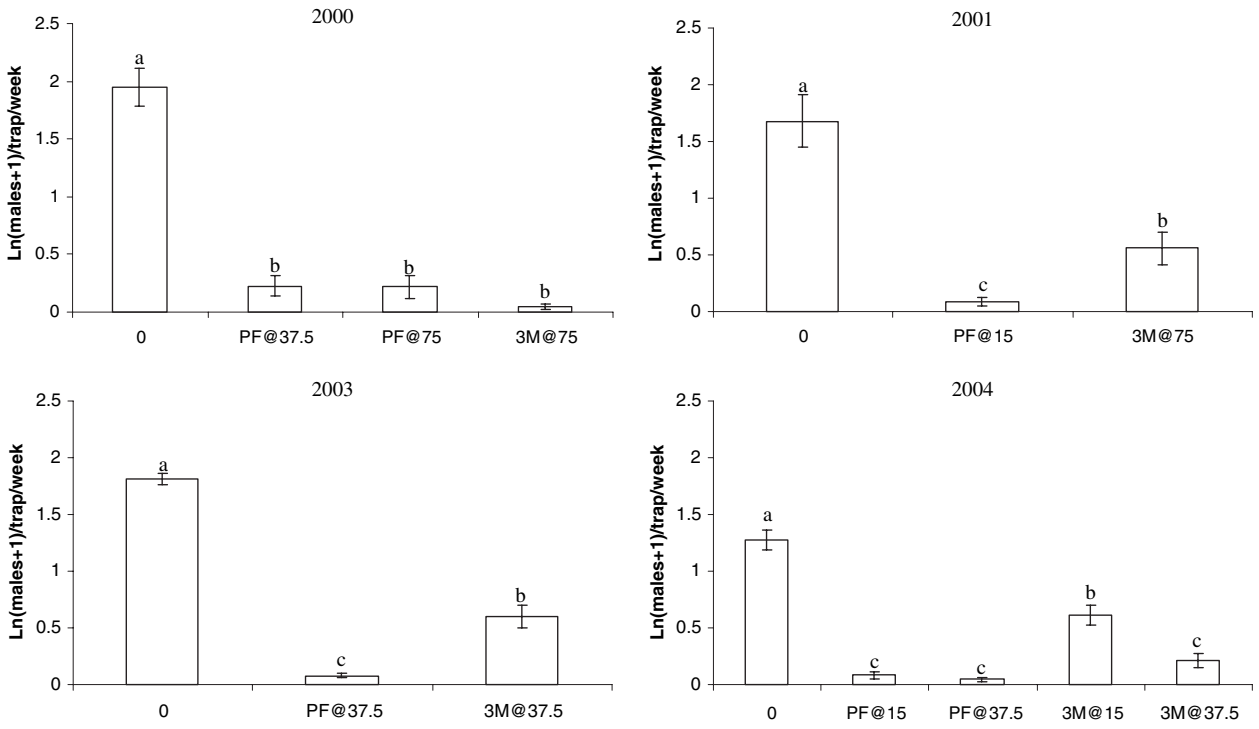

Fig. 3 Male gypsy moths [ln (*M* + 1)/trap/week ± SEM] captured in plots treated with various formulations and dosages of pheromone. Bars with the same letters are not significantly different, Tukey's HSD (α < 0.05).

Similarly, in 2003, mating success of females was significantly reduced in the experimental pots compared with the control plots (F = 22.04, P < 0.04, d.f. = 2, 2 fig. 2).

Male moth catches in the pheromone-baited traps were also significantly reduced with both treatments (F = 145.6, P = 0.007, d.f. = 2, 2, fig. 3). Again, the trap catches were significantly lower in plots treated with Hercon plastic flakes compared with the plots treated with the 3M^{TM} microcapsules. The results of ANOVA GLM also indicate significant effect of time (F = 6.67, P < 0.0023, d.f. = 5, 14) and of time and treatment interaction (F = 2.98, P < 0.03, d.f. = 10, 26) on the male moth catches in the pheromone-baited traps. The analysis of male moth catches in pheromone-baited traps over time showed that during the first 3 weeks, there was no significant difference between the male moth catches in pheromone-baited traps in plots treated with Hercon plastic flakes and 3M^{TM} microcapsules (fig. 4). However, a gradual decrease in pheromone effect was observed later in the

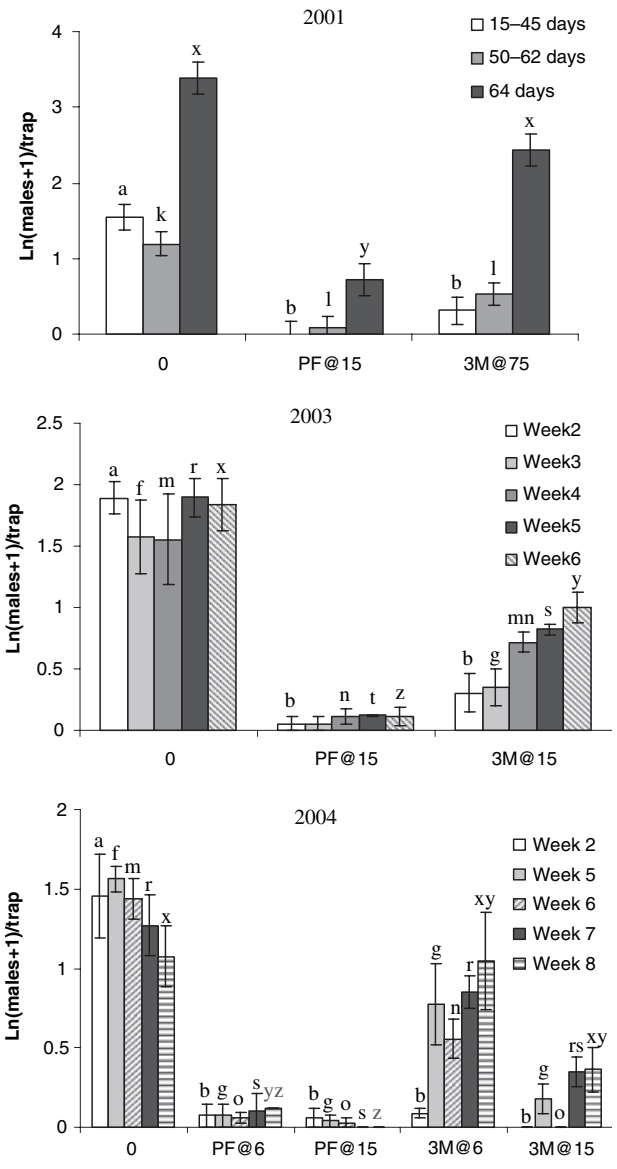

Fig. 4 Male gypsy moths [ln (M + 1) ± SEM] recaptured weekly in plots treated with various dosages of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA in 2001, 2003 and 2004. Bars within a series with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$). 2001: letters (a, b), (k, l) and (x, y) indicate significant differences between trap catches at 15–45, 50–62 and 64 days respectively. 2003: Letters (a, b), (f, g), (m, n), (r–t) and (x–z) indicate significant differences between trap catches at 2, 3, 4, 5 and 6 weeks respectively. 2004: Letters (a, b), (f, g), (m–o), (r, s) and (x–z) indicate significant differences between trap catches at 2, 5, 6, 7 and 8 weeks respectively.

season in the plots treated with the 3M^{TM} microcapsules.

In 2004, male moth catches in the pheromone-baited traps were significantly lower in treated plots compared with the control plots (F = 149.6, P = 0.0001, d.f. = 4, 4). However, trap catches in plots treated with 3M^{TM} microcapsules at 15 g a.i./ha were significantly higher than in the other treated plots, including the plots treated with Hercon plastic flakes at the same dosage (fig. 3).

The results of ANOVA GLM also indicate a significant effect of time (F = 2.9, P = 0.01, d.f. = 9, 32). The analysis of trap catches over time showed that in the beginning of the season the male moth catches in pheromone-baited traps were reduced significantly by all treatments. In the plots treated with Hercon plastic flakes, the trap catches were still significantly reduced 8 weeks after the treatments. In the plots treated with the 3M^{TM} microcapsules, the trap catches significantly increased during the seventh week following the applications (fig. 4).

Discussion

The goal of this study was to evaluate the new 3M^{TM} MEC-GM Sprayable Pheromone® formulation for its ability to disrupt mating in gypsy moth populations, and to compare its effect with that of the Hercon plastic flakes formulation currently used for operational mating disruption treatments against gypsy moth. In 2000, the 3M^{TM} microcapsule formulation was as effective as the Hercon plastic flakes applied at the same dosage and that it reduced mating success of females and male moth catches in pheromone-baited traps by >99%. In 2001, in plots treated with the 3M^{TM} microcapsules at 75 g a.i./ha the male moth catches in pheromone-baited traps were only reduced by 73% compared with a 98% reduction in plots treated with plastic flakes at 15 g a.i./ha. Mating success of females was reduced by >99% and 92% in plots treated with Hercon plastic flakes and the 3M^{TM} microcapsules respectively. These results show that Hercon plastic flakes applied at one-fifth the dosage of the 3M^{TM} microcapsules was more effective at disrupting mating in gypsy moth populations. In 2003, the trap catches in plots treated with microcapsules were reduced by 86%, whereas in plots treated with plastic flakes at the same dosage the trap catches were reduced by 99%. During the same period, mating success of females was reduced by 100% and 94.5% in plots treated with Hercon plastic flakes and the 3M^{TM} microcapsules respectively. In 2004, applications of 3M^{TM} formulations at 15 and 37.5 g a.i./ha reduced male moth catches in pheromone-baited traps by 80% and 95% respectively. The same dosages of

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pheromone formulated as plastic flakes reduced the trap catches by ≥99%.

For successful mating disruption the synthetic 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; Thorpe et al. 2006). The analysis of the time effect of the pheromone applied at different dosages in 2000 showed that Disrupt® II was effective throughout the entire flight period of gypsy moths at dosages tested in this study. The 3M™ microcapsules formulation was fully effective for about 3 weeks. In 2001, trap catches in plots treated with 3M™ formulation were significantly reduced for 9 weeks after the applications. In 2003, trap catches in plots treated with 3M™ microcapsules doubled during week four compared with the previous weeks and gradually increased from then on. In 2004, trap catches in plots treated with the 3M™ microcapsules at 15 g a.i./ha increased significantly 7 weeks after the applications. In the plots treated with the 3M™ microcapsules at 37.5 g a.i./ha, trap catches were significantly lower than in plots treated with the lower dosage of the same formulation. However, a gradual increase of male moth catches in pheromone-baited traps was also observed in these plots.

In the STS programme, the applied pheromone is required to be effective for a period of at least 8 weeks to cover the entire period of gypsy moth flight (up to 6 weeks) and to provide a safety margin for uncertainties associated with the logistics of treatment planning and with gypsy moth phenology. Thus, even though the 3M™ microcapsules significantly reduced season-long trap catches, its effects did not last long enough to satisfy the requirements for operational use in the STS programme. With a second aerial application in the middle of the season, the 3M™ microcapsules applied at 37.5 g a.i./ha could be used for operational treatments, but that would significantly increase the cost, making mating disruption treatments too expensive and logistically difficult. At the time these studies were initiated in 2000, the STS programme used a single application of Hercon Disrupt® II at the dosage of 75 g a.i./ha for most of its operational treatments. However, by 2004 dosages had been reduced to 15 g a.i./ha in a single application on the majority of the treated areas. Based on the results of the study reported here, the 3M™ MEC-GM Sprayable Pheromone® was unable to compete effectively with Hercon Disrupt ® II and was never integrated into the operational use in STS.

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