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Comparative efficacy of two controlled-release gypsy moth mating disruption formulations

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Abstract

The effects of aerial applications of the gypsy moth sex pheromone, disparlure, on mating disruption and suppression of growth of populations of the gypsy moth, *Lymantria dispar* (L.), were investigated. Two formulations of disparlure, plastic laminate flakes applied in a single application and polymethacrylate beads applied in two applications, were compared in two separate tests conducted in 1993 and 1994. The beads were applied in two applications spaced 2 weeks apart because preliminary tests had indicated that they released pheromone too rapidly to maintain adequate emission rates throughout the period of male flight. In 1993, the flakes were applied at a rate of 50 g a.i./ha, and the beads were applied at a rate of 15 g a.i./ha for each application. In 1994, the flakes were applied at a rate of 75 g a.i./ha and the beads were applied at rates of 32.5 and 42.5 g a.i./ha for the two applications. Beads with larger average particle size were used in 1994 to prolong disparlure release. The treatments applied in 1993 resulted in >97% reduction in mating and >82% suppression of population growth in the following year. Because of a 1995 collapse of gypsy moth populations in the vicinity of the tests, reliable population growth data were not available for the treatments applied in 1994, but significant mating disruption did occur under both treatments. Based on measurements of residual disparlure after field aging, the flakes released 32 and 48% of their disparlure content during the 6 weeks of male moth flight in 1993 and 1994, respectively. The smaller beads used in 1993 released 75% of their disparlure content, and the larger beads used in 1994 released 52% of their disparlure content, during the 6 weeks of male flight. The biological efficacy data suggest that the bead and flake formulations, as applied in these tests, have similar effects on gypsy moth mating disruption and subsequent population growth. Based on the observed release rates from both 1993 and 1994, a single application of the beads would provide emission rates equal to or greater than those provided by the flakes when applied at an equal dose.

Introduction

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is a serious pest of hardwood forests in northeastern United States. Defoliation, and the environmental degradation associated with it, has occurred to more than 22 million ha of United States forests since 1980 (Twardus, 1994). This pest is established in 15 states, and its range continues to expand to the south and west. It is estimated that at least 126 million ha of United States forest is dominated by trees that are susceptible to gypsy moth defoliation (USDA, 1995). To slow its spread, the United States Department of Agriculture (USDA) conducts a yearly program to eradicate isolated infestations of the gypsy moth in areas where it is not already established. Since 1988, eradication projects have been conducted on more than 122 000 ha in the United States (USDA, 1995). The gypsy moth sex attractant pheromone, (Z)-

7,8-epoxy-2-methyloctadecane, or disparlure (Bierl et al., 1970), is used as a mating disruptant in attempts to eradicate isolated gypsy moth populations. The optically active (+) enantiomer of disparlure is used extensively as an attractant in traps to detect and delimit gypsy moth populations, and in mass trapping efforts. Mating disruption activities employ racemic disparlure because of its lower cost and because it appears to disrupt mating as effectively as the (+) enantiomer (Kolodny-Hirsch & Schwalbe, 1990). Early attempts to disrupt gypsy moth populations with ground-applied pheromone dispensers failed to impact population growth, presumably because the pheromone did not sufficiently penetrate the canopy (Kolodny-Hirsch et al., 1990). Aerial pheromone application studies by Webb et al. (1988) established that mating success declined as the application rate was increased from 7.5 to 75 g of disparlure/ha. However, impact on subsequent population density or growth was not evaluated. Recently, Leonhardt et al. (1996) demonstrated that yearly aerial application of 75 g of racemic disparlure per ha formulated in plastic flakes suppressed gypsy moth populations compared to untreated controls, and that the suppression effect of a single application of 150 g/ha was measurable for 3 years following treatment.

Since 1989, most operational aerial applications of disparlure have used a plastic laminate flake formulation. This formulation is available commercially (Disrupt II, Hercon Environmental Corp., Emigsville, Pennsylvania). While this formulation has been used successfully to disrupt gypsy moth mating, it is difficult to apply from aircraft equipped with conventional spray equipment because of the large size and irregular shape of the flakes (Leonhardt et al., 1996). Specialized application equipment is required, and clogging is an occasional problem. Also, only 27–40% of the applied pheromone is released during the period of male moth flight, suggesting that a more efficient controlled-release formulation could allow the amount of disparlure applied per ha to be reduced without a reduction in efficacy (Leonhardt et al., 1996). Earlier tests involving a polymethacrylate bead formulation (Decoy GM Beads, biosys, Palo Alto, California) (Leonhardt et al., 1992), which can be applied with conventional spray equipment, suggested that the pheromone release profile may be more favorable than that of the flake's.

This paper reports the results of a 3-year (1993–1995) cooperative USDA effort to compare the release characteristics and efficacy of aerially-applied flake and bead formulations against low-density, leading-edge gypsy moth populations in Virginia woodlots. The objective in 1993 was to evaluate the effectiveness of low-dose applications of flakes and beads. Because of the extremely rapid release rates observed in preliminary tests with the bead formulation, it was decided that two applications of the beads would be needed to maintain disparlure emission rates comparable to those obtained with a single application of flakes (Reardon et al., 1998). Because the beads tend to release more of their disparlure during the flight season, the beads were applied at a lower total dose (30 g/ha) than were the flakes (50 g/ha) in 1993. To prolong the disparlure release from the beads, larger beads were applied in 1994, and both the beads and flakes were applied in the same total dose (75 g/ha) in 1994. In both 1993 and 1994, the total dose of beads was split between two applications. This work was conducted as a part of the USDA Forest Service Appalachian Gypsy Moth Integrated Pest Management Program (AIPM).

Materials and methods

Plot description. The test sites in 1993 were isolated woodlots (13–61 ha) located in Rockbridge Co., VA in an area which was considered at the time to be on the southern edge of the area generally infested with gypsy moth (Sharov et al., 1995). In 1994, test plots (26–52 ha) were delineated within continuously forested areas. Sampling was conducted within 8.1 ha core areas in the center of each plot. All plots had closed canopies of predominantly oak (*Quercus* spp.) with overstory trees reaching a height of 20–25 m. Individual plots were selected for inclusion in the study based on gypsy moth population density, as determined from male moth trappings and numbers of immature gypsy moth life stages found under burlap bands (Wallner et al., 1989), and the degree of isolation from other infested areas. Both tests utilized a randomized block design, with four blocks of three plots each. Plots were blocked according to pretreatment population density, as measured by egg mass counts and counts of the number of larvae beneath burlap bands, and proximity. Plots in both tests were monitored through 1995 to measure impacts on gypsy moth population trends.

Treatments. Separate tests were conducted in 1993 and 1994. Each test consisted of the following treatments: a single aerial application of flakes, two applications (2 weeks apart) of beads, and an untreated control. In 1993, the bead formulation was applied at a dose of 15 g a.i./ha/application and the flake formulation was applied at a dose of 50 g a.i./ha. Because some mating occurred at these doses in 1993, a higher rate of application was used in the 1994 test. In 1994, the bead formulation was applied at doses of 32.5 and 42.5 g a.i./ha for the first and second applications, respectively, and the flake formulation was applied at a dose of 75 g a.i./ha. The intended application dose for the bead formulation in 1994 was 37.5 g a.i./ha/application, but a mixing error resulted in a lower than intended dose on the first application. The second application was at a higher dose to bring the total dose up to the required 75 g a.i./ha.

Formulations. The plastic laminate flakes (1×3 mm) had 3-mil polyvinyl chloride (PVC) outer layers and an inner polymer layer with racemic disparlure (17.9% a.i.). The flakes and 4.7% diatomaceous earth (to reduce clogging) were blended 1:1 with a sticker (Gelva-1990 and Gelva-2333, Monsanto Co., St. Louis, Missouri) in 1993 and 1994, respectively. The polymethacrylate beads (34% a.i.) were prepared by suspension polymerization (Leonhardt et al., 1992). Beads (lot 35686-28) used in 1993 tests had a sieved particle size distribution of: <100 μm: 3.9%; 100–250 μm: 29.4%; 250–300 μm: 20.3%; 300–425 μm: 30.4%; 425–500 μm: 6.6%; and >500 μm: 9.4%. In 1994, the beads (lot RD419L) had a larger average particle size to prolong disparlure release: <100 μm: 2.1%: 100–250 μm: 1.4%; 250–300 μm: 10.8%; 300–425 μm: 24.3%; 425–500 μm: 21.7%; 500–600 μm: 30.4%; and >600 μm: 8.3%. Each year, beads were suspended at a rate of 15.4 g beads per liter of 90% water:10% sticker (Biogrip, Nalco Chemical Co., Naperville, Illinois).

Application. The flake formulation was applied with a Cessna 206 flying 8–15 m above the canopy at a speed of 193 km/h and with a lane separation of 14 m. Specialized application pods (Schweitzer Aircraft Corp., Elmira, NY) were used to dispense the flake formulation. The bead formulation was applied with a Cessna Ag-Truck flying 8–15 m above the canopy at a speed of 193 km/h and a lane separation of 18 m. The bead formulation was dispensed through six CP nozzles (C & E Enterprises, Mesa, Arizona) spaced at 203 cm intervals along the boom. The nozzles were set on the largest orifice diameter, the deflection plate was set to 90°, and the nozzles were oriented 90° into the slip stream. Boom pressure was maintained at 30–35 psi, and flow rate was 53.7 l/min. Both aircraft and pilots were provided by USDA, Animal Plant Health Inspection Service, Aircraft and Equipment Operations, Mission, Texas. Application dates were based on immature life stage development and occurred prior to moth flight as confirmed by male captures in pheromone-baited traps.

Release rate analysis. At the time of each application, 50 canvas spray cards (23×30 cm; Strathmore Paper, Westfield, Massachusetts), laid in an open area, were aerially treated with flakes or beads. The cards were then hung in a nearby wooded area for aging. Periodically, 3–5 replicate cards for each treatment were removed for determination of residual disparlure content in the applied flakes and beads. Flakes picked from spray cards in groups of 13–31 were counted and extracted with hexane. Groups of 100 or 200 individual beads (depending on the number available) were picked from the cards and extracted with 1:1 hexane and acetone. Disparlure content was measured by GC analyses (Model GC-9A GC, Shimadzu Instruments, Columbia, Maryland) on extracts (30-m SPB-1 capillary column; 0.75 mm i.d., set at 190 °C for 10 min and then programmed at 30°/min to 250 °C where it was held for 15 min).

Because the samples were left outdoors, they were subjected to rain and wind during the exposure period. Over time, some of the beads were lost from the sample cards. The larger beads, which were not bound as tightly to the cards by the sticker, were lost at a faster rate than were the smaller beads. Because larger beads contain a much greater volume of disparlure than smaller beads, the rate of loss of disparlure from the beads was partly confounded with the loss of larger beads. To adjust for this, the original (= pre-aging) disparlure content for each sample was estimated based on the weight of the beads and the original percentage of active ingredient (34%) in the beads. For each exposure period, the percent loss of disparlure was calculated by dividing the measured disparlure content of the bead sample by its estimated original disparlure content. Linear regression models were developed for the percent loss of disparlure as a function of the square root of exposure time (Kydonieus, 1977). These equations were used to estimate the daily emission rates resulting from the applications.

Efficacy assessment. Biological efficacy was assessed in the year of the treatment by measuring the effects of the treatments on the capture of male moths in (+)-disparlure-baited traps and mating success of monitor females. Impacts on population density and growth were measured by sampling egg masses and immature life stages in the year following the treatment. Populations were monitored for a third year in the plots treated in 1993, but because of a general, area-wide collapse in gypsy moth populations in 1995 these data were not included in the statistical analysis.

Male moth trapping. Standard USDA milk carton traps containing plastic laminate dispensers loaded with 500 μg of (+)-disparlure were placed in each plot at a density of one trap per 3 ha (five to 25 traps per plot) at a height of $\sim$2 m. Trap data from the year of the treatment were interpreted to indicate the effects of the treatments on the ability of males to locate and enter traps. A reduction in male moth capture indicates that the treatment interfered with normal gypsy moth chemical communication. Since trap catch is related to population density (Thorpe et al., 1993, Carter et al., 1994), trap data in years following the treatment indicate the effects of the treatment on gypsy moth population density. Trap data from the control plots were used to determine the duration and time of the peak period of male flight activity. All traps were monitored at least twice during the adult flight period and once after the end of male flight. In addition, one trap per plot was monitored daily to confirm that flight did not occur prior to treatment. Traps were removed from the plots or covered with plastic on the day of pheromone treatment to prevent contamination from aerially-applied pheromone formulations.

Monitor female mating success. Laboratory-reared females were placed in each plot to determine the effects of the treatments on mating success. Previous field evaluations have confirmed that the attractiveness of laboratory-reared females to wild males does not differ from that of wild females (V. C. Mastro, unpubl. results). Thirty virgin females, each one-day old, were placed in each plot three times per week during the period of male flight. Females were placed untethered on pieces of burlap fastened inside triangular cardboard mating stations which were distributed evenly throughout each plot at a height of $\sim$2 m. Because a previous study showed that the vertical distribution of flakes throughout the canopy was uniform (Reardon et al., 1998), we assumed that levels of mating disruption near the ground and in the canopy should be similar. The validity of this assumption has not been tested, and if it is false then the comparisons of treatment effects are valid only at ground level. The distribution of beads in the canopy has not been investigated. Females were generally placed in mating stations by 10:00 and retrieved, together with any egg masses, after two days. Females were held an additional 24 h and then discarded. Resulting egg masses were held 30 days in an outdoor insectary under ambient conditions and then examined for embryonation. Females producing egg masses which contained embryonated eggs were scored as ‘mated’ and those producing either no egg mass or all unembryonated eggs were scored as ‘not mated’. Leonhardt et al. (1996) provides a more detailed description of the procedure.

Life stages beneath burlap bands. Burlap bands (25 cm wide) were placed at a height of 1.4 m around the boles of four overstory host (mostly oak trees) at each node of a 50-m grid superimposed on each plot (140 to 852 trees per plot). Bands were checked at the completion of gypsy moth oviposition. Type and number of life stages were recorded and egg masses were collected, held under ambient conditions for at least 30 days, and then inspected for embryonation.

Egg mass density. Fixed-radius (0.01 ha) egg mass survey points (Liebhold et al., 1994) were conducted each year at the nodes of a 50-m grid superimposed over each plot (35 to 213 points per plot). When the total number of egg masses at a survey point was 50 or less, up to 30 egg masses within reach from the ground were collected and examined for embryonation. Otherwise, the number of egg masses collected and examined was limited to 10% of the total number of egg masses counted at the survey point.

Data analysis. Because of a 1995 general, area-wide collapse of gypsy moth populations that appeared to reverse developing population growth trends, data collected in 1995 were not included in analyses with data from previous years. The number of larvae and pupae beneath burlap bands prior to the application of a treatment was also excluded from multi-year analyses. For the 1993 test, an analysis of variance (ANOVA) was conducted on the 1993 and 1994 male trap catch and egg mass data using a repeated measures model (SAS, 1996; Proc Mixed). Single-year data were analyzed by the same procedure using an appropriate mixed model.

For each dependent variable, deviations from the assumption of variance homogeneity were detected by calculating the Spearman's correlation between the predicted values and the absolute values of the residuals (the actual minus the predicted response). When a significant correlation occurred, a logarithmic transformation of the form $Y_{\text{transformed}} = \log(Y + \text{constant})$ was performed (Berry, 1987). For each analysis, a constant resulting in the most homogeneous variance was used for the transformation (Carrol & Ruppert, 1988). Because many of the values from the 1994 test were zeros, the variance could not be stabilized by transformation. For these data, a nonparametric test was used in which data were ranked within blocks prior to analysis by ANOVA (Conover, 1980).

Results

Release rates. Amounts of residual disparlure in laminate flakes were similar in 1993 and 1994. Initially, the flakes contained 133 (1993) and 129 (1994) μg/flake; this amount declined to 91 and 67 μg/flake after 2 and 6 wk in the field in 1993, and to 91 and 64 μg/flake after 2 and 6 wk exposure in 1994. In 1993 the beads initially contained 0.90 μg/bead (1st and 2nd applications pooled). After 2 and 6 weeks in the field the disparlure content fell to 0.43 and 0.23 μg/bead, respectively. The larger beads used in 1994 initially contained 5.83 μg/bead (1st and 2nd applications pooled). Disparlure content fell to 4.01 and 2.78 μg/bead after 2 and 6 weeks of field exposure, respectively.

Figure 1 shows the change in residual content of disparlure in the flakes and beads over time expressed as a percentage of initial content. The smaller beads used in 1993 released disparlure at a faster rate than did the flakes. By day 16, the beads had released 50% of their disparlure content, while the flakes did not release 50% of their content until day 64. In 1994, the larger beads released their disparlure content at about the same rate as did the flakes.

Figure 2 shows the estimated daily amount of disparlure released per ha based on the calculated emission rates of the sampled flakes and beads. At the 1993 application dose of 50 g a.i./ha, the estimated release rate for the flakes ranged from 0.55 g disparlure/ha/day at ten days after treatment to 0.32 g disparlure/ha/day at 30 days after treatment (the period of peak male flight). Estimated release rates for the beads, which were applied at a dose of 30 g a.i./ha over two applications, ranged from 0.24 g disparlure/ha/day at ten days after treatment to 0.14 g disparlure/ha/day from the first application and 0.19 g disparlure/ha/day from the second application, for a total release of 0.33 g disparlure/ha/day at 30 days after the initial treatment. In 1994, flakes were applied at a higher dose of 75 g a.i./ha, which resulted in an estimated release rate that ranged from 1.04 to 0.63 g disparlure/ha/day at 10 and 30 days after application, respectively. The beads, which were larger in 1994 and which were applied at a dose of 75 g a.i./ha over two applications, resulted in an estimated release rate that ranged from 0.45 to 0.66 g/ha/day at 10 and 30 days after application, respectively.

Efficacy – 1993 treatment. The biological effects of the treatments applied in 1993 are shown in Table 1, and the results of the statistical analyses are shown in Table 2. Male moth capture in the year of treatment was 196.8 per trap in the untreated plots, compared to 3.4 and 1.3 per trap in the plots treated with beads and flakes, respectively, indicating that chemical communication was greatly affected by both treatments. Depression of trap capture continued into the next year. Since little residual disparlure remained in the formulations by the second year, especially in the beads, differences in trap capture in 1994 most likely reflected differences in gypsy moth population density due to treatment in the previous year. Numerical differences in trap capture between the untreated and treated plots continued into 1995, however the differences were not statistically significant.

In untreated plots, 28.4% of the monitor females were mated. In the plots treated with beads, no females were mated. In the plots treated with flakes, 0.2% of the females were mated. The reduction in mating success between the treated and untreated plots was statistically significant, but the difference between the plots treated with beads and those treated with flakes was not significant. No monitor females were deployed in these test plots in subsequent years.

The number of fertile egg masses per 100 burlap bands was significantly lower in treated plots than in untreated plots both during the year of treatment and the following year. In untreated plots, numbers of fertile egg masses per 100 bands increased from 28.8 in 1993 to 71.5 in 1994. The general collapse of populations that occurred in 1995 is reflected in the drop in the number of fertile egg masses per 100 bands in the untreated plots to 3.8. The number of fertile egg masses per 100 bands averaged 3.0 and 4.2 for the

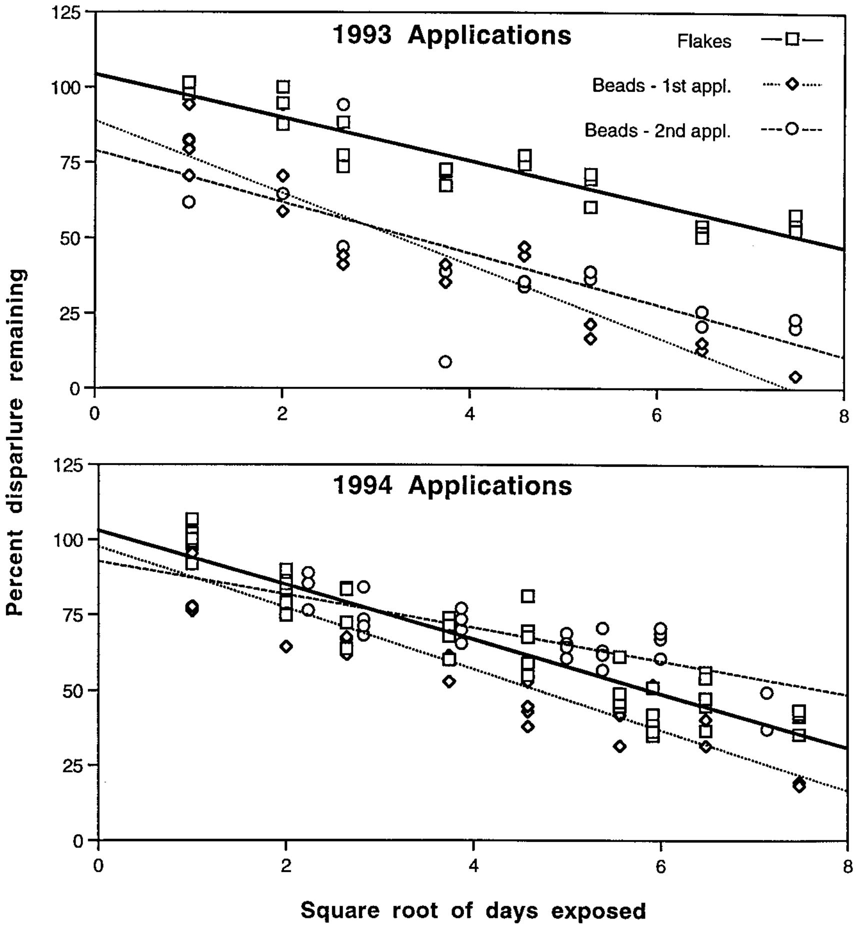

Figure 1. Relation between the percent of disparlure remaining in flakes and beads applied for gypsy moth mating disruption and the square root of the number of days of exposure, Rockbridge Co., Virginia, 1993 (A) and 1994 (B). Regression models for 1993 applications: $Y = -7.2 \cdot X + 104.3$; $R^2 = 0.86$ (flakes), $Y = -11.9 \cdot X + 88.8$; $R^2 = 0.83$ (1st appl. beads), $Y = -8.5 \cdot X + 78.9$; $R^2 = 0.59$ (2nd appl. beads). Regression models for 1994 applications: $Y = -9.0 \cdot X + 103.0$; $R^2 = 0.85$ (flakes), $Y = -10.1 \cdot X + 112.2$; $R^2 = 0.88$ (1st appl. beads), $Y = -5.5 \cdot X + 105.8$; $R^2 = 0.68$ (2nd appl. beads).

Table 1. Gypsy moth mating success and population growth in plots treated in 1993 with bead or flake formulations of aerially-applied racemic disparlure, Rockbridge County, VA

Year	Treatment	Life stages/100 bands	Males strap	Percent mating	Fertile egg masses/100 bands	Egg masses/ha
1993	Control	49.6 ± 25.8	196.8 ± 102.4 a	28.4 ± 9.8a	28.8 ± 12.7 a	68.7 ± 29.9 a
	Beads	27.8 ± 17.6	3.4 ± 3.0 b	0 b	3.0 ± 2.3 b	10.8 ± 9.4 b
	Flakes	34.1 ± 19.6	1.3 ± 0.6 b	0.2 ± 0.2	4.2 ± 2.4 b	16.6 ± 9.6 b
1994	Control	226.1 ± 116.3 a	401.6 ± 126.8 a	–	71.5 ± 36.4 a	156.7 ± 80.1 a
	Beads	20.8 ± 15.1 b	106.9 ± 77.9 b	–	10.8 ± 7.1 b	26.7 ± 20.8 b
	Flakes	27.5 ± 15.6 b	37.5 ± 20.9 b	–	12.7 ± 7.7 b	34.3 ± 20.5 b
1995	Control	50.6 ± 24.3 a	188.2 ± 69.3 a	–	3.8 ± 2.0 a	12.4 ± 11.1 a
	Beads	9.4 ± 5.0 a	47.9 ± 9.8 a	–	1.4 ± 0.4 a	0 a
	Flakes	24.9 ± 12.1 a	90.4 ± 38.1 a	–	2.4 ± 0.6 a	3.5 ± 1.7 a

Values are $\bar{x} \pm$ SE; means of treatments from the same year within a column followed by the same letters are not significantly different at a comparison-wise error rate of 0.05.

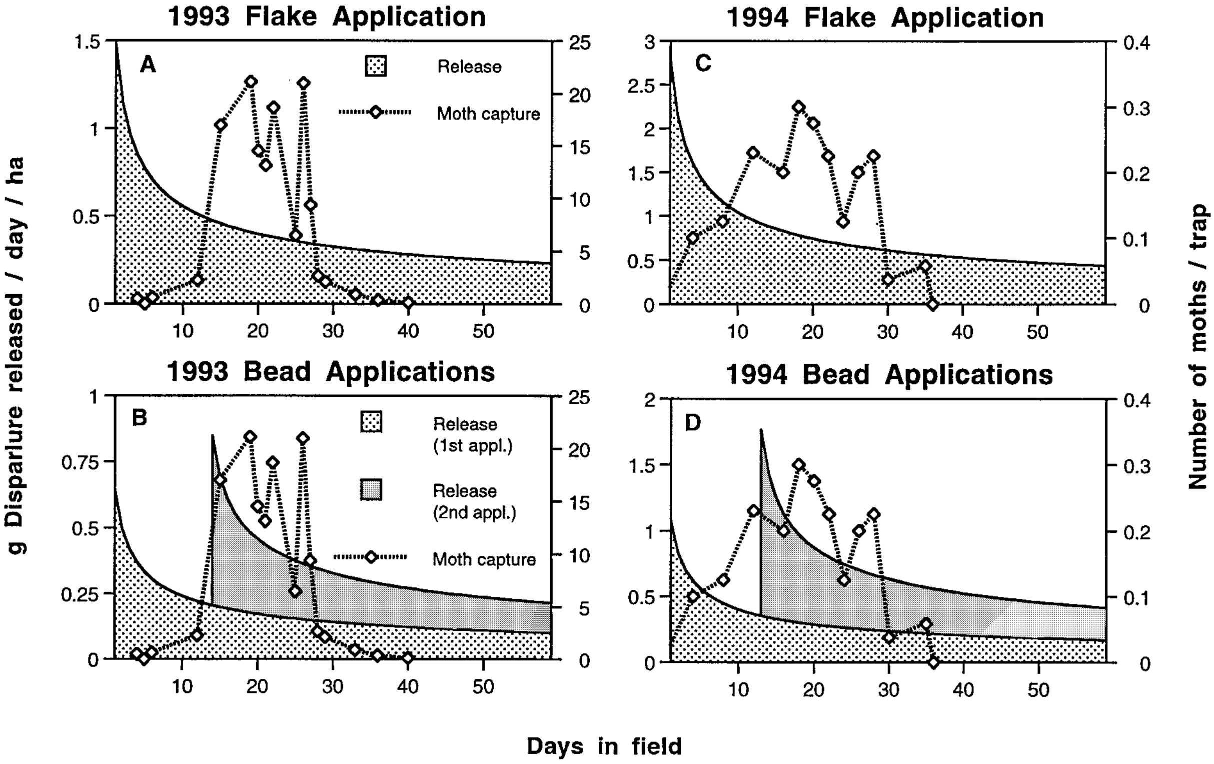

Figure 2. Disparlure emission rates from aerial applications of flakes (A,C) and beads (B,D) in 1993 (A,B) and 1994 (C,D). Applied doses were: 50 g a.i./ha (flakes, 1993), 15 g a.i./ha/application (beads, 1993, 2 applications), 75 g a.i./ha (flakes, 1994), 32.5 and 42.5 g a.i./ha for the 1st and 2nd applications, respectively (beads, 1994). Moth counts are from the untreated plots only, and are expressed as the average number per trap per day.

bead and flake treatments, respectively, in the year of the treatment, and increased to 10.8 and 12.7, respectively, the following year. Values for the treated plots were significantly lower than for the untreated plots both years, but the difference between the values for the flake- and bead-treated plots was not statistically significant. Egg mass density followed a similar trend, with the estimated number of egg masses per ha in untreated plots increasing from 68.7 in 1993 to 156.7 in 1994, and then dropping to 12.4 in 1995. In the treated plots, the egg mass densities were 10.8 (beads) and 16.6 (flakes) in 1993, increased to 26.7 and 34.3, respectively, in 1994 and then decreased to 0 and 3.5, respectively, in 1995 in the treated plots. Again, in the year of the treatment and in the following year, values for treated plots were significantly lower than for untreated plots, but egg mass density did not differ significantly between the flake- and bead-treated plots. No significant differences in egg mass density occurred in 1995.

Prior to the time of treatment, the number of immature gypsy moth life stages per 100 bands ranged from 27.8 to 49.6 in 1993. In 1994, the first year that these values could be affected by the treatments, the number of life stages per 100 bands increased to 226.1 in untreated plots but remained low at 20.8 and 27.5 in plots treated with beads and flakes, respectively. The value for the untreated plots was significantly higher than those for the treated plots, but the difference between the formulations was not statistically significant. In 1995, the number of life stages per 100 bands decreased in all plots, and the difference among the treatments was not statistically significant.

Efficacy – 1994 treatment. The biological effects of the treatments applied in 1994 are shown in Table 3, and the results of statistical analyses are shown in Table 4. Initial gypsy moth population densities in the plots used in the 1994 test were considerably lower than in the 1993 test. Male moth capture averaged 5.0 per trap in untreated plots, and 0 and 0.9 per trap in the plots treated with beads and flakes, respectively, in the year of the treatment. Each pair of means was significantly different. Male moth capture increased to

Table 2. Results of analysis of variance of the effects of 1993 applications of bead and flake formulations on gypsy moth mating success and population growth

Source	df	Life stages /100 bands (1994 only)	Males/trap[1]	Percent mating (1993 only)	Fertile egg[1] masses/100 bands	Egg masses[1]/ha
Year	1	–	0.047	–	0.01	0.20
Treatment	2	0.0002	0.001	$<$0.0001	$<$0.0001	0.0006
Year × trt.	2	–	0.45	–	0.75	0.72

Values are $P > F$.
[1]Analysis of data from 1993 and 1994 only.

Table 3. Gypsy moth mating succes and population growth in plots treated in 1994 with bead or flake formulations of aerially-applied racemic disparlure, Rockbridge County, VA

Year	Treatment	Life stages/100 bands	Males per trap	Percent mating	Fertile egg masses/100 bands	Egg masses/ha
1994	Control	0.3 ± 0.1	5.0 ± 1.2 a	3.4 ± 2.0 a	0.3 ± 0.1 a	0.2 ± 0.2 a
	Beads	0.4 ± 0.1	0 b	0.1 ± 0.1 a	0 b	0 a
	Flakes	0.7 ± 0.5	0.9 ± 0.6 c	0 a	0 b	0 a
1995	Control	1.5 ± 0.3 a	19.1 ± 5.7 a	–	0	0
	Beads	0 b	3.7 ± 1.3 b	–	0	0
	Flakes	0.4 ± 0.2 c	0.8 ± 0.1 c	–	0	0

Values are $\bar{x}$ ± SE; means of treatments from the same year within a column followed by the same letters are significantly different at a comparison-wise error rate of 0.05.

19.1, 3.7, and 0.8 the following year in plots that were untreated or treated with beads or flakes, respectively. However, as can be seen by the zero egg mass counts in 1995 in all plots, gypsy moth populations collapsed in all plots the year following the treatment.

Percent mating of monitor females appeared to be higher in the untreated than in the treated plots, but the difference was not statistically significant. No monitor females were deployed in 1995.

Numbers of fertile egg masses per 100 bands and numbers of egg masses per ha were very low in the untreated plots in 1994 (0.3 and 0.5, respectively), and were zero in the treated plots. These differences were statistically significant. No egg masses were found in any of the plots in 1995.

Numbers of gypsy moth life stages per 100 bands in all plots ranged from 0.3 to 0.7 prior to the application of the treatments in 1994. In 1995, the number of life stages per 100 bands in the untreated plots increased to 1.5 despite the overall population decline. This number was significantly higher than the values for the treated plots (0.4 and 0 for plots treated with flakes and beads, respectively). Numbers of life stages did not differ significantly between flake- and bead-treated plots.

Discussion

Preliminary tests, reported in Reardon et al. (1998), indicated that the polymethacrylate beads released disparlure at a rate that was too rapid to maintain sufficient disparlure concentration in the air to disrupt mating throughout the entire period of male flight. The release rates reported here for the beads were such that a single application should provide a disparlure concentration during the period of male flight equal to or greater than that provided by a single flake application. Figure 3 compares the hypothetical release rate of disparlure from flakes and beads if they were applied in a single application at a dose of 75 g a.i./ha. The release rate curves plotted in Figure 1 were applied to starting values of 75 g a.i./ha to calculate the emission rates for Figure 3. Data for the two bead applications within each year were combined to generate release rate curves for the beads. If applied in a single application at a dose of 75 g a.i./ha, the 1993 beads, which were smaller and released at the fastest rate, would emit the highest levels of disparlure over a 60-day period following application. The larger beads used in 1994 would emit less disparlure than the smaller beads, but would still emit more than the flakes based on the 1993 release rate. If the release rates from beads

Table 4. Results of anlaysis of variance of the effects of 1994 applications of bead and flake formulations on gypsy moth mating success and population growth

Source	df	Life stages/100 bands (1995)	Males/trap (1994)	Males/trap (1995)	Percent mating (1994)	Fertile egg masses/100 bands (1994)	Egg masses (1994)
Treatment	2	<0.0001	<0.0001	<0.0001	0.27	0.04	0.27

Values are P > F.

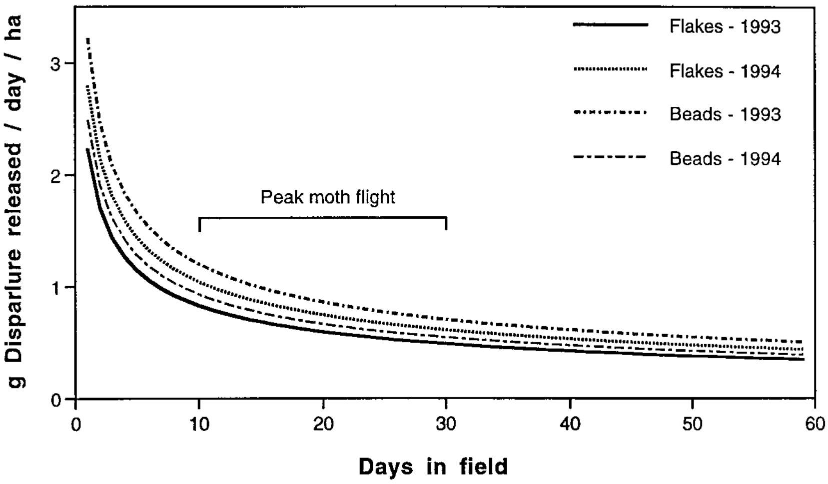

Figure 3. Hypothetical release rates for flakes and beads applied in a single dose of 75 g a.i./ha. Release rates are based on those observed in 1993 and 1994 bead and flake applications.

obtained in 1993 and 1994 could be achieved with future formulations, it appears that a single application could provide levels of disparlure emission during the entire period of male flight equal to or greater than those obtained with a single flake application.

Previous efforts to measure wash-off rates of beads and flakes indicated that the beads are more susceptible to wash-off than the flakes (Reardon et al., 1998). In 1993, both flake and bead formulations were sprayed on foliage to measure wash-off rates. After 13 days, 90 and 57% of the flakes and beads, respectively, still adhered to the foliage. After 47 days, 57 and 31% remained. The higher wash-off rate of the beads, which presumably resulted in the deposit of a higher proportion of the dispensers on the ground surface, may have reduced the concentration of disparlure in the canopy. However, if this did occur, it did not result in a measurable reduction in efficacy. Improved wash-off resistance should be a priority in the development of bead formulations for gypsy moth mating disruption.

Previous studies have demonstrated that gypsy moth mating can be suppressed by applying disparlure either from the ground (Schwalbe & Mastro, 1988; Kolodny-Hirsch et al., 1990; Webb et al., 1990) or air (Schwalbe et al., 1979, 1983; Webb et al., 1981, 1988). The range of gypsy moth population densities within which mating can be effectively disrupted with aerial applications of flake and/or bead formulations at the doses reported here is not presently known. Furthermore, the level of mating disruption required to achieve specific objectives (e.g., eradication of isolated infestations, population suppression, slowing the spread of the boundaries of the generally-infested area) is also yet to be determined. In a recent study, Leonhardt et al. (1996) reported the total elimination of mating among monitor females and the absence of fertile egg masses in plots after two applications of plastic laminate flakes at a dose of 75 g a.i./ha/application. They further reported an 83% reduction in the number of egg masses per ha in the year of treatment and a 99% reduction in the number of life stages beneath burlap bands in the year following treatment, as compared to untreated plots. Similar results were obtained in the current study with single aerial applications of disparlure-impregnated flakes at the same and lower doses (50 and 75 g a.i./ha in 1993 and 1994, respectively). While total suppression of mating did not occur in either of the two tests reported here, mating of monitor females was reduced by >97% and the number of fertile egg masses per 100 bands was reduced by >85%. In the plots treated in 1993, populations were reduced by >75% based on numbers of egg masses per ha in the year of the treatment and by >83% based on the numbers of life stages beneath bands in the year following treatment. In plots treated in 1994, the number of fertile egg masses produced in the year of the treatment was reduced to 0. These results were obtained over a range of initial gypsy moth population densities (33.4, 196.8, and 5.0 male moths per trap for the 1990, 1993, and 1994 applications, respectively).

Because a general collapse of gypsy moth populations occurred in the area of the tests in 1995, severely reducing the usefulness of the data from that year, only the treatments applied in 1993 provided useful information about the effects of the treatments in the year following the application. All variables measured for this test showed statistically significant effects on population growth in the year following treatment. This is in agreement with the results of a similar test with flakes conducted in 1990 on populations that were monitored for three additional years (Leonhardt et al., 1996), and which showed continued suppression of gypsy moth populations in years subsequent to mating disruption treatments.

Despite the different release characteristics of the bead and flake formulations, there was no evidence of a consistent difference in their biological effects. Both formulations significantly reduced mating success and suppressed population growth compared to untreated populations. The favorable release characteristics of the smaller beads used in 1993 and the larger beads used in 1994, the ability to apply the beads through conventional application equipment, and the demonstration of biological efficacy are all indications that the beads are operationally viable and should be developed further.

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