Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

Mating Disruption

A Review of the Use of Mating Disruption to Manage Gypsy Moth, *Lymantria dispar* (L.)

Kevin Thorpe, Richard Reardon, Ksenia Tcheslavskaia, Donna Leonard, and Victor Mastro

USDA U.S. Department of Agriculture

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Forest Health Technology Enterprise Team—Morgantown

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The Forest Health Technology Enterprise Team (FHTET) was created in 1995 by the Deputy Chief for State and Private Forestry, USDA, Forest Service, to develop and deliver technologies to protect and improve the health of American forests. This book was published by FHTET as part of the technology transfer series.

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Cover photos, clockwise from top left: aircraft-mounted pod for dispensing Disrupt II flakes, tethered gypsy moth female, scanning electron micrograph of 3M MEC-GM microcapsule formulation, male gypsy moth, Disrupt II flakes, removing gypsy moth egg mass from modified delta trap mating station.

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Forest Health Technology
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A REVIEW OF THE USE OF MATING DISRUPTION TO MANAGE GYPSY MOTH, *LYMANTRIA DISPAR* (L.)

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A Review of the Use of Mating Disruption to Manage Gypsy Moth, *Lymantria dispar* (L.)

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Preface

This review is published as part of a joint USDA program conducted by three of its agencies —Forest Service (FS), Agricultural Research Service (ARS), and Animal and Plant Health Inspection Service (APHIS)—to develop specific methods for managing sparse-density populations of the gypsy moth, *Lymantria dispar* (L.). The program is supported through the efforts of these agencies and private industry.

Field and laboratory studies are conducted to solve problems associated with the use of mating disruption to manage sparse-density gypsy moth populations. Also provided is technical assistance to improve the quality of operational programs involving the aerial application of pheromones for managing gypsy moth.

This publication (June 2006), A Review of the Use of Mating Disruption to Manage the Gypsy Moth, *Lymantria dispar* (L.), is an update of handbook FHTET-98-01 printed in January 1998. It contains all of the information included in the January 1998 handbook as well as the results of studies conducted through 2005.

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Contents

INTRODUCTION

Semiochemicals, also called behavior-modifying chemicals, are volatile chemicals emitted by organisms to transmit information to other individuals. Allelochemicals (e.g., kairomones, allomones) are a subset of semiochemicals that operate interspecifically, whereas pheromones are a subset of semiochemicals that operate intraspecifically. Pheromones that act as attractants cause an organism to move towards the chemical source.

Insect pheromones that act as sex attractants show promise for suppressing pest populations through mating disruption. The idea behind mating disruption is to create interference with the sex pheromone emitted by the female to a level at which the male has difficulty locating her.

Mating disruption is accomplished by adding artificial pheromone sources to the environment. The effect is creating an atmosphere concentrated with the attractive material or creating many odor sources. Consequently, the males become confused and are prevented from finding female moths.

Scientists first learned how to synthesize many pheromones in sufficient quantities for field evaluations in the early 1970s. In Europe experiments using pheromones to control major insect pests in fruit orchards were started in 1975. In the United States the first pheromone registered for use as a mating disruptant was for control of the pink bollworm, *Pectinophora gossypiella* (Saunders), in 1978. Ridgeway et al. (1990) did an in-depth review of a wide range of uses for pheromones and other behavior-modifying chemicals.

Preliminary demonstrations of the effectiveness of mating disruption were shown for several forest pests—the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough); western pineshoot borer, *Eucosma sonomana* Kearfott; ponderosa pine tip moth, *Rhyacionia zozana* (Kearfott); several bark beetles; and the gypsy moth, *Lymantria dispar* (L.).

In the late 1980s the trend towards using more environmentally friendly control agents and developing prevention strategies for managing the gypsy moth resulted in greater emphasis on developing mating disruption. Concurrently, the 5-year congressionally mandated Appalachian Integrated Pest Management (AIPM) Gypsy Moth Project was initiated in a 38-county area in West Virginia and Virginia. A major emphasis of this project was developing technology, including mating disruption, for managing sparse gypsy moth populations (less than ten egg masses per acre). The AIPM Project (1998 to mid-1993) was followed by the Gypsy Moth Slow-the-Spread Project (1998 to the present), which was implemented in ten states. The major emphasis of the Slow-the-Spread Project (STS) is to manage sparse gypsy moth populations.

This review is a compilation of historical and current information on the use of mating disruption to manage sparse-density populations of the European strain of the gypsy moth. Included is information on development, registration, and formulations of the synthetic pheromone disparlure, as well as effects on non-target organisms. Previously unpublished studies of efficacy, deposition, and residual activity of disparlure are reported.

MATING BIOLOGY OF THE GYPSY MOTH

In the European strain of the gypsy moth, the adult male is guided to the flightless adult female primarily by pheromone, identified by Bierl et al. (1970) as Z-7,8-epoxy-2-methyloctadecane, also called disparlure. Although zig-zag flight along the plume of disparlure is the primary mechanism for initial orientation, other behavioral cues are important for successful location and recognition of a mate. Visual cues, principally those presented by tree trunks, are important in inducing landing and the walking search for a female. Recognition at close range and mating are evoked by contact cues such as wing tip touching. Thus, although location of the female over long distances is mediated by pheromone, additional visual and contact stimuli are critical to successful reproduction (Charlton and Carde 1990).

The gypsy moth is univoltine, that is, has one generation per year, and the mating season occurs over a 3-6 week period in late summer. Some males mate more than once, and some of the males do not mate at all (Mastro 1995). A small portion (about 20%) of the females in dense populations, and an unknown but probably small percentage in sparse populations, mate more than once. Females are capable of high pheromone release rates for three days but then their ability to release pheromone and mate decreases. Once mated, females stop releasing pheromone and begin ovipositing. Unmated females near the end of their life will lay unfertilized eggs.

MATING DISRUPTION OF THE GYPSY MOTH

The synthetic version of the gypsy moth sex pheromone used in mating disruption is structurally similar to the pheromone produced by the female gypsy moth. A 50:50 mixture of the (+) and (—) enantiomers (molecular structures with a mirror-image relationship) of disparlure has been applied using ground and aerial application methods. In both methods dispensers impregnated with this racemic mixture of pheromone release it slowly into the environment.

The ideal formulation of disparlure would release the pheromone at a constant rate and discharge all of the active ingredient in a specified period of time. The physical characteristics of the formulation and tank mix adjuvants have an effect on the discharge of disparlure. Variable meteorological conditions such as temperature and relative humidity, however, can exert unpredictable influences on disparlure release from a formulation.

For mating disruption to be effective, the synthetic pheromone must be present in sufficient quantities for the entire mating period. Since disparlure is volatile, denser than air, and is dispersed by air currents, complete initial coverage of an area is probably not essential. Due to the vertical distribution of females on tree trunks, however, there is a need for vertical distribution of the pheromone.

Failure of males to locate mates in air permeated with disparlure, probably results from desensitization of the chemoreceptors in the males' antennae, as well as from disorientation by following false pheromone trails or leaving the pheromone treated area (Carde and Minks 1995).

The mating disruption technique is more effective as gypsy moth populations decrease in density, because males locate females primarily by the pheromone at low population densities (Beroza and Knipling 1972, Knipling 1979). At high population densities males can more easily locate females using visual cues, as well as by chance encounters.

The gypsy moth is not an ideal candidate for mating disruption due to the high fecundity of females producing 300-1200 eggs per mass, the polygamous nature of males, and the clumped or aggregated distribution pattern of adult females, which is probably due to their sedentary nature and clustering at suitable larval resting and pupation sites. Nevertheless, some characteristics of the European strain of the gypsy moth are suited to the use of mating disruption, including the flightlessness of females, limited dispersal of the majority of males beyond a few hundred meters, low mating success at sparse densities, and the characteristic of having one generation per year. Unlike the European strain, some recently introduced gypsy moth females of the Asian strain are capable of strong flight, and how this characteristic would influence the usefulness of the mating disruption technique is unknown.

ASSESSING BIOLOGICAL EFFECTIVENESS OF MATING DISRUPTION

The effectiveness of mating disruption of the gypsy moth is assessed biologically on the basis of using several techniques, alone or in combination: counting life stages under bands around tree boles, monitoring flight of males, monitoring females for mating success, examining eggs for embryonation, and conducting egg mass surveys.

Counting life stages under bands—Numbers of larvae, pupae and egg masses under bands (such as burlap) on tree boles at breast height are counted. A decrease in abundance or the absence of larvae and pupae as compared with levels in the previous year indicates effectiveness of mating disruption in the previous year. A decrease in abundance or the absence of viable egg masses in the treated area as compared with an untreated area indicates the effectiveness of mating disruption in the year of treatment. Bands around tree boles tend to bias numbers upward when compared to numbers of egg masses determined using 1/40 -acre plots.

Monitoring flight of males—Traps are baited with 500 µg of (+) - disparlure to attract males during the flight period and deployed at varying densities. If mating disruption is effective, resident (wild) or laboratory-reared (lab) males, with or without feeding on an artificial diet containing a red dye will not be caught because they will not be able to locate the pheromone in the trap just as they will not be able to locate females. In the year disparlure is applied, the number of males captured does not provide an estimate of population density but rather a measure of the effectiveness of communication disruption.

Monitoring females for mating success —Virgin 1-day-old females are placed untethered in shelters—such as modified delta traps (triangular cardboard traps usually used to capture adult male gypsy moths) or tethered and exposed on tree boles. The inability of males to locate monitor females in shelters or untethered on tree boles, or resident wild females from under bands, indicates effectiveness of mating disruption.

Examining eggs for embryonation—Egg masses are collected from monitor females and resident wild females under bands, held an additional 30 days in an outdoor insectary under ambient conditions, and then examined for the presence or absence of embryos. The absence of embryos indicates effectiveness of mating disruption.

Conducting egg mass surveys—A visual search for egg masses is conducted and egg masses are counted. Absence of egg masses or their presence in lower numbers indicates effectiveness of mating disruption.

Traps baited with disparlure have some competitive advantage over females in that traps emit pheromone continuously over the entire mating season. Traps do not, however, present all of the orientation and recognition cues that females do. Therefore a trap catches only 20-30% of the males that visit the area within 3 m of the trap (Carde 1996). Even though reduction in the number of males trapped after application of various disparlure formulations has been equated with reduction in mating success, monitor females provide a more direct measure of mating success. Such monitoring has not been directly related to changes in density of native egg masses, however, mainly because no accurate technique has been developed for quantifying egg mass densities in sparse populations.

DEVELOPMENT OF MATING DISRUPTION, 1971 THROUGH 2005

BEFORE 1989—INITIAL DEVELOPMENT AND APPLICATION

Federal and State agencies and private companies have attempted to use mating disruption to manage populations of the gypsy moth since 1971 (e.g., Granett and Doane 1975, Schwalbe et al. 1974, Stevens and Beroza 1972, Webb et al. 1988). Before 1989 various entities conducted mating disruption activities independently, with mixed results. Many research and operational trials of mating disruption were complicated by numerous problems, such as inconsistent formulations of disparlure, relatively insensitive evaluation techniques, and lack of data on seasonal release rates of disparlure. Nevertheless, the following generalizations were derived from these early efforts at mating disruption (Kolodny-Hirsch and Schwalbe 1990): (1) a direct dose-response relationship exists both for disruption of mating communication and for disruption of mating (Webb et al. 1988), (2) the degree of mating reduction is inversely related to male population density (Webb et al. 1988), and (3) a peak in mating occurs during peak male flight. In addition, in 11 of 15 mating disruption trials with the gypsy moth published between 1972 and 1988, evidence for mating disruption was based entirely on the reduction of male moths caught in traps and on the mating success of laboratory-reared or field-collected females placed in the test plots. In only one report were changes in native population trends statistically tested and shown to be significant (Beroza et al. 1974). In later trials, Webb et al. (1988) demonstrated more consistent efficacy by the use of 75 g AI per ha (30.4 g AI/acre) dispalure applied aerially to disrupt mating.

Numerous formulations containing the active ingredient (AI) disparlure were evaluated during these early years, for example, hollow plastic fibers (Conrel Inc.), gelatin microcapsules (National Cash Register Capsular Products, Stauffer Co., Penwalt Co.), and plastic laminated flakes (Hercon Environmental Inc.). In general, these formulations provided a relatively uniform distribution of pheromone under laboratory conditions. In field tests, however, the disparlure was inefficiently released, and major problems were encountered in the aerial application of these formulations due to the spray systems available for aircraft at the time.

In earlier trials, results were inconsistent and discouraging. From 1983 to 1989, only one commercial product containing racemic disparlure, the plastic laminated flakes Disrupt II (Hercon Environmental Inc., Emigsville, Pennsylvania), was registered by the US EPA for use in mating disruption. Therefore, operational use of mating disruption was limited to use of these layered plastic flakes.

1989—A TRANSITIONAL YEAR

In July 1989, an eradication program was conducted in Giles County, Virginia, with the aerial application of the bacterial insecticide *Bacillus thuringiensis* variety *kurstaki* (B.t.) and the growth regulator diflubenzuron as the primary treatments, and on approximately 2,500 acres disparlure was applied in the form of Disrupt II at a dose of 30.4 g AI/acre (Leonard et al. 1992). The cost of the Disrupt II with customer-supplied racemic disparlure was approximately $50/acre. The mating disruption technique was selected for that portion of the project area where the National Science Foundation had an ongoing study on behavior of dark-eyed junco (*Junco hyemalis hyemalis*). Since part of the diet of juncos is lepidopteran larvae, B.t. or diflubenzuron could not be used.

The project area included uninhabited forest land ranging in elevation from 1,000 to 1,300 m, with oak as the primary overstory vegetation and gypsy moth populations below 10 egg masses per acre. An adjacent untreated area was used for comparison.

The mating disruption portion of this eradication effort used previously developed technology for dispensing and evaluating disparlure but also acquired new data on vertical deposition and release rates of Disrupt II.

Specialized, unmodified equipment developed in the 1970s by Schweitzer Aircraft for applying flakes at the rate of 5-10 g AI/acre for control of pink bollworm and patented in 1984 was mounted, one pod on each side, under the wing of a Cessna 206 (Fig. 1). The dispensing rate of the flakes is controlled by an auger, and the sticker is controlled by a pump and tubing system. The flakes and sticker are mixed in a chamber then dispensed through a spinner (Fig. 2). During gypsy moth suppression projects using an aqueous formulation, this aircraft typically is assigned a 75-foot swath width, but due to the inability of the motor controlling the auger on each pod to deliver sufficient flakes, a 45-foot swath was assigned to realize the desired deposit rate of approximately 41 flakes per square meter (3.7 flakes per square foot).

(Fig. 1 here)

During characterization trials, the flake deposition pattern within the swath was uneven, with peaks occurring directly under the pods and valleys under the fuselage and wing tips. Additionally, the pod motors for augering the flakes and pumping the sticker malfunctioned periodically and bridging (binding) of the flakes in the hopper was a constant problem. During applications, a total of 165 g of flakes (dose of 30.4 g AI), talc powder to prevent bridging of the flakes, and 4 oz of the sticker-extender Gelva-1990 (Monsanto, St. Louis, Missouri) to adhere the flakes to the foliage were mixed and applied per acre. The flake treatment was initiated in July just before anticipated adult male flight. The size of the flake hoppers limited treatment to 125 acres per load at the 30.4 g AI dose.

(Fig. 2 here)

Efficacy. No egg masses were found before treatment (spring 1989) in the pheromone treated and untreated areas, while both areas had similar male moth catches (summer 1988). In 1989 after treatment, no males were captured in the treated area, and 26% of the traps captured male moths in the untreated area. Pheromone traps placed on a 250 m grid for two years after treatment (1990 and 1991) captured one moth on the edge of the pheromone treated area in 1991 and an average of four moths per trap in the untreated area. Both of these areas were trapped from 1992 through 1995 on a maximum spacing of a 1-km grid. In 1995 the pheromone treated area was still relatively free of male moths, while populations in the untreated area had received insecticide treatment in 1993.

Laboratory-strain 10K irradiated sterile gypsy moth females (male parent was irradiated with 10 Krads as a pupa) were used as monitor females. Sterile females were used to satisfy regulatory concerns because the project was located outside the quarantine regulated area. When mated with a normal male, these sterile females produce an egg mass that embryonates, but most eggs do not hatch. If these sterile females do not mate, an egg mass can be produced but it will not embryonate. Monitor females were deployed at 100 stations twice weekly for three weeks coinciding with male moth flight. They were left overnight and retrieved the next day. None of the monitor females were mated in the treated or untreated areas. This led to speculation that sterile females were not appropriate for use as monitors and plans to test an escape resistant mating station in which fertile females could be deployed when working outside of the quarantine regulated area were formulated.

Deposition. In addition to the efficacy results, a bucket truck and ground tarps were used to evaluate deposition of 10 times the normal application of flakes (1 x dose flakes containing disparlure and 9 x dose of blank flakes) applied to a 4 ha (10 acre) site within the disparlure-treated area. This high application rate was used to ensure detection of a sufficiently high number of flakes. Twenty overstory and twenty understory trees were sampled. Flakes were deposited throughout all layers of the canopy, including the understory foliage (Fig. 3). flakes were inventoried at 160 sampling points in the canopy and in 40 ground deposit nets. At application, only 10% of the flakes penetrated all levels of foliage and were deposited on the ground beneath the forest. Over six weeks, an additional 6% of the flakes fell to ground level indicating excellent performance of the sticker (Fig. 4).

(Fig. 3 here)

(Fig. 4 here)

Residual Activity. During aerial application, sections of black roofing paper were placed on the ground in open areas to collect flakes for release rate analysis. These flakes were weathered on the roofing paper placed beneath the forest canopy and collected weekly for eight weeks. Initially the flakes contained 17% disparlure by weight. Results of gas chromatographic analysis of the flakes indicated that they lost approximately 65% of their disparlure content over the first 10 days, 10% over the next 20 days, and 12% in the last 26 days of the test. Moth flight started approximately 15 days after application. This rapid initial release of lure from the flakes was unexpected and prompted additional sampling to confirm suspicions that elevated temperatures on the dark surface of the roofing paper from which the flake samples were collected had increased the release of disparlure. On day 100 after treatment, 20 flake samples were collected from foliage in the sun, foliage in the shade, and roofing paper. On average the flakes collected from foliage contained twice as much lure as the flakes aged on and collected from the roofing paper.

Summary of 1989 Results. The Giles County project was considered a success as only one male moth was recovered from the pheromone treated area for two years after treatment (1990 and 1991). These efficacy results were better than anticipated based on the inconsistent operability of the pods and the non-uniform distribution of flakes. More importantly, the Giles County project renewed interest in the use of mating dissruption to manage sparse- and low-desity populations of gypsy moth.

SECOND STAGE OF DEVELOPMENT AND APPLICATION, 1990-1997

Even though the Giles County project was considered a success, it was obvious that a large cooperative effort needed to be initiated for methods development and operational evaluation of mating disruption. This need led in fall 1990 to the formation of the Gypsy Moth Mating Disruption Working Group, which was composed of members from Federal, State, and county governmental agencies; and private companies.

The newly formed Working Group proposed a series of methods improvement projects for implementation over the next seven years. The broad objectives of these efforts were to (1) refine techniques for evaluating efficacy and equipment for applying Disrupt II, (2) quantify the lowest efficacious dose and number of applications of Disrupt II, and (3) develop an additional formulation of disparlure that could be applied through conventional boom and nozzle spray systems. Therefore, unlike the activities conducted from 1971 through 1989 by individual agencies, activities from 1990 through 1997 represented a coordinated effort among three USDA agencies (Agricultural Research Service, Forest Service, and Animal and Plant Health Inspection Service) and industry. Methods improvement trials are discussed by year in the following sections. Operational uses of Disrupt II are summarized in Table 1.

(Table 1 here)

1990—A New Bead Formulation

The studies initiated in 1990 had two specific objectives: (1) to evaluate the impact of a single application of disparlure applied yearly for each of four years and of an initial double application of disparlure on gypsy moth mating success and population trends over five years, and (2) to field test an additional commercial disparlure formulation that could be applied through conventional boom and nozzle systems. Rockbridge and Augusta Counties in Virginia were selected as the project area for these method improvement evaluations for these reasons: (1) this area was within the AIPM project 2-km grid of pheromone traps, and male moth data were available for 1989 (average 27 moths per trap, range from 11 to 200 per trap); (2) county technical support personnel were already in place; and (3) isolated woodlots were abundant.

Disrupt II

Three woodlots (each 35-160 acres) were treated with two applications of flakes (Disrupt II) in 1990 only, each at a dose of 30.4 g AI/acre per application. The cost of the Disrupt II with customer-supplied racemic disparlure was $25/acre. Treatments were applied just before antici-

Table 1. Acres of operational treatments using disparlure flakes, Disrupt II, applied at 30.4 g AI/acre to manage gypsy moth, 1989 through 1997.

Year	Location	Acres Treated
1989	Giles County, Virginia	2,5000
1990	Sequatchie County, Tennessee	200
1991	Roanoke/Bedford County, Virginia	2,900
	Allegheny/Botetourt County, Virginia	400
	Mercer County, West Virginia	1,700
Subtotal 1989-1991		*7,700*
1992	Bedford/Botetourt County, Virginia	4,829
	Floyd/Carroll County, Virginia	1,000
	Mercer/Monroe/Pocahontas County, West Virginia	4,230
Subtotal 1992		*10,059*
1993	Giles County, Virginia	2,600
	Mercer County, West Virginia	350
Subtotal 1993		*2950*
1994	Craig County, Virginia	775
	Monroe County, West Virginia	3,385
	Raleigh County, West Virginia	270
Subtotal 1994		*4,430*
1995	Pulaski/Giles County, Virginia	2450
	Mercer/Summers County, West Virginia	259
	Franklin County, Ohio	235
Subtotal 1995		*2,944*
1996	Halifax/Dare County, North Carolina	1,900
	Greensville/Southampton County, Virginia	2.926
	Giles/Craig/Botetourt/Roanoke County, Virginia	3,578
	Carroll County, Virginia	425
	Monroe County, West Virginia	3,887
	Mercer County, West Virginia	355
	Unicoi/Carter County, Tennessee	252
	Sheboygan County, Wisconsin	2,200
Subtotal 1996		*15,523*
1997	Southampton County, Virginia	560
	Floyd County, Virginia	567
	Roanoke County, Virginia	5,151
	Allegheny County, Virginia	1,227
	Greenbrier County, West Virginia	100
	Monroe County, West Virginia	1,156
	Summers County, West Virginia	2,052
Subtotal 1997		*10,813*
Total – All Years		**51,719**

pated initial male flight (June 17) and before anticipated peak flight (July 20). Three additional woodlots were treated just before male flight in 1990 and again every year from 1991 through 1993 with a single application of flakes (Disrupt II) at a dose of 30.4 g AI/acre. An additional three woodlots were not treated. All applications of flakes were applied at 165 g per acre with 4 oz of Gelva-1990 as sticker and talc to prevent bridging.

Density of gypsy moth populations were estimated yearly using three techniques: surveying pre- and post-treatment egg mass densities (1/40 acre subplots on uniform 50 m grids); surveying larval, pupal and egg mass densities under burlap bands (checked when most gypsy moth caterpillars reached late instars and pupal stages, and again after the male flight period); and trapping male moths (one trap per 15 acres, with a minimum of three traps in woodlots smaller than 45 acres). To monitor mating success, laboratory-strain females were placed in modified delta trap mating stations in each woodlot at the rate of 30 females three times a week for three weeks during the peak moth flight period. The modified delta trap (not coated on the inside with adhesive, ends open, and female placed on a piece of burlap inside) was found to be the most escape resistant as well as maintained the attractiveness of the female to males (Fig. 5). In addition to the laboratory-strain females placed in mating stations for monitoring, resident wild females under bands were monitored, and their egg masses collected and assessed for fertility within treated and untreated woodlots. A total of 1,165 1/40-acre subplots (@ 4.0% of total woodlot area) was established for egg mass surveys (Leonhardt et al. 1996).

(Fig. 5 here)

To assess relative competitiveness, laboratory-strain and resident wild females were deployed in a moderately dense population of gypsy moth located to the north of the study woodlots in Rockbridge County.

Efficacy. Population densities were significantly reduced by either the double application in 1990 or single application every year from 1991 through 1993. Compared with untreated woodlots, the abundance of immature life stages and fertilized egg masses under burlap bands, the percentage of mating success in monitored females, and the number of trapped adult males all remained low in woodlots treated yearly with one application of 30.4 g AI/acre (Leonhardt et al. 1996). The low degree of mating success in monitor and wild females indicated there was significant mating disruption in those treated woodlots in all four years of treatment. Although monitor females were not deployed in these woodlots in 1994, the year after the last pheromone treatment, all of the other measures of assessment showed that the gypsy moth population remained suppressed. In the woodlots receiving a double application, all measures of population density were low in the year of treatment (1990) and one year after treatment (1991). In 1992, however, the population began an upward trend although at a reduced rate of increase as compared with the untreated woodlots (Leonhardt et al. 1996). The evaluation of resident wild and laboratory-strain females in 1990 demonstrated similarity in their attractiveness, behavior and mating success. Therefore only laboratory-strain females were recommended for use in all future work.

Residual Activity. To evaluate consistency of Disrupt II over years, the release rates for the 1989 (used in Giles County) and 1990 (used in Rockbridge County) Disrupt II products were determined after multiple passes over 23 by 30 cm (9 by 12 inch) white canvas-coated paper cards (Strathmore Paper Co., Westfield, Massachusetts) placed on the ground in an open area at the airport. The cards were allowed to dry and were hung vertically on string beneath the forest canopy for aging. The amount of disparlure per flake was determined as a function of duration of exposure. Using this data, the calculated release rates for both products were slow and uniform over time and did not differ significantly (Fig. 6). Both the 1989 and 1990 Disrupt II products released only approximately 50% of their racemic disparlure content after 42 days, which is the approximate duration of the male moth flight period.

(Fig. 6 here)

Bead Formulation

AgriSense (Fresno, California) developed a new polymeric flowable bead formulation (beads were 400 to 800 microns in diameter) for mating disruption of pink bollworm in cotton. To determine the release characteristics of this bead formulation containing disparlure bound in an acrylic polymer matrix, laboratory evaluations were conducted by Leonhardt (ARS, Beltsville, Maryland) in 1990. The bead formulation released disparlure at a faster rate than did the flakes but field release and efficacy data were needed to support laboratory results.

Efficacy. A field test was proposed using this flowable bead formulation containing disparlure (AGRIS-1029). The bead formulation was to be applied to a 10-acre woodlot, to evaluate efficacy, and deposition and release rate profiles on foliage. A similar 10-acre woodlot was treated with flakes (1990 Disrupt II). The beads contained 40% AI (by weight), and ranged in diameter from 50 to 800 microns with a volume median diameter (VMD)—the droplet size that divides the spray volume in half—of 275 microns. The tank mix consisted of 75 g of beads, 124 oz water, 2% (by volume) Gelva- 1990, applied at 1 gal per acre to yield a dose of disparlure of 30.4 g AI/acre.

The application was attempted with a small fixed wing aircraft (Ag Cat) equipped with standard spray booms and flat fan nozzles. This aircraft spray configuration had been used effectively to apply the flowable bead formulation for pink bollworm, although the desired dose rate of 30.4 g AI/acre of disparlure (AGRIS-1029) required the application of between 21 to 53 times the amount of beads per gallon of tank mix as had been used effectively for pink bollworm.

Numerous problems were encountered during airport characterization trials: (1) beads collected on the flat fan diaphragms, and the nozzles would not shut off, (2) beads clumped together clogging the nozzles, boom and pump motor, and (3) beads would not stay suspended in the tank mix. Eventually the proposed 10-acre treatment had to be abandoned and a few trees near the airport were sprayed.

Residual Activity. To characterize release rates, approximately fifty 23 by 30 cm (9 by 12 inch) canvas coated paper cards were placed on the ground in an open area at the airport, and each group of 50 sprayed repeatedly with the bead or flake formulation. The cards were allowed to dry and were hung vertically on string beneath the forest canopy for aging. Samples (three to five cards) were taken after 0, 3, 7, 14, 21, 28, 42, and 56 days. Analyses were conducted on a Model 6C-9A gas chromatograph (Shimadzu Instruments, Columbia, Maryland) with results calculated as micrograms per flake or per bead. Residual disparlure in beads and flakes recovered from the spray cards showed that the bead formulation delivered about 3-4 times as much disparlure to the air over the 42 days of the test than did the flakes (Fig. 7). Although the beads released considerably more lure than the flakes, more than 70% of it was discharged prior to the start of male flight (day 10). The accumulated disparlure delivered by both formulations showed the beads released 22% of their content and the flakes 14% of their content during peak moth flight.

(Fig. 7 here)

Over 42 days, the beads released more than 90% of their total disparlure content, but the release was not at a constant rate. Over the same time period the flakes released 23% of their total lure at a slow but constant rate.

Deposition. During the airport trials, a few trees were sprayed with the bead formulation, to evaluate its adhesion to foliage. Of the total number of beads that originally adhered to foliage only approximately 20% remained 18 days after application.

Laboratory Evaluations. In fall 1990, an evaluation was conducted in the laboratory (APHIS Otis Methods Development Center) using the bead formulation to identify a suitable viscosity modifier (Soilserve, Surfix, Induce, Blendex, Penetration, Polyox, Nalquatic, StaPut, Van-Gel B, Mist Control, Rhodopol, and Natrosol) to suspend the beads in the tank and a sticker (Bivert, Poly AG, Clear Spray, No Foam, Bond, and Spray Fuse) to adhere the beads to foliage. Various tank mixes were applied to potted red oak seedlings and allowed to dry for various times before the application of rain. Before and between the rain events, the number of beads present within marked areas on replicate leaves were counted visually under a microscope.

The viscosity modifiers Nalquatic and StaPut (Nalco Chemical Co., Naperville, Illinois) provided favorable suspension of the beads. Nalquatic and StaPut acted as thickening agents and when each was combined with 2% Bond sticker (Loveland Industries, Greeley, Colorado) made the most promising tank mixes.

After the most promising tank mixes were identified, additional laboratory tests were conducted to determine if any of the tank mix additives significantly affected release of disparlure from the beads. All of the additives slightly reduced the initial release rate from the beads but there was no significant differences in release rates between the additives.

Additionally, the influence of bead size on release rate was investigated. As expected, the smaller beads released disparlure faster than did the larger beads.

Summary of 1990 Results. Results of trials conducted during 1990 indicated that the flakes were efficacious, releasing disparlure at a constant but slow rate. Problems persisted (e.g., bridging of flakes, uneven deposition of flakes beneath the aircraft) (Fig. 8) using the unmodified application equipment. The flowable bead formulation released disparlure erratically and too fast, probably because the majority of the beads were too small (106-205 microns). The rapid release of lure documented in 1990 led to the conclusion that double applications of beads, 10 to 14 days apart, would be required in all subsequent applications in order to cover the entire flight period with adequate disparlure to disrupt mating. The bead tank mix plugged standard aircraft boom and nozzle systems (e.g., accumulated under the nozzle diaphragm, and beads did not remain suspended). Subsequent laboratory tests identified 2% Bond sticker combined with either Nalquatic or StaPut as promising tank mixes. All additives slightly reduced the initial release of lure, but there were no differences in release among the tank additives.

(Fig. 8 here)

1991—Methods Development for the Bead Formulation

Airport Trials. In January 1991, a series of field trials were conducted at the APHIS Aircraft Operations facility in Mission, Texas. The objectives of the trials were to evaluate (1) tank mixes that provided favorable deposition, rainfastness and suspendability of beads in laboratory evaluations in 1990, and (2) various types of dispensers for application of the beads.

The viscosity modifiers Nalquatic (0.25%) and StaPut (20%) with Bond sticker (2%) provided favorable suspendability and deposition of beads when tank mixes were aerially applied to 23 by 30 cm (4 by 5 inch) Kromekote paper spray cards. Several nozzle systems (e.g., hollow cone, open pipes extending from the boom) were evaluated for sprayability of beads with CP nozzles (C and E Enterprises, Mesa, Arizona), performing with minimal clogging (Fig. 9).

(Fig. 9 here)

Efficacy. In June and July 1991, six blocks, each approximately 50 acres, were treated in Rockbridge County, Virginia: three blocks with a double application (12 days apart) of beads containing 40% racemic disparlure at a dose of 30.4 g AI/acre per application (75 g beads per acre per application) and three blocks with a double application of Disrupt II at 30.4 g AI/acre per application (165 g flakes per acre per application). The beads were applied using a Cessna 188 (AgTruck) equipped with six CP nozzles, 75 ft swath, and tank mix consisting of 20% StaPut, 3% Bond, and 77% water. A Cessna 206 equipped with two pods was used to apply the flakes using a 45 ft swath and tank mix consisting of 4 oz of Gelva- 1990 per acre. Three additional blocks were not treated.

Prior to 1991, the diatomaceous earth that Hercon adds to the flakes to reduce static electricity during the manufacturing process was sifted out prior to final packaging of the Disrupt formulation. Starting in 1991, some of the diatomaceous earth (12-14% by weight) was left in the flakes in order to prevent bridging of the flakes in the hoppers. This eliminated the need to add talc (12 oz. talc/10 Kg flakes) to the hoppers.

AgriSense adjusted the bead size so that there were fewer beads in the 106-250 micron range and more beads in the 300-425 micron range (Fig. 10). The objective was to reduce the release rate of disparlure from the beads (Fig. 11) without creating so large a bead as to cause clogging in the spray system as well as problems with large beads adhering to foliage.

(Fig. 10 here)

(Fig. 11 here)

The 1991 post-treatment efficacy results for the bead treatment indicated that it was effective, as no fertilized egg masses were found under burlap bands or in 1/40-acre subplots and none of the monitor females were mated. The bead treatment effect evident in 1991 was not evident in 1992, one year after treatment.

The 1991 posttreatment efficacy results for the flake treatment were complicated by gypsy moth populations expanding in the general area surrounding the northernmost block (fertile egg masses were recovered in and surrounding the treated block) while no fertile egg masses were recovered in the other two flake-treated blocks. Also, none of the monitor females in the flake-treated blocks were mated. In 1992, no treatment effect was evident.

Deposition. As part of the 1991 evaluations, two forested sites received 10 times the normal application of flakes or beads to allow recovery for determination of sticker performance on foliage. The formulations were applied in the same tank mixes used on the efficacy blocks. On day 12, coinciding with the start of peak flight, 79% of the flakes and 68% of the beads remained attached to foliage. On day 56 after application, only 59% of the flakes and only 28% of the beads remained attached to the foliage, due to failure of the sticker.

Residual Activity. Emission rates of disparlure from beads or flakes were essentially the same whether the sample was collected from foliage or canvas coated cards. On day 42 after application, the flakes still contained about 60-70% of their original disparlure content (Fig. 12A) and on day 88 in late September the flakes still contained 40% of their original lure content. Additional analysis showed that 2.5 µg per flake of disparlure or about 2% of the original dose remained after 12 months of exposure in the field. In contrast, on day 42 after application, the beads still contained about 10-15% of their original disparlure content (Fig. 12B). Although the release rate from the beads was relatively rapid, the new formulation of larger beads was effective in slowing the release of lure when compared with the 1990 formulation. The two applications provided high amounts of disparlure during peak male moth flight, however, the amount of disparlure released over 90 days was about threefold higher from the beads than from the flakes (Fig. 13A). At peak moth flight, the beads released a maximum of approximately 1.9 g per day per acre while the flakes released 0.6 g per day per acre (Fig. 13A). Although the beads are more efficient than flakes at discharging lure, the rate of discharge is not constant over time. The discharge rate from a single application (Fig. 13B) of beads drops from about 1.4 g per day per acre initially to about 0.8 g per day per acre after one month (June 17 - July 18). In comparison, the release rate from the laminate flakes over the same period in 1991 was nearly constant at 0.3 g per day per acre from a single application (Leonhardt et al. 1992).

Flake Dispersal System. In November 1991, a contract for $2,000 was awarded to K&K Aircreaft (Bridgewater, Virginia) to modify the flake dispensers or develop another system for use on larger aircraft.

(Fig. 12 here)

(Fig. 13 here)

Summary of 1991 Results. Results of trials conducted during 1991 indicated that standard spray booms with CP nozzles were usable for applying beads, and the 20% StaPut and 3% Bond tank mix provided improved suspendability and adhesion of the beads to foliage (over the Gelva-1990 tank mix), although additional carriers needed to be evaluated for use with the beads. The flakes were not sticking to foliage as well as in the past, probably due in part to the increased volume of diatomaceous earth (12-14% by weight) in the Disrupt II formulation, which was effective in preventing bridging. Also, the unmodified pod application equipment continued to perform erratically in spite of minor mechanical adjustments to upgrade the augering systems and in spite of the application of a Teflon coating to the spinner blades on each auger to prevent buildup of flakes and sticker. The larger beads produced for the 1991 season were effective in slowing the release of disparlure. The more efficient release profile of the beads compared with flakes means there is potential for reducing the total dose of beads while maintaining a daily emission rate equivalent to the standard lure (30.4 g AI) of flakes. Results of trials conducted during 1991 indicated that the flakes and beads were efficacious in the year of treatment but not one year after treatment.

Although the flakes still contained 40% of their disparlure after 88 days in the field, at the start of the next year they contained only 2% of their original disparlure content and were assumed not to emit enough disparlure to affect mating in the year after application. Sampling from cards yielded the same release rates as sampling from foliage, therefore all subsequent tests used card samples only.

All lab and field trials prior to and including 1991 used previously produced racemic dispalure provided to Hercon by ARS, APHIS, and the Virginia Department of Agriculture. The supply of "old" disparlure was depleted; therefore, in 1992, a contract was awarded to MTM Corporation to produce "new" racemic dispalure.

1992—Dose-Response Evaluations

Airport and Laboratory Trials. In April 1992, the previously used viscosity modifying agent StaPut at 20% volume with 3% Bond sticker and another sticker-extender, TX-7719 (Nalco Chemical Co.), at 10% volume without Bond were each mixed with the beads to evaluate ease of mixing, suspendability of beads, and sprayability and weathering on spray cards and potted oak seedlings. These studies were conducted at the APHIS Aircraft Operations facility. The results showed that TX-7719 provided suspension of the beads comparable to that provided by StaPut, but more importantly once the TX-7719 had dried wash-off was minimal (less than 5%) even with rainfall events of more than 1 inch. The effect of TX-7719 on the discharge of disparlure from beads was evaluated in the laboratory and results indicated that beads mixed with TX-7719 release disparlure at the same rate as when mixed with StaPut or water only when held in an environmental chamber at 35° C for 30 days. Plugging of the CP nozzles occured, and to prevent this, the CP nozzles had to be attached directly to the boom (no restrictors between the boom and nozzles).

Efficacy. A pre-season (June) evaluation of dose versus degree of mating disruption (dose-response) was set up to determine the minimum rate of disparlure (grams per day per acre) that is effective in disrupting mating. The doses of racemic disparlure were 30, 15, 6 and 3 g AI/acre, with one application per block per dose. One block was not treated. Of the average block size of 200 acres, only the center 20 acres were used for evaluation. The bead formulation (instead of the flake formulation) was selected for evaluation because of its rapid release characteristics and because it would provide more "point sources" of pheromone than would the flake formulation at a given dose. An AgTruck equipped with six CP nozzles was used to apply the beads in a 60 ft swath at 1 gal per acre (90% water and 10% Nalco TX-7719). Over the course of the 8-week evaluation a simulated population of 10 gypsy moth mating pairs per acre (10 mating pairs per acre at each of four times over a 2-week interval) was created by deployment of newly emerged adults from laboratory-strain pupae supplied by APHIS and held outdoors at the evaluation site until eclosion. Males were released on tree boles and females were placed in mating stations (Leonard 1994).

All doses appeared to suppress mating to a similar extent. Only 0.05 % of the egg masses recovered from the treated blocks were fertile while 24% of those recovered from the untreated block were fertile. These results were unanticipated and confusing based on the wide range of doses and previous efficacy results of other investigators, although the combination of an evenly distributed simulated low population versus a clumped or aggregate distribution in nature and cold rainy weather probably limited adult activity and confounded the sensitivity of the evaluation. Of the 720 males released into the untreated block only 67 (9.3%) were recaptured. No males were captured in any of the treated blocks.

Under more typical summer weather conditions, the test was repeated with essentially the same results.

A post-season (August) dose-response evaluation was repeated using doses of 30, 15, 6, and 3 g AI/acre. The percentage of fertile egg masses recovered was .69% in the treated blocks and 43% in the untreated block. Of the 720 males released into the untreated block only 139 (19.3%) were recaptured. No males were captured in any of the treated blocks.

An additional efficacy evaluation was conducted during the gypsy moth mating season (July) to determine whether two applications of beads at 6 g AI/acre would disrupt mating. This dose was selected based on 1991 release rates: an application of beads at 6 g AI/acre initially released disparlure at the same rate as an application of flakes at 30.4 g AI/acre and was more cost effective. A second application of beads, 10-14 days after the first application, was needed to augment the dose of racemic disparlure since the beads release their pheromone content quickly. Also, the 6 g AI/acre dose for each of two applications was considered economically competitive ($8 - $13 for AI and formulation for 12 g dose) with other operational control options ($15 per application per acre for the gypsy moth nucleopolyhedrosis virus product Gypchek and $5 per application per acre for B.t.). Six blocks (three treated and three untreated) were established in Rockbridge County, Virginia.

In 1992, fertile egg masses per acre were 0 in all three treated blocks compared with an average of 3.6 fertile egg masses in all three untreated blocks. The percentage of monitor females mated was substantially reduced by the treatment, but mating was not entirely disrupted as fertile egg masses were recovered from under burlap bands in one of the bead plots in the year of treatment. In 1993, two of the treated blocks had 0 fertile egg masses per acre and the other 12.6, whereas the untreated averaged 28 egg masses per acre.

Deposition. Sticker performance of two bead tank mixes (10% TX-7719, and 20% StaPut and 3% Bond) and one flake tank mix (Gelva- 1990 at an increased rate of 6 oz per acre) was evaluated on blocks, treated at 30.4 g AI/acre. Each tank mix was applied multiple times to ensure good coverage. Eight days after treatment and coinciding with the start of moth flight, 80% of the beads and TX-7719; 89% of the beads, StaPut and Bond; and 93% of the flakes remained adhered to foliage. At the conclusion of the test (44 days after treatment), 63% of the beads and TX-7719; 60% of the beads, StaPut and Bond; and 71% of the flakes remained adhered to foliage.

Residual Activity. Sets of 35 canvas coated cards were sprayed with beads mixed with 10% TX-7719, or with 20% StaPut and 3% Bond and hung in the woods for periodic collection, to determine disparlure release rates. The beads in both tank mixes released lure at approximately the same rate and at a slightly slower rate than in 1991, probably due to the fact that the bead size distribution again shifted slightly upwards. Both tank mixes released approximately 50% of the lure by day 20 and 70% of the lure by day 42.

Flake Dispersal System. In March 1992, the newly developed system was evaluated on a Twin-Beech aircraft. The deposition and functioning of the prototype unit was promising; therefore, another $2,000 was awarded to K&K Aircraft (April 1992) to continue the development of this prototype flake application system: an additional spinner unit needed to be added beneath the fuselage.

Summary of 1992 Results. In and out of season tests using simulated populations of 10 mating pairs per acre indicated that a 3 g AI/acre dose of beads was equally effective as a 30 g AI/acre dose in disrupting mating. The beads applied at 6 g AI/acre for each of two applications during the normal gypsy moth mating season provided mating disruption. Results of trials conducted during 1992 demonstrated that the sticker-extender TX-7719 provided adequate suspension of beads and provided equal adhesion of beads to foliage and release rates as did the 20% StaPut and 3% Bond mixture. For all subsequent tests with beads TX-7719 was used as the sticker-extender because less volume is required which results in less cost. The bead size shifted unpredictably upward again in 1992 bringing to attention the fact that different bead lots are not exactly repeatable in terms of bead size distribution or release rates.

The unmodified application pods used to apply the flakes continued to malfunction. The new system being developed by K&K Aircraft to apply the flakes from small (e.g., AgCat) and large (e.g., Twin Beech) aircraft was evaluated and, given promising results, an additional contract was awarded to K&K.

Flakes applied in 1992 were mixed with 6 oz (instead of 4 oz as in the past) Gelva-1990 per acre based on 1991 results that the flakes were not sticking as well as in the past, perhaps due to the 12-14% (by weight) diatomaceous earth, and now contained 5% (by weight) diatomaceous earth. This reduction in diatomaceous earth and increase in sticker volume improved the adhesion of flakes to foliage when compared with flakes applied in 1991.

Hercon Environmental received re-registration for its Disrupt II Gypsy Moth Mating Disruptant (EPA Reg. No. 8730-55) in March 1992. Previously registered Disrupt II (EPA Reg. No. 8730-46) was cancelled on October 10, 1989, for non-payment of fees. All applications of Disrupt II from 1990 through 1991 were performed under EUPs.

In 1992, costs for the operational use of Disrupt II were for sticker (18¢/acre), flakes (customer-supplied disparlure) ($7.20/acre), application costs ($4.23/acre), and an observation plane (50¢/acre).

1993—Reduced Doses of Disparlure

Efficacy. To determine the efficacy of reduced doses of disparlure, a trial was conducted in Rockbridge County, Virginia, using a total of 12 blocks: four blocks treated with beads applied at 6 g AI/acre (i.e., 15 grams of 40% AI formulation per acre) per application (i.e., using six CP nozzles without in-line screens, 90% water and 10% TX-7719 for a total spray of 1 gallon per acre) in two applications; four blocks treated with flakes at 20 g AI/acre with approximately 5% diatomaceous earth and 6% Gelva-1990 in one application; and four blocks untreated. The 6 g AI/acre per application dose of beads applied in two applications was used because results were favorable in 1992. These blocks used for the 1993 evaluations had been used as untreated blocks for previous pheromone evaluations (Thorpe et al. 1999). The treatments were blocked on the basis of population density (numbers of immature life stages under burlap just prior to treatment). Two of the four replicates in each treatment had more dense populations than would normally be selected for mating disruption.

Mating was disrupted in all treated blocks (greatest in the two replicates with lowest pre-treatment densities) when compared with untreated blocks, although fertile egg masses were recovered in two flake-treated blocks and three bead-treated blocks. An average of one male moth per trap was captured in the flake-treated blocks, three male moths per trap in the bead-treated blocks, and 200 male moths per trap in the untreated blocks. In general, there were no differences in efficacy between the bead or flake treatments. After treatment egg mass surveys conducted in 1993 yielded an average of 28, 6.7 and 4.4 egg masses per acre respectively in the untreated, flake and bead treated blocks (Thorpe et al. 1999). In 1994 the gypsy moth populations in the untreated blocks continued to increase above 1993 levels (average 402 males per trap and 63 egg masses per acre) and increases also occurred in the treated blocks (an average 38 males per trap and 13.9 egg masses per acre in flake-treated blocks, and an average 107 males per trap and 11 egg masses per acre in bead-treated blocks). In 1995 populations in all treated and untreated blocks decreased sharply, probably as a result of the increased incidence of the fungus *Entomophaga maimaiga* and nucleopolyhedrosis virus in the general area (Thorpe et al. 1999).

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In 1992, costs for the operational use of Disrupt II were: sticker, 18¢/acre; flakes (customer supplied disparlure), $7.20/acre; application costs, $4.23/acre; and observation plane, 50¢/acre.

Deposition and Residual Activity. Release rates were evaluated by aerially applying flakes with 6 oz of several sticker-extenders (Gelva-1990, Gelva-2333) and a Nalco product (RA-8554) to 23 by 30 cm (9 by 12 inch) canvas coated cards for analysis of residual disparlure content and to foliage for sticker performance. Monsanto management decided not to manufacture Gelva-1990 beyond this year; therefore, evaluation of other products was needed. Gelva-2333 is an acrylic multipolymer emulsion that has two major components: 1) an adhesive agent and 2) a surfactant. These components are designated “exempt from the requirement of tolerance when used as an inert ingredient in a pesticide formulation applied to growing crops,” which is an important consideration for the broad application of Disrupt II. The cards were hung in a nearby wooded area for aging. Periodically, three to five replicate cards for each treatment were removed for determination of residual disparlure content in the flakes and beads. Beads applied at 1 gal per acre with 10% TX-7719 were sprayed on canvas-coated cards for analysis of residual disparlure content as well as on foliage to evaluate adhesion.

Gelva-2333 was selected to replace Gelva-1990 based on good performance as a sticker and no noticeable changes in release characteristics. At 13 and 47 days after treatment, 90% and 84% of the flakes and Gelva-2333 were still adhered to foliage. This compared favorably with the flakes and Gelva-1990 mix where 88% and 72% were still adhered to foliage on days 13 and 47. The Nalco product (RA-8554) did not perform well, with only 51% and 28% of the flakes remaining at days 13 and 47. The beads and TX-7719 mix did not perform as well as in the past with only 57% and 31% of the beads still adhered 13 and 47 days after treatment.

Shin-Etsu Chemical Co. (Tokyo, Japan) provided a batch of an experimental slow release "gypsy moth powder" formulation and a commercially produced carrier for mixing with the powder in the aircraft hopper. The tank mix was foamy and the formulation powder appeared to float on top. The powder contained racemic disparlure (9.5% AI) and was evaluated only for release rates on canvas cards.

In April 1993, the original unmodified pod units were modified by Harold Miller of Harold's Flying Service (Leland, Illinois) and evaluated for eveness of deposition of flakes across the swath and uniformity of flow rate. Additional modifications to the system were needed prior to use in the field.

The K&K Aircraft prototype application system for dispensing the flakes, which now consisted of one augering unit mounted beneath each side of the front wing and one unit beneath the fuselage, was developed for use on a Twin Beech with the potential of carrying sufficient flakes to treat approximately 3,000 acres per aircraft load (Fig. 14). The system was evaluated at their facilities in February 1993. The swath width was 125 feet with peaks and valleys of deposition beneath the aircraft. This system needed 1) additional development to prevent clogging of the auger with flakes and sticker, and 2) larger holding tanks for the sticker in the fuselage.

(Figure 14 here)

Since Disrupt II was being applied to large acreages, a question was raised about its toxicity to aquatic invertebrates. In the original disparlure registration submission, dated 1 December 1978, Hercon had requested a waiver for the acute invertebrate (*Daphnia*) toxicity study. The registration was granted in August 1979 with the waiver request accepted. Hercon products containing disparlure that followed were based on the original submission. This includes Disrupt II, which does not have a *Daphnia* toxicity study.

Summary of 1993 Results. Results of trials conducted during 1993 demonstrated that lower doses of disparlure (beads applied at 6 g AI/acre in each of two applications and flakes applied at 20 g AI/acre in one application) suppressed mating when compared with untreated blocks but did not prevent the production of some fertile egg masses in the blocks with higher population densities. Therefore, there was a need to reevaluate these doses of disparlure against low density populations. Gelva-2333 was selected to replace the soon to be discontinued Gelva-1990 as a sticker for the flakes. Suspension of the Shin-Etsu powder when mixed with the carrier and water in the tank was unsatisfactory. Also, the pheromone release rate from the powder was more rapid than the release rate from beads. These unfavorable characteristics resulted in a request to Shin-Etsu to modify their formulation and carrier before the initiation of future trials. The prototype system for dispensing flakes from large aircraft developed by K&K Aircraft needed extensive modification (e.g., to widen the effective swath and achieve a more uniform distribution of flakes across the swath), but the modification was not pursued due to anticipated limited use of large aircraft to apply Disrupt II over large areas in the next five years. As a result, dispenser development was discontinued.

1994—Increased Doses of Disparlure

Efficacy. Twelve blocks, four per treatment, were established farther south in Rockbridge County, Virginia, where there were less dense gypsy moth populations as determined by the capture of fewer male moths. These blocks were not isolated woodlots as were used in the past, but part of the general forest. Each block was approximately 100 acres with only the center 20 acres used for evaluation. The treatments were (1) beads at 15.2 g AI/acre with 10% TX-7719 (now designated Biogrip or 93SD155) in 1 gal per acre for each of two applications; (2) flakes at 30.4 g AI/acre (165 g of flakes and 5 g of diatomaceous earth per acre) with 6 oz Gelva-2333 per acre for one application; and (3) untreated. Increased doses for both flakes and beads were used in 1994 because in 1993, even though lower doses of disparlure in both flakes and beads suppressed mating, fertile egg masses were produced in blocks with higher population densities. The beads manufactured for use in 1994 again increased in size. This emphasized the continuing problem of variability in bead size distribution between manufacturing lots.

The treatment blocks were established on the basis of the number of life stages found under burlap bands just prior to treatment.

The 1994 posttreatment results indicated that gypsy moth populations were effectively suppressed by both formulations; however, neither treatment was 100% effective in all blocks (Thorpe et al. 1999).

Deposition and Residual Activity. The release rate and stickability of the larger beads were compared with the 1993 beads for release rate of disparlure on canvas cards and for deposition on foliage. The beads were applied at the 30.4 g AI/acre dose in 1 gallon per acre with 10% Biogrip.

No differences were detected in the percentage of disparlure released over 30 days for “old” beads (77%) and “new” beads (74%). Also, there was no detectable difference in adhesion to foliage.

In 1994, some nozzle clogging was encountered using the larger beads. After each load the CP nozzles were cleaned, and usually at least one of the outer nozzles was completely plugged.

In 1994, the US EPA adopted a new policy allowing the testing of pheromones in solid matrix dispensers on no more than 250 acres to proceed without the need for an EUP (the previous limit being 10 acres). Unfortunately, pheromone products formulated in beads or flakes and intended for broadcast application are not covered by the rate because of specific environmental concerns.

Summary of 1994 Results. Results of methods improvement trials conducted during 1994 demonstrated that the bead and flake treatments suppressed population in the treatment year (1994) as determined by the number of fertile egg masses and egg masses per acre recovered in the treated versus untreated blocks (Leonhardt et al. 1995). There were no detectable differences between the 1993 beads and the 1994 beads based on release rates and adhesion to foliage. Unfortunately, the 1994 beads applied at 15.2 g AI with 10% Biogrip clogged CP nozzles. Preliminary thoughts concerning the cause of this problem were larger sized beads, unpublished change in the Biogrip additive, pH of the water in the tank mix, or a combination of these factors. Biosys is now the producer of the beads (Agris-1029), and AgriSense is a division of Biosys. The original unmodified pods manufactured for Hercon by Schweitzer aircraft in the 1970s were no longer reliable for use. The flake applications for the methods improvement trials were plagued with breakdowns, primarily of the motors and motor controls for the flake augers. This performance of the original unmodified pods was in contrast to more efficient performance of a much modified set of pods (i.e., with new motors and motor controls, a different design of augers, and larger flake hoppers) developed and used by Harold's Flying Service to apply the flakes to operational blocks. In spite of these improvements, the flake application system still needed additional modifications: 1) a larger capacity motor on the flake auger system so that it can put out a greater volume of flakes per minute; 2) additional volume capacity for the flake hopper; 3) a flake hopper that can slide back from under the aircraft for ease of loading, and 4) a lightweight sticker container to replace the present carboard box containers.

1995—Monitoring of Blocks Treated in 1994

No new efficacy and residue trials were initiated in 1995, and only one operational application, on 235 acres in Columbus, Ohio. The area was treated with Disrupt II applied using a Cesna 206 at 30.4 grams AI/acre with 6 oz of Gelva RA-1990. No egg masses were found inside the treatment area during the fall survey. Monitoring continued in blocks treated in 1994. The bead and flake treatments provided comparable population suppression (as compared with untreated plots), but results were complicated by an increased incidence of the fungus *Entomophaga maimaiga* and nucleopolyhedrosis virus, which also suppressed the gypsy moth populations in the treated and untreated blocks.

In response to plugging of CP nozzles by beads in 1994, a contract was developed with Schiffer Flying Service (Ovid, MI) to apply several tank mixes containing the bead formulation (now produced by Biosys) from the ground and air. Several nozzle/atomizers (e.g., flat fan, hollow cone, Micronair) were used to apply the bead formulation. Micronair AU-5000 atomizers (without screens) (Fig. 15) performed well and did not plug during ground and aerial trials. The other nozzle systems (including the CP nozzles) plugged during application using ground equipment that simulated spray boom and nozzle systems used for aerial application. Also, the Biogrip sticker droplets left a milky stain on car finishes that requires buffing or compounding to remove.

(Fig. 15 here)

Summary of 1995 Results. The increasing incidences of gypsy moth fungus and virus throughout Rockbridge and Augusta Counties, Virginia, further complicated the 1994 efficacy trials. It was decided not to initiate additional trials in 1996 until another area could be located with suitable low density gypsy moth population, modifications were completed to equipment for applying the flakes, and nozzles and tank mixes for applying the beads were reevaluated. AgriSense (United Kingdom) submitted a registration package to the US EPA for their bead formulation for gypsy moth, which was designated Decoy GM Beads. Biosys also provided a new microsponge formulation for evaluation, but it had suspendability problems. Shin-Etsu provided another chip formulation with release rates determined under laboratory conditions.

A contract was awarded to Sumitomo Corporation to produce racemic dispalure.

1996—Evaluation of Additional Tank Mixes

In the laboratory (APHIS Otis Methods Development Center), bead tank mixes were deposited on seasoned foliage of potted red oak seedlings, and the plants were exposed to accumulated rainfall totaling 5.0 inches. An additional tank mix was needed for the beads as the surfactant, TX-8815 (now designated Nalco-8815), that had earlier shown promise in preliminary tests in preventing clogging of nozzles and aiding in mixing did not by itself appreciably contribute to suspendability of the tank mixes and substantially increased wash-off of the standard tank mix

(Biogrip). Of the tank mixes tested, the following four combinations with beads were most resistant to wash-off: (1) Biogrip, Nalquatic, and Nalco-8815, (2) Biogrip and Nalquatic, (3) Bond and Nalquatic, and (4) Bond, Biogrip, Nalquatic, and Nalco-8815.

Airport Trials. At the airport (APHIS Aircraft Operations), the Bond and Nalquatic tank mix (with and without 0.5% Nalco-8815) were selected for evaluation in an aircraft based on performance during laboratory trials. Nalco-8815 did not aid in keeping beads in suspension once mixed but did aid in the initial mixing of beads into a slurry. The Micronair atomizers without cages did not malfunction using these tank mixes.

Field Trials. An AgTruck was used to aerially apply large volumes of several tank mixes to experimental blocks in Rockbridge County, Virginia, in July 1996. The Bond and Nalquatic mix was chosen as the basis for evaluation due to its higher resistance to wash-off as compared with the standard (Biogrip). Each tank mix was applied through CP nozzles and AU5000 Micronair atomizers. Mixes were evaluated with and without Nalco-8815.

Clogging did not occur in CP nozzles or Micronair atomizers with any of the tank mixes. Past efficacy trials in which clogging occurred used CP nozzles with stainless steel bodies. These trials used CP nozzles with plastic bodies. Flow dynamics of the bead mixes through plastic bodies is different than through steel bodies (discussion with C and E Enterprises, Mesa, Arizona). Further evaluation of the types of nozzle bodies is needed.

Both types of nozzles were configured to deliver a range of droplet sizes. Over an 8-week period after spraying, beads on foliage were counted to determine wash-off. None of the tank mixes applied through Micronair atomizers performed well. The best performing mix was 0.75% Nalquatic, 3% Bond, and 0.5% Nalco-8815 in water. When applied through CP nozzles set at maximum deflection and at maximum orifice size, this mix resulted in greater retention of beads on foliage than when applied through Micronair atomizers set at VRU of 12 and 90° blade angle with cages removed.

Retention of beads on foliage:

Days after application	CP nozzle	Micronair atomizer
14	90%	71%
28	80%	55%
42	50%	31%
56	35%	1%

The addition of Nalco-8815 surfactant did not cause undue foaming and appeared not to have increased wash-off of beads from foliage.

Operational Use. Operational treatments of 252 acres in eastern Tennessee, 1,900 acres in North Carolina, and 2200 acres in eastern Wisconsin were treated with one application of Disrupt II at 30.4 g AI/acre with Gelva-2333 sticker at 6 oz per acre.

Summary of 1996 Results. Increased incidence of the gypsy moth fungus dramatically reduced gypsy moth populations throughout Rockbridge County and continued to complicate evaluation of previous efficacy trials. Evaluations of various tank mixes showed that combinations of Nalquatic, Bond, and bead mixes in water, with and without Nalco-8815, could be applied through CP nozzles with plastic bodies and Micronair atomizers without cages with no clogging.

Wash-off data for the Nalquatic, Bond, and Nalco-8815 tank mix compared favorably with the original 1993 wash-off data for the Biogrip tank mix.

Harold's Flying Service redesigned and further modified the pheromone flake application equipment in anticipation of use during the operational program in Virginia (Fig. 16). The sticker containers and capacity of the flake containers that were modified previously needed a separate control unit for each sticker and flake unit so that they could be controlled and calibrated separately.

(Figure 16 here)

1997—Additional Trials of Flake Formulations

Efficacy. Because of concerns over anticipated delay of US EPA registration of the bead formulation, focus shifted back to evaluation of the flake formulation. Based on results of earlier tests with hand-applied pheromone dispensers (Kolodny-Hirsch and Schwalbe 1990), it was originally concluded that any successful pheromone dispenser must be distributed throughout the forest canopy to be effective. The effect of dispenser distribution on the efficacy of mating disruption, however, has never been determined experimentally for the Hercon flakes.

Twelve blocks that supported very low gypsy moth populations were established in the northern half of Rockbridge County, Virginia. Four of these blocks were treated with the standard flake tank mix, which consists of Disrupt II at 30.4 g AI in 6 oz of Gelva-2333 per acre and 3-5% diatomaceous earth. Four blocks were treated with flakes at the same dose and diatomaceous earth alone (without sticker), and four controls were left untreated.

USDA milk carton traps to catch males and delta traps to deploy monitor females were placed both at ground level (2.5 m) and in the forest canopy (approximately 19 m). Burlap bands and preseason and post-season 1/40-acre egg mass surveys were used to evaluate population levels and trends.

Communication between males and females was suppressed but not severed on treatment plots as measured by the number of males caught per trap per day. Average capture was 0.00013 males/trap/day on plots with sticker, 0.02 males/trap/day with no sticker, and 0.25 males/trap/day on control plots. Communication suppression was more successful in plots treated with sticker than in plots with no sticker. Mating success, as measured by the percent of fertile egg masses recovered from deployed females, was substantially higher in control plots (9.3%) than in either treatment (1.9% in plots with no sticker, and 1.4% in plots with sticker), and there appeared to be no difference between treatments. These combined data are ambiguous in that they do not show clear differences between the sticker and no-sticker treatments.

Alternative Tank Mix. Although the flakes have consistently been shown to adequately disrupt mating in efficacy trials in the past, the unmodified application equipment used to apply the flakes (dispenser pods developed in the 1970s by Schweitzer Aircraft and variously modified by Hercon Environmental, APHIS, and Harold's Flying Service) is expensive, specialized, and performs inconsistently. A slurry of flakes in an appropriate carrier that could be applied with conventional spray equipment would offer an advantage.

In 1997, field trials evaluated a tank mix slurry using a new carrier, LI108 (Loveland Industries, Greeley, Colorado)(Fig. 16). LI108 is both a thickening agent and adhesive; therefore, no other adjuvants were used in this mix. The slurry was pumped through (1) special pinch valves and 8050 Tee Jet flat fan tips fitted to adjustable stainless steel bodies and (2) large diaphragm check valves with 8085 Tee Jet flat fan tips and round orifices. Some clogging did occur in the stainless steel valve bodies, and boom pressures were lower than desired; however, the slurry passed through both nozzle configurations and there was positive shutoff. Unfortunately, when the flakes remain in the slurry there is a release of pheromone from the flakes into the slurry and, subsequently, to the air.

Summary of 1997 Results. Results of the sticker versus no-sticker efficacy tests were inconclusive, but provided some evidence that it may not be critical for pheromone dispensers (e.g., flakes) to be applied to a target area in such a way that they adhere to foliage. Under some circumstances it may be possible to simplify application procedures and reduce application costs by using a tank mix with no sticker. This was identified as a high priority for 1998.

The no-sticker mix was successfully applied using the pods except that, despite initial attempts to calibrate the system, the pods appeared to be putting out the total amount of material before the plot was actually finished. Although no specific factor could be identified as causative, a combination of factors could have contributed: 1) slight changes in calibration, 2) inaccurate acreage estimates, 3) delayed initiation or termination of pod auger operation or both, 4) flake dribbling after shutoff, and 5) overlap of previous swaths (the aircraft was fitted with a global positioning guidance system, but it was not functioning properly at the time of application).

Preliminary tests of an alternative tank mix (LI108-flake slurry) applied through specially designed nozzles attached to conventional spray equipment are encouraging, although the rapid leaching of pheromone from the flakes into the slurry needs to be resolved.

On January 20, 1997, Thermo Ecotek Corporation announced that its subsidiary Thermo Trilogy Corporation acquired Biosys Inc., including AgriSense-BCS, Ltd., a United Kingdom company. Discussions with Thermo Trilogy Corporation personnel indicated that they were going to continue to pursue the registration of the bead formulation with the US EPA.

THIRD STAGE OF DEVELOPMENT AND APPLICATION, 1998-2005

1998—Re-evaluation of the Need for Sticker

Efficacy of Flakes Without Sticker. Because the results obtained in 1997 were inconclusive, the efficacy of Disrupt II flakes applied without sticker was tested again in 1998 (Thorpe et al. 2000). Twelve 250-acre study plots were established within a continuously-forested area in Augusta and Rockbridge counties, Virginia. As before, four study plots were treated with Disrupt II at 30.4 g AI in 6 oz of Gelva-2333 per acre and 3% diatomaceous earth, four plots were treated at the same rate but without the Gelva-2333 sticker, and four plots were untreated controls. Biological efficacy was measured with standard USDA milk carton traps and daily deployment of laboratory-reared female gypsy moths. Traps and females were deployed at heights of 1.5 m above the ground and in the canopy using pulley systems. A total of 60 naturally-occurring egg masses were found and evaluated to determine if they were fertile.

In both 1997 and 1998, trap catch and mating success of deployed females were higher in the plots treated with flakes without sticker compared to the plots in which sticker was used, but the differences were not statistically significant (Figs. 17 and 18). When the results from both years were combined, trap catch was reduced by 67% compared to controls in plots treated with flakes without sticker and by 90% in plots treated with flakes with sticker. Mating success was reduced by 89% compared to controls in plots treated with flakes without sticker and by 99.5% in plots treated with flakes with sticker. Furthermore, 55% of the naturally-occurring egg masses that were found in the plots treated without sticker in 1998 were fertile, compared to 2.9% in the plots treated with sticker.

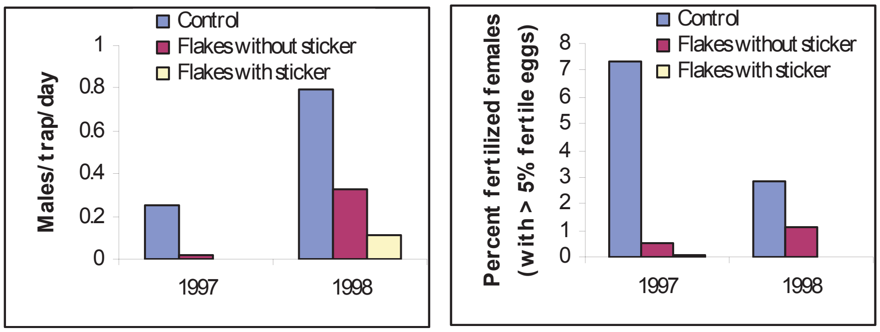

Figure 17. xxx

(Fig. 18 here)

While the addition of the sticker did not result in a statistically significant increase in treatment effectiveness, suppression of trap catch and mating success in the plots with sticker was greater each of the two years of the study. This trend is further supported by the finding in 1998 of substantially higher fertilization rates among naturally-occurring egg masses in plots treated without sticker. Therefore, to achieve maximum efficacy with flakes, a sticker should be used. However, in situations where the use of a sticker is problematic, such as in residential areas, a high level of mating disruption can be expected to occur with the application of flakes without sticker.

Flake Slurry Formulation. Efforts continued in 1998 to develop a slurry formulation that could deliver flakes using more conventional hydraulic application equipment. Andy Trent (USDA, Forest Service, Missoula Technology and Development Center, Missoula, Montana) developed an application system consisting of modified MicronAir AU5000 atomizers (Trent and Thistle 1999) (Fig. 18). Larger holes were drilled in both the inner and outer cages to prevent clogging. Initial tests were conducted at Covington, Virginia to assess swath patterns at different application volumes. At application volumes of 0.5 gallon and 1 quart per acre there was no clogging, and the distribution of flakes across a 75-foot swath was fairly uniform. Clogging did occur at an application volume of 1 pint per acre because of the low pressure (2 psi) required for the low volume output.

In June of 1998, four 250-acre plots in Rockbridge County, Virginia, were treated with the flake slurry formulation using the modified MicronAir system (two AU-5000 atomizers, 3-inch blades set for maximum RPM) at an application volume of 0.5 gallons per acre and at a dosage of 30.4 g AI/acre. Each nozzle was fed from the end of the boom through a 0.5-inch (inside diameter) plastic tube. This eliminated the possible build-up of air in the end of the boom (i.e., the material cannot be put out through the diaphragm check valve on the atomizers). Both cages need to be on the atomizer, but the inner one needs to be modified with larger openings. This eliminates the build-up of flakes and allows more sticker to adhere to the flakes (i.e., since the cages are turning at high RPMs, the skicker is spun off the flakes). An additional four plots were treated with a standard flake application at the same dosage using pods, and four plots were untreated controls. Standard evaluation methods were used to evaluate the biological efficacy of the treatments. Compared to the control plots, male trap catch was reduced by 85% in the standard flake plots and by 61% in the flake slurry plots (Fig. 19a). The percentage of deployed females producing fertile egg masses (with more than 5% fertile eggs) was zero in the standard flake plots and was reduced by 94% in the flake slurry plots (Fig. 19b). These results indicate that, while the flake slurry formulation is highly effective, there does appear to be a slight decrease in effectiveness compared to the standard flake application.

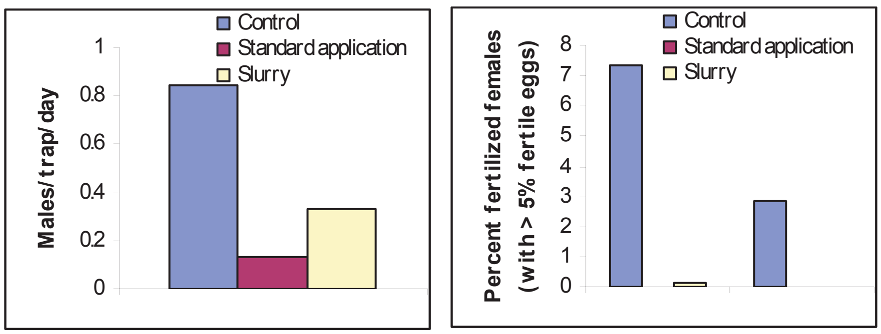

Figure 19. xxx

Deposition and Weathering. Because the flake slurry formulation used a different adhesive than is used in standard flake applications, tests were conducted to compare the deposit and weatherability of flakes applied using each of the systems. A 10x application of flakes using each of the application systems was made to oak trees along the edge of the Virginia Tech Shenandoah Valley Agricultural Research and Extension Center at Steeles Tavern, Virginia. A total of 250 flakes for each system was located, marked, and checked each week for eight weeks to determine washoff rate. At the end of the evaluation period, 149 flakes from those that were applied using the pods remained (40% loss) and 117 flakes that were applied with the slurry system remained (53% loss). In addition, a bucket truck was used to collect foliage from the canopies of plots treated with each of the application systems to compare the number of flakes remaining eight weeks after application. About 50,000 leaves were collected from foliage sprayed with each system. A total of 160 flakes were present on the foliage sprayed with the pods, and 112 were present on foliage sprayed with the slurry (Fig. 20a). This works out to about 0.003 flakes per leaf, or about 400 leaves per flake (Fig. 20b). The difference in the number of flakes between the two application systems is similar in the two tests (the number of flakes remaining after the slurry treatment is 21 and 31% lower than the number remaining after the pod treatment in the first and second tests, respectively). Clearly, while the weatherability of the flakes applied using the slurry system is good, there is greater loss of flakes applied using the slurry system.

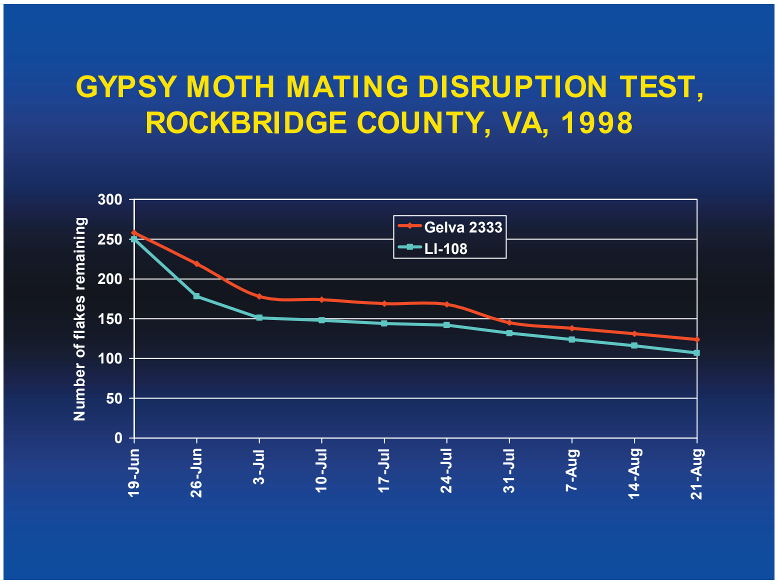

Figure 20a. xxx

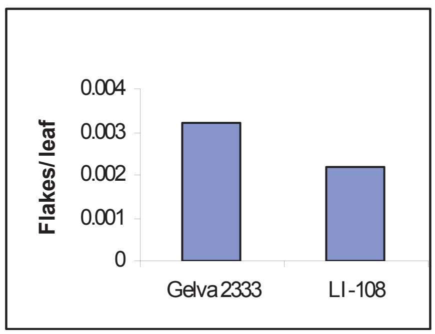

Figure 20b. xxx

Loss of Disparlure While in Contact With Sticker. During a standard outdoor release rate test it was noticed that the initial disparlure content of flakes mixed with the stickers Gelva 2333 (flake application using pods) and LI-108 (flake application in slurry) was lower than that in flakes not mixed with sticker. To determine the effect of exposure to stickers on disparlure content, a laboratory test was conducted. Flakes were mixed (1:1) with three aqueous dilutions (50, 75, and 100% sticker) of both stickers. Five time periods of exposure to the stickers were tested: 5, 20, 60, 240, and 1,440 minutes. After the flakes were removed from the sticker, excess sticker was wiped off and the flakes were placed on a screen to dry for 24 hours, after which time they were analyzed for disparlure content. There was no clear effect of exposure to any of the stickers or dilutions on disparlure content for the first four hours of exposure. However, the flakes exposed for 24 hours to 75% or 100% concentrations of both stickers had substantially less disparlure than did flakes not exposed to sticker. These results indicate that, under normal mixing conditions, there is no meaningful loss of disparlure from the flakes into the sticker. However, if the flakes are mixed with concentrated sticker but not used within the next four hours, there may be loss of disparlure into the sticker. If and at what rate the disparlure would be released from the dried sticker is unknown.

In 1998, Harold's Flying Service continued to improve the capacity and operability of his previously modified pods. Both the 502 and 503 (two seats) Turbine Air Tractors were given swaths of 75 feet at 140 mph.

Evaluation of a New Pheromone Flake Dispersal System. Al's Aerial Spraying of Ovid, Michigan, was awarded a three-year Technology Development contract ($100,000, 50% to be distributed in 1998, 25% in 1999, and 25% in 2000) in 1998 to develop a new pheromone flake dispersal system to replace the Hercon pods. Contract specifications required the design, develop, and demonstration of a new dispersal system for pheromone flakes. The new system should be similar to the pods in order to: 1) disperse the flakes and sticker at a specified rate, 2) coat the flakes with sticker to adhere to forest canopy, and 3) disperse the flakes and sticker uniformly over the treated area. In addition, the new system must be able to show 1) easy loading and cleaning of equipment, 2) increased flow rates that support wider swaths and faster airspeeds to increase production, 3) flakes must be deposited on at least 90% of the swath, and 4) the new system should be usable on more aircraft.

All rights, patents, and otherwise would be maintained in the public domain by both the contractor and federal government. A technical team of personnel were organized to review the progress of the contract: Dr. Gary VanEe, agricultural engineer at Michigan State University; Dr. Andy Trent, mechanical engineer at the Missoula Technology Development Center; and Win McLane and Tim Roland, Agriculturist and Chief Pilot for USDA-APHIS, respectively.

By November 1998, a prototype system based on an air blast spreader concept was designed, and preliminary airport trials were conducted (Fig. 21). The new dispersal system was attached to the boom area of an Air Tractor 402. The system consisted of eight ports (four on each wing): each port consisted of an open tube (1 inch diameter) with two flat fan nozzles directed toward the tube opening. The flakes were metered from the hopper to the manifold, which was divided into eight areas, each area connected to tubing. The flakes were pushed through the tubing by the air intake of the aircraft and out of the tube opening between the flat fan nozzles. One end of the tubing extended from the manifold area and the other end of the tubing was secured between the nozzles at each port on the boom. The sticker was metered by separate pumps to each wing through tubing to the flat fan nozzles at each port.

(Fig. 21 here)

Overall, the spray swath looked good (flakes were evenly distributed across the tarp with no gaps beneath the aircraft); however, there were problems getting the flakes coated with sticker. Al's Aerial Spraying realized that there wasn't sufficient sticker to coat the flakes and made arrangements to work with Andy Trent of Missoula Technology and Development Center to look at the dispersal of the flakes and sticker.

Non-target Concerns. In the fall of 1998, it was decided that—since the aerial application of Disrupt II to large forested areas was including streams, ponds, and other small bodies of open water beneath the hardwood foliage—a 48-hour static renewal acute toxicity test should be conducted with the Cladoceran *Daphnia magna* under Good Laboratory Practices. A contract was awarded to Wildlife International Ltd. (Easton, Maryland) to conduct this study using Disrupt II flakes, negative control, blank Disrupt II flakes (i.e., without racemic disparlure nor a chemical stabilizer to keep the pheromone from leaching out too rapidly), and racemic disparlure. The test concentrations were 39, 65, 108, 180, and 300 mg Disrupt II/liter of well water, and blank concentration of 300 mg blank flakes/L. The test was completed in April 1999. Daphnids in the negative and blank flake controls appeared normal and healthy. Daphnids in the 39, 65, 108, and 180 mg Disrupt II/L treatment groups also appeared normal and healthy. After 48 hours of exposure, mortality/immobility in the 300 mg Disrupt II/L treatment group was 80%. The results for the 300 mg Disrupt II/L treatment group were unexpected, but we suspected that the higher levels of the chemical stabilizer designed for the PVC compound to assist in weathering was the cause of the mortality. Following this effort, Hercon removed the stabilizer from the Disrupt II product. It was decided to re-run the test with the new Disrupt II product.

Summary. The slurry formulation of flakes is not an operationally friendly formulation (e.g., the slurry is hard to mix and clogging of the atomizers in encountered) and the release rate of pheromone into the sticker over time needs to be determined.

In the fall of 1998, disparlure was forwarded to 3M Canada Company (London, Ontario, Canada) to formulate into their Capsule Formulation for release rate evaluations in the laboratory. Their product contains 20% AI racemic disparlure.

1999—Portable Electroantennogram Devices

In 1999, 220 acres in Iowa were treated with flakes at 30.4 g AI/acre (16.5 g of flakes per acre) and 4 oz per acre of Gelva-2333 using the APHIS Cessna 206.

Since the start of the Slow-the-Spread Program in 1998, the use of mating disruption to manage low-density populations of gypsy moth has increased dramatically. Only one formulation, Disrupt II (flakes), is registered for use using aircraft application. It is applied using pods that have numerous mechanical problems (e.g., auger binding, motor failure, pump failure, flake bridging). Over the years, a number of slow-release formulations have been evaluated in the hopes that one with the desired release rate could be applied using standard agricultural spray systems. To date, none have met this requirement.

In March of 1999, Andy Trent (engineer at Missoula Technology and Development Center) used a digital camera with high speed flash in wind-tunnel tests to look at sticker and flakes being dispensed under near-operational conditions using the prototype system being developed by Al's Aerial Spraying. Photographs taken in the wind tunnel showed the flakes flowing through a sticker plume being sprayed by the nozzles. Also, evaluations were conducted to quantify the deposition and stickability of flakes on tarps and red oak seedlings.

Following these evaluations of the current prototype system, it still does not adequately coat the flakes with sticker. The flakes are not being coated when they pass through the sticker plume. Possible, the flakes might be moving too fast to get coated with sticker or the sticker is being atomized into such small droplets that they evaporate before coating the flakes.

In late 1999, Hercon Environmental was sold to the company's management and named Aberdeen Road Company, but it continues to do business as Hercon Environmental.

3M Canada Company Microcapsule Slow-Release Formulation

In March 1999, a microcapsule (20-50 micron) formulation of racemic disparlure developed by 3M Canada Company was tested for mixing and handling characteristics at APHIS Aircraft Operations (Fig. 22). The best equipment for applying the microcapsules were two flat fan nozzles with 8010 tips without nozzle or in-line screens (Fig. 23). A total of one pint per acre of total mix (153.8 ml 3M product and 319.4 ml water) resulting in 30.4 g AI/acre was evaluated.

(Fig. 22 here)

(Fig. 23 here)

Flake Slurry Formulation. An evaluation was conducted at APHIS Aircraft Operations to test the system using a mixture of flakes and sticker at an output of 1 pt/acre (1 qt/acre was used in 1998 tests). Basically the same hardware as before was used, but larger holes were drilled in the inner screen and Tygon tubing was run from the main pump outlet to the Micronairs (i.e., bypassing the boom), and the system performed well. A drawback of the system is the need to thoroughly mix the flakes and sticker so that no clumps can get lodged in the fittings.

Portable Electroantennogram Devices

Most attempts to measure the atmospheric concentration of insect pheromones resulting from mating disruption treatments have relied on the use of air sampling over a period of several hours or days to capture enough pheromone for analysis (Caro et al. 1981). Because of the extended time required to collect enough pheromone for analysis, estimates of pheromone concentration represent averages at the sampling location over the sampling period. Thus, any short-term fluctuations in pheromone concentration resulting from wind currents or other relatively rapid changes in atmospheric conditions are obscured. Rapid and sensitive detection of pheromone is possible with the use of an electoantennogram sensor (EAG), which uses amplified electrical impulses from chemoreceptors in a living insect antenna to indicate the presence of pheromones or other semiochemicals. At first, the only EAGs available were expensive and bulky, and not suited for use outside of the laboratory. Recently, a portable EAG was developed (Koch 1990) (Fig. 24) and used to measure atmospheric pheromone concentrations resulting from mating disruption treatments against a variety of pests in various crops (Sauer et al. 1992, Suckling et al. 1994, Färbert et al. 1997). The availability of a reliable and sensitive EAG could provide a direct means to measure atmospheric pheromone concentrations in test plots, which could reduce the need for lengthy and expensive biological evaluations of treatment efficacy. In 1999 two EAG devices were tested.

(Fig. 24 here)

Koch EAG

The development of a portable EAG device was pioneered by Dr. Uwe Koch, Department of Physics, University of Kaiserslautern, in Kaiserslautern, Germany (Koch 1990). Dr. Koch traveled to the United States with the device to participate in our 1999 mating disruption efforts. The objectives of the work with the Koch EAG were to determine if it was possible to detect disparlure in the air in treated plots and to examine the effects of height above the ground and meteorological conditions on pheromone concentrations. The EAG work was done from August 15-20, which was 55 days after the treatment was applied. To ensure adequate concentrations of pheromone in the air, a site that was treated with approximately 10 times as many flakes as in a normal application at 30.4 g AI/acre was used for this test. The site was at the Virginia Tech

Shenandoah Valley Agricultural Research and Extension Center at Steeles Tavern, Virginia. There was little undergrowth and a relatively low density of trees, with some openings in the canopy (avg. leaf area index = 2.1). To determine if detectable concentrations of disparlure were present under normal application conditions, samples were also taken in a plot treated with flakes with sticker and in a plot treated with an experimental microencapsulated formulation (3M Canada Company), both at a rate of 30.4 g AI/acre.

To operate the EAG, a male moth antenna was severed and placed across electrical contacts (Fig. 25). The electrical impulses from the antenna were input into a computer and analyzed in the field in real time. The readings from the EAG are expressed as values relative to an internal baseline generated by exposing the antenna to three different concentrations of pheromone. To sample at different heights above the ground, a rope was tied to the EAG and looped over a branch near the top of the canopy (Fig. 26). This permitted the EAG to be pulled to any desired height between 0 and 15 m. Meteorological conditions were continuously measured at a meteorological station erected adjacent to the EAG sampling site.

(Fig. 25 here)

(Fig. 26 here)

Average relative disparlure concentrations were 4.0 in the plot treated at 1x with microcapsules, 9.0 in the plot treated at 1x with flakes, and 25.5 at the site treated at 10x with flakes. These results demonstrated that it is possible to detect pheromone in treated plots with the EAG even eight weeks after the treatment. At the 10x site, EAG readings were consistently highest at a height of 30 cm regardless of wind speed. Readings dropped by almost 50% when the EAG was raised to 3 m, again independent of wind speed (Fig. 27). The EAG readings were further reduced at a height of 6 m under low wind speed conditions (less than 1 m/s), but not under higher wind speeds (greater than 1 m/s). At 10 m and 15 m, readings were twice as high under low compared to high wind speeds. In general, it appears that, under still conditions, pheromone concentrations are fairly uniform with respect to height, possibly with lower concentrations just beneath the canopy. When wind speed is greater than 1 m/s, pheromone concentrations tend to decrease with increasing height.

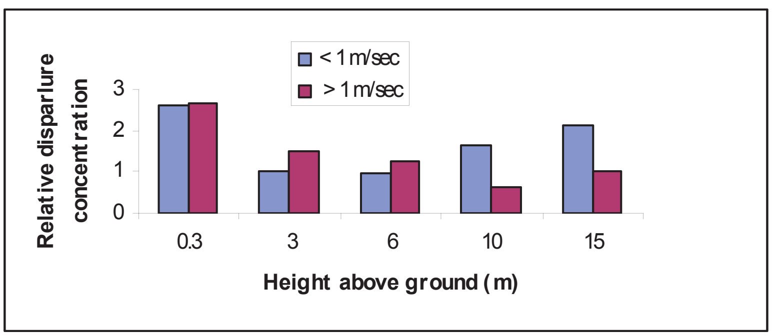

Figure 27. xxx

Syntech Portable EAG

While the EAG developed by Koch was portable and capable of providing reliable measurements of pheromone concentrations, it was bulky and its set-up and use required the attention of a specialist. A more compact and user-friendly portable EAG was introduced in 1998 (van der Pers and Minks 1998) (Fig. 28). This instrument was used in the present study to measure the atmospheric concentration of gypsy moth pheromone in forest plots treated with the Disrupt II. The objectives of this study were 1) to determine the feasibility of using this portable EAG to measure atmospheric disparlure concentrations in treated plots, 2) to determine the vertical profile of pheromone in a forest canopy, and 3) to attempt to correlate pheromone concentration with biological efficacy as measured by suppression of moth capture in pheromone-baited traps and female mating success.

(Fig. 28 here)

Six 250-acre plots were established in Rockbridge County, Virginia, in late June, 1999. Each plot received one of the following treatments: 1) Disrupt II flakes with sticker; 2) Disrupt II flakes without sticker; 3) Disrupt II flakes in a flake slurry formulation; 4) an experimental microcapsule formulation developed by 3M Canada Company and applied using conventional hydraulic nozzles; 5) Luretape GM (Hercon Environmental) applied by hand; and 6) an untreated control. All treatments were applied at a dosage of 30.4 g AI/acre. Treatments 1–4 were aerial applications. The Luretape was made from the same material as the flakes, but was cut into long, 1.3 cm wide ribbons. The ribbons were cut into 8-foot lengths, each containing 3 g of AI, and the individually stapled or tied to 25 trees per hectare (about 10 trees per acre) arranged in a uniform grid. Standard methods were used to evaluate biological efficacy of the treatments.

The EAG, provided by Jan van der Pers of Syntech (Hilversum, The Netherlands), weighed about 4 kg and measured [___ x ___ x ___] inches. A detailed description of the device can be found in van der Pers and Minks (1998). To prepare the EAG for use, a male gypsy moth antenna was severed and connected using electrically-conductive gel to electrical contacts in the EAG. When air samples are drawn past the antenna, electrical impulses from the antenna are recorded. The responses are expressed relative to baseline responses of the antenna when exposed to a standard reference chemical (hexanyl acetate). Vertical stratification of pheromone was measured at three heights (5, 10, and 20 m) in each of the plots from the extended bucket of a bucket truck.

The treatments reduced moth capture by greater than 98% in all treated plots compared to that in control plots. While no egg masses with greater than 5% fertile eggs were produced by deployed females in any of the treated plots, the percentage of fertilized females, as indicated by the presence of at least one fertile egg, varied from 0.6% to 2.6% in the treated plots (Fig. 29). Average relative atmospheric pheromone concentration ranged from 1.04 in the untreated plot to 1.95 in the plot treated with flakes with sticker. Pheromone concentrations were significantly higher in the flakes with sticker, flakes in slurry, and microcapsule treatments compared to the flakes without sticker, hand-applied Luretape, and control treatments. There was no significant difference among the three heights at which pheromone concentrations were measured. However, in each of the three treatments with the higher pheromone concentrations, the concentrations increased with increasing height. There was a significant negative correlation between average pheromone concentration in a plot and percent fertilization. The three plots with significantly higher pheromone concentrations (flakes with sticker, flakes in slurry, microcapsules) were also the plots with the highest levels of mating suppression.

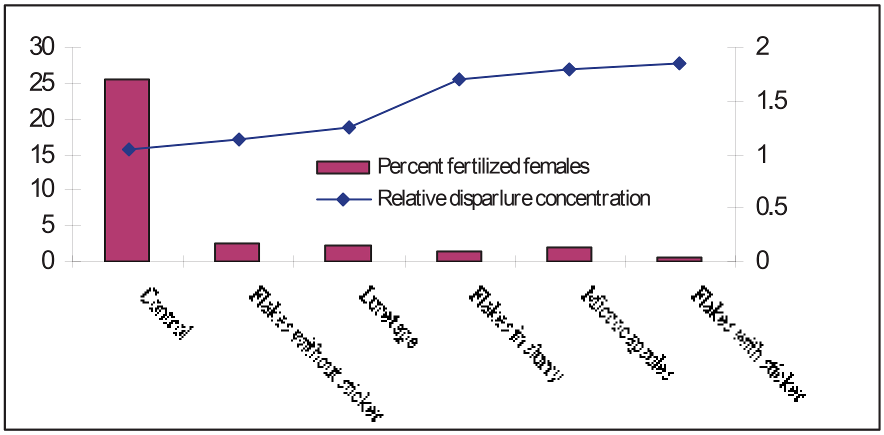

Figure 29. xxx

This test showed that the EAG sensor was capable of detecting biologically meaningful differences in pheromone concentrations resulting from different mating disruption formulations. It is important to note that all treatments eliminated the production of egg masses with more than 5% fertile eggs. This variable is now considered to be the best indicator of the success of a mating disruption treatment, because mated females that produce fewer than 5% fertile eggs make little biological contribution to the next generation. Therefore, this suggests that the EAG sensor was capable of detecting differences in pheromone concentration too small to be of biological significance. Therefore, it may be possible to use the EAG sensor to measure pheromone concentration in areas treated with mating disruptants and reliably predict biological effectiveness based on these measurements.

A pheromone-dispersion project was coordinated in an oak-hickory wooded area in the Appalachian highlands west of Staunton, Virginia, on 19-27 August 1999. The objective of the project was to attempt to understand the effective dispersion distance of a tracer gas released in the canopy trunk space. Sulfur hexafluoride (SF6) was used as the tracer gas as it is an easily detectable surrogate that can indicate dispersion patterns of insect pheromones. By measuring the horizontal dispersion of SF6, effective radii can be calculated for the dispersion of pheromone from passive dispersal release agents such as flakes or beads.

The study site was arrayed with 50 syringe samplers on three concentric circles with radii of 5, 10, and 30 m (Fig. 30). The syringe samplers recorded ½-hour samples for 4 ½ hours per trial. Nine trials were conducted, yielding nearly 4,000 1-hour chemical samples. Meteorological data and canopy architectural data were also collected. Preliminary analysis of the data indicated very narrow meandering plumes of SF6 with very steep concentration gradients near the plume edges, which were a function of both wind speed and stability (Fig. 31). (When the surface layer is stable, the tracer plume remains relatively concentrated and shows consistency in direction due to the suppression of turbulent mixing in the stable layer.) Also, there was regular near-field, canopy top venting in neutral to moderately unstable conditions and very low wind speeds (Thistle et al. 2004). This might lend itself to the idea that the gypsy moth sensors are not sophisticated enough to discern subtle gradients but use a sort of digital function that indicates either 'in' or 'out' of the plume.

(Fig. 30 here)

(Fig. 31 here)

An evaluation was conducted to compare the washoff of aerially applied flakes with and without sticker using plastic buckets under the canopy. The collected flakes were counted weekly for five weeks. As anticipated, there was less flake recovery for the flake with sticker block than in the flake without sticker block.

2000—A New Microcapsule Formulation

In April of 2000, 3M Canada Company sprayable microcapsules were evaluated for flow rate and overall handling characteristics at Al's Aerial Spraying. The microcapsules were applied through 8010 and 8015 nozzle tips and Maynard Lund (ML) nozzles (Lund Flying Service, Ritzville, Washington) at 1 pint (16 oz) per acre. All nozzle or in-line screens were removed. Also, an aging study utilizing 3M sprayable microcapsules on white canvas and aged in the greenhouse to determine if the 3M product has a release rate suitable for gypsy moth mating disruption, to compare it to the release rate of Disrupt II, and determine washoff.

The Hercon Disrupt II and 3M sprayable microcapsules, Phase III, are similar in their initial concentration (100 µg/flake or drop) and loss of disparlure through twenty-one days (Fig. 32). The Disrupt II peaks at a concentration near 50 µg disparlure/flake from day 14 through day 49, then continues to decrease. The 3M sprayable microcapsules show a steady decline in µg disparlure/drop throughout the entire 63-day monitoring period, from an initial average of 93.7 µg/drop to 1µg/drop.

(Fig. 32 here)

The 3M sprayable microcapsules, Phase III, has a faster release rate than the Hercon Disrupt II. This may result in too low of a concentration of disparlure during the end of adult gypsy moth flight. Experimental gypsy moth pheromone behavioral studies to be conducted in the summer of 2000 will aid in determining the efficacy of these products.

If the release rate of the 3M sprayable microcapsules proves to be sufficient for gypsy moth mating disruption it will provide an alternative treatment method. Currently, the only aerial application of gypsy moth pheromone done operationally is with Hercon Disrupt II. The Hercon product requires a unique application method (pods attached under the wing) or specially designed systems. The pods and special systems are limited to only a handful of applicators and are not commercially available.

The 3M sprayable microcapsules could be applied by any applicator through conventional aerial application systems. This would result in a greater number of applicators being able to bid on work in gypsy moth mating disruption projects and therefore result in a lower application cost.

Field Tests of 3M Sprayable Microcapsules and Reduced Rates of Hercon Disrupt II Flakes

Prior to 2000, all flake applications in STS were at a rate of 30.4 g AI/acre. This rate was based on earlier dose response tests conducted under male moth densities that were much higher than those targeted for mating disruption in STS (Webb et al. 1988). Therefore, a field test was conducted in 2000 to determine the efficacy of flakes applied at 15 g/acre (Tcheslavskaia et al. 2005b). As part of the same test, 3M sprayable microcapsules were tested at 30.4 g AI/acre. Twelve 60-acre (25-ha) plots were established in the George Washington National Forest near Millboro Springs, Virginia. Four plots were treated with each of the three treatments and four plots were used as untreated controls. The 3M sprayable microcapsules were applied using 4 ML tips directed straight back and applied at 1 pt/acre. Moth capture was reduced by more than 98% in all treated plots compared to that in untreated plots (Fig. 33). In the control plots, 19.9% of the females were mated. No mating occurred in any of the treated plots. These results indicated that there would be no loss in efficacy at a 15 g AI/acre flake application rate under moderate moth density conditions (the traps in the control plots caught an average of 135 moths over the season). The microencapsulated product tested in this experiment performed as well as the flakes.

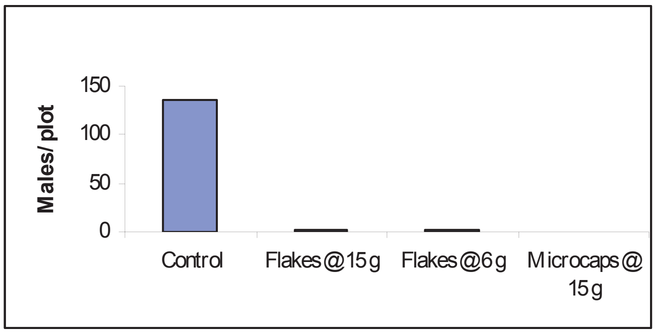

Figure 33. xxx

Tethered Females Versus Mating Stations. To measure the effects of mating disruption treatments on female mating success, laboratory-reared females are deployed in study plots and egg masses produced by those females are checked for the presence of fertile eggs. Since 1989, females had been deployed in mating stations consisting of modified delta traps. This method was favored because, once the delta traps were in place, it was quick and easy to deploy and retrieve females. However, concern was raised that the traps may impede the ability of males to find and mate with females, resulting in artificially low levels of mating success. To test this, females were deployed in two different ways in the control plots of the test described above (Tcheslavskaia et al. 2005b). In each plot, nine females were deployed in mating stations and nine were tethered using a thread with one end tied around the base of the forewing and the other attached to the bole of a tree with a push pin. To protect tethered females from predators, a circle of Tanglefoot pest barrier was applied around each female (Fig. 34). The mean fertilization rate of tethered females was 15.6%, which was about twice that of females deployed in mating stations. These results clearly show that the mating stations inhibit mating in untreated areas. Therefore, the use of mating stations for deployed females was discontinued in subsequent years.

(Fig. 34 here)

Treatment Effects Beyond Treated Areas. A study was initiated in 2000 to examine treatment effects on trap catch and mating success beyond the edges of treated areas (Sharov et al. 2002a). Six 37-acre (15-ha) plots treated with flakes at either 15 or 30.4 g AI/acre or microcapsules at 30.4 g AI/acre in the George Washington National Forest near Millboro Springs, Virginia were utilized for this study. The plots were along a valley (600 - 730 m altitude) between two ridges

(900 - 950 m altidude). A series of transects was placed from the edges of the treated plots to points either 1,800 m away (along the valley) or 500 m away (up the slopes). Standard USDA pheromone traps were placed every 200 m (along the valley) or 100 m (up the slopes). Lines of 10 tethered females were also deployed every 200 m on the transects along the valley. After the end of natural flight, laboratory-reared male moths were released along these same transects.

The effects of the treatment on rates of capture of both feral and released moths were evident up to 250 m from the edge of the treated plots (Fig. 35). On one transect, which was oriented along the valley, effects were observed as far away as 600 m. Female mating success increased gradually with distance from the edges of treated plots along the valley. There was a significant relationship between capture of males in traps and female mating success, and the relationship was similar to that which occurs in untreated areas (Sharov et al. 1995). These results indicate that mating is disrupted up to 250 m from treated areas, and in some cases effects can be seen at distances up to 600 m. The close agreement of the trap catch and mating success values in both treated and untreated areas provides evidence that trap catch alone provides a reliable measure of the effectiveness of mating disruption treatments.

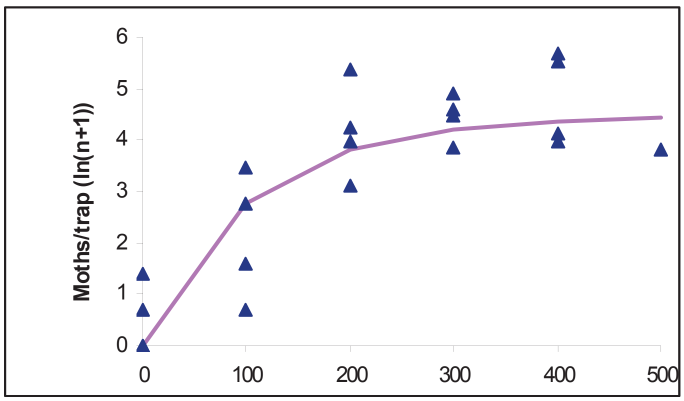

Figure 35. xxx

Trap Catch Versus Mating Success in Southern Wisconsin. The rate of spread of gypsy moths, as measured in the STS program, has to date been greater in Michigan and Wisconsin than in Virginia and West Virginia. It was hypothesized that the difference in rate of spread could be the result of higher mating success of females in northern areas, leading to higher rates of population establishment and growth. To examine this possibility, an experiment was conducted in southern Wisconsin (Tcheslavskaia et al. 2002) to determine if the relationship between trap catch and mating success there was different than the same relationship that had been determined previously in Virginia and West Virginia (Sharov et al. 1995). Seven plots were established in the Kettle Moraine State Forest and nearby forested areas in Waukesha and Walworth Counties. Each plot consisted of 20 tethered females and two USDA milk carton pheromone traps each placed 100 m from the line of females. Females were retrieved 24 hours after they were deployed and dissected to determine if they had mated. The relationship between trap catch and mating success did not differ significantly from that in the Virginia/West Virginia study, although mating success tended to be somewhat higher at a given trap catch value in Wisconsin (Fig. 36).

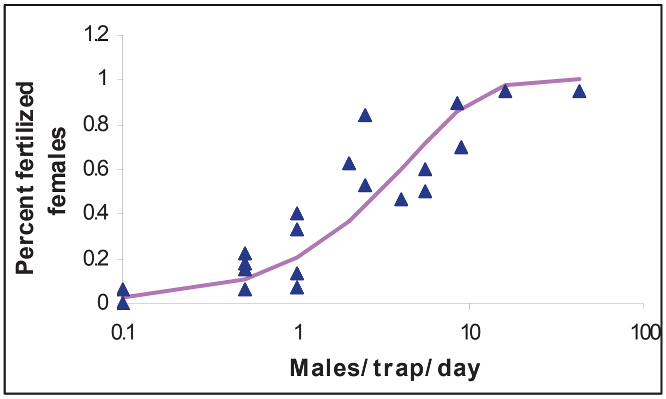

Figure 36. xxx

Portable EAG Measurements of Pheromone in Treated Plots

The Koch EAG was used again to measure atmospheric pheromone concentration in the plots described above that were treated with flakes at 30.4 and 15 g AI/acre. EAG measurements were taken during two consecutive days starting about 20 days after application. Measurements were taken both at ground level and in the canopy using a pulley system. Relative disparlure concentration was higher in the plot treated at 30.4 g AI/acre, and was higher in the canopy than at ground level (Fig. 37).

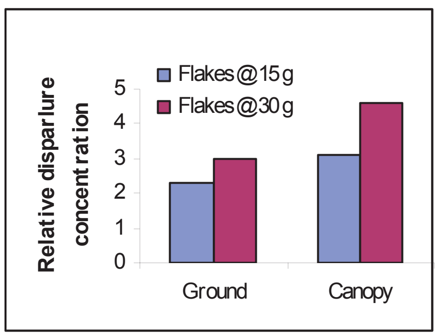

Figure 37. xxx

New Pheromone Flake Dispersal System. In 2000, the final evaluations were made of the new flake dispersal system being developed by Al's Aerial Spraying. Results from deposition trials conducted in June and September 1999 indicated that the problem persisted in that a high percentage of the flakes were without sticker, primarily in the center of the swath. Modifications were made to the system—the most significant being the replacement of the Duke metering gate (designed for larger quantities of materials; therefore, our desired flow rates were at the lower end of the calibration range) with a *[Transland]* metering gate (Fig. 38). This system (Prototype III) was evaluated in the field and there was an even distribution of the flakes across the swath, although it does not coat more than 25% of the total flakes, which negated its use as part of the new operational contract, requiring that 75% of the flakes need to be coated with sticker. The Prototype III system is a tremendous improvement over the unmodified Hercon pod system and is useable now in those situations where sticker is not required (e.g., residential areas) or with minimal additional technology (e.g., use of additives to break surface tension on the flakes) in those situation where 75% sticker is required.

(Fig. 38 here)

Implementation of the 'Slow the Spread of the Gypsy Moth' Project. Since its introduction into Medford, Massachussetts, around 1869, the gypsy moth infestation has expanded to the west and south at an average rate of 13 miles per year (Liebhold et al. 1992). The generally infested area currently extends northwest to Wisconsin and south to North Carolina (Sharov et al. 2002a). The expansion of its current range is expected to continue until the gypsy moth eventually occupies all areas of the U.S. containing favorable habitat. It has been estimated that the ultimate range of this pest will be three times greater than its current range (Liebhold et al. 1997). To address the economic and environmental impacts caused by the expanding range of the gypsy moth infestation, a national strategy was developed to manage gypsy moth populations along the leading edge of the infestation. The goal of this USDA Forest Service project, known as the 'Slow the Spread of the Gypsy Moth' (STS) program, is to intensively monitor populations along the leading edge and apply treatments such that the rate of expansion of the infested area is reduced.

2001—Dose Response Tests

In the winter, studies on aging of pheromone products on foliage were conducted—see results on the next two pages (Fig. 39).

(paper copies of graphs included with original markup; no electronic files)

Figure 39. xxx

Dose Response Test With 3M Sprayable Microcapsules

The results of previous field tests showed that trap capture and female mating are suppressed by mating disruption treatments down to 15 g AI/acre. To provide a full picture of the effectiveness of mating disruption treatments across a wide range of application rates, a dose response test was conducted in 2001. Since the effects of dosages of 15 g AI/acre and higher were known from previous tests, this test focused on the biological effects of low pheromone dosages. The 3M microcapsule formulation was used in this test because low dosages can be obtained by dilution without affecting application volume. Application rates of 0 (control), 0.06, 0.3, 1.2, 6, 15, and 30.4 g AI/acre were tested. Disrupt II flakes at 6 g/acre were also tested to provide a basis for comparing the two products. The tests were conducted in the Cumberland and Appomattox-Buckingham State Forests in central Virginia. Plots were 500 x 500 m (25 ha) and contained primarily favored gypsy moth host trees. Applications were conducted by Earl's Spray Service (Wheeler, Michigan) using an Air Tractor equipped with 4-D8 tips with #56 swirl plates pointed straight back. The 3M sprayable product was applied at the rate of 1 qt/acre. Laboratory-reared male moths were released as pupae (June 25 - July 27) or as adults (July 30 - August 13) to evaluate the effects of the treatments on trap catch and mating success.

During the first month after the treatments were applied, moth capture was greatly suppressed in all plots treated at rates greater than 1.2 g AI/acre (Fig. 40a). The effectiveness of the treatments declined with time, and by two months after treatment trap capture was relatively high at all dosages with the 3M formulation. Few moths were caught in the plots treated with flakes at 6 g AI/acre throughout the test. Female mating success at dosages of 1.2 or less g AI/acre was not different from that in untreated plots (Fig. 40b). Mating success was greatly suppressed at all dosages greater than 1.2 g AI/acre. The results of this test indicate that, with the 3M sprayable microcapsule formulation, pheromone dosages greater than 1.2 g AI/acre are required for effective mating disruption. However, by two months after treatment, the formulation is no longer effective at any dosage. This could have resulted from too rapid release of pheromone from the formulation, inadequate resistance to wash-off in rain, or both.

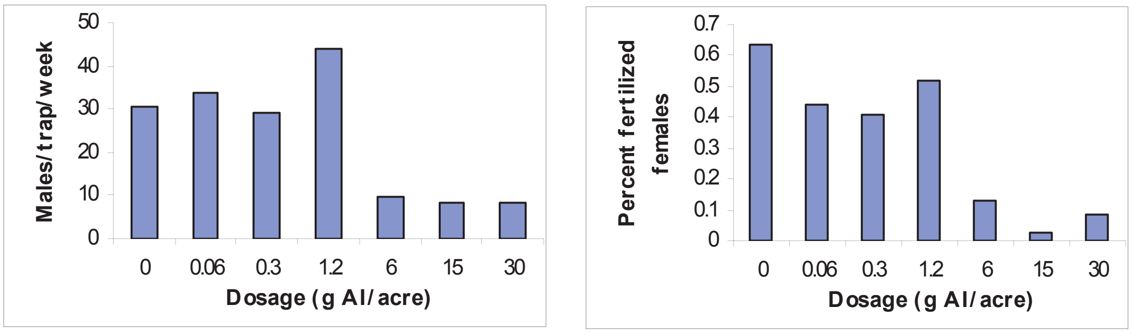

Figure 40. xxx

Skipped Swaths - 90 m Skips

The results of experiments started in 2000 indicated that the effects of mating disruption treatments extend well beyond the edges of treated areas. These findings suggest that it may not be necessary to apply mating disruption formulations uniformly over target areas, but rather that it may be possible to leave deliberate skips in coverage with no loss in efficacy. If so, this could lead to reductions in costs associated with fuel and flight time. To examine this, a series of field experiments was initiated in which Disrupt II flakes were applied by aircraft in alternating treated and untreated swaths. In the first test, two 50-ha plots (1 x 0.5 km) were treated at an overall rate of 15 g AI/acre by alternating a single 30-m swath treated at 60 g AI/acre with three untreated swaths. One of the plots was located at the Appomattox-Buckingham State Forest, Virginia (ABSF), and the other was in the Goshen Wildlife Management Area, Virginia (GWMA). Within the ABSF plot, a line of 32 male moth release points was laid out perpendicular to the aircraft flight path. Two lines of pheromone-baited traps were located parallel to and 30 m to the east and west of this line. Male moths were released and traps were checked weekly during July and twice each week during August. At GWMA, natural populations were higher so no males were released. Two lines of pheromone traps and four lines of deployed females were laid out perpendicular to the aircraft flight line.

In the ABSF plot (see Fig. 41), moth capture was significantly lower in the treated swaths than in the untreated area between treated swaths. While moth capture was also lower in the treated swaths at the GWMA, the difference was not statistically significant. There was no difference in female mating success between the treated and untreated areas. Despite the lack of difference in mating success in the plot in which it was measured, the higher rates of moth capture in the untreated areas between treated swaths raised concerns that the untreated area was too wide (90 m) for adequate suppression of mating over the entire plot. Therefore, plans were made to repeat the test the following year with less distance between treated swaths.

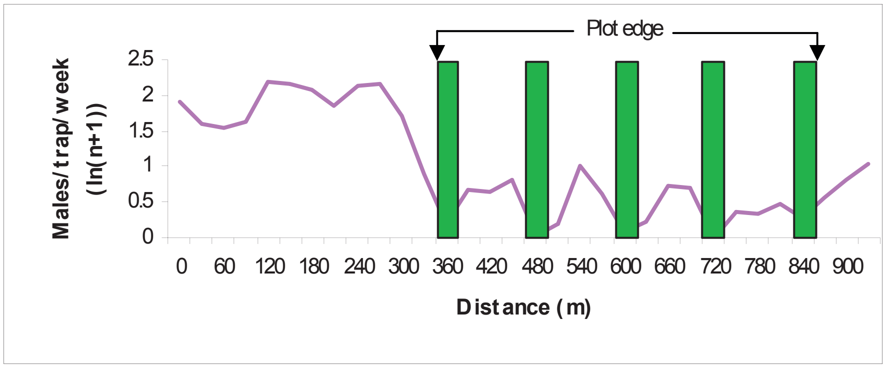

Figure 41. xxx

Treatment Effects Beyond Treated Areas: Part II

To support and extend the findings in 2000, additional tests were conducted in 2001. Study plots for the 3M sprayable microcapsule dose response test at the Appomattox-Buckingham State Forest (ABSF) (only plots treated at 30.4 g AI/acre were used) and the Skipped Swath test at the ABSF and the Goshen Wildlife Management Area (GWMA) (both plots were treated at an overall dosage of 15 g AI/acre) were utilized for this experiment. Laboratory-reared male moths were released at distances of 0, 100, 200, 500, and 1,000 m from the plot edges. Groups of four pheromone-baited traps were placed around every release point. The traps were located at a distance of 25 m from the release point. The results of this test were very similar to those obtained the previous year. The effects of the treatment were observable up to 250 m from the edges of the treated areas (Sharov et al. 2002a).

EAG Measurement of Pheromone Concentrations in Treated Plots

The Syntech EAG sensor was used to measure pheromone concentrations in the plots treated for the 3M sprayable microcapsule dose response test. These plots were treated at dosages of from 0 to 30.4 g AI/acre. Strong suppression of trap catch and mating success occurred at all dosages above 1.2 g AI/acre. Relative disparlure concentration was higher in the 15 and 30.4 g AI/acre plots (0.90 and 0.78, respectively) than in the plots treated at 6 g AI/acre or less (relative disparlure concentration less than 0.6 in all plots) (Fig. 42). However, the variability in the measurements was very high, and the differences were not statistically significant. Based on these results, it seems likely that the Syntech EAG may not be sensitive enough to detect and quantify pheromone concentrations in plots treated at rates of less than 15 g AI/acre. This is a serious limitation, since an application rate of 6 g AI/acre is used operationally in STS.

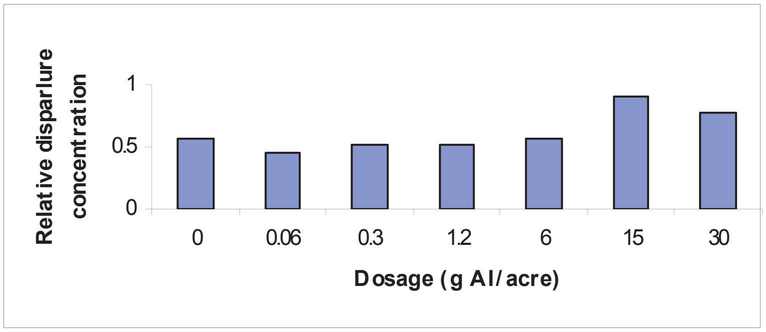

Figure 42. xxx

In 2001, another 48-hour static-renewal acute toxicity test was conducted by Wildlife International (Easton, Maryland) on *Daphnia magna* using a negative control, blank flakes (417 mg/L), and Disrupt II (130, 216, 360, 600, 100 mg/L). The only mortality (100%) was recorded for the blank flakes, which was surprising and thought to be due to the leaching of an ingredient in the flakes (increased to compensate for the loss of the racemic). The 80% mortality recorded in the 1999 test using 300 mg loaded flakes/L was now 5% for 1000 mg-loaded flakes/L.

2002—Skipped Swath Tests

Dose Response Test With Disrupt II Flakes

The dose response test conducted in 2001 used an experimental 3M sprayable microcapsule formulation. Since the only gypsy moth mating disruption product currently registered with US EPA is the Hercon Disrupt II flakes, another dose response test was conducted, this time with the flakes (Tcheslavskaia et al. 2005b). The study was conducted in the Cumberland and Appomattox-Buckingham State Forests in central Virginia. One plot (500 x 500 m) in each state forest was treated at a rate of 0 (control), 0.06, 0.3, 1.2, 6, or 15 g AI/acre. The 6 and 15 g AI/acre rates were obtained by adjusting the flow rate to deliver the specified volume of flakes per acre. Because of mechanical limitations, it was not possible to further reduce the flow rate to obtain the lower application rates. Instead, blank (without disparlure) flakes were mixed with loaded flakes to obtain the specified application rate when applied at the same flow rate as used for the 6 g AI/acre application. The effects of the treatments on recapture of released moths was measured in all plots. In addition, females were deployed to measure mating success in plots treated at 6 and 15 g AI/acre. Extra emphasis was placed on data collection in the 6 and 15 g AI/acre plots because in 2002 STS began operational use of flakes at 6 g AI/acre.

Male moth capture was reduced significantly in all treated plots compared to controls (Fig. 43). Moth capture was very low at both 6 and 15 g AI/acre, and increased with decreasing dosage. Moth capture increased with time since application at all dosages, but the increase was more pronounced at dosages less than 6 g AI/acre. Female mating success was nearly eliminated at both 6 and 15 g AI/acre. Based on these results, the efficacy of flake applications at 6 g AI/acre should be similar to that at 15 g AI/acre. However, application rates below 6 g AI/acre may not be effective, especially as the time since application increases.

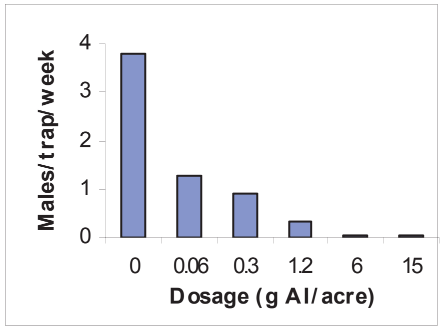

Figure 43. xxx

Shin-Etsu Sprayable Formulation

Two study plots for evaluating a liquid sprayable formulation developed by Shin-Etsu (Fig. 44) were established and incorporated into the flake dose response test. The material was applied at 6 and 15 g AI/acre using *[...application parameters...]*. Recapture of released male moths was used to evaluate treatment efficacy. During the first two weeks after application, moth capture was very low at both dosages, and was not significantly different from that in plots treated with flakes at the same dosages. However, after two weeks moth capture increased in the plots treated with the Shin-Etsu formulation, and by 50 - 56 days after application moth capture was significantly higher in the Shin-Etsu plots compared to the flake plots at the same dosages. It was clear that improvements in this Shin-Etsu formulation were needed to extend its period of effectiveness to a full eight weeks.

(Fig. 44 here)

Skipped Swaths - 30 m Skips

Two plots (500 x 500 m) were treated at the Goshen Wildlife Management Area, Virginia, with Disrupt II flakes at an overall dosage of 15 g AI/acre by alternating treated swaths (30 g AI/acre) with untreated swaths. Two untreated plots of similar size were left untreated and served as controls. Three lines of 35 to 37 pheromone traps spaced 30 m apart were placed across each of the four plots. In the treated plots, the trap lines were perpendicular to the aircraft flight path. Traps were checked once each week for three weeks. Male moths were not released, so only native moths were used to evaluate the treatments.

Trap catch was reduced by 96% in the treated swaths and by 93% in the untreated swaths compared to trap catch in the untreated plots (Fig. 45). The difference in trap catch between treated and untreated swaths was not significant. It appears from this experiment that mating will be effectively disrupted in plots treated with flakes at 15 g AI/acre with alternating treated and untreated swaths (Tcheslavskaia et al. 2005a).

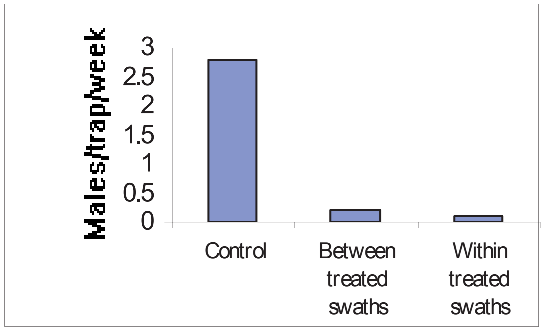

Figure 45. xxx

In April of 2002, Thorpe and Reardon visited 3M Canada Company in London, Ontario to discuss the results to date with their sprayable microcapsule product. Grant Oliver of 3M mentioned that the Phase III 3M sprayable microcapsules that we have been using for gypsy moth is the product that the company will register with the US EPA.

2003—Diffusion of Pheromones in a Forest Canopy

In 2003, 3M Canada Company obtained a registration for its commercial product (3M MEC-GM Sprayable Pheromone for Gypsy Moth) from the US EPA, but it has only been applied to small acreages for methods development and not used operationally.

Screening of Experimental Formulations

It has long been recognized that the addition of alternative mating disruption formulations could benefit STS by reducing costs and increasing flexibility in awarding contracts to applicators. In 2003, a number of candidate experimental formulations became available for field testing. The following six formulations were tested, each at application rates of 6 and 15 g AI/acre (Table 2):

1. Standard flakes (Hercon Environmental, Emigsville, Pennsylvania). This is the EPA-registered, commercially-available formulation (Disrupt II) consisting of plastic laminate flakes (1 x 3 mm) applied with a sticker and used operationally in STS.
2. Modified flakes (Hercon Environmental). This formulation is identical to the standard flakes except for proprietary changes in the manufacturing process.
3. Micro-flakes (Hercon Environmental). This formulation consists of smaller plastic laminate flakes mixed into a liquid slurry intended for application by helicopter.
4. Hollow fibers (Scentry Biologicals, Billings, Montana). This formulation consists of hollow plastic fibers containing pheromone and mixed with a sticking agent (Fig. 46).
5. Granules (Valent Biosciences, Long Grove, Illinois). This is a granular formulation intended for application by helicopter (Fig. 47). A sticking agent was used at the 6 g rate but not at the 15 g rate.
6. 3M MEC (3M Canada Company, London, Ontario, Canada). This is a sprayable microcapsule formulation containing pheromone in a liquid carrier intended for application through conventional spray equipment.

Table 2. Formulations for comparison.

Code	Formulation	%AI	Mix	Rate (gm AI/acre)	Method	Sticker
H015	Hercon – old flake	17.9	Neat	15	Flake	Gelva 2333
HN15	Hercon–ESO flake	17.9	Neat	15	Flake	Gelva 2333
HMN15	Herdon–ESO microflake*	17.9	Neat	15 (85 gms/flakes)	Flake	Gypmone & Gelva 2333
VG15	Valent (VBC 60052	.33	Neat	15 (10 lbs/acre)	Granule	Pypmone & Gelva 2333
SBC15	Scentry (center sealed)	10	Neat	15	Fiber	Bio-tac
3MF15	3M (MEC-GM) phase III	20	(Diluted water)	15	2µl drop	None
3MF6	3M (MEC-GM) phase III	20	(Diluted water)	6	2 µl drop	None

Time (days): 0, 3, 7, 14, 28, 21, 28, 35, 49, 60, 74
Substrate: Canvas paper (or petri dish)
Replicates: Four canvas papers/formulations/time
All canvas papers (or petri dish contents) will be placed in glass vials for shipment to Devilbiss
7 formulations x 10 times x reps/for/time = 280

(Fig. 46 here)

(Fig. 47 here)

The study was conducted in the Cumberland and Appomattox-Buckingham State Forests in central Virginia. Study plots (500 x 500 m) contained primarily favored gypsy moth host trees and were separated by a minimum distance of 1 km. Each treatment was replicated twice - once on each state forest. Two untreated plots served as controls. Laboratory-reared male moths were released twice each week for eight weeks at the center and at points 150 m to the north and south of the center of each plot at a rate of approximately 50 males per release point. The north and south release point each had four standard milk carton pheromone traps positioned around them at a distance of 25 m. All released males were coated with a fluorescent powder so that they could be distinguished from feral males (Fig. 48). Only released males were used in the analyses. Traps were checked and emptied twice per week.

(Fig. 48 here)

More than five moths per trap per release were caught in the untreated control plots (Fig. 49). In plots treated with standard flakes, 0.25 and 0.09 moths per trap per release were caught at the 6 and 15 g rates, respectively. This was a 95 and 98% reduction compared to controls. Similar results were obtained for the modified flakes, indicating that the manufacturing changes did not affect efficacy. In the plots treated with the micro-flakes, traps caught 0.25 and 0.63 moths per release at the 6 and 15 g rates, respectively. No obvious explanation could be found for the increased moth capture at the higher application rate. Trap catch in the plots treated with plastic fibers was 0.38 and 0.26 per trap per release at the 6 and 15 g rates, respectively, or 93 and 95% reductions compared to controls. In the plots treated with granules, trap catch was 1.35 and 1.76 per trap per release at the 6 and 15 g rate, respectively. This represents a reduction compared to controls of 74 and 67%. The greater trap catch at the 15 g compared to the 6 g rate may have been because the formulation was applied without sticker. Trap catch in plots treated with the 3M MEC formulation were 4.32 and 0.92 at the 6 and 15 g rates, respectively. Reductions in trap catch with this formulation compared to the controls were 18 and 83%. There were a number of heavy rainfall events in the weeks after the formulations were applied, which may have reduced the efficacy of some of the formulations.

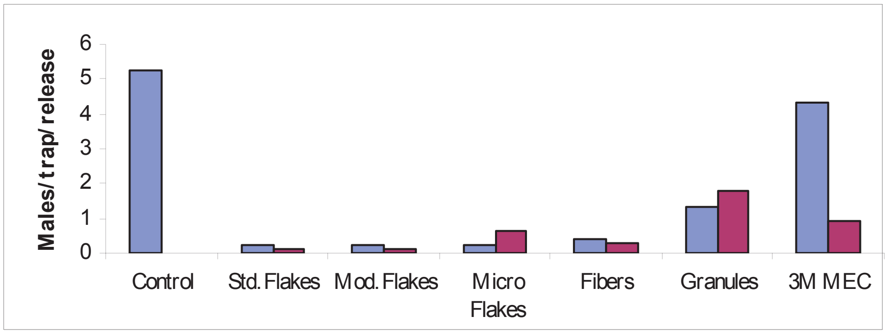

Figure 49. xxx

The experimental formulations that were field tested were evaluated in the greenhouse to determine the residual pheromone levels. Twenty-five granules/drops/flakes/fibers were applied on canvas paper (or Petri dishes). A subset of the canvas papers were not protected from rain (see Table 3).

Table 3. Results of gypsy moth pheromone aging studies in the Otis Greenhouse at 0 days and 21 days post-treatment. Compiled on March 23, 2001.

Material	On Paper		On Red Oak Foliage	
	0 Days	21 Days	0 Days	21 Days
Hercon Flakes	125µg	78 µg	145 µg	85 µg
	Loss: 38%		Loss: 41%	
Shin-Etsu	63 µg	29 µg	63 µg	40 µg
	Loss: 54%		Loss: 37%	
3M	43 µg	18 µg	44 µg	13 µg
	Loss: 58%		Loss: 70%	

Average of three samples for each treatment.

Trap Capture Versus Mating Success in Northern Wisconsin

Gypsy moth spread rates continued to be higher in the north compared to the south. Within the state of Wisconsin, spread rates increased from south to north. To collect additional data on the relationship between trap catch and mating success, and to search for differences in this relationship that could be related to differences in spread rates, an experiment was conducted in northern Wisconsin. This experiment was a repeat of experiments conducted in Virginia and West Virginia in 1994 (Sharov et al. 1995) and southern Wisconsin in 2000 (Tcheslavskaia et al. 2002). Based on male moth catches in pheromone traps, six plots were established in the Chequamegon-Nicolet National Forest and nearby forested sites during August at various distances from the advancing gypsy moth front. At each plot, female mating success was measured from tethered females deployed on trees and male trap catch was measured with two milk carton pheromone traps each located 100 m from the nearest deployed female. Females were retrieved and traps checked about 24 hours after deployment. Average trap catches ranged from 0 to 50 per trap per day. Mating success increased with increasing trap capture. The relationship was similar to that obtained in the other two studies, except that there was more variability and female mating success was somewhat higher at a given level of trap catch than previously. In the three studies, the ratio of mating success to trap catch increases from south to north (Virginia to northern Wisconsin), which coincides with increasing spread rates. Further work will be needed to determine if these similar trends are caused by the same factors.

Diffusion of Pheromone in a Forest Canopy

Ongoing research on the movement of insect pheromones within a forest canopy (Thistle et al. 2004) uses a tracer gas (sulfur hexafluoride, SF6) as a model. To confirm that the tracer gas is a reliable surrogate for gypsy moth pheromone, a test was conducted in a mixed hardwood/conifer forest at the University of Michigan Biological Station, Pellston, MI during August. An SF6 gas generator was co-located with a source of disparlure (Hercon Luretape). An SF6 detector and a Syntech portable EAG sensor were co-located at a downwind distance of 5 m from the sources (Fig. 50). The SF6 detector was a modified gas chromatograph (Benner and Lamb 1985) capable of detecting the tracer gas at a minimum concentration of about 30 parts per trillion. The EAG used the intensity of responses from a severed male moth antenna to estimate the concentration of pheromone in the air. Ten 30-minute experiments were conducted in all. There was good agreement between the two sensors in detecting the presence of plumes of SF6 and pheromone, and in the distribution of normalized gas concentration values. The results of this study suggest that the SF6 tracer gas is a realistic model for the diffusion of gypsy moth pheromone in a forest canopy (Smith et al. 2004).

(Fig. 50 here)

2004—Effect of Female Moth Deployment Method on Mating Success

Mating Disruption With ExoSex Dispensers

An experimental ground-based gypsy moth mating disruption system provided by ExoSect Limited (Southampton, United Kingdom) was field tested in 2003 (Fig. 51). Dispensers, which were similar in size and shape to delta traps, contained a waxy powder loaded with 0.1% (w/w) +-disparlure. The dispensers were designed to attract males and contaminate them with the powder containing the pheromone. The expected mechanism of mating disruption was auto-confusion of male moths by their contamination with pheromone, rendering them unable to locate females, and by the contaminated males serving as additional pheromone point sources to confuse uncontaminated males. The dispensers were deployed at a density of 10 per ha in two 10-acre plots in the Cumberland and Appomattox-Buckingham State Forests in central Virginia. Other plots from other experiments in the same vicinity that were set up and monitored in exactly the same way served as negative controls (untreated) and positive controls (plots treated with Disrupt II flakes at 6 and 15 g AI/acre) (see Screening of Experimental Formulations section, above).

(Fig. 51 here)

Mating success in the plots treated with the ExoSex dispensers was 22.3%, which was nearly the same as that in the untreated plots (25.3%) (Fig. 52). Mating was reduced to 0.2% and 0 in the plots treated with Disrupt II flakes at 6 and 15 g AI/acre, respectively. Based on these results, there is no evidence that the ExoSex mating disruption system as deployed in this experiment disrupted gypsy moth mating.

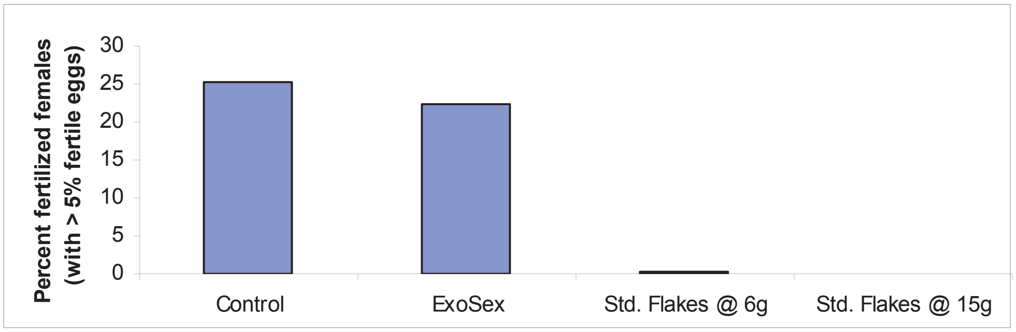

Figure 52. xxx

3M MEC-GM Sprayable Pheromone for Gypsy Moth

A sprayable, microencapsulated gypsy moth mating disruption formulation that can be applied through conventional hydraulic aerial application equipment has been under development by 3M Canada Company for several years. In 2003, an EPA registration was granted for 3M MEC-GM Sprayable Pheromone for Gypsy Moth. This formulation was tested in study plots in 2003. Results were poor at the 6 g but better at the 15 g AI/acre rate. Another test of the 3M product was conducted in study plots in 2004 and evaluated using both male trap catch and female mating success. The test was conducted in the Cumberland and Appomattox-Buckingham State Forests in central Virginia, and the standard study plot protocol was used (500 x 500 m plots, laboratory-reared males released two times per week for eight weeks at three release points per plot, four pheromone traps around two of the release points and a 50-m radius circle of deployed females around the remaining release point. The 3M product was applied at 6 and 15 g AI/acre using *[...application parameters...]*. Also included in the test were treatments with flakes at 6 and 15 g AI/acre and an untreated control. Males were reared on artificial diet containing a red dye which is visible in the adults so that released males could be identified. Only data from released males were included in the male trap catch results.

Moth capture in the plots treated with flakes at 6 and 15 g/acre was 2.6 and 0.7% of controls, respectively (Fig. 53a). With a single exception (6 g/acre treatment on July 21), all values were reduced by more than 90% compared to controls. In the plots treated with 3M MEC, average moth capture at 6 and 15 g/acre was 38.5 and 9.1% of controls, respectively. Reduction in moth capture was less than 90% in most plots treated at 6 g/acre, but only on four dates was the reduction in moth capture compared to controls less than 90% in the plots treated at 15 g/acre. The first of these dates did not occur until about seven weeks after pheromone application, suggesting that the product's release rate may have been lower during the later part of the evaluation period. While the results at 15 g AI/acre are encouraging, there is still a need to increase the effective life of the product to a full eight weeks. Unfortunately, after the completion of the test 3M announced that it will not pursue further development and marketing of the product.

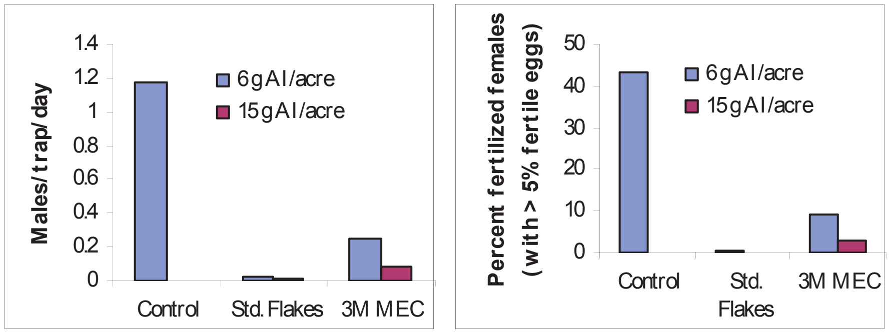

Figure 53. xxx (no text reference to Fig. 53b)

Effect of Moth Density on Effectiveness of Mating Disruption Treatments

The standard evaluation protocol that is used in conjunction with study plots to test dose responses and efficacy of aerially-applied experimental formulations provides consistent and reliable results. Briefly, it involves the release of adult male gypsy moths that were reared in the laboratory on artificial diet containing a red dye. Males are released two times per week for eight weeks at three release points in each plot. One of the release points, located in the center of the plot, is surrounded by a 50-m radius circle of trees on which laboratory-reared females are deployed. Females are placed untethered on the boles of trees on which a single barrier band consisting of duct tape coated with Tangletrap pest barrier has been placed at a height of about 2 m. The barrier prevents the female from ascending the tree. Females are retrieved 24 hours after deployment and placed, along with any eggs they may have laid, in a paper bag. The bags are stored at room temperature for at least 30 days, after which time they are examined for evidence of embryonation which indicates that the egg is fertile and the female has mated. The other two release points are each located 150 m from the plot center. Each is surrounded by four milk carton pheromone traps at a distance of 25 m from the release point. The traps are checked two times per week. During flight, males are checked for the presence of dye and only released males are included in the data used to evaluate the treatment.

This evaluation protocol is assumed to simulate low-density gypsy moth populations such as are targeted in STS. However, there is no direct way to relate the level of moth density simulated in this study to that in the real world. As a preliminary step toward relating the results obtained using the standard evaluation protocol to those in STS, an experiment was conducted to determine the sensitivity of the standard protocol to changes in numbers of released moths. The study was conducted in plots (500 x 500) in the Cumberland and Appomattox-Buckingham State Forests in central Virginia. Four plots were treated at 6 g AI/acre, four were treated at 15 g AI/acre, and four were left as untreated controls. In half the plots males were released at the standard rate of 50 moths per release point per release. In the other half, the moth release rate was tripled to 150 moths per release point per release.

Moth recapture in untreated plots averaged 1.2 and 3.5 per trap per day at the low and high moth release rate, respectively (Fig. 54a). Moth recapture from plots treated with flakes at 6 g AI/acre was about 2.5% of untreated controls regardless of the release density (Fig. 54b). Moth recapture from plots treated at 15 g AI/acre was about 0.6% of untreated controls regardless of release density. Percent fertilized females (with >5% fertile eggs) was 43.1 and 77.3 in the untreated plots at the low and high release rate, respectively. Percent fertilization was reduced by greater than 99% compared to controls at both moth densities at both dosages. Over the range of moth release rates used in this experiment (50 - 150 per release point per release) there was a proportionate increase in the numbers of moths captured and percent fertilization, but moth capture and fertilization rates as a percentage of controls remained constant.

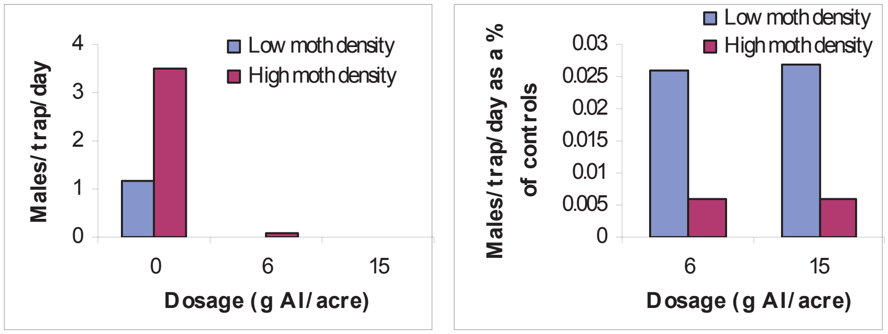

Figure 54. xxx

Residual Effects of Previous Year's Treatment

In July 1991, (Leonhardt et al. 1996) collected flakes from the forest floor in a plot that had been treated in July of 1990 and analyzed them for disparlure content. They were found to contain 1.8% of their original content. To determine if biological effects from this residual disparlure content could be detected in the year after treatment, two study plots that had been treated the previous year with flakes at 15 g AI/acre were evaluated. Male trap catch in these plots was 62% of that in the untreated controls and percent fertilization (>5% of eggs fertile) was 37% of that in the untreated controls (Fig. 55a and b). Clearly, biological effects, while weak, were still present the year after treatment. This is of concern in STS because the success of operational treatments is determined based on trap catch in treatment blocks the year after treatment. This evaluation method relied on the assumption that reductions in trap catch the year after treatment were the result of reductions in moth reproduction. However, based on this study it appears likely that reductions in moth catch may also be due, at least in part, to residual effects from the previous year's treatment.

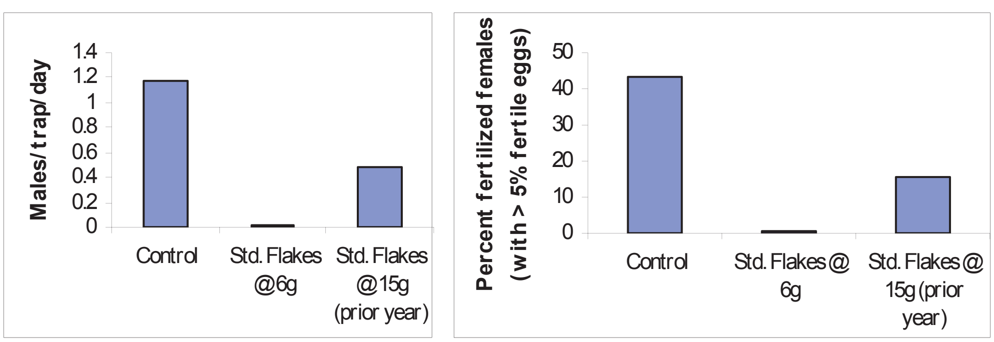

Figure 55. xxx

Efficacy of Mating Disruption Treatments in Open Landscapes

Currently, mating disruption is rarely used for eradication treatments, because of a lack of data to support this use and because the treatment prevents the use of pheromone traps to monitor the infestation in the treated area in the year of treatment. However, as a non-toxic, species-specific control tactic, there is interest in using mating disruption to eradicate isolated infestations. Based on discussions with program managers in states conducting eradication activities, several questions were raised. The first was a concern that mating disruption might not be effective in open landscapes, such as parks and residential areas. Possible reasons for lack of effectiveness include the excessive deposit of flakes on the ground and the concentration of male searching activity on a small number of trees. To examine this issue, an experiment was conducted at the Goshen Wildlife Management Area, Goshen, Virginia. Two areas were selected that had been clear cut the previous year. Each clear cut contained several isolated, mature trees surrounded by a cleared area. One clear cut, and an adjacent forested area, were treated with flakes at 15 g AI/acre. The other clear cut, together with an adjacent forested area, were left untreated. Females were deployed on the boles of the trees beneath a Tangletrap pest barrier which was placed at a height of 2 m. Males were released from four points around each tree, each at a distance of 25 m from the tree, at a rate of 15 males per point per release. Females were retrieved after 24 hours and their fertilization status was determined from their eggs.

The average fertilization rate (>5% fertile eggs) of females in untreated areas was 33.9% in the clear cut and 24.2% in the forested area (Fig. 56). In the treated area, the average fertilization rate was 0.5% in the forested area (one female was mated) and no females were mated in the clear cut area. It appears that an aerial application of flakes at 15 g AI/acre will effectively shut down mating in forested areas or open landscapes.

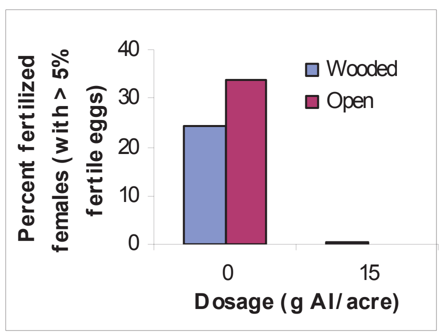

Figure 56. xxx

Efficacy of Mating Disruption Treatments When Male and Female Gypsy Moths Emerge Close Together

Another question that was raised relative to the use of mating disruption to eradicate isolated infestations was how effective the tactic would be in a situation where males and females emerge close together in space and time. To address this question, an experiment was conducted in the Cumberland State Forest in central Virginia. Four plots (500 x 500 m) were treated with flakes at dosages of 0, 0.06, 6 and 15 g AI/acre. In each plot, 12 sites were established at a minimum distance of 10 m from each other. Each site consisted of a pair of trees separated by a distance of about 1 m. A Tangletrap pest barrier was placed on one of the trees at each site at a height of 2 m. Laboratory-reared females less than 24 hours old were placed on the tree with the pest barrier. A laboratory-reared male that had emerged from its pupal case within the previous 30 minutes was placed in one of three positions:

1. Next to the female (within 5 cm)
2. On the same bole as the female but near the ground
3. On the other tree (about 1 m away)

Females were retrieved 24 hours after they were deployed.

In the untreated plot, the percentage of fertilized females (>5% eggs fertile) was above 50% regardless of the initial positions of the moths (Fig. 57). In the treated plots, fertilization rates of females initially placed next to males ranged from 52% at 0.06 g AI/acre to 23% at 15 g AI/acre. When males were released on separate trees, fertilization rates dropped to 58% in the untreated plot, 21% at 0.06 g, 4% at 6 g, and no females were fertilized at 15 g AI/acre. Fertilization rates of females placed near the ground but on the same tree as males were about mid-way between these two values. These results suggest that, while mating disruption treatments will greatly reduce mating regardless of how close males and females emerge in space and time, they will not adequately reduce mating if females and males emerge close together on the same tree at the same time. The significance of this finding depends on how often males and females emerge together in space and time. Future work will be needed to address this question.

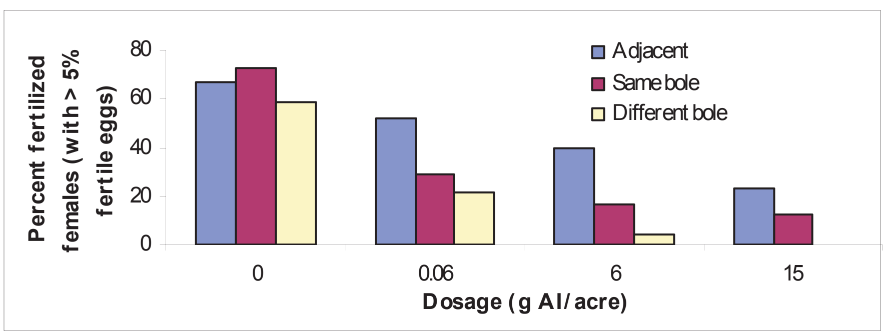

Figure 57. xxx

Effect of Female Deployment Method on Mating Success

The deployment and recovery of laboratory-reared female gypsy moths in study plots is a critical part of the biological evaluation of mating disruption treatments. Prior to 2000, females were deployed in modified delta trap mating stations. These were used because they were convenient, easy to install, and resulted in good rates of female recovery. However, as part of the study plot research activities in 2000, a direct comparison was made of mating success of females deployed in mating stations and females that were tethered with a thread and pinned to tree trunks (Tcheslavskaia et al. in press). Mating success of tethered females averaged about twice that of females deployed in delta traps in untreated plots. Tethered females were used in some subsequent study plot work, but before long the process of tethering deployed females was discontinued because it was too time consuming and tedious. For a short time the use of tethered females was replaced by the deployment of females in arenas constructed on the boles of trees. These arenas consisted of duct tape coated with Tangletrap pest barrier and stapled to the tree to confine the female and reduce predation by ants. To catch females that fell, a tray constructed out of aluminum flashing was stapled to the bottom of the arena in such a way that the female could climb back up on the trunk if she fell (Fig. 58). Rates of mating success using these arenas were never compared to those of tethered females. Later, female deployment was further simplified by placing untethered females directly on tree trunks beneath a barrier made of duct tape coated with Tangletrap pest barrier. The barrier prevented the females from ascending the tree, and falling females could climb back up the trunk from the ground. Again, mating success using this method of female deployment relative to that using tethered females was not known.

(Figure 58 here)

To obtain information about how these methods of female deployment compare, an experiment was conducted in the Goshen Wildlife Management Area, Goshen, Virginia. Five different methods of female deployment were tested:

1. modified delta trap
2. duct tape arena with flashing tray
3. single trunk barrier
4. double trunk barrier (above and below female)
5. tethered female

Rates of recovery and mating success of deployed females were measured. The five different methods were arranged in [___] m radius circles around points at which laboratory-reared males were released. The study was conducted in an untreated area and in a plot that had been treated with flakes at a dosage of 0.06 g AI/acre - high enough to affect mating success but low enough that mating would not be eliminated.

Recovery rates were slightly lower in the treated area, except for the delta trap mating station, for which recovery rates were not affected by the treatment. Recovery rates varied from 61% with the single band method in the treated area to 91% in the delta trap mating station in the treated area (Fig. 59). Mating success was substantially lower using all deployment methods in the treated plot compared to the untreated area. In untreated areas, mating success was lowest in the delta trap mating station (14%) and highest with tethered females (41%). Mating success using the flashing and single and double band methods was about mid-way between that using delta traps and tethered females. In the treated plot, mating success was lowest with the delta trap mating stations (2%) and was approximately equal among the other methods (from 9 - 12%). The cost of each method was estimated based on initial time to set up the mating station and recurring costs associated with female preparation (e.g. tethering), time actually deploying females, and time needed to maintain the mating stations. The single band method was the least expensive and tethering was the most expensive. The other methods were intermediate in cost. Based on the above information, it appears that the single band method of deploying females was the most cost effective. It was the least expensive, it resulted in relatively high levels of mating success, and, even though it had the lowest recovery rates, the recovery rates were still reasonably high (>60%). While tethering is superior to the single band method both in recovery rates and mating success, experience has shown that the cost of this method is unacceptably high.

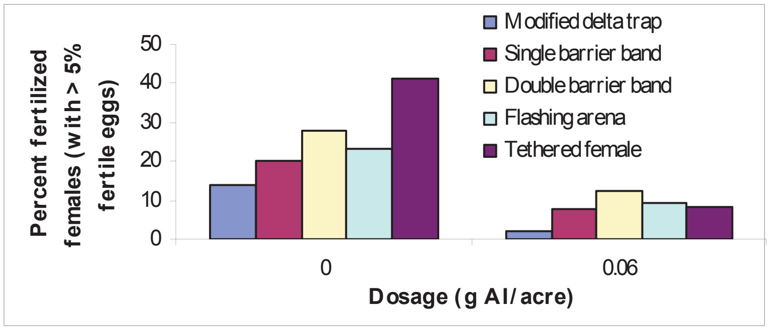

Figure 59. xxx

Air Sampling of Disparlure

During conversations with scientists from Shin-Etsu Chemical Co., Tokyo, Japan, they mentioned that they routinely used charcoal filters to sample airborne concentrations of pheromone applied for control of pink bollworm, peach fruit moth, tea tortrix, and other insect pests. To determine if similar air testing for disparlure was feasible, Shin-Etsu provided 12-volt pumps and glass tubes packed with activated charcoal to sample the air in plots treated for gypsy moth mating disruption. The pumps were originally designed for use in aquariums and provided a flow rate of 8 - 10 liters/minute. Sampling was conducted in plots treated with flakes at 6 and 15 g AI/acre. A pulley system was erected in each plot so that pumps could be raised into the canopy (Fig. 60). Sampling was conducted for 12 continuous hours each week for eight weeks after application. In each plot, one pump was positioned at 2 m from the ground and another in the canopy. The charcoal filters were washed with solvent and analyzed by USDA, ARS, Beltsville, Maryland using GC-mass spectroscopy. No disparlure could be detected in any of the samples. Efforts are ongoing to determine if the charcoal failed to capture all of the disparlure that passed through the sample tubes or if 12 hours was insufficient time to accumulate detectable amounts of disparlure.

(Figure 60 here)

In 2004, another 48-hour static-renewal acute toxicity test using *Daphnia magna* was conducted by Wildlife International. The same lot number samples that were tested in 1999 and 2001 were re-evaluated along with the 2004 version of Disrupt II flakes. Also, the 3M Canada Company product MEC-GM was evaluated both loaded with racemic disparlure and loaded with a vegetable oil (the chemistry of the capsule formulation would not allow water to be used instead of an oil) and vegetable oil (control). Also, tested was the technical racemic disparlure provided by Shin-Etsu. There was zero mortality for the loaded flakes up to and including 1000 mg/L for all samples. There was mortality for 3M sprayable microcapsules for 27 mg/L and 300 mg/L (80 and 60% respectively) after 48 hours. The mortality was probably due to the oily surface layer. Mortality for the ISP and Shin-Etsu technical racemic disparlure at 300 mg/L and 1000 mg/L was 100%, again due to the oily surface layer.

Screening tests (96 hour) with bluegill (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*) were conducted using the Disrupt II block (1000 mg/L), standard flakes (300 and 1000 mg/L), ESO flakes (300 and 1000 mg/L) and the Shin-Etsu and ISP technical (1000 mg/L). There was zero mortality for each species.

2005—Multi-year Residual Effects of Mating Disruption Treatments

3M Sprayable Pheromone

The 3M Canada Company announced on 4 January 2005 its intention to exit the 3M-branded Sprayable Pheromone business for both agriculture and forestry markets effective immediately.

This was a major disappointment as numerous trials were conducted with their product and it was a viable option to Disrupt II for operational programs.

Shin-Etsu Sprayable Formulation

A Shin-Etsu sprayable gypsy moth mating disruption formulation that was tested in study plots in 2002 did not provide adequate release rates throughout the season. In 2005, a modified formulation was tested in study plots in the Cumberland and Appomattox-Buckingham state forests in central Virginia using the standard study plot protocol. The product was applied at 6 and 15 g AI/acre using [application parameters …]. Disrupt II flakes at 6 and 15 g/acre were applied to plots at the same time, and untreated plots were monitored as controls. Only data from released males (distinguished by a red dye fed to larvae) are included in the male trap catch results.

Moth capture in plots treated with flakes at 6 and 15 g/acre was 2.5 and 0.1% of controls, respectively (Fig. 61). In the plots treated with the Shin-Etsu product, moth capture averaged 8.6 and 4.6% of controls, respectively. Mating success in plots treated with flakes at 6 and 15 g/acre was 1.1 and 0.7% of controls, respectively. In the plots treated with the Shin-Etsu formulation it was 0.3 and 0.4% of controls, respectively. At both application rates, trap capture increased gradually over time, indicating that the product may have been releasing too rapidly to provide uniform coverage throughout the full 8-week evaluation period. However, mating was essentially shut down at both application rates and the product's performance was much improved compared to the formulation tested in 2002.

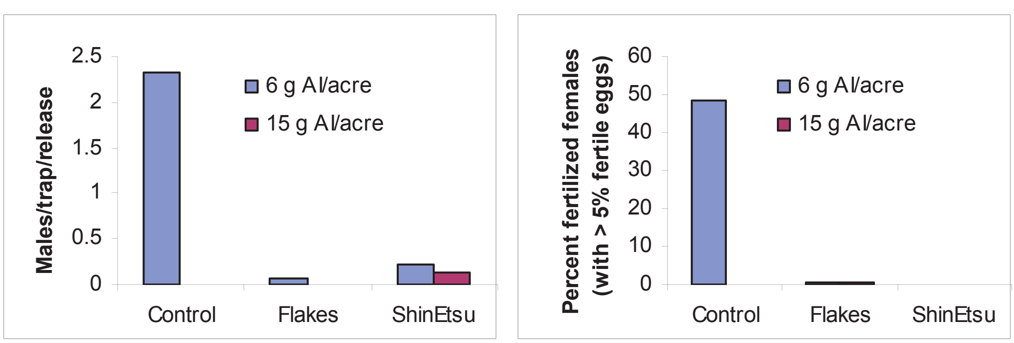

Figure 61. xxx

A serious concern with the Shin-Etsu product tested in 2005 was that it was difficult to wash it from surfaces and to clean application and mixing equipment. Once it hardened, it was extremely difficult to remove even from stainless steel surfaces. Shin-Etsu agreed to try to modify their product to make it easier to clean up.

Multi-year Residual Effects of Mating Disruption Treatments

Monitoring during 2004 of plots treated with Disrupt II at 15 g/acre in 2003 indicated that suppression of both trap catch and mating success persisted into the year following treatment. To confirm and expand on those findings, plots treated with flakes in 2003 and 2004 at 15 g/acre and in 2004 at 6 g/acre were monitored in 2005. The plots were located in the Cumberland and Appomattox-Buckingham state forests in central Virginia. Standard monitoring protocols as described previously were used. Trap catch and mating success resulting from releases of laboratory-reared males in these plots were compared to those in plots treated with flakes at 15 and 6 g/acre in 2005 and untreated control plots.

Trap catch and mating success in plots treated the previous year at 15 g/acre were reduced by 52.7 and 81.7%, respectively, compared to controls (Fig. 62). Trap catch and mating success in plots treated two years previously at 15 g/acre were reduced by 18.9 and 34.2%, respectively. Trap catch and mating success in plots treated the previous year at 6 g/acre were reduced by 40.2 and 93.1%, respectively, compared to controls. These results confirm those from 2004 for plots treated at 15 g/acre in 2003, and indicate that there may be continued suppression into the year following treatment. These findings also suggest that trap catch and mating is suppressed the year following a treatment at 6 g/acre.

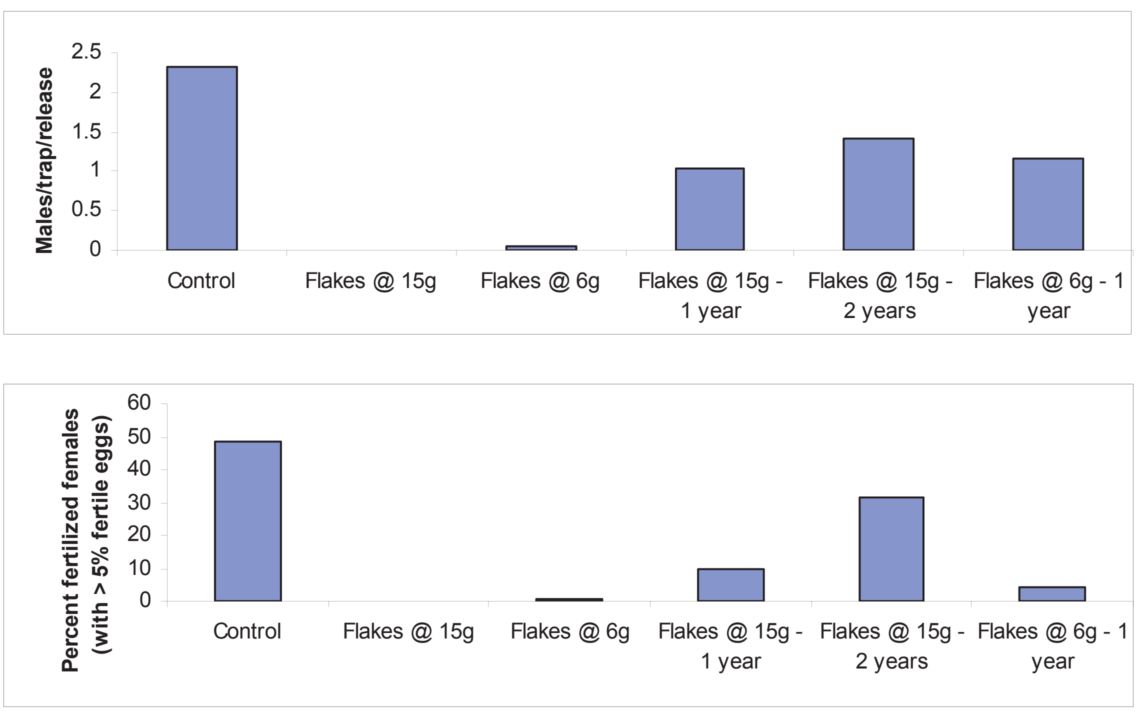

Figure 62. xxx

Season-Long Mating Success Versus Trap Catch

While previous efforts have quantified the relationship between gypsy moth mating success and daily trap capture (Sharov et al. 1995; see section 2000 – Trap Catch Versus Mating Success in Southern Wisconsin and section 2003 – Trap Capture Versus Mating Success in Northern Wisconsin), these studies were not conducted through the entire gypsy moth flight period, so they did not provide a means to relate mating success to male density through time. Also, it is not possible from these previous studies to relate mating success to season-long trap capture (SLTC), which is important because all gypsy moth population density data from the STS program is expressed in terms of season-long capture of males in pheromone-baited traps. Therefore, a study was conducted in 2005 to measure mating success and daily male trap capture throughout the entire flight period at locations that ranged in population density from very low to high and for which SLTC was measured.

The study was conducted in the Appomattox-Buckingham State Forest near Dillwyn, Virginia (20,000 acres). Ten plots consisting primarily of oaks with a canopy height of approximately 30 m were established at various locations within the state forest. At each plot, a trunk barrier consisting of duct tape with a narrow bead of Tanglefoot Pest Barrier (The Tanglefoot Company, Grand Rapids, Michigan) was applied to 15 trees arrayed in a 100-m circle. Two standard USDA milk carton pheromone traps were placed opposite each other and about 250 m from the plot center. Laboratory-reared female gypsy moth pupae were shipped to Virginia from the USDA-APHIS Pest Survey, Detection, and Exclusion Laboratory in Massachussetts, and reared to adults. Starting just prior to the beginning of male flight in the area, female moths were placed on the boles of the trees below the trunk bands. Only females less than 24 h old were used. Females and any egg masses they produced were collected 24 hours later, placed in a paper bag, and held for at least 30 days. After 30 days the eggs were checked for embryonation, which indicates fertilization. Females were placed on trees daily (except weekends) until just after flight ended. Traps were checked each day that females were deployed or collected.

Data from two of the plots are shown below. Circle 2 is from a relatively high gypsy moth population (SLTC = 64), and is typical of other locations with SLTC > 60. While no life stages other than males were found at this location, the high SLTC indicates that populations are probably too high to be suitable for the use of mating disruption. Daily trap capture through the flight period is shown by the solid line (Fig. 63a). Flight was first detected on June 30 and peaked on July 8 with a daily catch of 25, and the last day of flight was July 19. At these high population densities, mating success rapidly rose to 80% or greater early in the flight curve and stayed high until the very end of flight. These data suggest that there was a sufficient number of males to achieve high levels of mating success throughout the entire flight period.

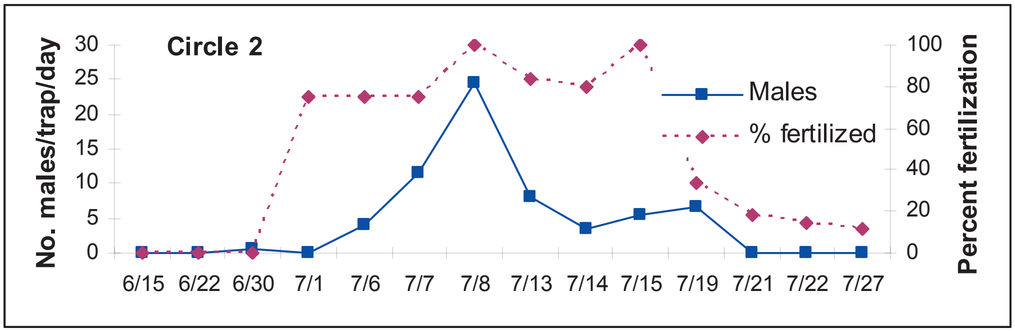

Fig. 63a. xxx

Circle 10 is typical of data from lower population density sites. Under these conditions, mating success more closely tracks changes in daily trap catch. Even at the peak of male flight, mating success remained below 70% Fig. 63b). These data suggest that, under these conditions, mating success is limited by the lower numbers of available males.

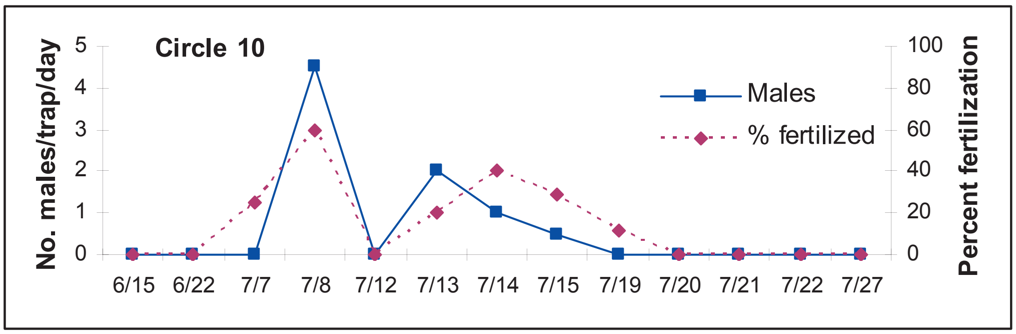

Figure 63b. xxx

The figure below shows combined data from each of the 10 plots (Fig. 64). Male trap catch (X-axis) is expressed as SLTC. Mating success (Y-axis) is expressed as the average of the highest three mating success values recorded for that plot. Fitting a logarithmic curve to the data results in a model that relates SLTC to mating success. Future work is planned to collect more data at lower population densities (below SLTC = 30) and to study the relationship between SLTC and mating success in other geographic areas, especially in the North Central states.

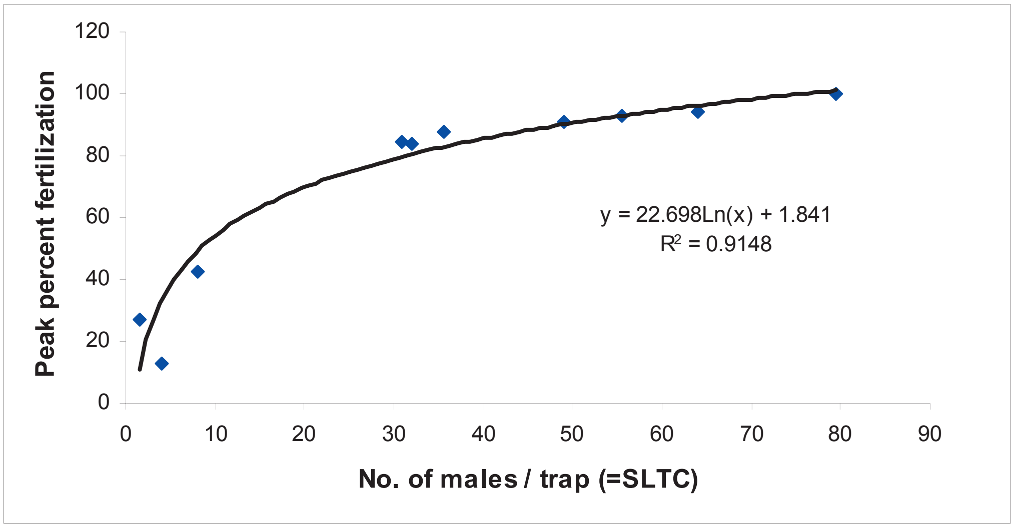

Figure 64. xxx

Finally, the above data were useful in providing an answer to a recurrring question concerning simulated gypsy moth populations in field study plot tests. Over the past several years, tests have been conducted in field plots in which laboratory-reared gypsy moth males were released in plots treated with various formulations and dosages of mating disruptants. Released males, rather than naturally occurring males, were used so that moth density among plots could be closely controlled. However, because of the way that male moths were trapped in the plots, there is no way to relate moth density based on trap capture in the study plots to naturally-occurring gypsy moth density, so it has not been possible to estimate the population density that is being simulated in the study plots. Because mating success is measured in exactly the same way in the study plots and in the above experiment with naturally-occurring moth populations, the measurement of mating success provides a quantitative link between simulated and natural population densities. Mating success in the untreated study plots generally lies between 40 and 60% (see section 2004 - 3M MEC-GM Sprayable Pheromone for Gypsy Moth; section 2005 – Shin-Etsu Sprayable Formulation). Based on the model shown in the above figure, this range of mating success is associated with season-long trap capture values between 5 and 13. This is important information, because the study plot work is intended to apply to population densities that are low enough to be appropriate for mating disruption treatments, and it appears that the releases of males in the study plots simulate appropriate population densities.

Mating Success of Irradiated Females

Future mating disruption work at low gypsy moth population densities may have to be done in areas outside of the gypsy moth quarantine. If laboratory-reared gypsy moths are required for this work, it may be necessary to use sterilized insects. USDA-APHIS has developed a procedure for irradiating female pupae that will produce only sterile adults. In 2005, an experiment was conducted at the Appomattox-Buckingham State Forest in central Virginia to compare the mating success of irradiated and normal females within the context of the standard study plot biological evaluation protocol. Female pupae were irradiated with 40 Krads of gamma radiation, which renders them incapable of producing fertile progeny. In preliminary tests, 74.9% of the eggs produced by females irradiated at this dose and mated to normal males became embryonated. Embryonation is important because it is used in most of the studies reported here to distinguish mated from unmated females. Four 100-meter-radius circles of 15 trees each were selected and Tangletrap pest barrier rings were applied at a height of 2 m around each of the trees. Normal and irradiated females were alternated on each of the trees and males were released at the center of each of the circles at a rate of 50 per release. A release was made each day that females were deployed. Females were retrieved 24 hours after they were deployed and any eggs produced by the females were checked for fertilization. Mating success was 74.0% among normal females and 64.6% among irradiated females (Fig. 65). Therefore, irradiation has little or no effect on mating success of laboratory-reared females.

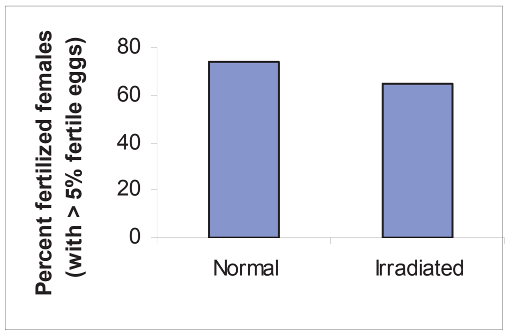

Figure 65. xxx

Ground Application of Disrupt II Flakes

Tests conducted in 2004 confirmed that aerial applications of Disrupt II flakes to individual, isolated trees prevented mating (see 2004 - Efficacy of Mating Disruption Treatments in Open Landscapes). A study was conducted in 2005 in the Goshen Wildlife Management Area, Goshen, Virginia, to test the feasibility and effectiveness of applying flakes to individual trees using ground-based equipment. The manufacturer of Disrupt II flakes, Hercon Environmental, provided a modified hydroseeder for this test. Four trees that were growing in the open were selected for treatment and four more were left untreated. As a positive control, eight trees were selected that were within a forested area but were close to a road. Four of these were selected for treatment and four were left untreated. The tank of the hydroseeder was filled with 28 gallons of water, 2.5 gallons of Gelva-2333, 1.25 pounds of guar gum, and 1650 g of flakes (=300 g active ingredient). The hydroseeder had a gasoline engine-driven pump that recirculated the mixture to keep the flakes well-suspended. The pump was calibrated to deliver 1.75 liters each time the trigger was pressed. Each tree was sprayed once from each of four positions around the tree to get uniform coverage. This delivered 7 liters of tank mix per tree, or 18.5 g AI/tree. The spray only reached about 20 feet in height, so it was only possible to reach the bottom of the canopy. Most of the spray mixture fell back to the ground. Females were deployed on the boles of the trees beneath a Tangletrap pest barrier which was placed at a height of 2 m. Males were released from four points around each tree at a distance of 25 m and a rate of 15 males per point per release. Females were retrieved after 24 hours and their fertilization status was determined from their eggs.

The average fertilization rate (>5% fertile eggs) of females in untreated areas was 42.2% in the clear cut and 32.3% in the forested area (Fig. 66). In the treated area, the average fertilization rate was 1.1% in the forested area (one female was mated) and no females were mated in the clear cut area. It appears that ground-based application of flakes to individual trees at an application rate of 18.5 g AI/tree shuts down mating in forested areas or open landscapes, even though the majority of the product fell to the ground beneath the trees.

Figure 66. xxx

Because the sticker (Gelva-2333) was diluted, about 100 flakes on leaves of treated trees that were within reach from the ground were marked and checked after 45 days to determine retention. After 45 days, 76% of the flakes remained.

Paint Ball Mating Disprution Formulation

In 2005, a novel method was tested for applying racemic disparlure. A proprietary formulation called "SPLAT" (3% AI), (ISCA Technologies, Inc., Riverside, California), was injected into paint balls to be applied using paint ball guns. Twelve 1-acre plots (63 x 63 m) were established in the Appomattox-Buckingham State Forest in central Virginia. Because of the small size of the plots, the standard evaluation protocol had to be modified. A central male moth release point was established in the center of each plot. The nearest tree to the center point and three trees in each cardinal direction, spaced approximately 5 m, apart were selected for female placement. A ring of Tangletrap pest barrier at a height of 2 m was applied to each of these trees. Two standard milk-carton pheromone traps were each placed about 20 m from the center to the west and east. Two paint ball guns powered by compressed CO2 gas were used to apply the formulation to 100 trees in eight of the plots to achieve an application rate of ____ g AI/acre. To achieve uniform coverage, the shooters worked out from the plot center to each of 25 regularly spaced points and shot at four trees in cardinal directions from each point. The shooters attempted to shoot at large trees at a distance of 5 m and at a height of 2 m. Eight of the plots were treated and four were untreated. The formulation was applied on July 27 and was evaluated for three weeks. Males were released at the plot center every day, and the treatment was evaluated either with the two pheromone traps per plot or by deploying females and retrieving them on the following day. On the days that females were deployed the traps were removed and sealed in plastic bags.

Trap catch in the treated plots was reduced by 96.4% compared to controls and mating success was reduced by 95.7% (Fig. 67a and b). Because the treatment was deployed so late in the season, these results represent only the first three weeks after application. To assess the longevity of the product, an additional evaluation was conducted eight weeks after application.

The evaluation consisted of male release and recapture in pheromone traps. Trap catch suppression compared to controls was 79.3% after eight weeks, suggesting that the application had lost some effectiveness.

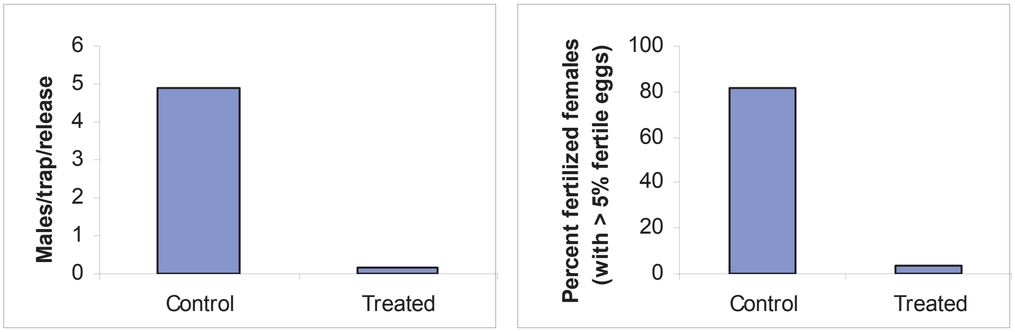

Figure 67. xxx

With two shooters using two guns, it took 15-20 minutes to treat 1 acre with the paint balls. This is quite fast compared to other methods of deploying mating disruption dispensers from the ground. The speed of application and the promising efficacy results make this formulation a candidate for further evaluation as a ground-based mating disruption treatment.

QUALITY ASSURANCE AND CONTOL OF PHEROMONE PRODUCTS

Because of the increasing use of pheromone trapping and disruption systems in decision-making, the consistency and reliability of each product are of paramount importance. Currently, no standardized label is used for the packaging of pheromone products for sale. For example, the data of production is important but not available on the product package. Also, there is no agency that has the responsibility to certify that the label information is within accepted limits of accuracy; for example, for purity of the technical and formulated product.

CURRENT DISPARLURE FORMULATIONS AND USE

DISRUPT II

The current formulation of disparlure flakes, modified Disrupt II (Hercon Environmental Co., Emigsville, Pennsylvania), was granted full registration by the US EPA under the original registration (Reg. No. 8730-55) granted in 1992. It contains a different plasticizer than the earlier formulation. The current label specifies application of 6-40 g AI/acre to forested and residential areas (more than one house occurring per 10 acres).

The standard and modified Disrupt II controlled release formulations of disparlure consist of multilayered plastic flakes or confetti, each 1/32 by 3/32 inch (1 by 3 mm) (Fig. 68). The flakes contain 18.5% AI of racemic disparlure. The active ingredient is implanted and protectively sealed in a layer between outer polymeric layers. The inner layer serves as a reservoir of the active ingredient, which migrates continuously through the permeable barrier layer. The flakes themselves contain 18.5% AI but they are packaged with diatomaceous earth (3% by weight); therefore, the label for the final product is 17.9% AI. The flakes are mixed and applied with 4 oz per acre of the sticker-extender Gelva-2333. This mix is applied using an augering system modified from the system originally developed by Schweitzer Aircraft.

(Figure 68 here)

The flakes are applied at 6 and 15 g AI/acre in one application at the estimated start of adult male emergence. On average the flakes release approximately 30-40% of their pheromone by day 42 after treatment. At the 30.4 g AI/acre dose, 0.3 g per acre per day is released over 2 weeks. The release rate is consistently low during the male moth flight period; therefore, much of the pheromone is released after the flight period and is wasted. The acrylic multipolymer resin emulsion Gelva-2333 is the sticker-extender mixed with the flake formulation. This sticker-extender has two major components: 1) an adhesive agent to adhere the flakes to foliage or other plant surfaces and 2) a surfactant. The specific components are found on the inert ingredient list provided by US EPA and are considered exempt from the requirement of a tolerance when used as an inert ingredient in a pesticide formulation applied to growing crops. This sticker-extender performs well: in general, about 80% of the flakes deposited remain adhered after 42 days of exposure.

Disrupt II is delivered in plastic bags (approx. 18.7 lb or 8.5 kg) each containing sufficient flakes to cover 100 acres at the dose of 15 g AI/acre. The cost to incorporate the disparlure into and manufacture the plastic flakes is approximately $8 to $20 per acre, depending on the quantity ordered, which does not include the cost of the racemic disparlure (also approximately $12 to $20 per acre). The plastic components of the flakes can persist in the environment for 10-15 years, but usually are not noticed due to their small size, green color, and minimal deposition (average 2 per square foot). When applied using the system modified by Harold's flying Service, each load will treat approximately 400 acres.

DECOY GM

An application to register the Decoy GM bead formulation of disparlure was submitted by Biosys (Columbia, Maryland) to the US EPA in February 1995. In 1997, Thermo Trilogy Corporation (Columbia, Maryland) acquired Biosys and stated that they were pursuing registration of the bead formulation. Unfortunately, the company did not pursue the registration and the product is no longer available.

OPERATIONAL USE

Although the standard Disrupt II flake product has been used operationally since 1990, many of the minor problems encountered with this product then still exist today. One of the problems is the slow release of the pheromone during the application year—specifically, during the male moth flight period. Then much of the pheromone is released after male moth activity and is consequently wasted. This problem requires the application of a high dose (15 g AI/acre) of disparlure with its associated high costs. The application equipment performs consistently due to upgrades in the motors for the flake augering system and enlargement of the flake hoppers. The pods still mandate special aircraft requirements: at least a 24-volt electrical system, high wing for pilot to observe proper functioning and FAA approval. The persistence of the three-layer plastic laminate in the field continues to be an environmental concern about the application of flakes to residential areas. Hercon has evaluated a slow release flake formulation consisting of biodegradable plastics in the laboratory, but results have not been promising.

EFFECTS ON NONTARGET ORGANISMS

The toxicity of insect pheromones to mammals is relatively low, and the US EPA requires less rigorous testing of these products than it requires of insecticides. Therefore the toxicity data on disparlure is limited (Beroza et al. 1975, USDA 2004a). Data regarding the toxicity of disparlure to animals or humans after subchronic or chronic exposures were not found in the available literature. Moreover, the acute toxicity of disparlure for endpoints other than mortality is poorly characterized (USDA 2004b). Cameron (1995) reported an apparent persistence of disparlure in the human body based on attractancy to male gypsy moths for a minimum of 16 years.

In one laboratory exposure study, concentrations of racemic disparlure greater than 100 mg per liter of water resulted in some mortality of the test population of rainbow trout and bluegill (USDA Fish and Wildlife Service 1972). This should not be interpreted to mean that racemic disparlure is toxic to fish when used for mating disruption of the gypsy moth, only that excessively large doses might be toxic.

CONCLUSIONS

The mating disruption technique should be used only to manage isolated or area-wide low density populations of the European strain of the gypsy moth. The exact biological parameters for its successful use have not been identified, although since 1990 there have been many successes in reducing populations (compared with untreated areas) within a range of low level populations. Criteria currently recommended for its use are these: 1) traps should capture no greater than 30 male moths per trap, and the average capture should be less than 15 per trap in the year before treatment, 2) populations should be well delimited (i.e., at least nine traps per square mile), 3) the treated area should be at least 5 miles from a source of large numbers of migrating male moths, and 4) the treated area should be large enough to offset anticipated male moth migration (e.g., at least 2,500 ft on a side). In operational uses, monitoring for treatment effectiveness is the same as that used with traditional insecticides on eradication projects (e.g., delimiting grids of at least nine traps per acre deployed for at least two years after treatment). However, with mating disruption, 0 captures in the year of treatment does not necessarily equate with successful mating disruption. Rather, the captures in the year after treatment are used to evaluate effectiveness.

Costs associated with the manufacture and application of racemic disparlure and Disrupt II are high compared with costs associated with aerial application of traditional insecticides.

Additionally, the cost of monitoring in methods development areas (e.g., Rockbridge County) is quite high. The use of females obtained as pupae from natural populations or laboratory-strain to monitor mating success is labor intensive in holding the pupae until adult emergence, in placing and collecting one-day-old females at monitoring locations, and in determining mating and embryonation of the collected females and egg masses. Laboratory experiments and field evaluations indicate that monitor females, both laboratory-strain and wild, are comparable in attractiveness to wild male populations.

SUMMARY

The use of pheromones to manage pest species has proven an effective technique in agriculture and forestry. Insect pheromones that act as sex attractants are used to suppress pest populations through mating disruption. This publication is a compilation of historical and current information on the use of mating disruption to manage sparse-density populations of the European strain of the gypsy moth.

MATING BIOLOGY OF THE GYPSY MOTH

The mating disruption technique is more effective as populations decrease in density since at higher population densities males can locate females visually, by chance encounter, as well as by following plumes of pheromone emitted by females. The gypsy moth has one generation per year, and the mating season occurs over a 3-6 week period in late summer. Adult flight activity and mating are temperature mediated but most mating normally occurs daily between 1000-1700 hours.

MATING DISRUPTION OF THE GYPSY MOTH

The identification and production of the synthetic gypsy moth sex pheromone (Bieri et al. 1970) or disparlure provided the opportunity to manage gypsy moth populations by mating disruption. Failure of males to locate females in air saturated with disparlure probably results from desensitization of the chemoreceptors in the males' antennae, as well as from disorientation by following false pheromone trails or leaving the pheromone-treated area (Carde 1996). The gypsy moth is not an ideal candidate for mating disruption due to its high fecundity. In addition, males are highly polygamous, and natural distribution patterns of adult females are not random but clumped or aggregated. Good characteristics of the European strain of the gypsy moth for mating disruption include flightless females, low mating success of females at sparse densities, limited dispersal of the majority of males beyond a few hundred meters, and one generation per year. Because some recently introduced females of the Asian strain of the gypsy moth are capable of flight, this strain may be less suited to the use of mating disruption.

DEVELOPMENT OF MATING DISRUPTION, 1971 THROUGH 2005

Since 1971, many attempts have been made to use mating disruption to manage populations of the gypsy moth. Kolodny-Hirsch and Schwalbe (1990) reviewed the results of research and operational trials before 1989. In general, these results were inconsistent in terms of efficacy and formulation performance, and disparlure release profiles were not monitored during the treatment year.

In 1989, an eradication program was conducted on 2,500 acres in Giles County, Virginia, using the Disrupt II plastic flake slow release formulation (Hercon Environmental Inc.). The flakes were applied at a dose of 30.4 g AI/acre of racemic disparlure in one application using a small fixed-wing aircraft. The efficacy results were excellent as no adult male moths were captured within the treated block from 1989 through 1991 (male moths were recovered in an associated untreated block). In spite of the favorable efficacy results, the application equipment which intermittently malfunctioned and produced an uneven deposition pattern of flakes across the

swath raised concerns for future use. Also, the flakes released only a small percentage of their disparlure content during male moth flight.

In 1990, a replicated study was conducted in Rockbridge County, Virginia, to evaluate three treatments: 1) two applications of flakes in 1990 only, each at a dose of 30.4 g AI/acre, 2) a single application of flakes in 1990 and again every year from 1991 through 1993 at a dose of 30.4 g AI/acre, and 3) untreated. The efficacy results over all years (1990 through 1994) showed that gypsy moth populations were significantly reduced by either type of flake treatment (Leonhardt et al. 1996). The flake application equipment continued to malfunction and, based on anticipated high costs to replace or redesign the equipment, an effort was made to locate another controlled-release formulation that could be applied through conventional booms and nozzles.

In 1990, AgriSense (formerly biosys and now Thermo Trilogy, Columbia, Maryland) developed a polymeric flowable bead formulation containing disparlure (Decoy GM). Release rate evaluations showed that the bead formulation released disparlure at a faster rate than the plastic laminate flake formulation.

In 1991 through 1994, various trials were conducted using the beads applied in two applications at doses from 6 to 30.4 g AI/acre per application in 1 gal of tank mix per acre per application. Although efficacy results were inconsistent over the various doses, in general results were favorable at doses as low as 6 g AI/acre per application for two applications. A bead formulation containing a greater portion of larger diameter beads was manufactured in an effort to slow the disparlure release rate.

The flakes were used operationally in several states. The operability of the application equipment for the flakes was improved by upgrading the motors for turning the augers and enlarging the holding capacity of the flake hoppers.

CURRENT DISPARLURE FORMULATIONS AND USE

The plastic flake formulation Disrupt II is registered by the US EPA and used operationally to manage low density populations of the gypsy moth. The plastic flakes are applied at 30.4 g AI/acre of disparlure for one application using pods mounted on the underside of the aircraft wing. The polymeric bead formulation Decoy GM is not registered by the US EPA, but an application for registration was submitted in February 1995. The beads are applied at 15.2 g AI/acre per application in two applications, using CP or Micronair AU-5000 atomizers with screen cages removed and attached to standard spray booms. Formulation costs are approximately $8 per acre for the flakes and $3 per acre for the beads. The active ingredient which is not included in the cost of formulation costs between $400 - $700 per kg, which equates to $12 - $20 per acre for a 30.4 g AI dose.

The exact biological parameters for the successful use of the mating disruption technique have not been identified although the technique should be used only to manage isolated or area-wide (to reduce the possibility of insect movement into the treated area) low density populations of the European strain. The technique is specific for the gypsy moth and has no known impacts on nontarget organisms.

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