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CONTROL OF LOW-DENSITY GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) POPULATIONS BY MATING DISRUPTION WITH PHEROMONE

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Abstract-This four-year study demonstrated that low-density populations of the gypsy moth, *Lymantria dispaf* (L.), were effectively suppressed by annual aerial application of 75 g of racemic disparlure per hectare formulated in plastic laminate flakes. These tests also showed that, when plots were treated with 150 g of pheromone per hectare in 1990 only and left untreated for the following three years, populations continued to be suppressed in 1991-1993 as compared with the controls. Although none of the plots were treated in 1994, population assessment continued and showed that the gypsy moth population density remained low in the plots that had been treated annually for the preceding four years. The laminate flakes released an average of 0.48 g disparlure per day per hectare from each of the two applications in 1990, and 0.72 g per day per hectare from the single application in each of the following three years (1991-1993). Only 27-40% of the applied pheromone dose was emitted during male moth flight.

Key Words-Gypsy moth, *Lymantria dispar*, disparlure, pheromone, mating disruption, laminate flakes, forest pest, aerial application.

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INTRODUCTION

The gypsy moth, *Lymantria dispar* (L.), is a major pest of forests in the eastern United States, Michigan, and parts of Canada. In addition, over the last 10 years, approximately 32 isolated infestations per year have been detected and eradicated in states outside of the generally infested area (McGovern, 1994). Studies on the use of the sex attractant pheromone, (2)-7 ,8-epoxy-2-methyloctadecane or disparlure (Bierl et aI., 1970), for disruption of mating in low-density populations have been carried out for the past 15-20 years (Cameron, 1981). Tests of aerially applied, controlled-release formulations of disparlure conducted in the late 1970s (Webb et aI., 1981) showed that gypsy moth mating was reduced by approximately 70-85% or more depending upon the population density and the applied dose (Schwalbe et aI., 1979, 1983). Dose-response studies (Webb et aI., 1988) showed that the degree of mating disruption increased as the amounts of disparlure in plastic laminate flakes was increased from 7.5 to 75 g/ha in aerially treated plots.

Ground applications of disparlure at rates of 5, 50, and 500 g/ha, dispensed from plastic laminate tapes in plots with high-density gypsy moth populations, resulted in 6.5, 34, and 84% decreases in mating success, respectively, as measured against controls in small plot tests (Schwalbe and Mastro, 1988). Similar studies with disparlure applied in plastic laminate tape (plastic rope) showed that doses as high as 250 g/ha were insufficient to disrupt mating in isolated, high-density populations in which pretreatment surveys showed that immature stages of gypsy moths averaged over 40 per burlap-banded tree (Webb et aI., 1990). A five-year evaluation of the same technology (50 g disparlure/ha applied every other year in plastic laminate tapes) demonstrated biological activity for two seasons as measured by reduced mating success and male moth trap capture. However, these ground treatments were not effective in dampening the rate of population increase as measured by egg-mass densities over the five-year study (Kolodny-Hirsch et aI., 1990). Part of the lack of efficacy was attributed to inadequate permeation of the ground-applied disruptant throughout the upper canopy of the trees. This study found a significant increase in mating success of females deployed at a height of 15 m as compared with those placed at a height of 1.5 m. These results suggest that, for mating disruption to be effective, the pheromone must be distributed vertically throughout the canopy and remain there for the duration of adult activity. An aerially applied laminate flake formulation of disparlure resulted in the deposit and adherence of the flakes throughout all strata of the canopy and yielded season-long mating disruption in Giles Country, Virginia (Leonard et aI., 1992).

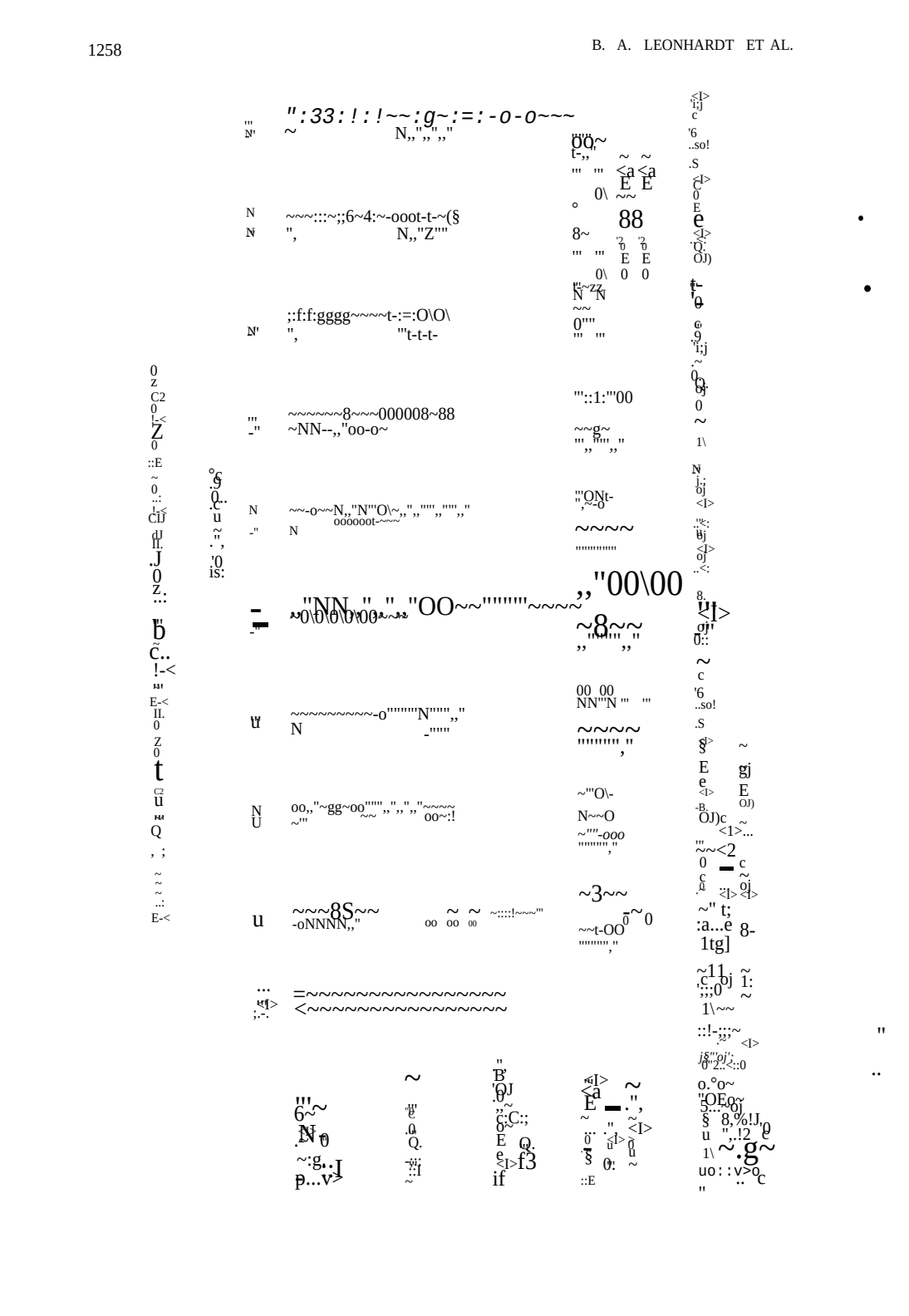
This paper reports the results of a five-year (1990-1994) U.S. Department of Agriculture (USDA) cooperative program within the Forest Service's Appalachian Gypsy Moth Integrated Pest Management Program (AIPM) to disrupt

mating in low-density gypsy moth populations in relatively large woodlots in Virginia. These tests compared plots aerially treated with one application of a laminated plastic flake fonnulation of disparlure in each of the first four years with plots treated with two applications of the flakes only in 1990 followed by no treatment in succeeding years. Efficacy and fonnulation release rates were detennined for each treatment.

METHODS AND MATERIALS

Treatments. The test sites were isolated woodlots (each 13.8-63.9 ha, Table 1) in Rockbridge County, Virginia, in an area that was newly infested with the gypsy moth. It was considered to be part of the southern leading edge of the generally infested area. In 1989, the year prior to treatment, captures of male moths in USDA milk carton survey traps (2 km spacing) averaged 27 males/trap in the area encompassing the test plots. Nine individual woodlots, each surrounded by agricultural fields, were selected on the basis of this male moth survey data and stand composition (greater than 70% basal area oak, *Quercus* spp.). At the beginning of the study, the nearest dense gypsy moth population was approximately 20 km from any of the test plots. The woodlots were randomly assigned to one of three treatments for these mating disruption studies (Table 1). Three plots, C1, C2, and C3, were untreated controls. Three plots, ILl, 1L2, and 1L3, were treated annually (1990-1993) with a single application of 75. g disparlure/ha just prior to initial male moth flight. The final three plots, 2Ll, 2L2, and 2L3, were treated with two 75 g disparlure/ha applications only in 1990; one treatment was applied just prior to initial male moth flight and the second treatment was prior to peak flight. Treatment dates were selected based on the development of immature life stages and expected male eclosion. Monitoring of male trap catches confinned that application dates occurred within the desired time period. For 1990 through 1993, initial treatments were applied, respectively, on June 26, 17, 30, and 23. The second treatment of pheromone to plots 2Ll, 2L2, and 2L3 in 1990 was applied on July 16. All pheromone treatments were made using specialized equipment (Schwalbe et aI., 1983) attached to a Cessna 206 aircraft and calibrated to deliver 75 g active ingredient (a.i.) in 405 g flakes per hectare per application. Two of the nine study plots were no longer available in 1994 due to actions by the property owners; control plot C3, defoliated by high-density gypsy moth populations in 1993, was treated with chemical insecticide and plot lL3 was clear-cut. None of the remaining plots was treated with pheromone in 1994.

The plastic laminate flakes (1 x 3 mm) had 3-mil PVC outer layers and an inner polymer layer containing racemic disparlure (Disrupt II; Hercon Environmental Co., Emigsville, Pennsylvania). The flakes (18.5% a.i.) were pack-



aged with 4.7% diatomaceous earth to prevent clogging in the application appardtus. This mixture was blended during application with a sticker (Gelva 1990, Monsanto Corp., St. Louis, Missouri) in a I : I ratio by volume to provide adhesion to leaf surfaces.

Population Sampling Procedures. Because low-density gypsy moth populations are difficult to characterize using counts of egg masses (Wilson and Fontaine, 1978), several techniques were selected to evaluate the impacts of treatments on populations and to follow the population trends through the course of the study. These included: burlap bands for sampling abundance of immature life stages, pheromone trapping of adult males, mating success of monitor females, and wild egg mass abundance and fertility.

Immature Life Stages. Relative abundance of immature life stages (Table I) was monitored in the plots using burlap bands (25 cm wide) fastened around host trees approximately 1.4 m above the ground (64-844 bands per plot). Sampled trees were spaced uniformly throughout the plots by selecting host trees closest to points on a 50-m grid. Burlap bands have been shown to be useful for sampling low-density gypsy moth populations (Wallner et a\., 1989, 1990). Bands were checked once when gypsy moths were in the late-instar or pupal stages and a second time after adult flight when shed pupal cases and egg masses were present. Type and number of life stages were recorded at each sampling period. Egg masses were collected and held under ambient conditions for at least 30 days; following mechanical dehairing, they were inspected for evidence of embryonation.

Male Moth Trapping. USDA milk carton traps baited with the standard pheromone dispenser were used in all plots (Table 1) to monitor adult male moth flight (Schwalbe and Mastro, 1988). Generally one trap was placed every 6.2 ha (3-17 traps per plot); three traps were placed in the one plot (C2) that was smaller than 18.6 ha. 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. Trap data from the control plots were used to determine the duration and peak period of male flight activity. Traps were removed from the plots or covered with plastic on the day of pheromone treatment to prevent contamination from aerially applied flakes.

Mating Success of Monitor Females. To monitor mating success, laboratory-reared females were placed in each plot because few wild females were projected to be available for sampling, particularly in the early years of the study. Normally, 30 one-day-old virgin female moths were placed in each plot three times a week. When sufficient numbers of I-day-old females were not available, they were supplemented with 2-day-old females. In these circumstances, care was taken to uniformly distribute 1- and 2-day-old females among all plots. Females were shipped as pupae four to eight days after pupation from

the rearing location at the Otis Methods Development Center in Massachusetts to an outdoor insectary in Rockbridge County. Therefore, the pupae were exposed to the local light and thermal regimes prior to eclosion and placement in the field. Females were placed untethered on pieces of burlap fastened inside triangular mating stations, and 30 of these stations were spaced uniformly throughout each plot. These mating stations were constructed from USDA delta traps without adhesive and with open ends. Females were generally placed in these stations by 10: 00 AM and retrieved, together with any egg masses, after two days. Most females or their egg masses were recovered (Table I). The recovery rate from a single plot in a given year ranged from 78 to 98 %; over all plots and all years, the recovery rates averaged 89 %. Collected females were held individually an additional 24 hr to complete any oviposition and then discarded. Resulting egg masses were held an additional 30 days in an outdoor insectary under ambient conditions and then examined for embryonation. Females producing egg masses that contained embryonated eggs were scored as mated, and those producing either no egg mass or an egg mass containing all unembryonated eggs were scored as not mated. Monitor females were not placed in the 2L plots in 1992-1993 or in any of the seven plots in 1994 due to a lack of resources.

Wild Egg Mass Abundance and Fertility. Egg-mass surveys were conducted in O.Ol-ha subplots spaced uniformly (50-m grid) on each plot. The number of subplots sampled on each plot ranged from 34 to 242, corresponding to 2.5-4.6% of the total plot area (Table I). Egg-mass surveys were conducted in the 0.0 I-ha subplots prior to treatment in 1990 and late in the year thereafter during the five years of the test. All egg masses found were collected and examined for embryonation with the exception of the 1993 and 1994 control plots. In these plots, because of high populations, a 10% subs ample of the egg masses found in O.Ol-ha surveys were collected and examined for embryonation. The fertility of egg masses was determined from the wild egg masses retrieved both from the burlap bands and from trees in the 0.01 ha subplots.

Data Analyses. Data were analyzed by a repeated-measures analysis of variance [PROC GLM, SAS version 6.08 (SAS Institute, 1985)]. For each variable, the degree of spherical covariance across years was tested based on Mauchly's sphericity criterion using an approximate chi-square test for orthonormal contrasts (Mauchly, 1940). If the sphericity criterion was not met, the degrees of freedom used for the test of the significance of the treatment x year interaction were adjusted by the Greenhouse-Geisser epsilon value (Greenhouse and Geisser, 1959). The average number of larvae beneath burlap bands in 1990 was included as the covariate in all analyses of dependent variables (except itself) to remove differences in pretreatment gypsy moth density among the plots. This covariate was chosen because the treatments, which were first applied in 1990, could not have affected larval density in that year. Variance homogeneity for each dependent variable was tested by determining the Spearman

correlation between the predicted values and the absolute values of the residuals (the actual minus the predicted response). A significant correlation coefficient was interpreted as an indication that a transformation was needed to stabilize variance. This was accomplished using a logarithmic transformation of the form $Y_{transformed} = \log(Y + \text{constant})$ (Berry, 1987). For each analysis, a constant resulting in the most homogeneous variance was used for the transformation (Carrol and Ruppert, 1988). Data collected in 1994 are presented but were not included in the analysis.

Release Rate Analyses. At the time of flake application to the test plots, approximately 50 white canvas spray cards (23 x 30 cm; Strathmore Paper Co., Westfield, Massachusetts) were aerially treated with flakes by multiple passes of the aircraft. The cards were then hung in a wooded area near the test plots for aging. Periodically during the field test, three to five replicate cards for each treatment were removed for determination of the residual disparlure content in the applied flakes. In 1990, flakes were also removed from foliage for analysis at similar intervals of field aging. To determine how much of the pheromone remained in the flakes after a year of exposure in the field, leaves containing flakes from the 1990 treatment were retrieved from the forest floor in October 1990 and sequestered in a wire mesh enclosure in a nearby location. Flakes were removed from these leaves in July 1991 for analysis.

Flakes picked from each of the spray cards (or foliage) for each of the sampling periods were counted and extracted overnight with 1 ml of hexane. Gas chromatographic (GC) analyses were conducted on a model GC-9A GC (Shimadzu Instruments, Columbia, Maryland) using l-{tl injections of the extracts on a 30-m SPB-1 capillary column (0.75 mm ID) set at 190°C for 10 min and then programmed at 30° Imin to 250°C where it was held for 15 min to clear high-molecular-weight materials extracted from the polymer. Disparlure contents were measured using an external standard, and peak areas and results were calculated as micrograms per flake.

RESULTS

Immature Life Stages. In the first year (1990), the burlap band survey prior to treatment found similar small numbers of larvae and pupae in the nine plots (Table 2). The mean number of gypsy moth immatures in the control plots was 4.6/100 bands in 1990 and increased each year thereafter. In the plots treated annually for the first four years (1L), the number of immatures per hundred bands remained low throughout the study, and the treatment effect was significant ($P = 0.05$). An LSD test, conducted to compare treatment means pooled over all years, indicated that immature counts in the lL plots were significantly ($P = 0.02$) lower than in control plots. In plots treated only in 1990 (2L),

TABLE 2. MEAN NUMBERS OF GyPSY MOTH LIFE STAGES IN UNTREATED CONTROL PLOTS AND PLOTS TREATED YEARLY WITH ONE ApPLICATION OF LAMINATE FLAKES CONTAINING RACEMIC DISPARLURE (lL PLOTS) AND Two ApPLICATIONS ONLY IN 1990 (2L PLOTS)"

TreaImeni	1990	1991	1992	1993	1994
Mean numbers (SE) of immatures (pupae plus larvae) per 100 burlap bandsb					
Control	4.6 :f: 4.0	30.2:f: 23.9	217.3 :f: 123.6	708.6:f: 456.7	2696.7 :f: 2453.0
Yearlyapplic.(1L)	2.7:f:1.4	0.2:f:0.2	1.4:f:0.7	32.2:f:14.8	17.6 :t 1.3
1990 applic. only (2L)	0.8:f: 0.4	0.8 :f: 0.4	16.6:f: 0.1	160.9 :f: 64.2	1445.8 :t 506.0
Mean numbers (SE) of male gypsy moths captured in pheromone-baited Irapsc					
Conirol	33.4 :f: 18.2	130.4:f: 62.1	635.2 :f: 240.1	1960.0:f: 905.2	715.6 :f: 234.7
Yearly applic. (lL)	0.0	0.0	0.2 :f: 0.1	0.6 :f: 0.3	8.5 :t 0.5
1990 applic. only (2L)	0.3:f: 0.1	0.5 :f: 0.2	76.9:f: 12.6	458.1:f: 91.9	534.4 :t 136.6
Mean perceniages (SE) of monitor females matedd					
Control	10.3 :f: 2.7	2.5 :f: 2.7	67.7:f: 6.5	52.8 :f: 9.6	
Yearly applic. (lL)	0.0	0.0	0.7 :f: 0.4	0.1 :f: 0.1	
1990 applic. only (2L)	0,0	0.2 :f: 0.2			
Mean numbers (SE) of fertile wild egg masses found per 100 burlap bands after moth flight'					
Control	3.5 :f: 2.8	21.4:f: 18.7	70.7:f: 35.4	593.6:f: 267.3	299.3:f: 213.0
Yearly applic. (lL)	0.0	0.1 :f: 0.1	0.0	3.9 :f: 2.3	5.5 :t 1.0
1990 applic. only (2L)	0.0	0.3 :t 0.1	7.0 :f: 1.8	105.0 :t 30.3	490.2 :t 132.6
Mean numbers (SE) of fertile wild egg masses per hectare found in postseason O.OI-ha subplots!					
Control	10.1 :t 5.2	14.3:t 12.8	177.2:t 85.5	3136.8:f: 1485.2	2384.3 :f: 2105.8
Yearly applic. (IL)	1.7:f: 1.5	0.3 :t 0.3	0.0	13.8:f: 10.3	13.2 :t 4.1
1990 applic. only (2L)	0.0	0.3 :t 0.3	9.1 :f: 4.9	285.5 :f: 97.0	1267.5:t 441.6

aTests were conducted from 1990 to 1994 in Rockbridge County, Virginia.

bValues were transformed to log $(x + 0.1)$ prior to analysis. Test for sphericity: chi-square $= 6.6$; $df = 5$; $P = 0.26$. Treatment x year interaction effect: $F = 1.6$; $df = 6, 17$; $P = 0.2$. Treatment effect: $F = 4.9$; $df = 2, 6$; $P = 0.05$. Results of LSD tests on treatment means: control versus IL, $P = 0.02$; control versus 2L, $P = 0.12$; lL versus 2L, $P = 0.27$.

cTest for sphericity: chi-square $= 44.4$; $df = 5$; $P < 0.0001$. Treatment x year interaction effect: $F = 6.78$; $df = 6, 15$ [unadjusted]; $P = 0.03$; Greenhouse-Geisser epsilon $= 0.38$.

dTest for sphericity (control versus IL-all years): not performed due to insufficient degrees of freedom. Treatment x year interaction effect (1990 versus 1991): $F = 21.0$; $df = 2, 5$; $P = 0.004$. Treatment x year interaction effect (control versus lL, all years): $F = 51.0$; $df = 3,9$; [unadjusted]; $P = 0.005$; Greenhouse-Geisser epsilon $= 0.34$.

'Values were transformed to log $(x + 1)$ prior to analysis. Test for sphericity: chi-square $= 9.6$; $df = 5$; $P = 0.08$. Treatment x year interaction effect: $F = 9.9$; $df = 6, 16$; $P < 0.0001$.

!Test for sphericity: chi-square $= 9.0$; $df = 5$; $P = 0.11$. Treatment x year interaction effect: $F = 4.8$; $df = 6, 15$; $P = 0.007$.

immature life-stage counts were lower than in control plots throughout the study, but an LSD test of these data did not show ($P = 0.12$) significantditferences between the 2L and control plots.

Densities of immature stages in 1994 in the two remaining control plots averaged 2697 life stages/lOO bands. This represents approximately a fourfold

increase in counts of immatures compared to the 1993 numbers (Table 2), even if the 1993 data are adjusted to exclude data from the control plot that was treated with insecticide in 1994. Such adjustment gives a mean immature density of 80 I life stages/IOO bands in 1993 instead of *709.*

Counts of immatures under burlap bands in 1994 in the two remaining IL plots averaged 17.6 life stagesll 00 bands. This appears to be a decrease in density as compared with the 1993 data (Table 2) even when the 1993 data are adjusted to remove the data for the woodlot lost to clear cutting. The adjusted mean number of life stagesllOO bands in 1993 for the IL plots is 46.4. This suggests that the gypsy moth population did not rebound in 1994, the first year that these plots were not treated with pheromone. In the three woodlots treated only in 1990 (2L), counts of immatures remained relatively low in the two years following treatment (1991 and 1992), but then increased about IO-fold in each of the next two years to a level where they were similar to those found in control plots.

Male Moth Captures. Captures of male moths in all of the plots in the first year of treatment (1990) were relatively low, but the mean male captures in the control plots (33.4 males/trap) was higher than those in either the IL *(0.0* males/trap) *or* 2L *(0.3* males/trap) plots (Table 2). The numbers of male moths captured in the control plots were also higher in each of the following four years than the numbers captured in treated plots and increased to a mean of 1960 males/trap in 1993. In 1994, male captures in control plots (716 males/trap) were lower than the 1993 captures even if data from the control plot that was lost in 1994 (due to pesticide application) are excluded from the 1993 captures; with that adjustment, the two remaining control plots had a mean capture of 1104 males/trap in 1993.

Although gypsy moth populations adjacent to the experimental plots also had become very high, the mean number of males captured per trap in the IL plots remained less than one per trap in each of the first four years of treatment. In 1994, the first year with no treatment, the number of males captured in the IL plots was still very low ($\bar{X}$ = 8.5/trap) when compared with the control. Once more this suggested that the populations in the IL plots remained suppressed, even though treatment ceased in 1993. Similarly, the numbers of males captured per trap in the 2L plots remained low ($\bar{X}$ = 0.5 males/trap) in the first year following treatment (1991), but increased each year thereafter to 534.4 males/trap in 1994 (Table 2). The data analysis showed that numbers of males captured over time differed significantly (P = 0.03) among the treatments.

Mating Success of Monitor Females. In 1990, none of 1961 monitor females recovered from the six treated plots (1 Land 2L) was mated, but an average of 10.3% of the 931 females recovered from the control plots were mated. This relatively low mating success in the control plots may be attributed to low male mating pressure before and after peak male emergence. Female placements were

initiated a week prior to anticipated male flight and were continued until no males were trapped in control blocks for three consecutive days. During the two weeks of peak male flight (July 16-30, 1990), the percentage of mated monitor females in the control plots was substantially higher at 17.4 % compared to 5.5 % before and 4.8% after peak flight. In 1991, there was still no mating of the monitor females in the annually treated plots (1 L) and only 0.2 % mating (2 of 966 females) in the 2L plots. In contrast, 2.5 % of the 937 females placed in the control plots in 1991 were mated, and 4.2% of those placed in the two weeks of peak male flight were mated. In 1992 and 1993, 0.7% and 0.1 %, respectively, of the monitor females were mated in the 1L plots as compared with 67.7% and 52.8%, respectively, in the control plots. Data analysis for all three treatments for the first two years (1990 and 1991) showed a significant ($P = 0.004$) treatment X year interaction effect. This indicated that mating success changed differently for each of the three treatments and that these differences were significant. A separate analysis showed that mating success in the control plots over 1990-1993 was significantly ($P = 0.005$) different from that in the lL plots. Monitor females were not placed in any plots in 1994.

Wild Egg Mass Abundance and Fertility. Analyses of numbers of fertile egg masses under burlap bands ($P = 0.0001$) and numbers of fertile egg masses discovered in surveys ofO.Ol-ha subplots conducted late in the year ($P = 0.007$) indicated that the density changes over time were significantly different for the three treatments.

Survey of the O.OI-ha subplots prior to treatment in the first year of the test revealed only one egg mass and that in plot C3. Numbers of fertile egg masses found under burlap bands in control plots increased over the first four years of study (Table 2). After the adult flight period in 1990, burlap bands in control plots averaged 3.5 fertile egg masses/lOO bands. These numbers rose steadily for the next three years and averaged 594 fertile egg masses!loo bands in 1993. This constitutes a l70-fold increase over four years. Over the same period, the egg mass surveys in the O.Ol-ha subplots indicated that an even greater increase in the fertile egg-mass density had occurred in the control plots than was indicated by the burlap-band survey. Mean numbers of fertile egg masses per hectare found in the control plots during these surveys rose from 10.1 in 1990 to a high of 3137 in 1993 (a 31O-fold increase). In the control plots, 99% of all of the egg masses found in 1993 under burlap bands or in the postseason surveys were fertile. The .1994 egg mass counts from burlap-banded trees in the control plots apparently decreased even when the 1993 data are adjusted to remove data for the control plot that was lost in 1994 (adjusted 1993 mean is 443.2 egg mass!l()() bands). Density estimates from sampling O.OI-ha subplots in control plots also indicated a decrease in egg mass density in 1994 (Table 2); however, when the 1993 data are adjusted to eliminate those from the lost replicate, the 1993 number of fertile egg masses per hectare becomes

2292, which is similar to the 1994 number. The contrast between this information and the 1994 decline in egg mass density indicated from the burlap band counts might suggest that, in low density populations, burlap bands are more sensitive for detecting egg masses than are the standard 0.0 I-ha subplot surveys. However, in high populations, the burlap bands can become saturated and the counts fail to indicate the true population level.

In plots treated annually from 1990 to 1993 with pheromone (lL), the numbers of fertile egg masses found under the burlap bands remained low throughout the years of treatment (Table 2). Although the mean number of fertile egg masses in the lL plots was 3.9/100 bands in 1993, more egg masses (mean of 13.4/100 bands; data not shown) were actually found but only 29% of these were fertile. Postseason egg mass sampling in O.OI-ha subplots showed low numbers of fertile egg masses per hectare in 1990-1992 in the 1L plots but, in 1993, this number increased to 13.8 fertile egg masses per hectare reflecting the pressure from the surrounding high-population areas (Table 2). In 1994, the first year in which these lL plots were not treated, the number of fertile egg masses under burlap bands and in the O.Ol-ha subplots remained similar to the 1993 levels (Table 2). This assessment does not change markedly when the data for the replicate that was lost in 1994 are eliminated from the calculation of the 1993 means. The adjusted numbers of fertile egg masses are 5.3/100 bands and 18.7/ha for 1993, and these means remain comparable to the numbers obtained in 1994. As with other population measures, the fertile egg mass data suggest that the gypsy moth populations remained low in the year after the treatment was terminated.

In plots treated only in 1990 (2L), the mean number of fertile egg masses per 100 bands was very low (0.0) in the year of treatment as well as in the year following treatment (0.3), but rose thereafter (Table 2). Similarly, the mean number of fertilized egg masses per hectare found in the O.OI-ha surveys on the 2L plots were 0.0 and 0.3 in 1990 and 1991, respectively, but rose to 9.1 in 1992 and eventually to 1268 in 1994. Counts under burlap bands as well as in the O.OI-ha surveys increased approximately fourfold between 1993 and 1994 (Table 2). It appears that populations in these plots that had only been treated in 1990 were rapidly approaching densities observed in control plots.

Release Rates. Results of analyses for residual disparlure content in flakes were used to generate linear regressions of residual content with the square root of aging time (Figure 1). The rate at which disparlure was delivered by the flakes declined over the period of male moth flight (about six weeks). For each of the 1990 applications, the average release rate over the first six weeks of the test, calculated from the regression equations, was 0.94 ILg/day/ftake with mean rates during the first and sixth week of 2.31 and 0.49 ILg/day/ftake, respectively. Flakes picked from foliage gave similar values as those from spray cards (data not shown). For the 1991-1993 applications, the calculated average rate was

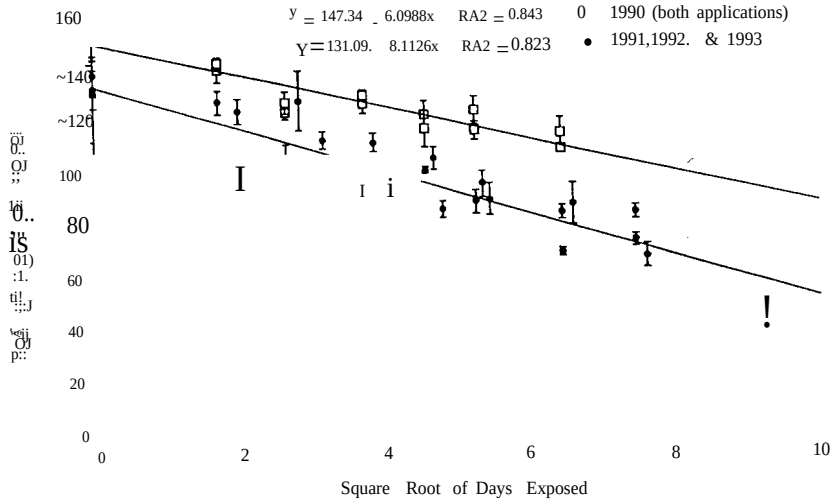


FIG. I. Residual quantity of disparlure in flakes retrieved from spray cards in 1990-1993 field tests in Rockbridge County, Virginia. Linear regression of disparlure content with square root of time (days) exposed. ElTOr bars show standard errors of sample means.

1.25 I-tg/daylflake over the first six weeks with rates during the first and sixth weeks of 3.07 and 0.53 I-tg/day/flake, respectively.

The release rates were used to calculate the amount of disparlure delivered per day per hectare over the course of male moth flight (Figure 2). Since the 2L plots received two applications in 1990, the additive effect is shown in Figure 2A. Each of the two 1990 applications delivered a total of 20.1 g of disparlure over the first six weeks after application for an average rate of 0.48 g/day/ha per application; this is 27% of the applied 75-g dose. The 1991-1993 applications (Figure 2B) each released 30.1 g or 40% of the applied pheromone per hectare over the first six weeks of the test for an average rate of 0.72 g/day/ha. Since male moth flight in Rockbridge County Virginia virtually ceases by the end of July, pheromone released from the flakes six weeks after mid-June application does not contribute to the treatment impact and is essentially wasted. Based on these release rates, about 60-73% of the applied pheromone in the laminate flakes was not released during moth flight in the year of application.

Analysis of flakes retrieved a year later from the 1990 application showed that 2.5 I-tg/flakeof disparlure or about 1.8% of the original dose remained after 12 months of exposure in the field. Therefore, during moth flight in 1991, a

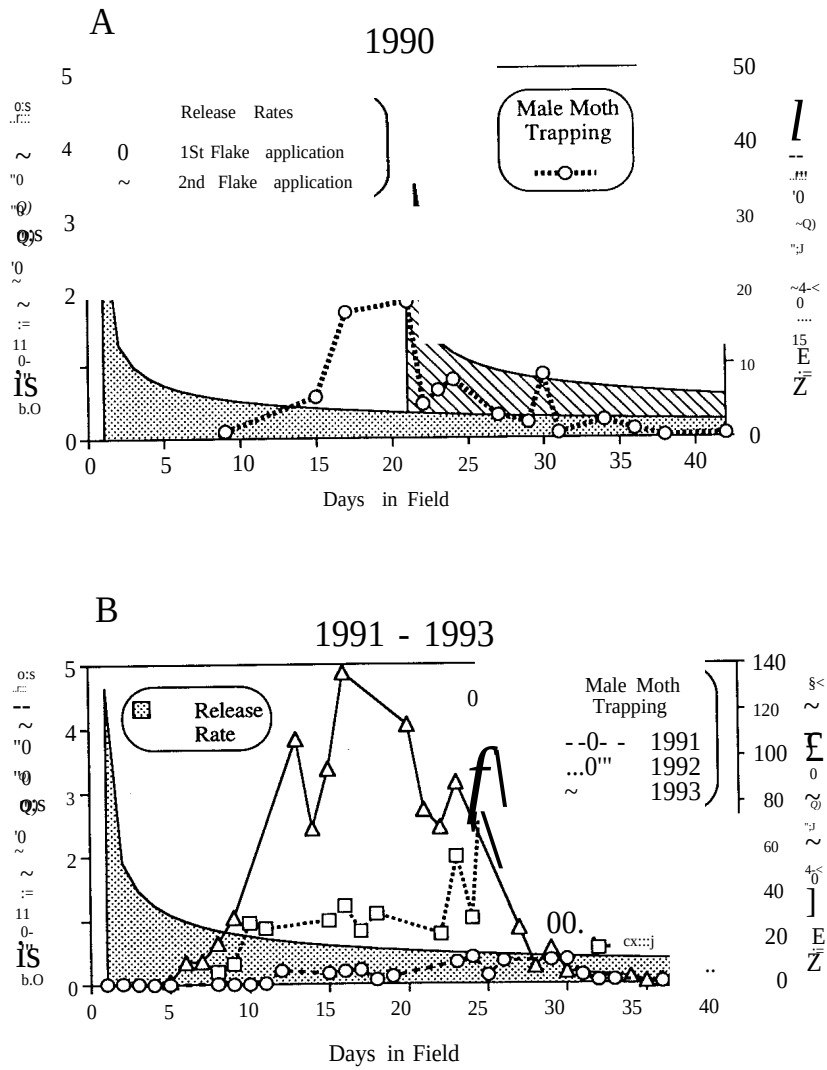


FIG. 2. Amount of disparlure delivered per day per hectare over the course of male moth flight. (A) Single and double applications in 1990. (B) Annual single applications in 1991-1993. Times of male moth flight relative to pheromone application dates are illustrated by overlayed line graphs of trap captures in control plots.

small amount of disparlure remained in the 2L plots that had been treated only in 1990.

DISCUSSION

Previous mating disruption studies with the gypsy moth have attempted to show an impact on populations only in the year of treatment. They often demonstrated that male moth captures in traps could be reduced or entirely eliminated and that mating success could be reduced, although seldom eliminated, in plots treated with pheromone. **In** this study, an attempt was made to study the longer-term impacts on population trends from a single treatment and from yearly treatments. The results of this study demonstrated that population densities were impacted by either type of treatment. Compared to control plots, the abundance of immature life stages and fertilized egg masses, the percentage of mating success of monitor females, and the number of trapped adult males all remained low in plots treated yearly with one application of 75 g disparlure/ha (lL plots). The reduction of monitor female and wild female mating successes indicated there was significant mating disruption in these treated plots in all four years of treatment. Although monitor females were not deployed in these plots in 1994, the year following the last pheromone treatment, the measures of population density showed that the gypsy moth populations remained suppressed.

In the 2L plots, all measures of population density were low in the year of treatment (1990) as well as in the succeeding year (1991). However, in 1992, all measures indicated an upward trend in population density. Nevertheless, it appears that the double application of pheromone in these 2L plots in 1990 resulted in populations that were lower than those in the control plots in 1992 and 1993. Meanwhile, surrounding populations and populations in control plots increased rapidly and eventually reached defoliating levels. Although the woodlots chosen for this study provide some degree of isolation from migrant males and perhaps from ballooning first-instar larvae (McManus, 1973), these plots are not totally isolated. Therefore, it is surprising (and encouraging) that the 2L plots treated only in 1990 continued to show depressed populations for one to three years as compared with the control plots.

In past studies, the absence of trap captures of males has not been equated with the absence of female mating success. It has been speculated that females are more attractive because of chemical or visual cues. Visual cues have been shown (Richerson et at, 1976; Richerson, 1977; Charlton and Carde, 1990) to be important in male close-range orientation. However, more time is generally required for a male moth to enter a trap than to find an exposed female on a tree bole (Mastro et ai., 1977). The longer the required searching time, the higher the probability that a male will discontinue the search and move on. **In**

this study, the monitor females were placed in a sheltered location more like native female sites in a low-density gypsy moth population. However, the shelter did not expose the females and required males to spend more time searching. While mating success of these females may have been greater if they had been exposed, it has recently been shown that mating success in low-density populations is normally low (Sharov et aI., 1995).

Results of this study indicate that low-density gypsy moth populations can be substantially suppressed with annual application of 75 g disparlure/ha. It is yet to be determined what population level (as indicated by egg-mass surveys, numbers of immatures under burlap bands, or male moth trapping) can be targeted for control by the mating disruption technique. The data in this study suggest that, in the year of pheromone application in treated plots, male moth captures may be somewhat unreliable for determining the level of mating success. For example, male trap captures were low in the lL plots in 1993 (0.6 malesltrap), yet an average of 3.9 fertile egg masses/lOO bands and 13.8 fertile egg masses/ha were found. Even low male captures in traps may act as a warning that a treatment did not entirely eliminate mating. Based on the four years of this study, it appears that if a pretreatment survey on O.Ol-ha subplots shows less than two fertile egg masses per hectare, and if the number of immatures (larvae + pupae) per 100 burlap bands averages approximately five or less, it is highly likely that application of pheromone will effectively control female mating.

Male moths are highly mobile and attempts to relate trap captures to other measures of population density have not been successful under all conditions (Liebhold et aI., 1995). Therefore, the mean numbers of immatures and fertile egg masses under burlap bands and the numbers of fertile egg masses found in late-season O.Ol-ha subplot surveys were used to obtain a population index for all nine plots during the five years of this study. The sum of the means of these three population measures (immatures per 100 bands, fertile egg masses per 100 bands, and fertile egg masses per hectare) were plotted versus year (Figure 3) to indicate the population changes over time for the three treatments. From this graph, it is apparent that the single pheromone application in 1990 (2L plots) delayed the population increase by one to four years. Annual pheromone application controlled the population for three years and slowed the rate of increase thereafter.

SUMMARY

This study showed that the population represented by an average male moth capture of 27/trap in the vicinity of the test plots in 1989 was effectively controlled for at least three years by annual pheromone application until neighbor-

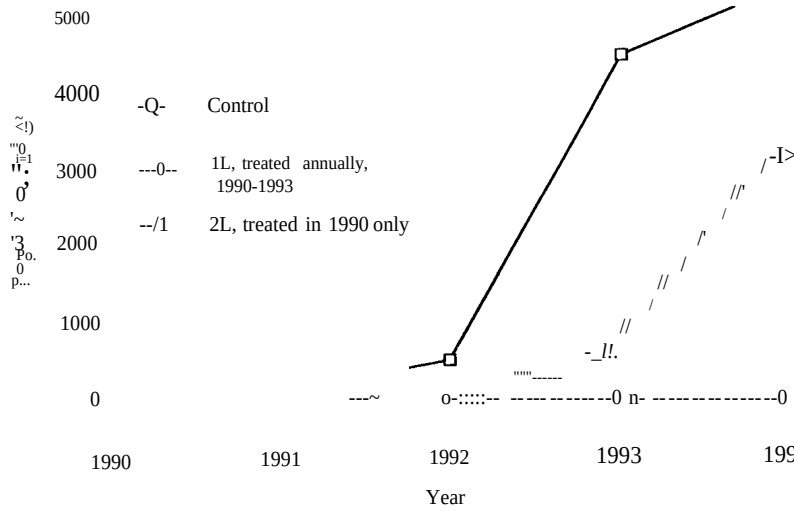


FIG. 3. Population index for all nine study plots (sum of mean number of immatures/ 100 bands + mean number of fertile egg masses/lOO bands + mean number of fertile egg masses/ha) plotted by treatment for each of the five years of the study,

ing, uncontrolled populations exerted too high an influence. However, despite this population pressure from surrounding areas, the single application of 75 g of pheromone/per hectare significantly disrupted mating in each of the four treatment years. This study also showed that treatment with pheromone in only one year (albeit a double application) substantially suppressed the population increase as compared with the controls for three subsequent years. Thus, in properly selected populations, mating disruption with aerially applied pheromone is an effective control method.

The release profile of disparlure from the laminate flakes is chamcteristic of a laminated structure (Kydonieus, 1980) and has the advantage that exact timing of the application just before male flight is not criticaL However, the fact that only 27-40% of the applied pheromone was released during male flight suggests that a controlled-release formulation, which more efficiently delivers its pheromone dose, would allow the total amount of applied pheromone to be reduced by perhaps a factor of two without loss of efficacy. Such a formulation would reduce treatment costs. Work is now being carried out to develop such a delivery system.

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REFERENCES

BERRY, D. A. 1987. Logarithmic transfonnations in ANOVA. *Biometrics* 43:439-456.

BIERL, B. A., BEROZA, M., and COLLIER, C. W. 1970. Potent sex attractant of the gypsy moth: Its isolation, identification, and synthesis. *Science* 170:87-89.

CARROL, R. J., and RUPPERT, D. 1988. Transfonnation and Weighting in Regression. Chapman and Hall, New York.

CAMERON, E. A. 1981. The use of disparlure to disrupt mating, pp. 554-559, *in* C. C. Doane and M. L McManus (eds.). The Gypsy Moth: Research toward Integrated Pest Management. USDA Tech. Bull. 1584, U.S. Department of Agriculture, Washington, D.c.

CHARLTON, R. E., and CARDE, R. T. 1990. Orientation of male gypsy moths, *Lymantria dispar* (L), to pheromone sources: The role of olfactory and visual cues. *J. Insect Behav.* 3:443-469.

GREENHOUSE, W. S., and GEISSER, S. 1959. On methods in the analysis of profile data. *Psychometrika* 24:95-112.

KOLODNY-HIRSCH, D. M., WEBB, R. E., OLSEN, R., and VENABLES, L 1990. Mating disruption of gypsy moth (Lepidoptera: Lymantriidae) following repeated ground application of racemic disparlue. *J. Econ. Entomo/.* 83:1972-1976.

KYDONIEUS, A. F. 1980. Fundamental concepts of controlled release, pp. 1-19, *in* A. F. Kydonieus (ed.). Controlled Release Technologies: Methods, Theory, and Applications, Vol. I. CRC Press, Boca Raton, Florida.

LEONARD, D. S., LEONHARDT, B. A., McLANE, W. H., GHENT, J., PARKER, S. K., ROLAND, T. J., and REARDON, R. C. 1992. Aerial application of racemic disparlure to manage low-level populations of gypsy moth, Giles County, VA, 1989. USDA Forest Service, Northeastern Area State & Private Forestry Tech. Pub!. 04-92, U.S. Department of Agriculture, Atlanta, Georgia.

LIEBHOLD, A. M., ELKINTON, 1. S., ZHOU, G., HOHN, M. E., ROSSI, R. E., BOETTNER, G. H., BOETTNER, C. W., BURNHAM, c., and McMANUS, M. L. 1995. Regional correlation of gypsy moth (Lepidoptera: Lymantriidae) defoliation with counts of egg masses, pupae, and male moths. *Environ. Entomo!.* 24:193-203.

MASTRO, V. c., RICHERSON, J. V., and CAMERON, E. A. 1977. An evaluation of gypsy moth pheromone-baited traps using behavioral observations as a measure of trap efficiency. *Environ. Entomo/.* 6: 128-132.

MAUCHLY, J. W. 1940. Significance test for sphericity of a nonnal n-variate distribution. *Ann. Math. Stat.* 11:204-209.

MCGOVERN, T. E. 1994. USDA, APHIS-PPQ Gypsy Moth Treatment Data Base. U.S. Department of Agriculture, Hyattsville, Maryland.

McMANUS, M. L 1973. The role of behavior in the dispersal of newly hatched gypsy moth larvae. USDA Forest Service Research Report NE-267 , U.S. Department of Agriculture, Atlanta, Georgia.

RICHERSON, J. V. 1977. Pheromone-mediated behavior of the gypsy moth. *J. Chern. Eco!.* 3:291-308.

RICHERSON, J. V., CAMERON, E. A., and BROWN, E. A. 1976. Sexual activity of the gypsy moth. *Am. Mid. Not.* 95:299-312.

SAS INSTITUTE. 1985. *SAS/STAT* guide for personal computers, version 6. SAS Institute, Cary, North Carolina.

SCHWALBE, C. P., and MASTRO, V. C. 1988. Gypsy moth mating disruption: dosage effects. 1. *Chern. Eco/.* 14:581-588.

SCHWALBE, C. P., PASZEK, E. c., WEBB, R. E., BIERL-LEONHARDT, B. A., PLiMMER, J. R., MCCOMB, C. W., and DULL, C. W. 1979. Field evaluation of controlled release formulations of disparlure for gypsy moth mating disruption. *J. Econ. Enlama/.* 72:322-326.

SCHWALBE, C. P., PASZEK, E. c., BIERL-LEONHARDT, B. A., and PLiMMER, J. R. 1983. Disruption of gypsy moth (Lepidoptera: Lymantriidae) mating with disparlure. 1. *Ecan. Enlomo/.* 76:841-844.

SHAROV, A. A., LiEBHOLD, A. M., and RAVLlN, F. W. 1995. Prediction of gypsy moth (Lepidoptera: Lymantriidae) mating success from-pheromone trap counts. *Environ. Enlomo/.* 24: 1239-1244.

WALLNER, W. E., DEVITO, A. S., and ZARNOCH, S. J. 1989. Regression estimators for late-instar gypsy moth larvae at low population densities. *For. Sci.* 35:789-800.

WALLNER, W. E., JONES, C. G., ELKINTON, J. S., and PARKER, B. L. 1990. Sampling low density gypsy moth populations, *in* Proceedings USDA Gypsy Moth Research Review. Annapolis, Maryland.

WEBB, R. E., MCCOMB, C. W., PLiMMER, J. R., BIERL-LEONHARDT, B. A., SCHWALBE, C. P., and ALTMAN, R. M. 1981. Disruption along the "leading edge" of the infestation, pp. 560-570, *in* C. C. Doane and M. L. McManus (eds.). The Gypsy Moth: Research toward Integrated Pest Management. USDA Tech. **Bull.** 1584, U.S. Department of Agriculture, Washington, Dc.

WEBB, R. E., TATMAN, K. M., LEONHARDT, B. A., PLiMMER, 1. R., BOYD, V. K., BYSTRAK, P. G., SCHWALBE, C. P., and DOUGLASS, L. W. 1988. Effect of aerial application of racemic disparlure on male trap catch and female mating success of gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Enlomo/.* 81:268-273.

WEBB, R. E., LEONHARDT, B. A., PLiMMER, J. R., TATMAN, K. M., BOYD, V. K., COHEN, D. **L.,** SCHWALBE, C. P., and DOUGLASS, L. W. 1990. Effect of racemic disparlure released from grids of plastic ropes on mating success of gypsy moth (Lepidoptera: Lymantriidae) as influenced by dose and by population density. *J. Econ. Enlomo/.* 83:910-916.

WILSON, JR., R. W., and FONTAINE, G. A. 1978. Gypsy moth egg-mass sampling with fixed-and variable-radius plots. USDA Handbook No. 523. U.S. Government Printing Office, Washington, D.C.