Mating Success in Low-Density Gypsy Moth Populations

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Abstract

Field studies were conducted to evaluate the effect of mating disruption on the mating success of the gypsy moth, *Lymantria dispar* (L), in low-density populations. The gypsy moth is an insect pest of hardwood forests in many regions of the world. The discovery of the sex pheromone disparlure (cis-7,8-epoxy-2-methyloctadecane) produced by females marked the start of a new era in the control and management of gypsy moth populations. Sex pheromones, like disparlure, have been used for detecting new populations, monitoring the spread of populations and for population control based on the disruption of mating communication. Although mating disruption has been used against populations of insect pests in agricultural and forest systems, considerable information about the use of this method for managing gypsy moths is still lacking. Studies, therefore, were designed and carried out specifically to improve current understanding of the mechanism of mating success, to evaluate existing techniques for mating disruption, and to develop methods that would improve the application of pheromone used for mating disruption so as to reduce the costs associated with the use of this management tactic.

The first study was conducted to compare the mating success and mortality of gypsy moth females in low-density populations in Virginia and Wisconsin because of differences, which have been observed in the population dynamics and the impact of management strategies between these two states. The results suggested that the higher rate of population spread in Wisconsin might be due to the increased mating success of females compared with Virginia, which may be due in part to increased long-distance dispersal of males and increased longevity of females.

The effect of artificial pheromone applied at various doses and formulations on mating success in low-density gypsy moth populations also was studied. Dose-response relationships were obtained for pheromone doses ranging from 0.15 to 75 g a.i./ha. The doses of 37.5 and 15 g a.i./ha of pheromone were shown to effectively disrupt mating and, therefore, have been recommended for operational use. The results also showed that the disruption of mating and

attraction of males to pheromone-baited traps as a result of application of pheromone formulated in plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA) was stronger and lasted longer than for the pheromone formulated as microcapsule (3M Canada Co., London, Ontario, Canada) and in liquid (Shin-Etsu Chemical Co. Ltd, Tokyo, Japan).

Another study was carried out to improve the use and efficacy of the pheromone for mating disruption by reducing the amount of pheromone that was sprayed and the flight distance during aerial application. This study showed that in mountainous landscapes the effect of disparlure along the valley between mountains could be observed at a larger distance (633 ± 63 m) from the treated area than across the valley (104 ± 22m). In a relatively flat area, the effective distance for mating disruption was similar to the effective distance across the valley in a mountainous area (67 ± 17m). These dispersal characteristics of the pheromone provided further evidence that it could be used effectively in mating disruption treatments.

Finally, a portable Electroantennogram (EAG) device was evaluated for its ability to detect disparlure sprayed for mating disruption in gypsy moth populations. The study found no relationship between the dose of artificial airborne pheromone and response of gypsy moth antenna as measured by the voltage ratio. The inability to detect differences between airborne pheromone concentrations in the plots treated for mating disruption might have been due to high variability among antennae and also by the inability of the EAG device to detect the low concentration of airborne pheromone used operationally for mating disruption. Further studies are required to improve the sensitivity of the portable EAG device before it can be recommended for use in the field.

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Table of Contents

List of Figures

List of Tables

1. Introduction

The gypsy moth, *Lymantria dispar* (L), is a pest of hardwood forests. In the late 1860s the insect was introduced accidentally to Medford, MA (Liebhold et al. 1989) and since that time has expanded its range gradually in North America. Today, *L. dispar* is one of the most important forest pests in the eastern United States (Doane and McManus 1981). Gypsy moth populations can spread due to wind-borne larval dispersal. However, the spread of this species over large areas is due mainly to accidental transportation of different life stages by humans (Mason and McManus 1981). Gypsy moth can also rapidly increase its density and cause serious damage to forested areas (Schoëner 1988). Insecticides such as carbaryl (Sevin®), acephate (Orthene®), and diflubenzuron (Dimilin®) have been used effectively against gypsy moth populations. However, the application of pesticides is limited by both the cost of treatment and negative community reaction against pesticide usage (Plimmer et al. 1982).

Recently, the method of mating disruption has been recommended for use as an alternative to pesticides for eradication of low-density populations. Mating disruption requires the use of synthetic pheromone for modification of the behavior of males in their search for females. This method is used widely against many different pests including the gypsy moth. Synthetic pheromones were shown to be ecologically safe due to their low toxicity to mammals and rapid degradability (Bierl et al. 1976, Reardon et al. 1998). In gypsy moth, mating disruption is effective only at relatively low population densities and is used widely for eradication of isolated infestations and to control the spread by suppressing low-density populations that become established beyond the main population front (Schwalbe et al. 1983, Webb et al. 1990).

The goal of this project was to evaluate the use of mating disruption on the mating success of gypsy moth in low-density populations. Specifically, the study focused on understanding the mechanism of mating success, and on the evaluation and improvement of existing techniques for mating disruption. Three products, Hercon plastic laminated flakes, 3M microcapsules and Shin-Etsu sprayable pheromone were evaluated for their ability to disrupt mating. Hercon is currently used operationally for mating disruption treatments. 3M and Shin-Etsu are new products that were developed taking in consideration all the previous problems with Hercon plastic flakes such as costly manufacturing, complexity of application technology, and slow release rate. Another aspect of the improvement was an attempt to lower the cost of treatment by lowering the dose of pheromone recommended for operational use and by making

the application process more economical. In this relation, dose-response experiments were conducted to find the threshold dose that effectively disrupts mating. Attempts also were made to study the distribution of sprayed artificial pheromone in time and space.

2. Literature Review

2.1. Gypsy Moth Biology

The species name of the gypsy moth, *dispar* (to separate, Lat.), describes well the sexual dimorphism between male and female gypsy moths. Gypsy moth males have plumose antennae and are mottled brown with black markings on the wings. Females are white or yellowish, with black markings on the wings and have serrate antennae (Leonard 1981, Andreeva et al. 1999).

The gypsy moth is distributed throughout most of the Northern Hemisphere from North Africa, throughout Europe, China, Korea, Asia Minor and Japan (Marcovic` et al. 1998). In Europe it occurs from the West Coast of the mainland to the Urals; in the north it is limited by a line stretching from central Sweden through Moscow, and the oak growth zone limits it in altitude (Grijpma 1988). In the south, gypsy moth is found in Morocco and Tunisia (http://www.fs.fed.us/ne/morgantown/4557/gmoth/world/). The gypsy moth has one generation per year. Moths emerge from pupae in midsummer with males usually emerging 1 or 2 days before females. In North America and Europe females do not fly, but in Asian populations (Baranchikov and Sukachev 1989, Sun 1988) both males and females are able to fly.

Adults of *L. dispar* live for about a week during which time they do not feed. Although adults can imbibe moisture, their digestive system is not functional. Under natural conditions gypsy moth females mate only once (Doane 1968), but in dense populations re-mating may occur (Cardé and Hagaman 1984). The reproductive period is less than a week (Leonard 1981). After a female has mated she stops releasing pheromone and begins to lay eggs. Unmated females, however, may lay unfertilized eggs near the end of their lives (Reardon et al. 1998). Females deposit eggs in a single cluster or mass that is covered by a dense coating of hair from their abdomen. The number of eggs that a female lays varies from less than 100 to more than 1,000. Generally most egg masses are found on tree trunks and branches, but may also be found on rocks, foliage, vehicles and outdoor household articles. Egg embryonation begins soon after oviposition and larvae are fully developed inside the egg in about a month (Leonard 1981). In spring, larvae hatch from eggs at the time when trees are beginning to produce new leaves. These larvae can be recognized by the presence of 5 pairs of blue warts on the front back part of the body (*anterior dorsum*) and 6 pairs of red warts on the hind segments (Leonard 1981, Andreeva et al. 1999).

Because females are unable to fly, the primary natural mechanism of dispersion is wind-borne movement of 1st instars (Mason and McManus 1981). During the first two instars larvae remain mostly on leaves, but after the third instar they usually hide in crevices on the trunk and under the loose bark during the daytime when they are not feeding. Larvae feed on a wide variety of species (Liebhold et al. 1995). *Quercus spp*. are among the preferred host plants, but the gypsy moth may also feed on *Populus spp*., *Larix*, *Salix*, and many other trees (Mosher 1915, Martinat and Barbosa 1987). Late instar larvae feed readily on the needles of *Tsuga sp*., *Picea sp*., and *Pinus sp*. Before pupation, feeding stops, larvae void their guts, surround themselves by a sparse silken net, and begin to contract in length. This prepupal stage lasts for about 2 days. Pupae remain in their cocoons for about two weeks. When development has been completed the adult emerges. After several hours, the wings expand, cuticle hardens, and the lifecycle is repeated (Leonard 1981).

2.2. Dispersal and Population Growth

The gypsy moth normally occurs in low densities for many years, but for unknown reasons numbers may increase rapidly leading to extremely high population densities (Liebhold and Elkinton 1988, Elkinton and Liebhold 1990, Liebhold et al. 2000). Consequently, the insect can spread over large areas and cause serious damage to the forest (Schönherr 1988). According to Leonard (1971) gypsy moth populations first build in small foci and in response to high density and food limitation newly hatched larvae disperse out of the area on silk threads in air currents. It has been shown that gypsy moth larva can be blown several miles from the source of infestation (Collins 1915), but the most important way of dispersion is the accidental transportation of egg masses and other life stages by humans (Mason and McManus 1981). Several models have been developed to describe gypsy moth population spread. The one currently used for slowing the spread of the insect (Sharov and Liebhold 1998) considers three zones: an infested zone, which is a source for long-distance dispersal; a transition zone, where new small colonies become established; and an uninfested zone where no colonies exist. New colonies become established at various distances from the population front, which is the boundary between infested and transition zones. Newly established colonies are small and have a

low density, but as they grow, they coalesce and merge with the primary colony, thus expanding the infested area.

Gypsy moth populations can increase rapidly due to their high reproductive potential and wide adaptation to environmental conditions. Caterpillars feed on the foliage of numerous plant species (Liebhold et al. 1995a, Marovic´ et al. 1998). During an outbreak the gypsy moth can expand its food preferences and the number of host plants species increases up to 500 (Lance 1983). Between outbreaks the insect feeds only on its primary hosts (Rossiter 1992).

2.3. Natural Enemies

There is a complex of natural enemies that may limit population growth of the gypsy moth. This complex includes parasitoids, predators and pathogens. Parasitoids and one predacious beetle were introduced into North America by the U.S. Department of Agriculture for classical biological control. Gypsy moth predators include insects, spiders, birds and small mammals. Also gypsy moth can be infected by a number of microorganisms.

2.3.1. Parasitoids

Most gypsy moth parasitoids were not introduced into the United States along with the pest. Therefore, the U.S. Department of Agriculture in cooperation with several of the states that were affected by the pest, conducted programs to introduce natural enemies into the U.S. This classical biological control activity resulted in establishment of 10 species of parasitoids and one predacious beetle (Leonard 1981).

Most gypsy moth parasitoids are from the insect orders Diptera and Hymenoptera. Four introduced species of parasitic flies attack gypsy moth larval stages. *Parasetigena silvestris* (Diptera: Tachinidae) and *Blepharipa pratensis* (Diptera: Tachinidae) are the univoltine species. *P. silvestris* can develop in several hosts including the gypsy moth (Burgess and Crossman 1929). It attacks large larvae and lays a solitary egg in the intersegmental fold near the head region (Leonard 1981, Burgess and Crossman 1929). The parasitic larva molts, penetrates the host, and begins its development. Emergence can occur from the host larva, prepupa, or pupa (Leonard 1981). *B. pratensis* deposits large numbers of eggs on the leaves of trees. Volatiles released from freshly chewed leaves attract this species and increase the probability of

oviposition near feeding larvae (Leonard 1981). Eggs must be swallowed by gypsy moth larvae before they can hatch. However, development only begins after the host reaches the larval or pupal stage (Hoy 1976). Larvae emerge only from gypsy moth pupae.

The two important multivoltine species of parasitoids are *Compsilura concinnata* (Diptera: Tachinidae) with 2-4 generations per year and *Exorista larvarum* (Diptera: Tachinidae). *C. concinnata* has a host range of more than 200 species (Webber and Schaffner 1926). The adult fly impales the host and deposits a larva rather than an egg (Leonard 1981). Adults emerge from large gypsy moth larvae or pupae (Hoy 1976). *E. larvarum* is a polyphagous species that requires alternate hosts for overwintering (Hoy 1976, Leonard 1981). It lays eggs on large gypsy moth larvae.

Aphantorhaphopsis (=*Ceranthia*) *samarensis* (Diptera: Tachinidae) is a promising natural enemy for classical biological control of the gypsy moth in North America. Experimental studies in Europe showed that *A. samarensis* causes considerable mortality of gypsy moth at a low density and responds very quickly and effectively to local increases in the gypsy moth population density (Mills and Nealis 1992). *A. samarensis* parasitizes mainly intermediate instars of gypsy moth (Quednau 1993). However, it can attack and develop in late instars (Maier 1990). It was shown that *A. samarensis* is very host-specific and that the probability of it attacking native Lepidoptera other than Lymantriids approaches zero (Fuester et al. 2001).

Parasitoids in the insect order Hymenoptera attack gypsy moth eggs, larvae and pupae. The parasitic wasps *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) and *Anastatus disparis* (Hymenoptera: Eupelmidae) attack gypsy moth eggs. *A. disparis* attacks only unembryonated eggs. The female parasite is wingless thus the natural spread of the parasite is very slow. *O. cuvanae* attacks both embryonated and unembryonated eggs and has several generations in fall and one spring generation. Because the ovipositor of this wasp is too short to penetrate the lower layers of an egg mass, it only parasitizes the outer layer of the egg mass (Weseloh 1972, Tigner 1974, Leonard 1981).

Two species of parasitic wasps attack small gypsy moth larvae. *Cotesia* (=*Apanteles*) *melanoscelus* (Hymenoptera: Braconidae) can respond to chemicals (kairomones) produced by the gypsy moth (Leonard 1981). This species has two generations per year. The first generation attacks first and second instars, and the second generation attacks third and fourth instars. The host mortality does not necessarily result in parasitoid emergence. Parasitoid-induced

nonemergence mortality was shown to be a significant part of overall mortality caused by *C. melanoscelus* (Thorpe et al. 1990). The effectiveness of the parasitoid is limited by high mortality during overwintering, by hyperparasites, and also by its poor synchronization with its host (Weseloh 1975). *Phobocampe disparis* also parasitizes early-instar gypsy moth larvae, but it is relatively rare and is less important (Hoy 1976, Leonard 1981).

There are also two parasitoids that are known to attack gypsy moth pupae. Like *C. melanoscelus*, *Brachymeria intermedia* responds to kairomones produced by gypsy moth (Leonard 1981). *B. intermedia* was the last introduced parasitoid to become established and spread rapidly. *B. intermedia* prefers open sunny areas and, therefore, the highest rate of parasitism occurs in open and defoliated areas. The second parasitoid is *Monodontomerus aureu,* which attacks both gypsy moth and browntail moth and also is a hyperparasitoid of other hymenopteran and dipteran parasitoids. The value of *M. aureu* is very low because of its rarity as a gypsy moth parasite and its negative impact on other parasitoids (Leonard 1981).

2.3.2. Pathogens

Gypsy moth can be infected by a number of microorganisms including viruses, bacteria, fungi, and microsporidia. The most important agent is the nucleopolyhedrosis virus (NPV) *Borralinivirus reprimens*, which causes polyhedrosis or wilt disease (Reardon and Podgwaite 1992, Hajek 1998). The origin of gypsy moth NPV in North America is unclear. However, it could have been present in the samples brought from Europe by Leopold Trouvelot in 1864 (Liebhold et al. 1989) or could have been introduced with shipments of parasitoids (Leonard, 1981). Rapid spread of the disease usually occurs when larval populations are high (Doane 1970, Woods and Elkinton 1987). Larvae become infected with NPV when chewing their way out from the egg also ingest virus from egg chorion or from the hairs that cover an egg mass. When these first instars die their bodies disintegrate allowing the spread of the virus on foliage. Other larvae that consume this foliage also become infected, die, disintegrate, and spread the viral particles. NPV was shown to be density-dependent, which means it was only active in high-density populations (Webb et al. 1999). This pathogen can be cultured in the lab and used for biological control. The product was registered under the name of Gypchek (Lewis et al. 1979, Leonard 1981, Reardon and Podgwaite 1992).

The bacterium, *Bacillus thuringiensis* var *kurstaki* Berliner (*Bt*) was discovered from diseased flour moth caterpillars in 1915, but it was not until 1950 that any attempts to use this bacterium against gypsy moth occurred. The first *Bt* product was registered by 1961 and used to control caterpillar pests, mainly in agricultural crops. *Bt* causes mortality of gypsy moth larvae both in the laboratory (Cantwell et al. 1961, Lewis and Connola 1966) and in the field (Dunbar and Kaya 1972). The mode of action of Bt is generally as follows. The bacterium produces a crystal protein toxin that kills the cells lining the insect gut (Shetlar et al. 2002). When ingested, the bacterial cell wall is digested and toxins are released. Toxic molecules oligomerize to make a pore, which results in influx of water and ions causing cell swelling and lysis.

Entomophaga maimaiga is a fungal pathogen that was introduced into the United States from Japan in 1910 and 1911, but was not detected until 1989 (Reardon and Hajek 1993, Hajek 1998). The pathogen has a life cycle that is well synchronized with the life cycle of gypsy moth, and as such it can kill large numbers of larvae. Resting spores overwinter in the soil and germinate in spring of the following year, usually about two weeks prior to gypsy moth egg hatch. The resting spores produce conidia that can infect and kill gypsy moth larvae within 7-10 days. Fungal hyphae formed in a dead body produce short-lived conidia (in spring and early summer) or the resting spores (starting from mid-June) or both. Cadavers of larvae killed by this disease remain hanging on tree boles with their heads down (Weseloh and Andrealis 1991). In contrast to NPV, *E. maimaiga* is equally active in both low- and high-density populations, and can significantly decrease host population density (Webb et al. 1999).

2.3.3. Predators

Gypsy moth predators include insects, spiders, birds, and small mammals. *Calosoma sycophanta* (Coleoptera: Carabide) is the only introduced and well-established insect predator (Leonard 1981). Both larvae and adults feed on the contents of gypsy moth larvae and pupae using their large mandibles to tear and open their prey. *C. sycophanta* can reach high numbers, but its distribution is spotty and it does not appear in the northern regions of gypsy moth's distribution.

Predation on gypsy moth life stages by birds is generally low (Liebhold 1998, Elkinton and Liebhold 1990). Most predation occurs on early-stage larvae. Very few birds consume egg masses, adults, and late-stage larvae (Forbush and Fernald 1896, Brown and Cameron 1982).

Rodents, particularly the white-footed mouse, *Peromyscus leucopus*, are very important mortality factors in sparse gypsy moth populations (Leonard 1981). Gypsy moth tends to pupate on or near the ground when the population density is low (Campbell and Sloan 1976). The behavior of pupating and resting in the litter could developed in Europe, where mortality caused by avian predators and Tachinid parasitoids is higher compared with that caused by small mammals (Campbell and Sloan 1976). In North America, small mammals predation is the most important factor in preventing outbreaks in areas where the gypsy moth populations are at low levels for a number of years (Bess et al. 1947, Campbell and Sloan 1977, Elkinton et al. 1989, Elkinton et al. 1996).

2.4. Mating Behavior of Gypsy Moth Females

Gypsy moth females attract males with a pheromone released by rhythmic protrusion and retraction of the last abdominal segments (Doane 1968). Several hours after emergence, females release a sex pheromone, called Disparlure (Leonard 1981). The pheromone initiates male flight toward flightless adult females. The primary mechanism for initial orientation is the zigzag flight along the pheromone plume. However, other behavioral cues are also important for successful location and recognition of a mate. Calling behavior and pheromone emission do not occur continuously, but occur simultaneously and periodically with a circadian rhythm (Cardé et al. 1975a). It has been shown that during mid-afternoon females are more attractive to males than are pheromone-baited traps, but in the early evening pheromone traps are more attractive to males than females (Cardé et al. 1974).

Virgin females are positively phototropic and tend to move to shiny locations on the tree trunks (Doane 1968). This fact may explain why in dense populations, where sight is important, males can easily discern the ultraviolet silhouettes of females (Brown 1974). After mating females become active and negatively phototropic (Doane 1968).

Females are ready to mate within two hours after emerging (Doane 1968) and are most attractive during the first three days; by the third day after emergence the attractiveness of females decreases rapidly (Collins and Potts 1932, Richerson and Cameron 1974). Mating, however, is possible even in 8-day old females. Sometimes females will begin laying eggs without mating. Females that lay more than 10 unfertilized eggs are less likely to mate, and even

if they mate, are not likely to lay fertilized eggs (Richerson et al. 1976a). Female attractiveness increases sharply after the third day (Holbrook et al. 1960) from 3% at 16-24 hours after emergence to 28% at 64-72 hours.

Fertilized females store sperm received from the male in their spermatheca. Therefore, proof that a female has been fertilized can be obtained by dissection and spermatheca analysis. In fertilized females, the spermatheca is filled with sperm and is white in reflected light. In unfertilized females, the spermatheca is transparent (Sharov et al. 1995). Oviposition begins one hour after mating and during this process females are difficult to disturb (Doane 1976). Fertilized females typically lay 100-1000 eggs per egg mass (Andreeva et al. 1999). Several studies showed that when population density decreases, females deposit more egg masses on man-made objects than on trees (Campbell et al. 1976). However, a later study showed no significant differences in the distribution of egg masses between trees, houses, and man-made objects in high and low-density populations (Thorpe and Ridgway 1992).

2.5. Flight and Mating Behavior of Gypsy Moth Males

Odor plumes form when the wind disperses molecules from their source. The structure of a plume, therefore, depends on the atmospheric conditions. The farther the plume moves away from the source, the more it expands, and the lower the concentration of the molecules becomes. There are two processes contributing to the expansion of the cloud: molecular diffusion that causes slow and gradual dispersal of molecules in a plume, and turbulent diffusion that tears apart, tumbles, and mixes large air masses by forces in the earth's boundary layer (Murlis et al. 1992). As wind speed and direction vary, the plume randomly wanders around over a large area. The length of the plume near the source, where molecular diffusion is most significant, depends on the source size and the speed of the wind (Elkinton and Cardé 1984, Cardé 1984, Murlis et al. 1992).

The long distance orientation of gypsy moth males toward virgin females was described as an upwind zigzag flight following the scent produced by the female (Collins and Potts 1932, Charlton and Cardé 1990). This behavior was termed as odor-regulated, optomotor anemotaxis and reversing anemomenotaxis (upwind zigzag flight) (Kennedy 1978, Kennedy and Marsh 1974, Marsh et al. 1978). A model for male mating behavior was constructed (Cardé 1979, Cardé

and Hagaman 1979, Cardé et al. 1977) based on both field and wind tunnel experiments. If a male is introduced to the pheromone, it starts moving, wing fanning, walking, and finally flying. He flies upwind and remains in the plume by a zigzag flight. Frequent contact with the pheromone results in relatively straight flight, which is interpreted as a string of upwind surges, whereas flight in the plume where the contact with the pheromone is less frequent results in cast-surge-cast response (Mafra-Neto and Cardé 1996). During the flight, males use visual cues to regulate their speed. When a male locates a female it lands, walks with wings fanning, makes contact with the female, and copulates. It was reported that this behavior occurs in sparse populations (Doane 1976), and that mating behavior of males in dense populations may be different. In dense populations males may orient to vertical silhouettes (e.g., trees) rather than directly to the calling females (Richerson et al. 1976a,b). Males search surfaces at ground level (rocks, foliage, litter) as well as up and down tree trunks, limbs and foliage (Doane 1976). However, they mainly search around lower parts of trees. As a result, moth catches decrease when traps are positioned high. For example, the greatest number of males was caught in traps placed 0.5 to 1.5 m above the ground and fewer at 3 m and higher. Traps located 10-20 cm above the ground caught 3 times more males than those placed at 1 m (Cardé et al. 1975b). The highest catch also occurred at 0.5 m from trunks compared to 1, 3 and 10 m (Granett 1974).

In areas where pheromone concentration is below a specific behavioral threshold, a male probably flies randomly without searching, but once it enters an "active space", where pheromone concentration is enough to cause a behavioral response, the male starts orienting toward some or all trees within this space (Richerson et al. 1976b). Several authors observed males passing closely to calling females, sometimes even touching them, but no mating occurred (Hidaka 1977, Richerson et al. 1976a, Doane 1976). Therefore, males are able to follow the plume and thus locate a female even in the absence of visual cues at any population density, though there are differences in their behaviors at different population levels (Webb 1982).

2.6. History of Control in USA

The gypsy moth was introduced into the U.S. in late 1860s and by the end of summer of 1869 it was established and attracted much public attention (Burgess and Baker 1938). In 1890 the Massachusetts Legislature allocated $25,000 for managing gypsy moth populations by

applying creosote or acid to egg masses, burning infested trees and shrubbery, banding trees with burlap and sticky material to trap larvae or to prevent them from climbing trees, and spraying chemical insecticides (Kirkland 1905, Burgess 1930). These tactics, however, had little effect on gypsy moth population increases. From 1906 to 1912 the Federal Government and the state of Massachusetts jointly financed the importation of natural enemies of the gypsy moth from Europe and Japan (Brown and Sheals 1944). During this period intensive research on larval dispersal (Burgess 1913, Collins 1915), viral diseases (Glazer 1915) and on managing infested areas (Clement and Munro 1917) were conducted. In order to prevent shipment of infested materials a quarantine against gypsy moth was established in 1912. In 1924 a barrier zone was created along Hudson River valley extending from Canada to Long Island to prevent the westward spread of the gypsy moth. Spot infestations that were found each year within a barrier zone were reduced or eradicated, but a serious infestation occurred in Pennsylvania in 1932. Despite all of the eradication efforts the gypsy moth persisted in Pennsylvania and by 1939 the entire barrier zone became infested (McManus and McIntyre 1981). Several insecticides were applied to control gypsy moth (e.g., cryolite, DDT). This period of insecticide application marked the start of the development of modern methods of application such as aerial spraying and mist blower (Nichols 1961). At the time, DDT was the most widely used insecticide against the gypsy moth - over 1.2 millions ha were sprayed aerially with DDT in 1957. Also, at that time, questions about pesticide persistence and residues in crops and food began to raise public awareness (Carson 1962). As a result, DDT was replaced by carbaryl (Sevin®). Research on alternative types of control, such as *Bacillus thuringiensis* (*Bt*), natural nucleopolyhedrosis virus (NPV), sterile male releases, and the synthetic pheromone were being conducted in 1961 - 1970. In 1971, the U.S. Department of Agriculture, Forest Service and Agricultural Research Service started developing and evaluating a new synthetic sex attractant disparlure (McManus and McIntyre 1981).

The sex pheromone of gypsy moth females, disparlure, was identified as (+)-enantiomer of cis-7,8-epoxy-2-methyloctadecane (Bierl et al. 1970, Iwaki et al. 1974). After synthetic disparlure became available, it was used as a bait in pheromone traps to evaluate population abundance and to predict outbreaks (Schwalbe 1981, Grijpma 1988). Currently the (+) enantiomer of disparlure is used throughout the U.S. as a bait in traps to detect new infestations (Schwalbe 1981, Plimmer et al. 1982). It was shown that (+) disparlure is a better attractant than

racemic disparlure (mixture of (+) and (-) enantiomeres) (Miller et al. 1977, Plimmer et al. 1977). The (-)-enantiomer is not active as a pheromone, but is known to inhibit the response of gypsy moth males to (+)-disparlure (Yamada et al. 1976, Cardé et al. 1977, Miller et al. 1977, Plimmer et al. 1977). However, it is the racemic disparlure that is used most commonly for mating disruption since it is cheaper and was shown to be an effective mating disruptant (Kolodny-Hirsch and Schwalbe 1990).

Beginning in the late 1980's there was increasing interest in ecologically safe methods for managing gypsy moth populations. This resulted in greater emphasis being placed on the method of 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. The major goal of this project was to apply technology, including mating disruption, for slowing the spread and reducing the impacts of gypsy moth populations (Reardon et al. 1998).

In 1993, the USDA Forest Service Slow-The-Spread (STS) project was initiated. The goal of the STS project is to reduce the rate of expansion of gypsy moth populations in the US via detection and suppression of low-density isolated colonies that are located just beyond the expanding population front of the infested area (Campbell 1981, McFadden and McManus 1991, Leonard and Sharov 1995). Currently, participating states include Indiana, Illinois, Kentucky, Michigan, North Carolina, Ohio, Virginia, West Virginia, and Wisconsin. In 1998, the STS project became operational (Sharov 2001). As the result of both AIPM and STS programs, the rate of spread in the Appalachian Mountains has declined by 35% from 1988 to 1994 (Sharov et al. 1996).

In order to assess the effectiveness of the STS project, a model was developed to describe the gypsy moth population spread (Sharov and Liebhold 1998). This model assumes that isolated colonies become established beyond the expanding population front at various distances from the front. The velocity of progression of the population front can be calculated using a traveling wave equation. The model of population spread predicted that eradication of isolated colonies beyond the population front would result in reduction of spread rate by 54%, which was very close to the actual spread rate reduction; since 1990 when eradication of isolated colonies started, the rate of spread has declined by 59% (Sharov and Liebhold 1998).

2.7. The Use of Pheromone for Monitoring And Management

The gypsy moth is spreading gradually in North America to the South and West. Monitoring this spread is necessary for planning preventive methods and areas of domestic quarantine, and also for evaluating the effects of population management on the rate of gypsy moth spread (Sharov et al. 1997). Three methods of monitoring are available; aerial maps of forest defoliation, counts of overwintering egg masses (Kolodny-Hirsch 1986) and counts of males in pheromone-baited traps (Talerico 1981, Ravlin et al. 1987). Sharov et al. (1997) showed that the front of the defoliated area is very unstable in space and time compared with population boundaries derived from moth captures in pheromone traps and egg mass counts. The boundary of 10 moths per trap was shown to be the most stable and, therefore, the most reliable for monitoring population spread.

The egg mass sampling is a technique for monitoring medium- and high-density populations (Ravlin et al. 1987), whereas counts of male moths in pheromone-bated traps are mostly used for detecting isolated colonies (Schwalbe 1981a). In the continuously infested areas, moth catches in traps were found not to be correlated well with defoliation (Liebhold et al. 1995, Sharov et al. 1996). Rates of defoliation spread were shown to be significantly different from rates of spread estimated using counts of gypsy moth males in traps and egg masses (Sharov et al. 1996).

Pheromone-baited traps are used widely for monitoring several pest species including the gypsy moth. The proper use of pheromone traps requires knowledge about the phenology of gypsy moth male flight. This information is used to determine the correct time for deployment of traps before the beginning of moth flight and retrieval after flight termination (Régnière and Sharov 1998). Each pheromone trap has an "active space" or "trapping zone" - the area within which an insect responds to the pheromone sufficiently to be trapped (Wall and Perry 1978). When active spaces overlap, interference or competition between traps may occur. To prevent this interference, the active space of particular traps needs to be estimated and large enough distance between traps should be used (Minks 1977).

Pheromone traps have several advantages compared with other monitoring methods. Pheromone traps are inexpensive, easy to use, standardized, catch mostly the target species, and are effective in extremely low population densities (Elkinton and Cardé 1981). However, there are two problems that may arise with pheromone traps. First, trap efficiency declines as it fills up

with males. This decline occurs partially because of the reduced volume of filled traps. Trapped males might be able to more easily locate entrance holes and escape from a trap when it is filled or half-filled with insects. The presence of decomposing moths also suppresses catches, probably because of odors associated with decomposition (Elkinton 1987). Trap efficiency drops drastically when the number of males reaches 500. These traps can hold 2,000 males in contrast to Delta sticky traps, which can capture only up to 25 males (Elkinton 1987, Plimmer et al. 1982). Second problem with pheromone traps is the possible decrease of attractiveness in the presence of wild females. Pheromone traps compete with females and thus catch fewer males if the pheromone bait is not strong enough (Webb 1982).

Pheromone traps are helpful for locating isolated gypsy moth populations, delineating areas for egg mass sampling (Kolodny-Hirsch and Schwalbe 1990), for predicting subsequent population density (Thorpe et al. 1993), and for measuring population spread rate (Sharov et al. 1995). The rate of population spread can be quantified using population boundaries, which are lines that separate areas where population densities are generally below or above a specific threshold (Sharov et al. 1995). Disparlure-baited traps can also be used to evaluate the effect of mating disruption by comparing male moth catches in treated and untreated areas in the year of treatment.

The exact mechanism of mating disruption is unknown. Several possible mechanisms of mating disruption were hypothesized (Granett 1976). One of the hypotheses suggests that the method of mating disruption is based on the idea of adding artificial pheromone sources to the environment and thus creating a background level of pheromone high enough to confuse males in their search for females (Reardon et al. 1998). As a result, females remain unmated and, therefore, do not lay any eggs or lay unfertilized eggs. It was shown that artificial disparlure disrupts mating mostly in sparse populations (Beroza and Knipling 1972, Beroza et al. 1974, Knipling 1979). However, in a plot with high pretreatment catches (401 males in 4 traps per day) treated with 500 g/ha of disparlure, female mating was very rare. This indicates that even in high-density populations mating can be disrupted successfully (Schwalbe and Mastro 1988). The suggestion was made to increase the dose of pheromone when using it against high-density populations of gypsy moth (Plimmer et al. 1982), but later results indicated that the increase in pheromone concentration above 75 g a.i./ha did not result in a proportional increase in mating disruption (Webb et al. 1988). As most studies have shown, mating disruption is most effective

in low-density gypsy moth populations. Therefore, the method is currently used to suppress or eradicate low-density populations by reducing the number of fertile eggs that are laid (Plimmer et al. 1982).

The mechanism of mating disruption is still not fully understood. There are several possible effects of synthetic pheromone on gypsy moth males (Granett 1974). First, synthetic pheromone sources compete with females. Second, males may become habituated to the high levels of pheromone in the air and do not respond to it. Third, the artificially high level of pheromone may cause abnormal behavioral responses in males so that they become overstimulated and are not able to orient and find females (Granett and Doane 1975, Richerson et al. 1976a). Fourth, it is hypothesized that the presence of artificial pheromone in the environment changes the copulatory behavior of gypsy moths. Based on the above propositions, it was thought that high airborne levels of pheromone cause males to leave treated areas (Granett and Doane 1975). More recent data showed that the density of male moths was slightly higher in treated areas than in control plots and that the duration of precopulatory and copulatory periods was similar for all females regardless of disruptant treatment (Schwalbe and Mastro 1988).

For successful disruption, the artificial pheromone must be present in the air in sufficient concentration throughout the mating period (Reardon et al. 1998). This can be achieved by using controlled release technologies. Controlled release is a regulated transfer of an active agent from a reservoir, which is usually a polymeric matrix, to a target surface to maintain a predetermined level of concentration for a specific period of time (Zedi et al. 1982). Two or three polymeric layers are laminated, where the central layer contains active agent (e.g., pheromone) and the outer layers control the release of the active agent from the central layer.

Currently there are several products used for mating disruption. These include Disrupt® II (Hercon) and Luretape Gypsy Moth, which are registered for "general use", and Luretape Plus, which does not require EPA registration. Generally, pheromones, which are used as lures, do not have to be registered by EPA, unless they are used as disruptants (http://infoventures.com/e-hlth/pesticide/dispar.html). Two controlled-release formulations of disparlure registered with the EPA are the three-layered plastic laminated tape, Hercon Luretape GM and granulated flakes, Hercon Disrupt® II (Kolodny-Hirsch and Schwalbe 1990, Reardon et al. 1998).

Hercon Luretape GM (Health-Chem Corporation, New York, NY) is 3.8 cm wide, has 2 PVC outer layers, and contains racemic disparlure at a concentration of 12.9 mg/cm^2 (Kolodny-Hirsch et al. 1990). This formulation is usually used for ground application.

Hercon Disrupt® II is a formulation of polymeric 3-layer laminated flakes 1 mm x 3 mm. The flakes contain racemic disparlure at a concentration of 3.1 mg/cm^2 (Health-Chem Corporation, New York, NY). A sticker is also incorporated in the formulation. Use of Disrupt® II without use of sticker significantly disrupts mating in gypsy moth populations; however, sticker should be used in order to achieve the maximum effect of the formulation (Thorpe et al. 2000). Disrupt® II is usually applied aerially using special equipment (Plimmer et al. 1982).

The 3M Corporation of Canada has developed a liquid microencapsulated pheromone product "Sprayable Pheromone". The 3M sprayable pheromone contains 20.0% of racemic disparlure (Cowan and DeVilbiss 2001, unpublished). This formulation is still in the process of development and has not been registered with the EPA and, therefore, is not available commercially.

2.8. Biological Effectiveness of Mating Disruption

A combination of several techniques is used to assess the biological effectiveness of mating disruption. Counting life stages (e.g., pupae, larvae and egg masses) is a way to detect a change in the abundance. A decrease in numbers of larvae and pupae under the burlap bands placed on tree boles indicates the effectiveness of mating disruption in the previous year, while the decrease in the abundance of egg masses shows the effectiveness of applied pheromone in the year of application. Monitoring male flight is usually assessed with pheromone-baited traps. Low catches or no catches, as well as the inability of males to find females in delta traps placed for monitoring, indicate successful disruption of pheromone communication. The absence of embryos in the eggs collected from females indicates successful mating disruption. Finally, low numbers or the absence of egg masses in a test plot compared with control also indicates the effectiveness of mating disruption (Reardon et al. 1998).

The effectiveness of mating disruption can be discerned from the following examples. In 1980, a laminated plastic flake formulation of disparlure was applied aerially at the doses of 0, 7.5, 30 and 75 g a.i./ha in Cecil County, MD. A steep reduction in trap catches was observed as

dose increased from 0 to 75 g a.i./ha (Webb et al. 1988). In 1981, laminated plastic tape was applied manually in a grid at the doses of 0, 25, and 250 g a.i./ha. Significant decrease in male trap catch and in mating success of females was observed with increasing the dose of disruptant (Webb et al. 1990) In 1989, disparlure (Disrupt® II) was applied on approximately 1 ha at a dose of 75 g a.i./ha as a part of an eradication program in Giles county, Virginia (Leonard et al. 1992). No moths were captured in the treated area after pheromone application, whereas in the untreated area 26% of traps captured male moths. Two years after treatments, one male moth was captured on the edge of the treated plot and an average of four males per trap were captured in the untreated area. In 1995, the treated area had much lower trap counts males than in the untreated areas (Reardon et al. 1998). In 1990, three plots were treated with double application of Disrupt® II at a dose of 75 g a.i./ha/application. Another three plots were treated every year from 1990 through 1993 with a single application at the same dose of 75 g a.i./ha. Both types of treatments significantly reduced population densities compared to untreated areas (Leonhardt et al. 1996). Thus, mating disruption was shown to be an effective method of suppressing low-density gypsy moth population and now it is used operationally in several states for eradication of isolated populations and in the Slow-The-Spread project.

2.9. Objectives of the Study

This study is a part of a research project targeted on evaluating and improving mating disruption treatments against gypsy moth. The goal of this study is to understand the mechanisms of mating success and to reduce the cost of the tactic by improving methods for application of pheromone used for mating disruption. The efficiency of pheromone treatments can be optimized by determining the lowest dose of pheromone that will effectively disrupt mating, and by defining the maximum distance between sprayed swaths that still result in effective mating disruption. Knowledge of spatial and temporal dynamics of aerially applied pheromone will allow for more rational use of the pheromone. Therefore, the objectives are:

1. To compare mating success and mortality of adult gypsy moth females in Virginia and Wisconsin
2. To study the effect of various pheromone treatments on mating success of gypsy moth females

3. To evaluate the effect of the pheromone sprayed for mating disruption beyond treated areas
4. To evaluate an EAG device for its ability to detect gypsy moth pheromone sprayed in the field for mating disruption

3. Mating Success and Mortality of Gypsy Moth Females in Virginia and Wisconsin

3.1. Introduction

The gypsy moth, *Lymantria dispar* (Linnaeus), is a pest of hardwood forests. The insect was introduced accidentally into the United States near Boston, Massachusetts from France (Liebhold et al. 1989) and has spread at a rate of approximately 20 km/yr to the south, west and north from where it was introduced (Liebhold et al. 1992). In 1993 USDA Forest Service initiated the gypsy moth management program. An objective of the program is to slow the spread of the insect by suppressing solitary low-density populations (McFadden and McManus 1991, Leonard and Sharov 1995), which establish beyond the population front, grow, and contribute to the main population and to the movement of the front (Sharov et al. 1998).

Very few studies have been devoted to understanding the population dynamics mechanisms specific to low-density gypsy moth populations. One such mechanism is mating success of females, which appears to be a critical inverse density-dependent factor (Sharov et al. 1995). Mating success depends mainly on the local abundance of adult males and is correlated highly with the rate of male moth capture in pheromone traps (Sharov et al. 1995). Mating success, therefore, can be predicted from male moth capture in pheromone-baited traps. Sharov et al. (1995) used this approach to study the mating success of gypsy moths in the Appalachian Mountains, Virginia. Another important factor that affects low-density gypsy moth populations is predation, e.g., bird predation on adult males. However, ants were shown to be the most important predators of adult gypsy moth females (Sharov et al. 1995). Ant predators decrease the mating probability of females by decreasing their longevity.

The male moth catches in pheromone-baited traps in Wisconsin range from <1 in the Western and middle part of the state to 30 – 300 in the East (Figure 3.1). The spread of gypsy moth populations in southern Wisconsin also occurs much faster than in the Appalachian Mountains, Virginia (Sharov 1998). It has been hypothesized that this difference in the rate of spread between the two regions may be due to the higher mating success of females and subsequent higher rate of growth in low-density populations in Wisconsin (Sharov et al. 1999).

In contrast to the Appalachian Mountains, male moth counts in pheromone traps in Wisconsin tend to decrease more gradually with increasing distance from the population front (Régniére and Sharov 1998). This suggests that male moth dispersal may be more extensive in Wisconsin than in the Appalachian Mountains. In this paper we describe the study of the relationship between mating success of gypsy moth females and male moth captures in pheromone traps. In order to understand the mechanism of mating success of gypsy moth females in Wisconsin, we compared this mechanism with the mating success observed in the study done previously by Sharov et al. (1995) in the Appalachian Mountains, Virginia.

The goal of this objective was to study the relationship between mating success of gypsy moth females and male moth captures in pheromone traps. In order to understand the mechanism of mating success of gypsy moth females in Wisconsin we compared this mechanism with the mating success observed in the study done previously by Sharov et al. (1995) in Appalachian Mountains, Virginia.

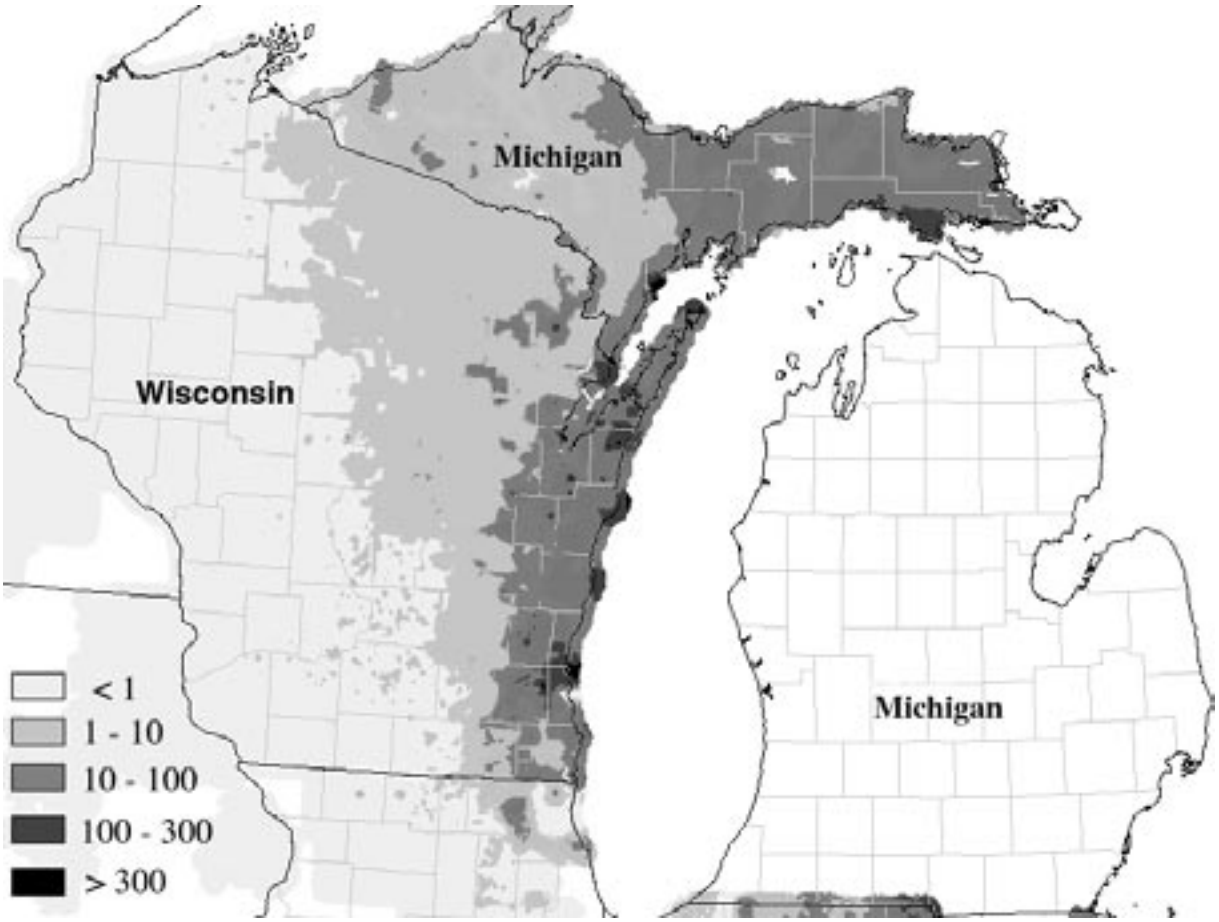

Figure 3.1: Male gypsy moth counts in traps in WI, 1999

3.2. Materials and Methods

A study was conducted in Kettle Moraine State Forest and nearby forested sites in Wisconsin between July 25 and August 6, 2000. Based on male moth catches in pheromone-baited traps (Figure 3.1), seven study plots were established at various distances from an advancing gypsy moth front (Figure 3.2). Three plots were located in the Waukesha County and four plots in the Walworth County.

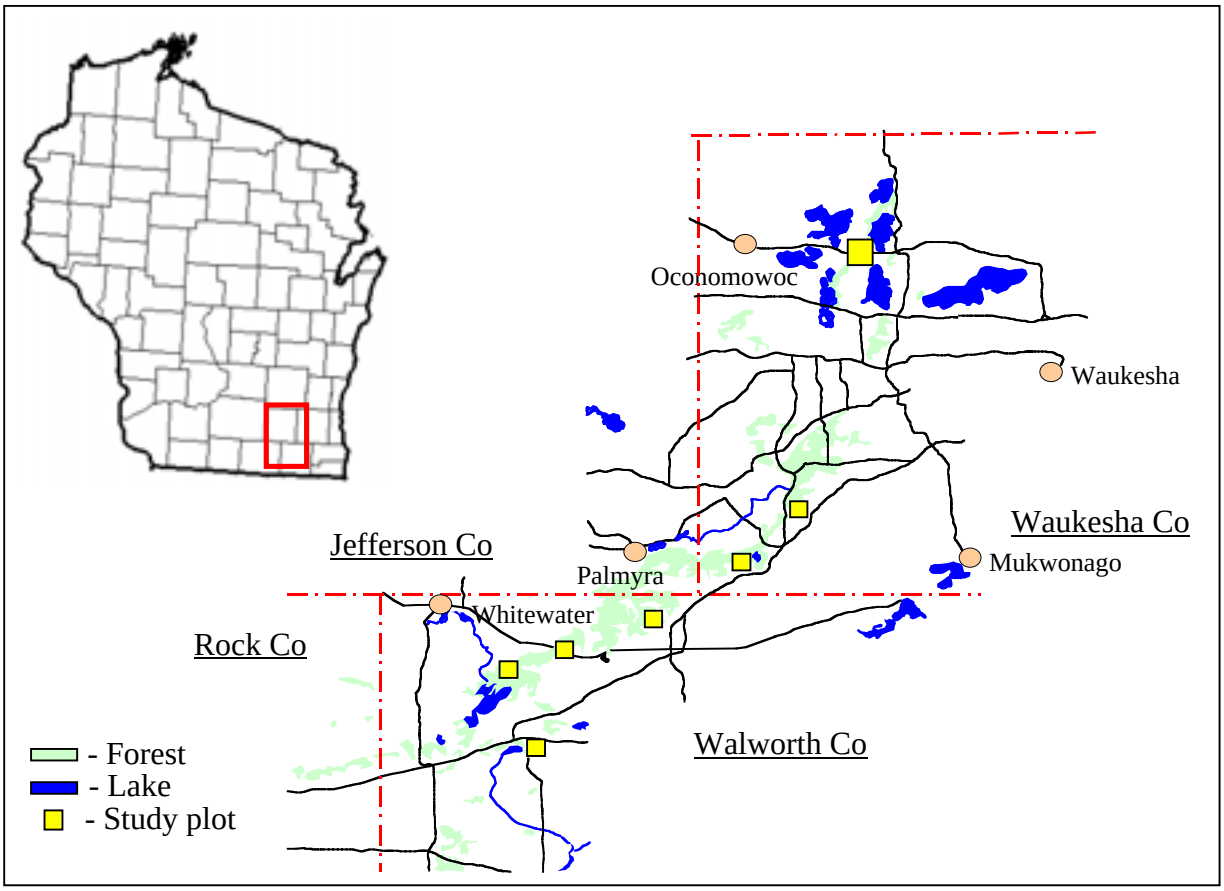

Figure 3.2: Map of experimental plots in WI, 2000

Mating success of gypsy moth females was evaluated using tethered virgin females. Gypsy moth females were raised from pupae obtained from USDA-APHIS Otis Methods Development Center (Otis, MA). Virgin 1-day old females were tethered by a 10-15 cm thread tied to the base of the front wing and attached to a tree by a pushpin (Sharov et al. 1995). Two lines of 10-13 tethered females per line were set up in each plot. The lines were separated by a distance of 20 m, and the distance between females in a line was 20-25 m (Figure 3.3). A barrier of tanglefoot glue was applied in the radius of 25 cm around females in some of the plots to protect individuals from natural predators (e.g., ants). Females were left on trees for 24 hr, after which they were removed and their fertilization status was determined via dissection. In some plots, where male trap catches were ≤ 0.5 males per trap, females were left on trees for two days. Females that were collected were stored in the vials for 24 hours and then dissected to check for

the presence of sperm in the spermatheca. Egg masses also were analyzed later for embryonation in those cases where we were uncertain about whether mating had occurred. Non-fertilized eggs do not develop but remain white, whereas, fertilized eggs become dark and a larva can be observed inside the egg under a dissecting microscope.

Male moth abundance was determined using pheromone-baited traps. Two pheromone-baited traps were placed in each plot at a distance of 100 m from the lines of females to avoid competition between the two sources of pheromone (Figure 3.3). Larger distances could not be used because of the fragmented forest landscape. The pheromone traps were checked before placing tethered females on trees and at the time females were collected.

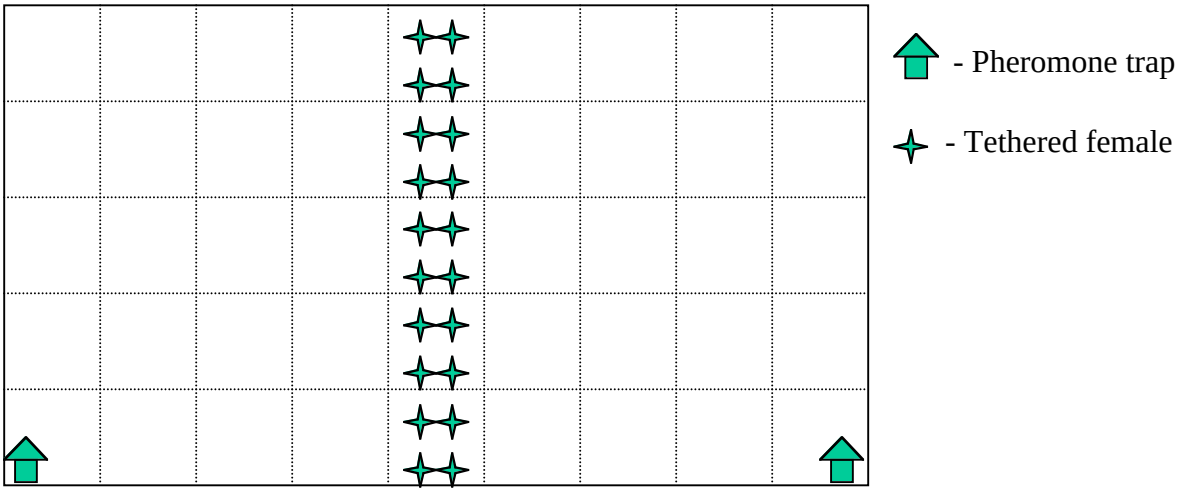

Figure 3.3: Layout of traps and tethered females in a study plot in WI, 2000

Statistical analysis. The relationship between mating success of females and male moth capture was described by the exponential model (Sharov et al. 1995):

$$P(t) = 1 - \exp(-s \cdot t \cdot M) \qquad \textbf{(3.1)}$$

Where *P(t)* is the proportion of females that are mated during time *t* (days), *M* is the male catch per trap per day, and *s* is a parameter that can be interpreted as a relative attractiveness of virgin females to males compared with pheromone-baited traps. The parameter *s* was estimated using nonlinear regression of *P(t)* versus ($t * M$) (least squares method). The fit of the model was checked using the coefficient of determination (R^2).

Predation rate on gypsy moth females in Wisconsin was estimated by counting the proportion of females removed by predators per day among individuals not protected with a tanglefoot glue barrier. A Chi-square (χ^2) test was used to compare the mortality of gypsy moth females in Wisconsin with that in Virginia using the data collected by Sharov in 1995 (Sharov et al. 1995).

3.3. Results

In Wisconsin, the mortality of gypsy moth females from predation ranged between 0 to 35% per plot per day (Table 3.1) with an average of 14.2 ± 2.5%. The average trap capture rate in the study plots in Wisconsin, ranged from 0.1 to 42.5 male moths per trap per day; the mating probability of gypsy moth females ranged from 0 to 0.95 (Figure 3.4). Mating success of females increased with increasing male moth captures in traps. The relative attractiveness of females compared with pheromone traps, *s*, was 0.23 with a 95% confidence interval of 0.16 to 0.30 (R^2 = 0.816).

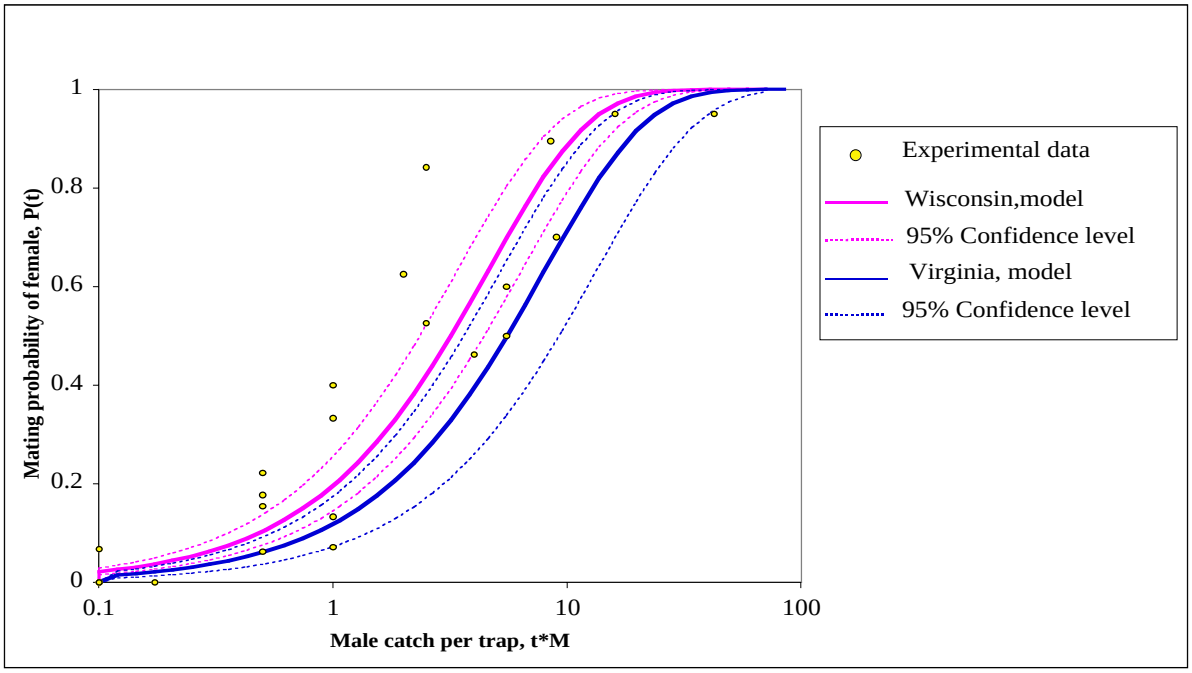

Figure 3.4: Mating probability of gypsy moth females in Virginia and Wisconsin

Table 3.1: Mortality of females in Wisconsin, 2000

date	plot#	Females analyzed	% Mortality
29-Jul-2000	13	20	4.76
31-Jul-2000	1	20	0
31-Jul-2000	2	20	0
31-Jul-2000	4	23	4.16
1-Aug-2000	16	13	35
2-Aug-2000	12	13	35
2-Aug-2000	16	15	25
3-Aug-2000	12	16	20
3-Aug-2000	14	17	15
4-Aug-2000	1	20	0
4-Aug-2000	12	19	5
4-Aug-2000	13	19	4.76
4-Aug-2000	14	16	20
5-Aug-2000	12	20	0
5-Aug-2000	14	15	25
5-Aug-2000	16	14	30
6-Aug-2000	1	20	0
6-Aug-2000	2	18	10
6-Aug-2000	4	18	10
6-Aug-2000	13	19	9.5
6-Aug-2000	14	17	15
6-Aug-2000	16	15	25

Mean = 14.2

Standard Error = 2.5

3.4. Discussion

This first study of gypsy moth mating success in Wisconsin indicates that the mating probability of females can be predicted from male moth capture in pheromone traps. This may be important for determining the extent to which moth captures are associated with stable reproducing isolated colonies of the gypsy moth in the areas beyond the expanding population front. The threshold number of male moths captured per season that is associated with a stable population was determined using the model (Sharov et al 1995):

$$1 - \exp(-s * t * M) = \exp(-r) \qquad \textbf{(3.2)},$$

Where s is the parameter that describes the relative attractiveness of virgin females to males compared with pheromone-baited traps, t is female calling time (days), and M is male moth catch per trap per season. The calling time t of females depends on the dynamics of pheromone emission but also may be reduced by predation. Females are usually attractive during the first three days of their lives, and thereafter their attractiveness declines sharply (Collins and Potts 1932, Richerson and Cameron 1974). Considering the predation rate of 14.2% per day as the only mortality cause, the average survival time of females in Wisconsin would be -1/ln(1-0.142) = 6.53 days. This time is longer than the calling time (3 days). Thus, predation does not appear to limit female calling time in Wisconsin.

The threshold number of males associated with a stable population, M, was also calculated using average population growth rate r = 1.65 (Sharov et al. 1995). This yielded M = 0.23 males per day. Assuming a 3-week average flight period, this is equivalent to 4.8 males/trap/season. Thus, male moth catches below 5 moths per trap are usually not associated with a reproducing population in this particular location. However, for monitoring purposes, a catch of 5 moths per trap may be an indication of a reproducing population that is located at some distance from the trap (e.g., 1-2 km away).

The results of the study of the mating success of gypsy moth and male moth capture in the pheromone-baited traps in Wisconsin were compared with the results of an analogous study conducted in Appalachian Mountains, Virginia. In the study in Virginia, s was estimated as 0.15 with a 95% confidence interval of 0.09 - 0.23 (Sharov et. al 1995). The confidence intervals of s in Wisconsin and in Virginia overlap (Figure 3.4), suggesting that there is no significant difference in the relationships of mating success of gypsy moth females and male moth counts in traps in the two regions.

Female mortality from predation in Virginia ranged from 30 to 94% per day with an average predation of 52 ± 5%. Mortality of gypsy moth females from predation, therefore, was significantly lower in Wisconsin than in Virginia ($p < 0.001$). The threshold number of males associated with a stable population, M, was estimated to be lower in Wisconsin (M = 4.8 males/trap/season) than in Virginia (M = 7 and 15 males/trap/season with and without predation respectively). Therefore, the rate of population spread in Wisconsin is higher than in Virginia.

In the study in Virginia, predation considerably reduced the waiting time of females t = 1.36 days compared to Wisconsin, where t = 6.53 days (Sharov et al. 1995). This suggests that females live longer and have a higher chance of mating in Wisconsin than in Virginia.

In the light of the above discussion we may conclude, that the higher rate of population spread in southern Wisconsin compared with the Appalachian Mountains, Virginia may be due to the increased mating success of females, which is probably caused by the increased long-distance dispersal of males and the longevity of females.

3.5. Conclusions

No significant difference was found in relationships between mating success of gypsy moth females and male moth counts in traps in Virginia and Wisconsin. The mortality of females from predation was significantly higher in Virginia than in Wisconsin. Therefore, the higher rate of population spread in Wisconsin may be due to the increased mating success of females, which is probably caused by increased long-distance dispersal of males and increased longevity of females.

4. Influence of Various Pheromone Treatments on Mating Success of Gypsy Moth Females

4.1. Introduction

The use of mating disruption to control populations of gypsy moth, *Lymantria dispar*, has been attempted since 1971 (Stevens and Beroza 1972, Schwalbe et al. 1974, Granett and Doane 1975). Extensive research has been conducted to find an appropriate formulation and concentration of pheromone for use in mating disruption. Dose-response experiments have been used widely in toxicological studies and, therefore, should also be useful in field studies of the effect of pheromone release rate on disruption of mating in insect populations. The first dose-response experiment on mating disruption in gypsy moth was conducted using the Hercon Disrupt® (Health Chem Corporation, New York, NY) formulation of pheromone, applied at doses of 7.5, 30 and 75 g a.i./ha (Webb et al. 1988). A strong negative relationship between pheromone dose and population response was shown. Average catches were 633, 101, and 57 males per plot, respectively compared with 1,976 males in the control area. The high number of males that were trapped suggested that the study was conducted in an area with a high population density of gypsy moths. Mating success of both lab and wild females also was shown to decrease as pheromone dose was increased from 0 (control) to 75 g a.i./ha.

In a later study, two additional doses (25 and 250 g a.i./ha) were tested using Hercon Luretape® (Hercon Laboratories, South Plainfield, PA) (Webb et al. 1990). Male moth captures in pheromone-baited traps and mating success of females decreased significantly as the dose of applied pheromone increased from 0 (control) to 250 g a.i./ha. This study also was conducted in a high-density gypsy moth population. In another dose-response experiment conducted in an area with a dense gypsy moth population using Hercon Luretape at doses of 5, 50 and 500 g a.i./ha, there was a gradual decrease of male captures in traps and mating success of females with increasing doses of the pheromone (Schwalbe and Mastro 1988). Kolodny-Hirsch and Schwalbe (1990) and Kolodny-Hirsch and Webb (1993) studied the effect of high rates (50 and 700 g a.i./ha) of racemic disparlure on low-density gypsy moth populations and also observed a dramatic decrease in male moth captures in traps and in the mating success of females.

For mating disruption to be successful the pheromone must be present in the air in sufficient quantities (Reardon et al. 1998). The synthetic disruptant should be formulated so that

it would release pheromone slowly at a constant rate for a specific period of time. Therefore, studies have been conducted to find an ideal formulation of pheromone (e.g., Plimmer et al. 1982, Thorpe et al. 1999). Three types of controlled release formulation were tested in 1982, including microcapsules (National Cash Register Corporation, Dayton, Ohio), Hercon Disrupt® laminated polymeric flakes (Health-Chem Corporation, New York, NY), and Albany International hollow fibers (Needham Heights, MA) (Plimmer et al. 1982). The three formulations were compared at 2 and 20 g a.i./ha. At 2 g a.i./ha, microcapsules, Hercon Disrupt® and hollow fibers reduced male catches in pheromone-baited traps by 44.6, 42.9, and 46.2%, respectively. At 20 g a.i./h there were 63.4, 75.7, and 91.6% reductions in male catches in traps by the same formulations. Mating success of females was also reduced by 33.6, 38.4 and 40.3% at 2 g a.i./ha and by 62.6, 88.3, and 90.9% at 20 g a.i./ha. The study showed that Hercon Disrupt® and Albany International are equivalent in their ability to disrupt mating. Later studies also confirmed the effectiveness of Hercon Disrupt® (Webb et al. 1988).

Hercon Disrupt® II formulation of disparlure was the only product registered by EPA from 1983 to 1989 and available for operational use (Reardon et al. 1998). In the late 1990s a flowable bead formulation of the pheromone was developed and tested by AgriSense (Fresno, CA). Laboratory tests and some of the field studies showed that the release rate from the bead formulation was too rapid and that this formulation caused problems with application equipment (Reardon et al. 1998). Thorpe et al. (1999), however, found that a single application of flowable beads disrupted mating as well as Hercon Disrupt® formulation, which disagreed with the previous results.

Because most dose-response studies were conducted using high doses of pheromone, no information is available on the effects of low doses of disparlure on gypsy moth populations. Also, the densities of populations that have been tested were usually high and the range of racemic disparlure doses was not wide enough. Therefore, one of the objectives of this study was to conduct dose-response experiments in low-density gypsy moth populations using a wider range of pheromone doses. A second objective of the study was dictated by the fact that Hercon Disrupt® II was still the only formulation of disparlure available for aerial applications. Therefore, experiments were conducted to compare the relationship between plots treated with various doses and formulations of pheromone and mating disruption as measured by mating success of females and male moth catches in pheromone-baited traps.

4.2. Materials and Methods

4.2.1. Dose-response Experiment: 2000

Sixteen plots, each 500 by 500 m in size and separated by at least 1 km, were selected in Millboro Springs (Bath Co), VA [UTM 637052 E, 4223294 N to 614250 E, 4192715 N]. Four plots were used as controls and left untreated, and the remaining 12 plots were treated with various doses and formulations of pheromone, which was applied by airplane, as follows: microcapsules (3M Canada Co., London, Ontario, Canada) at 75 g a.i./ha (3M75), plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA) at 37.5 g a.i./acre (PF37.5), and plastic flakes at 75 g a.i./ha (PF75). Thus, each treatment was replicated four times. Previous studies showed that the dose of disparlure of 75 g a.i./ha disrupts mating (Webb et al. 1990). Therefore, this dose was used to compare the two formulations (3M and PF).

Gypsy moth mating success was evaluated by deploying laboratory-reared virgin females. Half of the females were put into mating stations attached to a tree trunk and the other half were tethered. Each mating station was a delta trap with neither glue nor pheromone that contained a female. Females were tethered around the base of a front wing using a 10-15 cm thread and attached to a tree by a pushpin (Sharov et al. 1995). To protect females from natural predators (e.g., ants), a tanglefoot glue barrier was applied in the radius of 25 cm around each individual. Male moth capture was determined using milk-carton pheromone traps baited with 500 μg of (+)-disparlure in twine dispensers (Schwalbe 1981a, Leonhardt et al. 1992).

Each study plot had 9 tethered females, 9 females in mating stations, and 4 pheromone-baited traps (Figure 4.1). Two females (one in a mating station and one tethered) were placed near the center of the plot, approximately 25 m from each other. Four pairs of females were set up symmetrically in the north, south, east and west directions 50 m from the central point. Pheromone traps were placed 25 m from each of the four pairs and four pairs of females were placed 25m from each pheromone trap. Females were left on trees for 24 hr, after which they were removed and their fertilization status was determined via dissection.

Statistical Analysis. The proportion of fertilized females was calculated for each treatment. The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) were used to test the difference in moth counts between

groups of traps located in plots treated with various doses and formulations of pheromone. Log-transformed total moth counts per trap per week for each type of pheromone treatment, ln(N+1), was modeled as a function of week and dose without interactions of factors. Male moths catches in the pheromone-baited traps were also analyzed for three time intervals: June 30 - July 7 (1 – 8 days after pheromone application), July 10 – July 13 (11 – 14 days after pheromone application) and July 18 – July 28 (19 - 29 days after pheromone application).

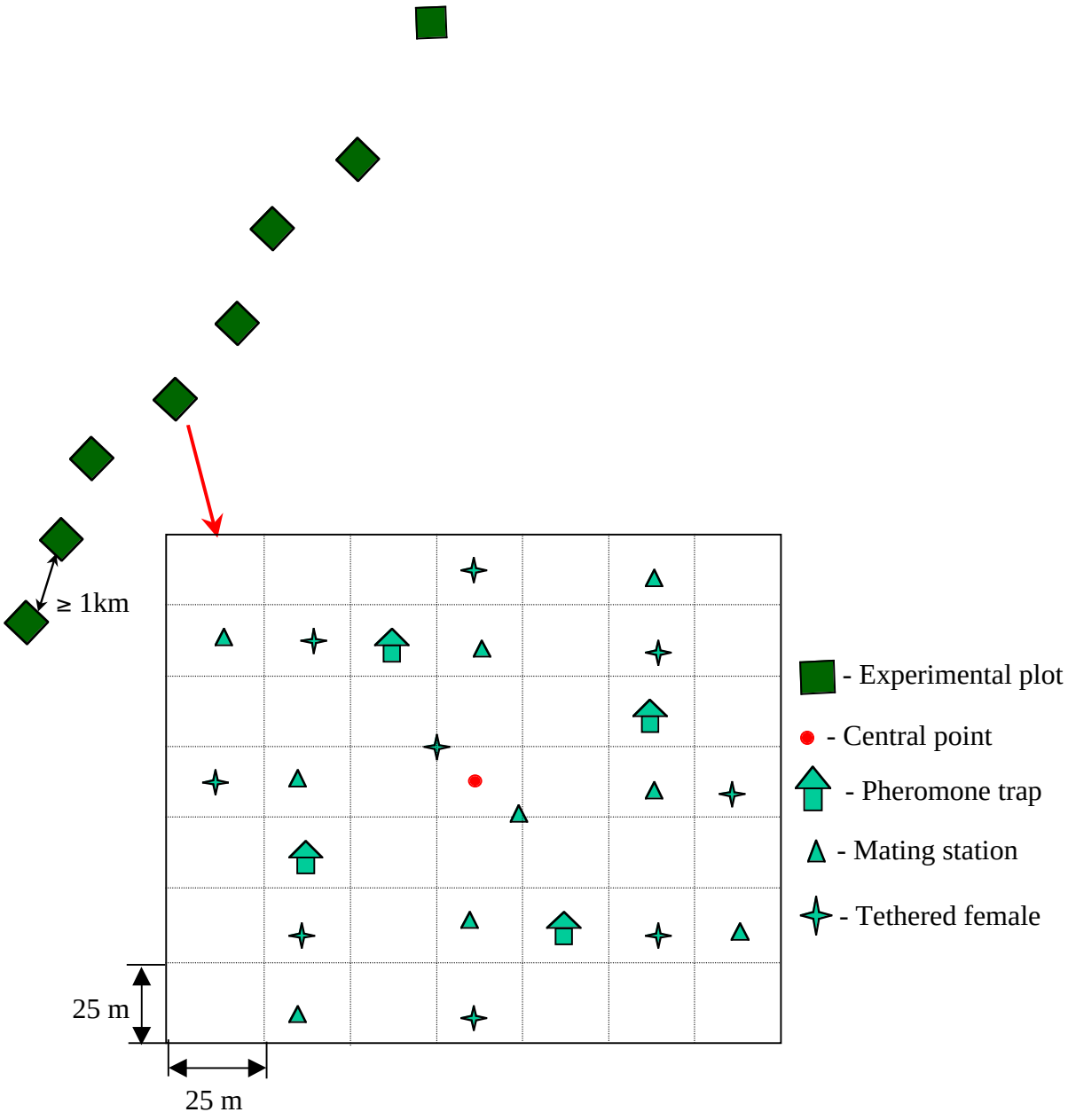

Figure 4.1: Layout of traps, mating stations, and tethered females around the center of a plot in Millboro Springs, VA, 2000.

4.2.2. Dose-response Experiment: 2001

An experiment was conducted in Appomattox-Buckingham (Appomattox and

Buckingham Co.) and Cumberland (Cumberland Co) State Forests, VA [UTM 746246 E, 4166292 N to 700180 E, 4136389 N]. Seven experimental plots were chosen in each of the areas. Racemic disparlure was applied aerially in two formulations, microcapsules (3M Canada Co., London, Ontario, Canada) and plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA). One plot in each state forest was used as a control and the remainder of the plots each were treated either with 3M microcapsules at 0.15, 0.75, 3, 15, 37.5, or 75 g a.i./ha or with plastic flakes at 15 g a.i./ha. All doses were replicated twice, except for doses of the 3M formulation at 15 and 37.5 g a.i./ha, which were unreplicated.

Because the density of the resident population was very low, mating disruption was evaluated by deploying laboratory-reared tethered females following the release of laboratory-reared males. Disruption was measured by the rate of fertilization of females and by recapturing males in USDA milk-carton traps baited with 500 μg of (+)-disparlure in twine dispensers (Schwalbe 1981a, Leonhardt et al. 1992). Each study plot had 2 male moth release points, 15 tethered females placed in a circle around the release point at the center of the plot, and 7 pheromone-baited traps. Four traps were placed 150 m to the north of the center of the plot; the distance between traps was 25 m. One trap also was placed 150 m to the south, east and west of the central release point (Figure 4.2). Adult females were placed on tree boles for 1 day and protected from ant predation by a band of the tanglefoot glue. Fertilization, as determined by the analysis of egg embryonation, was used as an indicator of mating.

Male gypsy moths were obtained as pupae from USDA APHIS Otis Methods Development Center, Massachusetts. Pupae were kept in laminated paper cups with plastic lids before they were transferred to release cups, which were stapled to the trunks of trees in the field. The release cups were the same types of cups used for rearing males but with several openings cut at mid-height to allow emerging males to escape. Tanglefoot (The Tanglefoot Co., Grand Rapids, Michigan) glue was applied in circles around the tree trunk. Fluorescent powder dye was added to the cups to mark emerging male moths. Each week, the same number of males (~150) was released at each release point. Male moths captured in pheromone traps were removed and stored in the freezer. The moths were later examined under the microscope with a UV light for the presence of fluorescent powder on wings, antennae or body to distinguish between released and natural moths. Natural population density was evaluated by counting pupae under burlap bands attached to the trunks of 100 trees in plots that did not have traps or females.

Statistical Analysis. Trap catches and mating success of females were analyzed using the General Linear Model ANOVA procedure (SAS 1996, Proc GLM). Arcsine-transformed proportion of fertilized females ($\arcsin\sqrt{N}$) was modeled as a function of week and dose with interactions of factors. Male moth catches in the pheromone-baited traps were analyzed both using data from the entire period of the study and separately using data from each of the three time interval: June-July (15 – 45 days after pheromone application), August 1 – August 13 (50 – 62 days after pheromone application) and August 15 (64 days after pheromone application). The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to analyze the pooled data to test for significance of differences in moth counts between groups of traps located in plots treated with various doses and formulations of pheromone. The log-transformed total moth counts per trap per week for each type of pheromone treatment, $\ln(N+1)$, was modeled as a function of week, dose, and state forest without interactions of factors. For the first interval (June - July), $\ln(N+1)$ was modeled as a function of week, dose, and state forest without interactions of factors. For the other two intervals (August 1 – August13 and August 15), $\ln(N+1)$ was modeled as a function of the dose and state forest without interactions of factors.

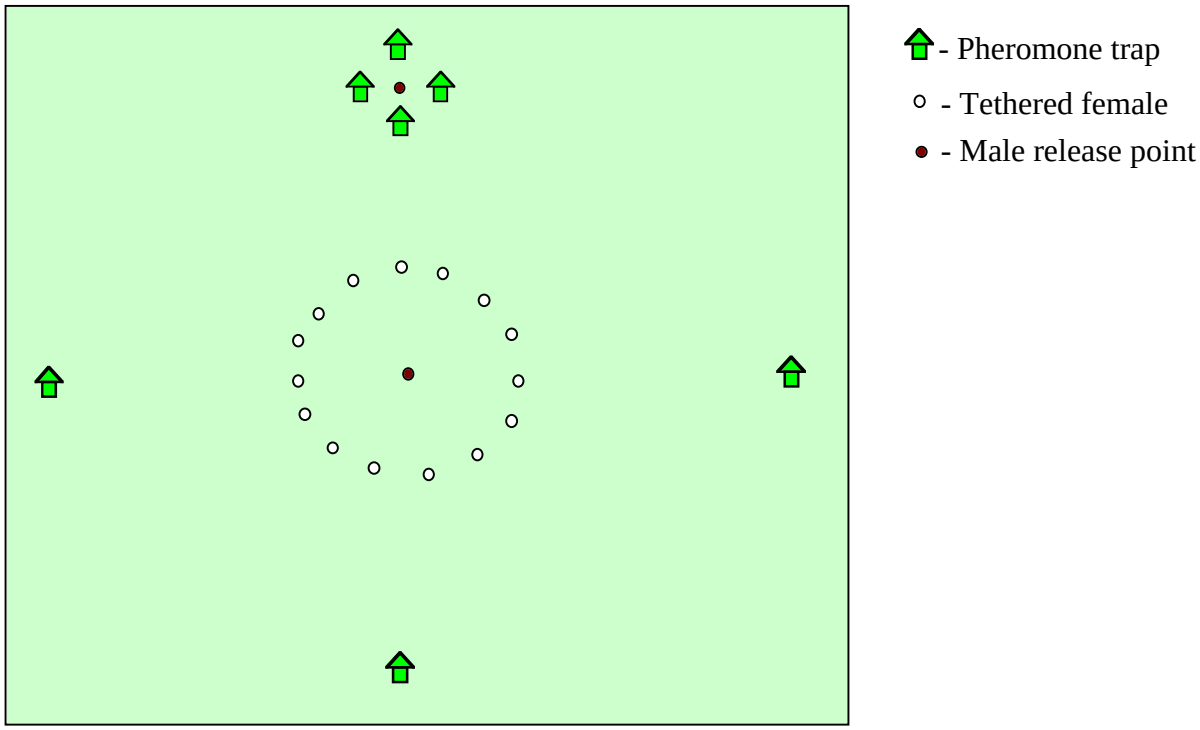

Figure 4.2: Layout of tethered females, traps, and adult male release points in a plot in Cumberland and Appomattox-Buckingham State Forests, Virginia, 2001.

4.2.3. Dose-response Experiment: 2002

A study was conducted in Appomattox-Buckingham (Appomattox and Buckingham Co.) and Cumberland (Cumberland Co.) State Forests, VA [UTM 746246 E, 4166292 N to 700180 E, 4136389 N]. Twelve experimental plots were chosen in Appomattox-Buckingham State Forest and nine plots were chosen in Cumberland State Forest. Racemic disparlure was applied aerially in two formulations: a liquid formulation (Shin-Etsu Chemical Co. Ltd, Tokyo, Japan) and plastic flake formulation (Disrupt® II, Hercon Environmental, Emigsville, PA). One plot in each state forest was used as a control and the remainder of the plots each were treated either with plastic flakes at 0.15, 0.75, 3, 15, or 37.5 g a.i./ha or with liquid formulation at 15 or 37.5 g a.i./ha. The low doses of plastic flakes formulation (0.15, 0.75 and 3 g a.i./ha) and liquid formulation at 15 g a.i./ha were replicated twice, the liquid formulation at 37.5 g a.i./ha was replicated three times, and the plastic flakes at 15 and 37.5 g a.i./ha were replicated four times.

Mating disruption was evaluated by recapture of released laboratory-reared males in

USDA milk-carton traps baited with 500 μg of (+)-disparlure in twine dispensers (Schwalbe 1981a, Leonhardt et al. 1992). Each study plot had 3 male moth release points and 8 pheromone-baited traps (Figure 4.3). The release points were established at the center of each plot and 150 m to the north and south of the plot center. Northern and southern release points were surrounded by 4 pheromone-baited traps, which were placed 25 m to the north, south, east, and west from the release point.

Male gypsy moths were shipped as pupae from USDA APHIS Otis Methods Development Center, Massachusetts. Pupae were kept in laminated paper cups with plastic lids and emerged adults were released in the field. Fluorescent powder dye was added to cups with pupae to mark emerging male moths. Each week, the same number of males (~150) was released at each release point. Male moths were removed from the pheromone traps and stored in a freezer. Later they were examined under the microscope with a UV light for the presence of fluorescent powder on wings, antennae or body to distinguish between released and natural moths.

Statistical Analysis. Male moth catches in pheromone-baited traps were analyzed both using data from the entire period of the study and separately using data from each of the three time intervals: 8 – 14 days after pheromone application, 15 – 49 days after pheromone application, and 50 – 56 days after pheromone application. To analyze pooled data, a General Linear Model ANOVA with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to test the significance of differences in moth counts between groups of traps located in plots treated with various doses and formulations of pheromone. The log-transformed total moth counts per trap per week for each type of pheromone treatment, $\ln(N+1)$, was modeled as a function of week, dose and state forest without interactions of factors. For the first (8 – 14 days) and third (50 – 56 days) intervals, $\ln(N+1)$ was modeled as a function of dose and state forest without interactions of factors. For the second interval (15 – 49 days), $\ln(N+1)$ was modeled as a function of week, dose and state forest without interactions of factors. The cumulative percent of male catches was used to generate a phenology plot.

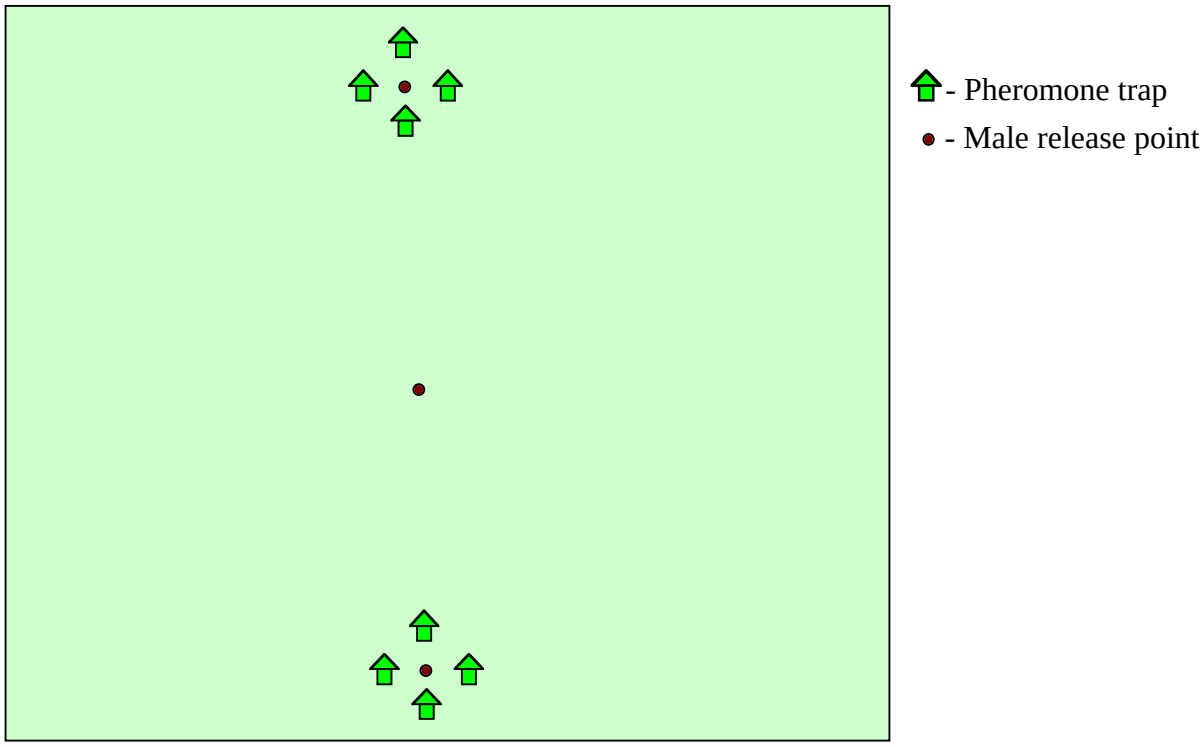

Figure 4.3: Layout of pheromone-baited traps, and adult male release points in a plot in Cumberland and Appomattox-Buckingham State Forests, VA, 2002

4.3. Results

4.3.1. Dose-response Experiment: 2000

Mating success of laboratory-reared females in plots treated with pheromone was reduced significantly compared with females in control plots. In the control plots 19.9% of females were fertilized while in all treated plots 100% of females remained unmated (Figure 4.4).

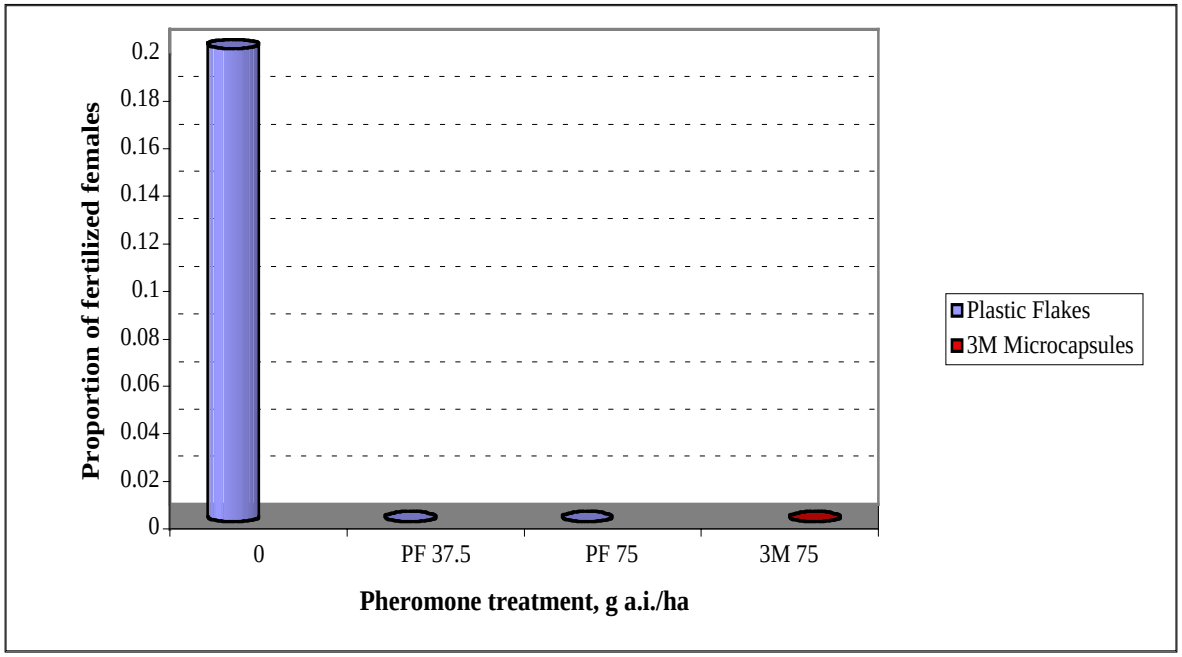

Figure 4.4: Proportion of fertilized females in plots treated with various doses of pheromone in Millboro Springs, VA, 2000

Male trap catches were also suppressed by the pheromone formulations and doses that were applied. Season-long male catches averaged 135.25, 1.83, 2.06, and 0.38 males per plot at the doses of disparlure (g a.i./ha) of 0, 37.5 (plastic flakes), 75 (plastic flakes), and 75 (3M microcapsules), respectively. There was also a significant time effect ($F = 2.08$, d.f. = 2, $P < 0.05$) and a strong treatment effect ($F = 101.37$, d.f. = 3, $P < 0.001$) of the pheromone formulations.

Male moth catches in traps were high during the third week after pheromone application (July 18 – July 28) (Figure 4.5), which coincides with the peak of male flight (Figure 4.6). Trap catches in treated plots were significantly lower than in control plots (Figure 4.7). Trap catches in plots treated with microcapsules at 75 g a.i./ha were lower than in plots treated with the same dose of plastic flakes, but the difference was not statistically significant.

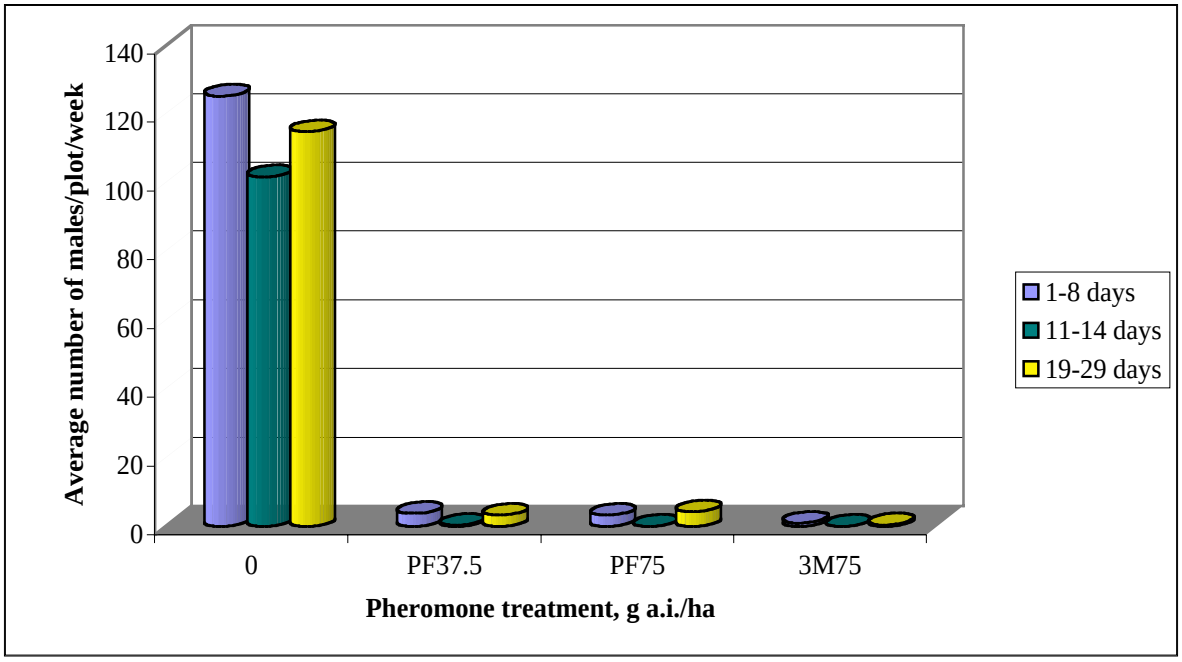

Figure 4.5: Weekly male moths catches on plots treated with various doses of pheromone in Millboro Springs, VA, 2000

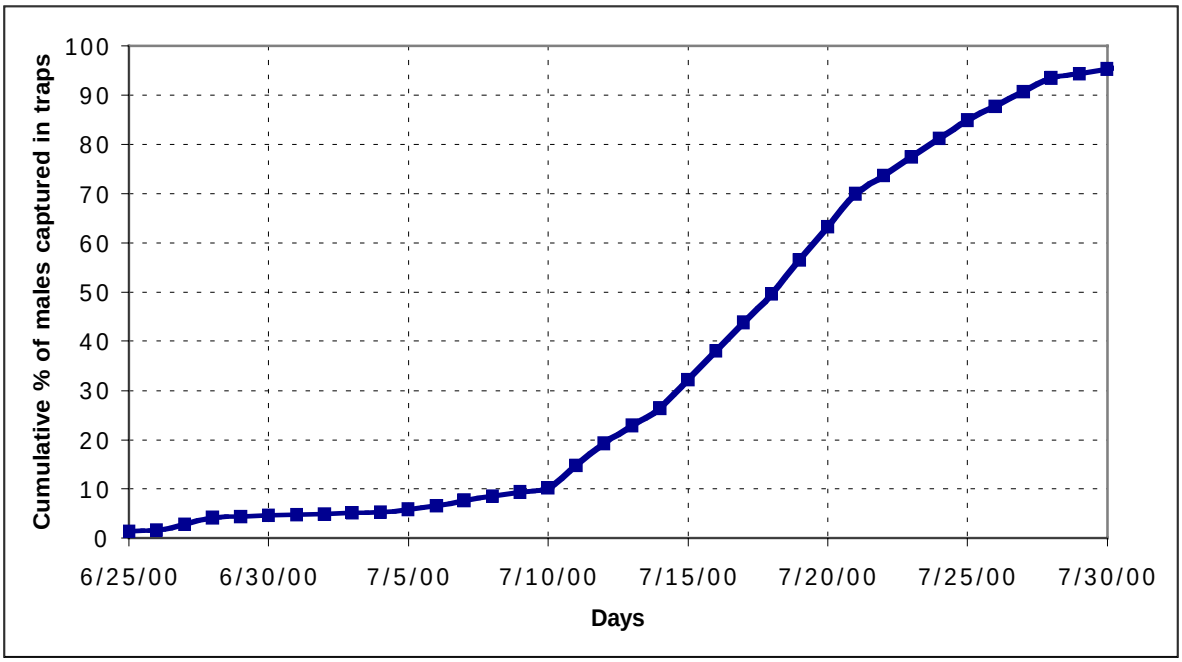

Figure 4.6: Phenology of gypsy moth males in Millboro Springs, VA, 2000

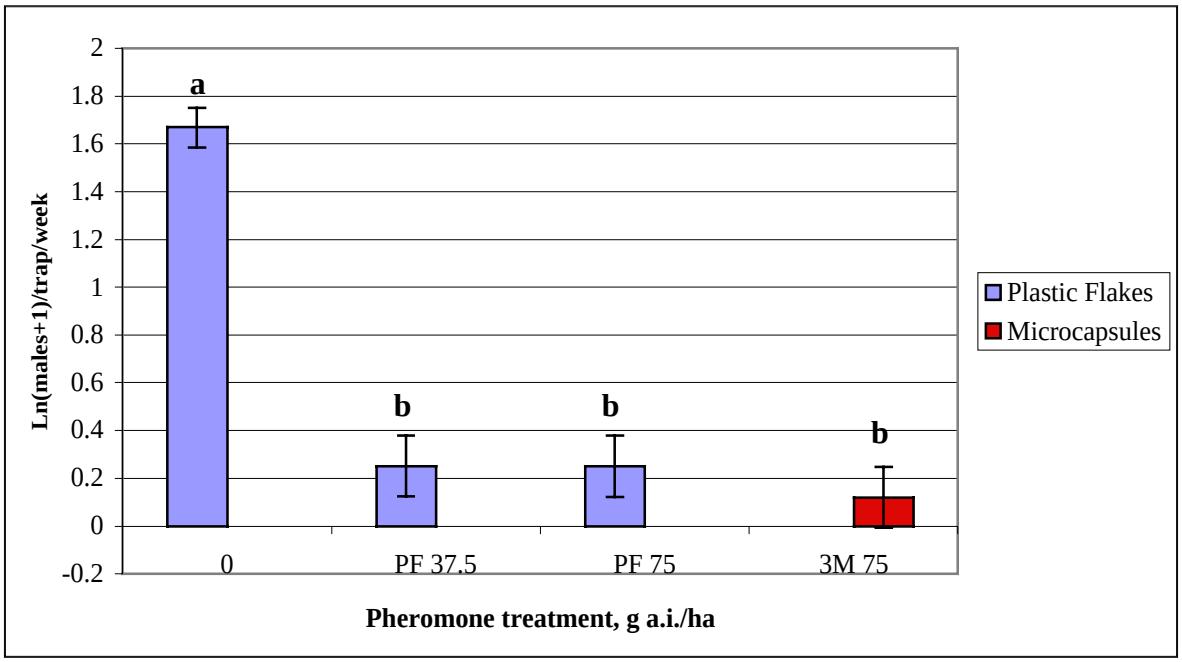

Figure 4.7: Log male moths (ln(N+1) ± SD) captured in plots treated with various doses of pheromone in Millboro Springs, VA, 2000. Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

4.3.2. Dose-Response Experiment 2001

Mating success of laboratory-reared females was suppressed significantly in the plots treated with 3M microcapsules at 15, 37.5, and 75 g a.i./ha and with plastic flakes at 15 g a.i./ha ($F = 25.64$, d.f. = 7, $P < 0.01$) (Figure 4.8).

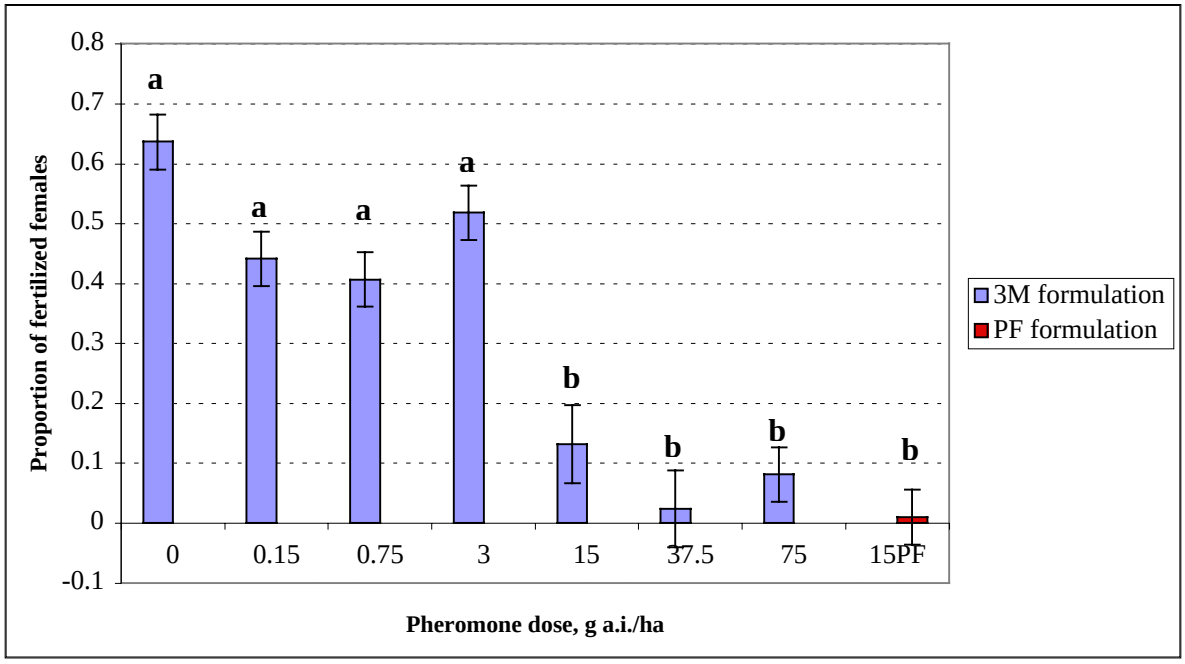

Figure 4.8: Proportion of fertilized females (arcsin $\sqrt{N}$ ± SD) in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001. Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

Season-long male catches (from June 27 to August 15) averaged 460.5, 508, 437.5, 659, 148.5, 124.5, 124, and 11 males per plot at the doses of disparlure (g a.i./ha) of 0, 0.15, 0.75, 3, 15, 75, and 37.5 (3M microcapsules) and 15 (plastic flakes), respectively.

Overall male moth recapture in pheromone traps were significantly lower in plots treated with plastic flakes at 15 g a.i./ha and 3M microcapsules at 37.5 and 75 g a.i./ha compared to all other treatments (Figure 4.9). Male moths recapture in pheromone traps was also studied over periods of 15-45, 50-62 and 64 days after pheromone treatment. At 15-45 days after treatment, catches on plots treated with plastic flakes at 15 g a.i./ha, and with 3M formulation at 37.5 g a.i./ha and 75 g a.i./ha were lower than catches on all other plots ($F = 30.58$, d.f. = 7, $P < 0.01$; Figure 4.10, Figure 4.11). A gradual decrease of pheromone effect was observed later in the season together with the change in relative trap catch among plots treated with various doses of pheromone (Figure 4.10, Figure 4.12,

Figure **4.13**).

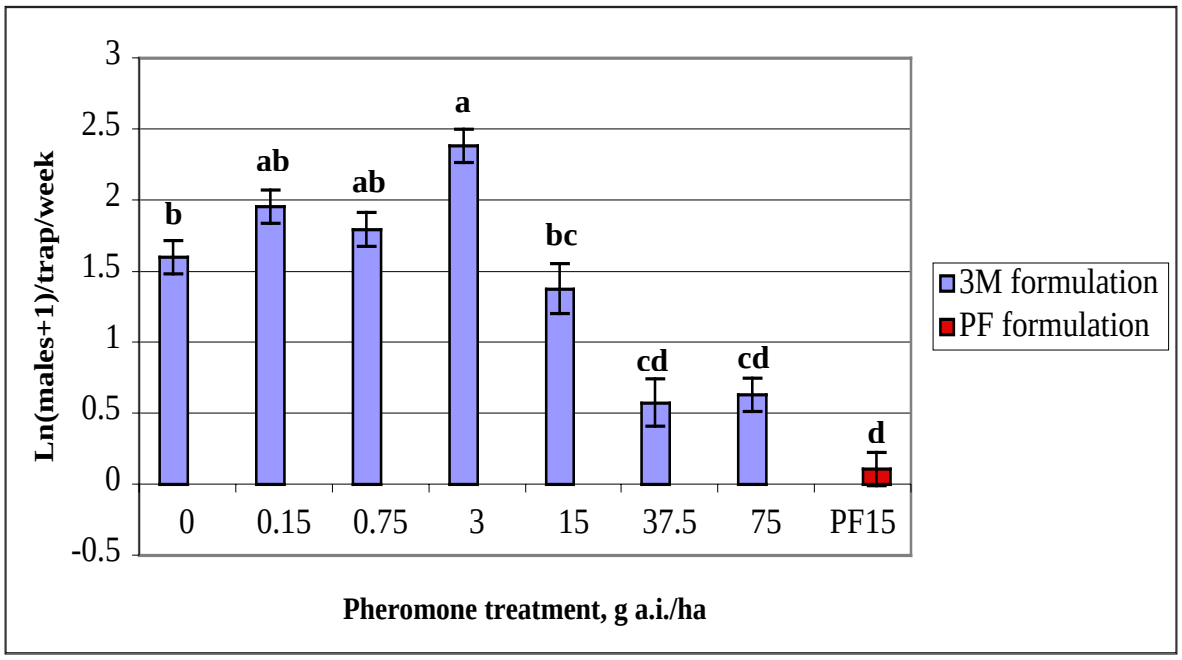

Figure 4.9: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001. Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

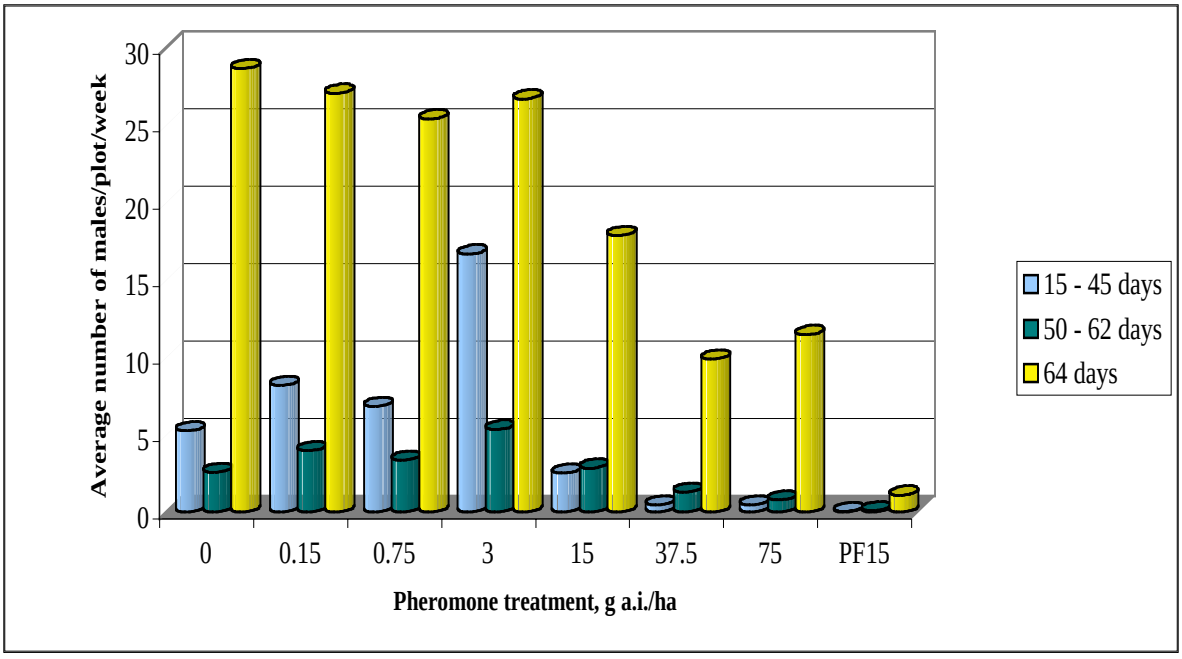

Figure 4.10: Average male moth captures in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

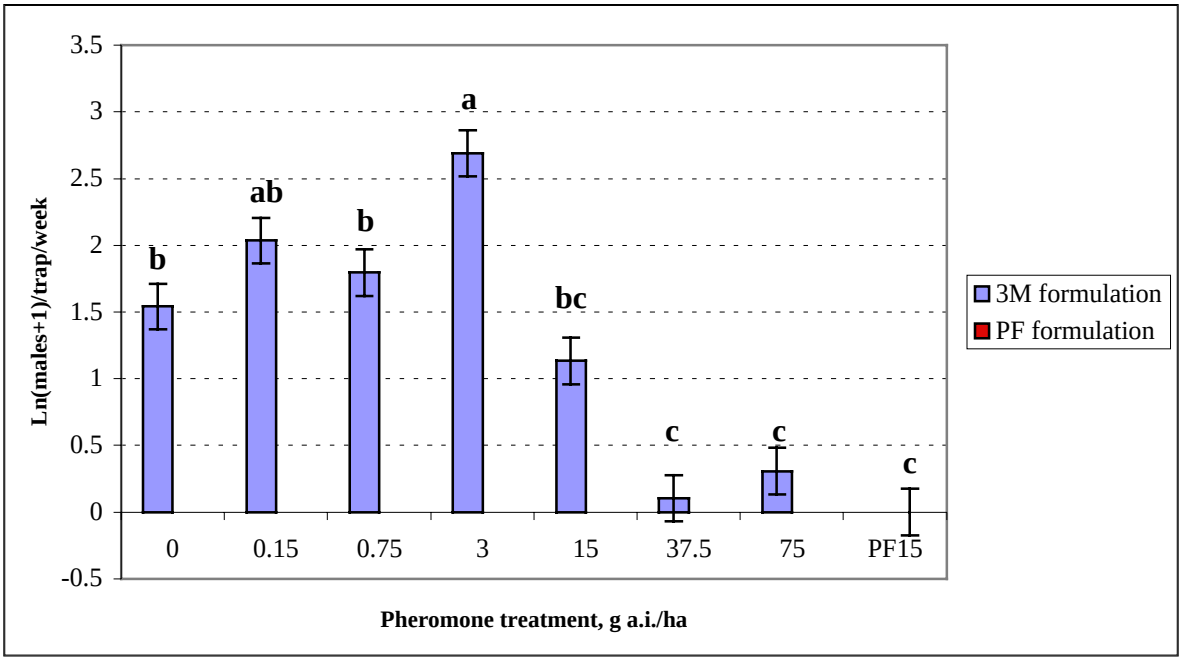

Figure 4.11: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001 (15 – 45 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$).

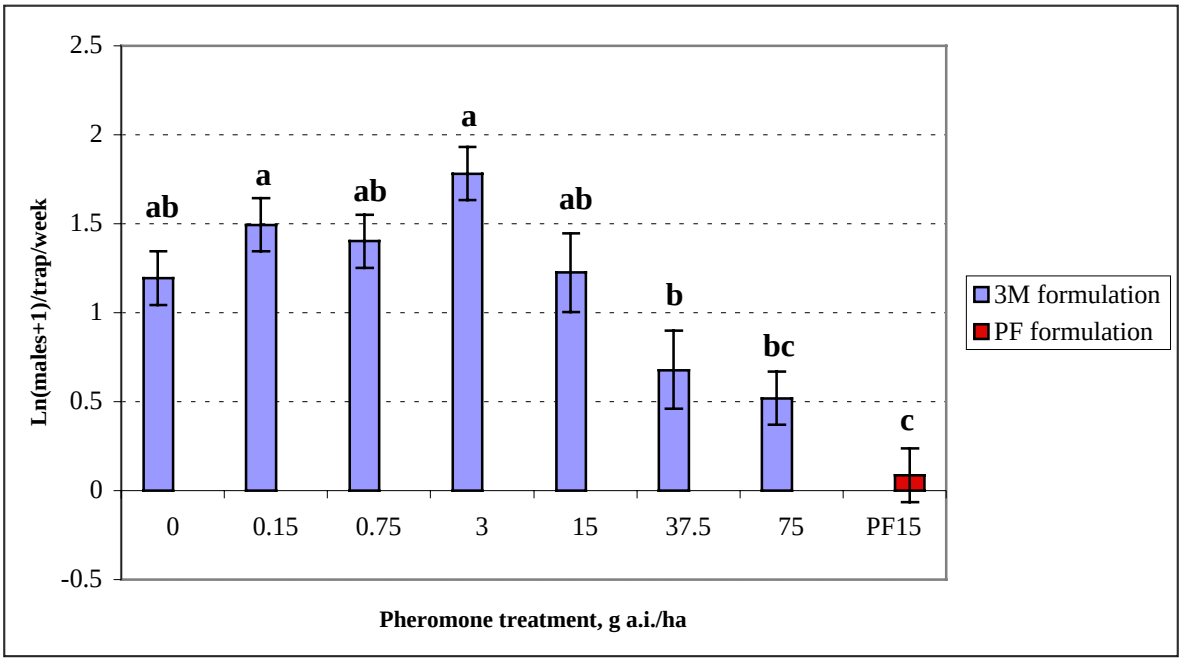

Figure 4.12: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001 (50 – 62 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

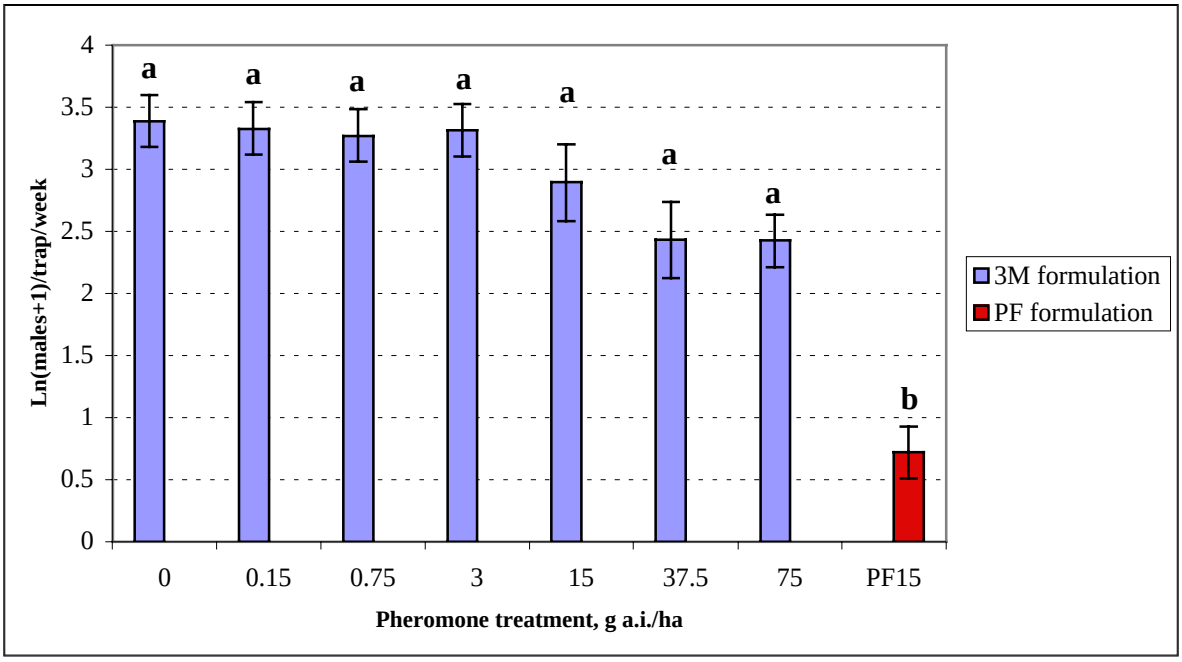

Figure 4.13: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2001 (64 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

Male catches in plots treated with plastic flakes were significantly lower than in other plots. Catches in plots treated with 3M formulation at 37.5 and 75g a.i./ha were higher than catches in plots treated with plastic flakes, but were much lower than in the plots treated with all other doses ($F = 13.85$, d.f. = 7, $P < 0.01$). On August 15, male catches in all plots treated with 3M formulation of pheromone were significantly higher than in plots treated with plastic flakes ($F = 18.19$, d.f. = 7, $P < 0.01$).

4.3.3. Dose-response Experiment: 2002

Season-long male catches (from June 25 to August 6) averaged 273.5, 91.5, 65.5, 23.5, 2.7, 2.5, 27.5, and 15.7 males per plot at the doses of disparlure (g a.i./ha) 0, 0.15, 0.75, 3, 15, 37.5 (plastic flakes), 15 and 37.5 (liquid pheromone), respectively.

Overall male moth recaptures were lower in plots treated with plastic flakes at 15 and 37.5 g a.i./ha compared with the plots treated with liquid formulation at the same doses of synthetic

pheromone, but the differences were not statistically significant (Figure 4.14). Male moth recaptures were studied over the periods of 8–14, 15–49, and 50 –56 days after pheromone application. At 8–14 days catches in the treated plots were significantly lower than in control plots ($F = 10.1$, d.f. = 7, $P < 0.001$; (Figure 4.15, Figure 4.16). Trap catches in the plots treated with plastic flakes at 0.15 and 3 g a.i./ha were higher than in plots treated with plastic flakes and liquid pheromone at 15 and 37.5 g a.i./ha, but the difference was not statistically significant.

At 15 – 49 days after the pheromone application, trap catches decreased with the increase of the dose of the applied pheromone formulated as plastic flakes ($F = 53.75$, d.f. = 7, $P < 0.001$; Figure 4.15, Figure 4.17). Trap catches in the plots treated with liquid formulation at 15 and 37.5 g a.i./ha were higher than in the plots treated with the same doses of plastic flakes.

At 50 – 56 days after treatment, trap catches in the plots treated with plastic flakes at 15 and 37.5 g a.i./ha were significantly lower than in the rest of the plots ($F = 26.18$, d.f. = 7, $P < 0.001$; Figure 4.15, Figure 4.18). Trap catches in the plots treated with liquid pheromone were not significantly different from the catches in the plots treated with low doses of plastic flakes.

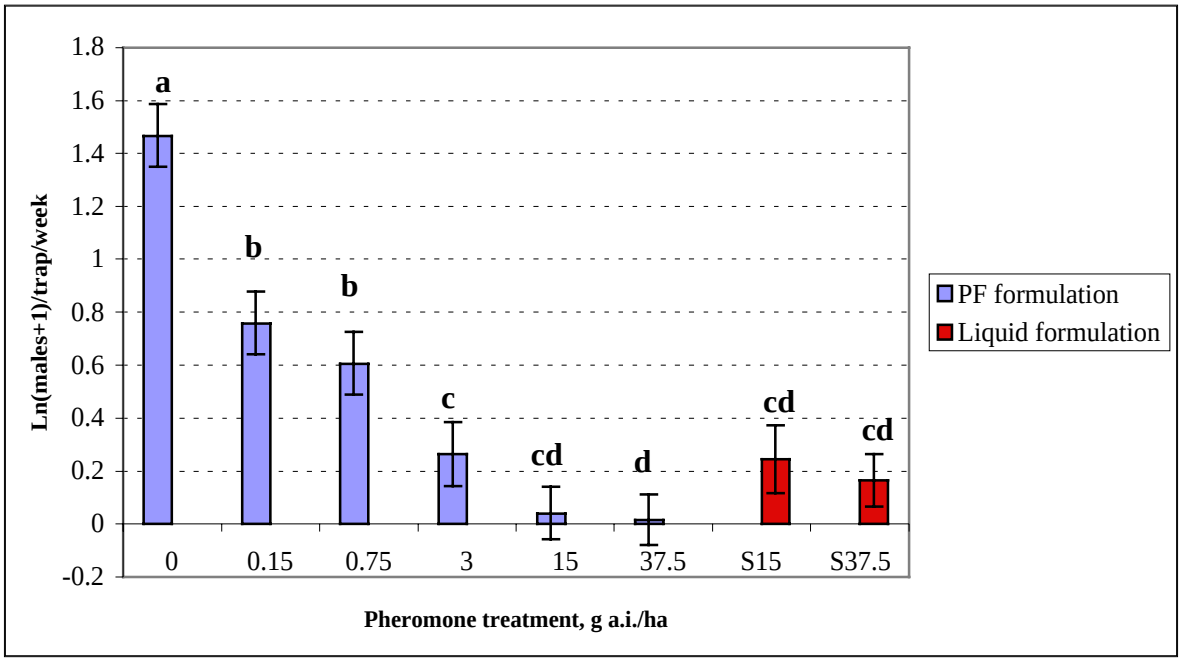

Figure 4.14: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2002. Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

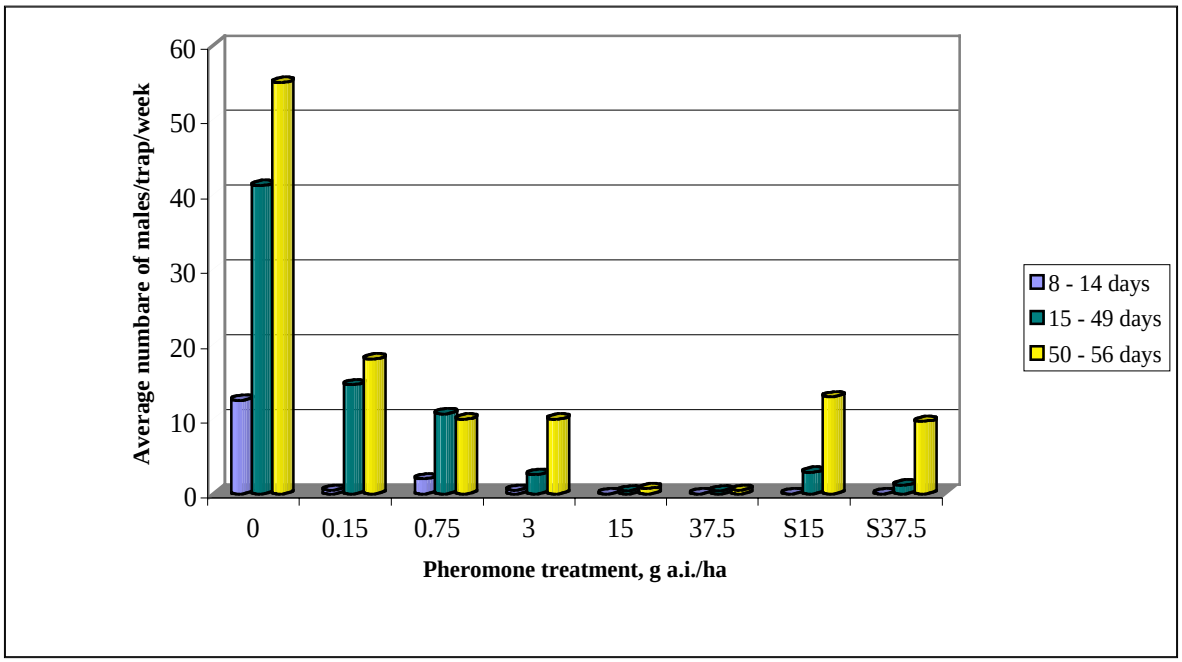

Figure 4.15: Average male moth recaptures in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2002

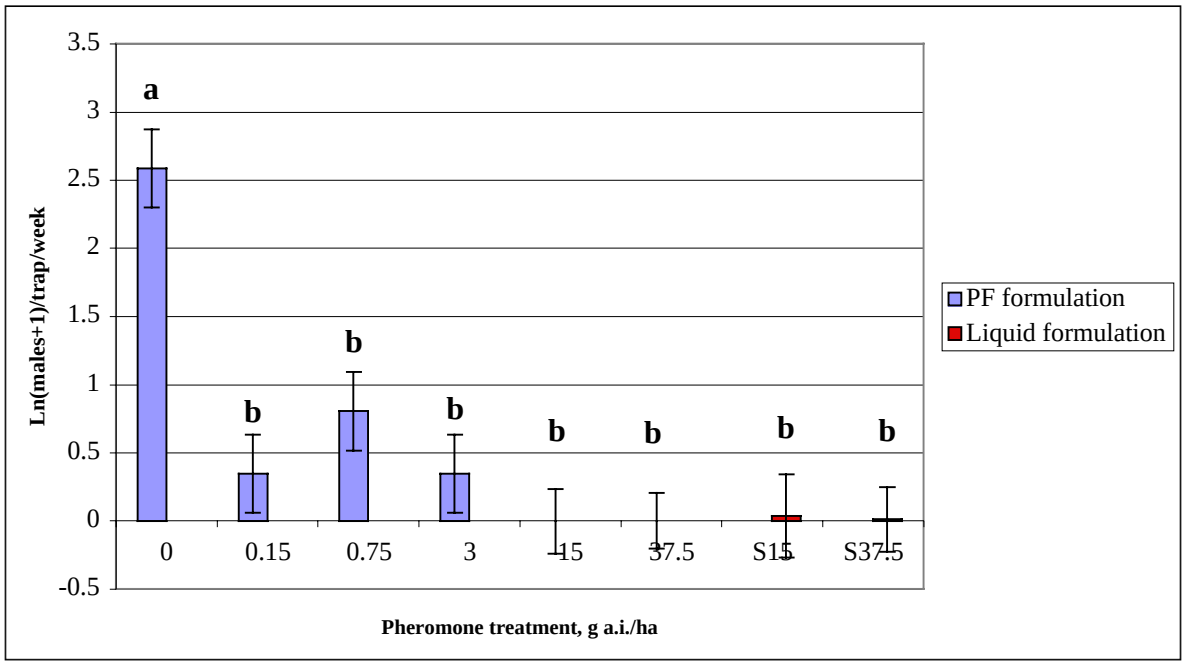

Figure 4.16: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2002 (8 – 14 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

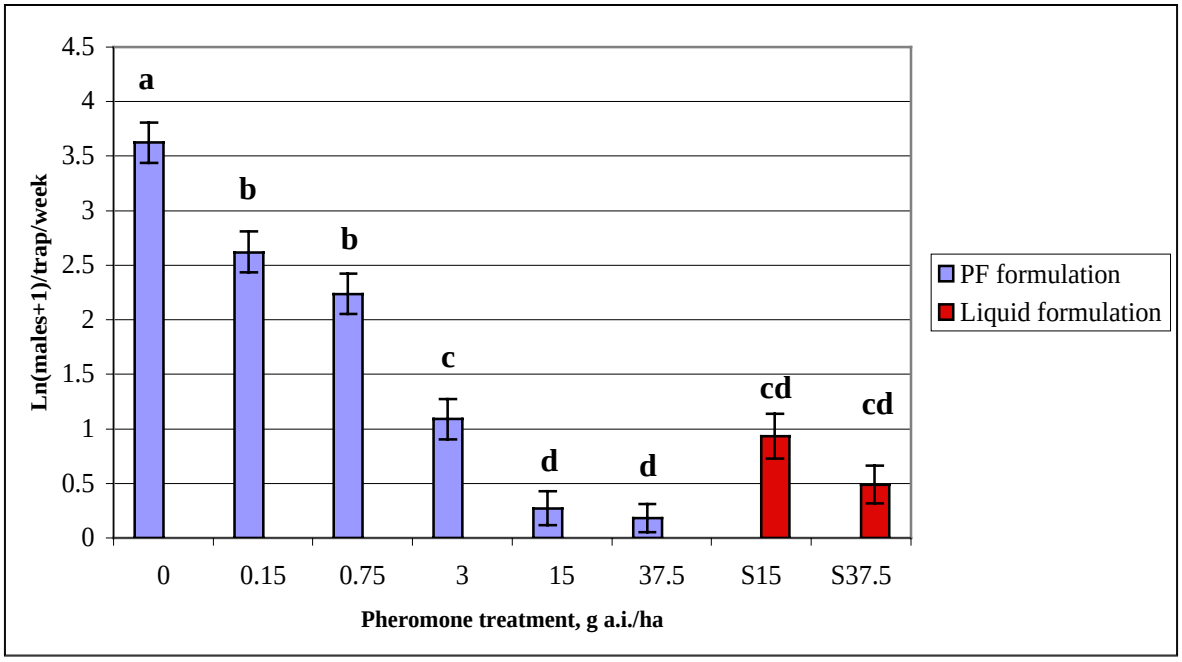

Figure 4.17: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2002 (15 – 49 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$)

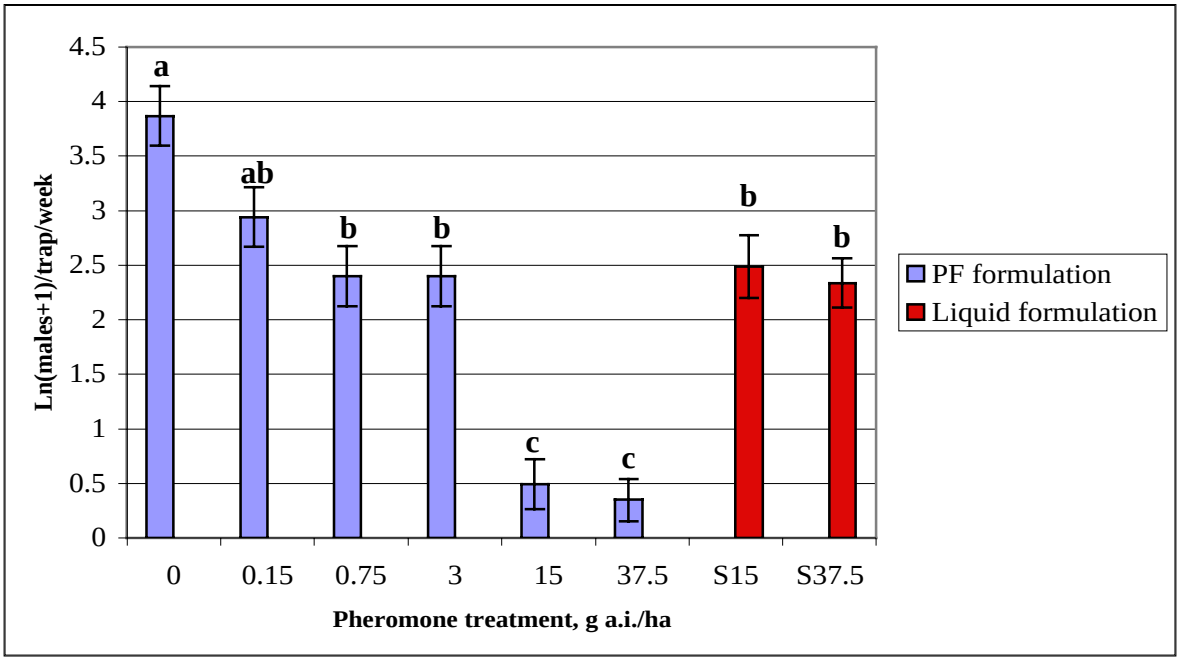

Figure 4.18: Released moths (ln(N+1) ± SD) recaptured in plots treated with various doses of pheromone in Cumberland and Appomattox-Buckingham State Forests, VA, 2002 (50 – 56 days after treatment). Bars with the same letters are not statistically significant, Tukey's HSD ($\alpha < 0.05$).

4.4. Discussion

Previous studies and operational trials showed that there is a direct dose-response relationship in the disruption of mating communication and mating success (Webb et al. 1988, Webb 1990). In the studies conducted in 2000–2002, a dose-response relationship in pheromone treatments also was observed. For example, male moth catches in traps and the proportion of fertilized females were lower in plots treated with relatively high doses of disparlure (15, 37.5 and 75 g a.i./ha) compared with plots treated with low doses of the pheromone (0, 0.15, 0.75 and 3 g a.i./ha). In addition, the effects of high doses of the pheromone on mating disruption were found to be similar. The absence of any significant effects of high doses of pheromone suggests that an increase in pheromone concentration above a certain dose (such as 15 g a.i./ha applied in this study) will result in proportionally less of an increase in mating disruption. This phenomenon also was observed by Webb et al. 1990, but for a much higher dose of pheromone (50 g a.i./ha). Differences between the results obtained in this study and that of Webb et al. (1990) could be due to differences in the number and range of doses that were tested.

Previous studies have also shown that there is a complete disruption of mating and absence of fertile egg masses in experimental plots treated with double applications of plastic flakes (Leonhardt et al. 1996). Thorpe et al. (1999) also found that mating success of females could be reduced by >97% after single aerial applications of plastic flakes at 50 and 75 g a.i./ha. In the studies conducted in 2000, single aerial applications of plastic flakes at 37.5 g a.i./ha and 75 g a.i./ha reduced mating success of females and male moth catches in pheromone-baited traps by 100 and >97%, respectively. Therefore, the pheromone dose of 37.5 g a.i./ha was shown to be as effective at decreasing mating success of females and male moth catches in traps as the dose of 75 g a.i./ha. The dose of 37.5 g a.i./ha subsequently was recommended for operational use.

In similar experiments conducted in 2001, mating success of females and male moth catches in the pheromone-baited traps were reduced significantly in the plots treated with the pheromone in plastic flakes at the dose of 15 g a.i./ha compared with control plots and with plots treated with 3M microcapsules at higher doses (37.5 and 75 g a.i./ha). In plots treated with plastic flakes at 15 g a.i./ha, mating success of females was reduced by >98% compared with the control plots, and by 92.6% and 91.2% compared with plots treated with microcapsules at 37.5 and 75 g a.i./ha, respectively. Male moth catches in pheromone-baited traps were reduced by

97.6% compared with control plots, and by 58.4% and 87.7% compared with plots treated with microcapsules at 37.5 and 75 g a.i./ha, respectively. These studies suggest that the dose of 15 g a.i./ha of disparlure in plastic flakes was as effective as the lowest dose that was tested in 2000 (37.5 g a.i./ha). The results of the study conducted in 2002 agreed with those of the experiments conducted in 2001. All of the studies confirmed that the dose of 15 g a.i./ha could be used operationally to disrupt mating.

The plastic flakes formulation has been used operationally since 1981 (Schwalbe 1981b, 1982) and was evaluated by Webb et al. (1988) and shown to provide season-long mating disruption. Numerous formulations that contained disparlure as an active ingredient were also evaluated for use in the mating disruption tactic. However, the plastic flake formulation of the pheromone has been the only commercially available product containing disparlure, which has been used for mating disruption in gypsy moth populations (Reardon et al. 1998). The present study clearly demonstrates the strong effect that the microcapsule formulation of the pheromone has on mating success of laboratory-reared females and male moth catches in the pheromone-baited traps. However, the study also showed that pheromone formulated in plastic flakes has a much stronger and longer lasting effect. The reason for this may be because the effect of disparlure formulated in microcapsules changes over the season. For example, in the beginning of the season (15–45 days after pheromone application), trap catches in plots treated with plastic flakes at 15 g a.i./ha and 3M microcapsules at 37.5 and 75 g a.i./ha were similarly low. By the end of the season (64 days after pheromone application), however, trap catches in plots treated with microcapsules at 37.5 and 75 g a.i./ha were as high as catches in the control plots and plots treated with microcapsules at lower doses. At the same time, trap catches in plots treated with plastic flakes at 15 g a.i./ha were still much lower compared with the control plots. These findings suggest that the rate of pheromone release from the microcapsule formulation was much higher than the release rate of the pheromone from plastic flakes. As a result, there was insufficient pheromone left in microcapsules to disrupt mating at the end of the flight season.

The effect of pheromone in the liquid formulation also changed over the season. For example, there was a significant reduction in trap catches in the beginning of the season (8–49 days after pheromone application) compared with trap catches in control plots. However, during the same period, male moth catches in traps in plots treated with liquid and plastic flakes formulations were similar. By the end of the season (50–56 days after pheromone application)

trap catches in plots treated with liquid formulation at 15 and 37.5 g a.i./ha were much higher than in plots treated with plastic flakes at 15 g a.i./ha. These results suggest that like pheromone in the microcapsule formulation, the release rate of pheromone in the liquid formulation may have been too rapid to allow the pheromone to persist in the air during the entire flight period of gypsy moth.

The studies suggest that, so far, the pheromone formulated in the plastic laminated flakes is the most effective of all controlled-release formulations of disparlure used for aerial applications. These results agree with the results of previous studies (Webb et al. 1988, Reardon et al. 1998). The plastic flake formulation of the gypsy moth pheromone, Disrupt® II (Hercon Environmental, Emigsville, Pennsylvania), consists of polymeric 3-layer laminated flakes that contain the pheromone, disparlure, at 3.1 mg/cm^2 (20 mg/in^2). Studies have shown that the emission rate of the pheromone from the flakes is affected by the concentration of the pheromone, the thickness of outer layers of the flakes, and by environmental factors such as temperature and air movement (Bierl et al. 1976, Caro 1982). Environmental factors, however, have been found to affect the emission rate of pheromone in plastic flakes in a predictable manner. This makes it possible to use the plastic flake formulation to provide a uniform concentration of the pheromone in the forest atmosphere throughout the flight period of the gypsy moth (Bierl et al. 1976). Similar studies need to be carried out to understand the factors that affect the dynamics of emission from other formulations of pheromone, such as in 3M microcapsules and liquid.

Finally, this study confirms that male moth catches in pheromone-baited traps could be used as the single measure of the effectiveness of pheromone treatments on gypsy moth populations. In earlier studies, the evaluation of mating disruption in treated areas was based on reduction of male trap catches. However, several studies since then showed that the frequency of female mating provided a more accurate measure of mating disruption (e.g., Plimmer et al. 1982, Schwalbe and Mastro 1988, Kolodny-Hirsch and Webb 1993). Later Sharov et al. (1995) found that the proportion of fertilized females was related closely to the moth capture rate in nearby pheromone traps and, as such, they developed a model of the relationship between the proportion of fertilized females and male moth capture in pheromone traps. The studies conducted in 2000 and 2001 support the findings of Sharov et al. (1995). These studies showed that the proportion of fertilized females was reduced much more by lower doses of pheromone than it took to reduce

trap catches. This phenomenon may be explained by the fact that a female is a weaker source of the pheromone compared with a pheromone-baited trap used for evaluation of mating disruption tests and, therefore, females are more difficult for a gypsy moth male to locate than pheromone-baited traps. As such, a significant reduction in male catches would suggest that there also would be a significant reduction in mating success of females. Therefore, male moth catches in traps alone can be used to evaluate disruption of mating communication in treated areas.

4.5. Conclusions

The dose-response relationship was obtained for the wider range of pheromone doses than was previously mentioned in the literature. In all experiments, the doses of 37.5 g and 15 g a.i./ha were shown to effectively disrupt mating and, therefore, were recommended for operational use. The effect of pheromone in the plastic flakes formulation was stronger and lasted longer than the effect of pheromone in both microcapsule and liquid formulations.

5. Mating Disruption Effect Beyond Treated Areas

5.1. Introduction

Gypsy moth females use a sex pheromone to attract males. The pheromone, disparlure, and was identified as (+) enantiomer of cys-7,8-epoxy-2-methyloctadecane (Bierl et al. 1970, Iwaki et al. 1974). Shortly after a synthetic form of the pheromone became available, attempts were made to use it to disrupt mating in gypsy moth populations (Doane and McManus 1981). The method of mating disruption is based on the idea that permeating the atmosphere with the artificial pheromone disrupts males in their search for females (Reardon et al. 1998). This method is most effective against low-density populations, which usually become established at various distances away from the population front (Schwalbe 1982, Webb et al. 1990, Sharov and Liebhold 1998). Newly established colonies are small but can grow and coalesce, thus contributing to the spread of the main population. In 1993, the USDA Forest Service initiated the Slow-the-Spread (STS) project, the goal of which was to reduce the rate of gypsy moth spread in the U.S. Slowing the spread requires the detection and suppression of isolated low-density populations (Leonard and Sharov 1995, Sharov et al. 2002).

The only product that is available commercially and used operationally for mating disruption in gypsy moth populations, is Disrupt® II (Hercon Environmental, Emigsville, Pennsylvania) (Reardon et al. 1998). Disrupt® II is formulated as polymeric 3-layer laminated flakes that contain disparlure at 3.1 mg/cm^2 (20 mg/in^2). The emission rate of the disparlure from the flakes is controlled by its concentration, the thickness of outer layers and other factors such as temperature and air movement. However, environmental parameters affect the emission rate in a predictable manner and, therefore, it is possible to provide a uniform concentration of pheromone in the forest atmosphere throughout the flight period of the gypsy moth (Bierl et al. 1976). Disrupt® II is applied aerially using special equipment (Plimmer et al. 1982). Experiments conducted to evaluate Disrupt® II showed that deposition of the flakes occurred through all layers of canopy, including the understory foliage, and only 16% of the flakes reached ground (Reardon et al. 1998). However, not much is known about the horizontal distribution of the pheromone after application. Information on the horizontal distribution of the pheromone is important because of the economic costs associated with gypsy moth control using pheromone treatments. The cost of using the tactic consists of the costs of pheromone and

application. The cost of the pheromone in turn includes the pheromone itself, its formulation, and the dose that must be applied. The cost of spraying depends on the sticker and on the area that must be covered by the aircraft. Therefore, any improvements in the methods for application of the pheromone used for mating disruption are likely to reduce the cost of the tactic.

The use of pheromone for mating disruption can be improved by reducing the amount of pheromone that is sprayed and by reducing the flight distance of the aircraft. In the past, pheromone was distributed evenly in non-overlapping swaths in the same manner as pesticides. In 2000, mating disruption in the areas just outside treated plots was discovered accidentally during our dose-response experiments. The question this discovery raised was how far from treated plots does the mating disruption work? If mating can be disrupted at some distance away from sprayed area, it might be possible to apply pheromone effectively by using wide-swath aerial treatments and leaving larger gaps between sprayed swaths. This method of application would decrease the cost of the mating disruption considerably. Therefore, the objective of this experiment was to determine whether the effect that was observed for the gypsy moth sex pheromone outside treated area could be used in mating disruption treatments.

5.2. Materials and Methods

Experiments were conducted during summers of 2000 and 2001. In 2000, experimental plots were selected near Millboro Springs (Bath Co., VA), and in 2001, plots were located in Appomattox-Buckingham (Buckingham and Appomattox Co., VA) and Cumberland (Cumberland Co., VA) State Forests (Figure 5.1). Plots were treated aerially with racemic disparlure from a fixed-wing aircraft. Two formulations of synthetic disparlure, Disrupt® II (Hercon laminated plastic flakes) and 3M (experimental microcapsules), were tested at doses of 37.5 and 75 g a.i./ha. USDA pheromone-baited milk carton traps were used to test the effect of sprayed disparlure on male moths capture at various distances from treated plots.

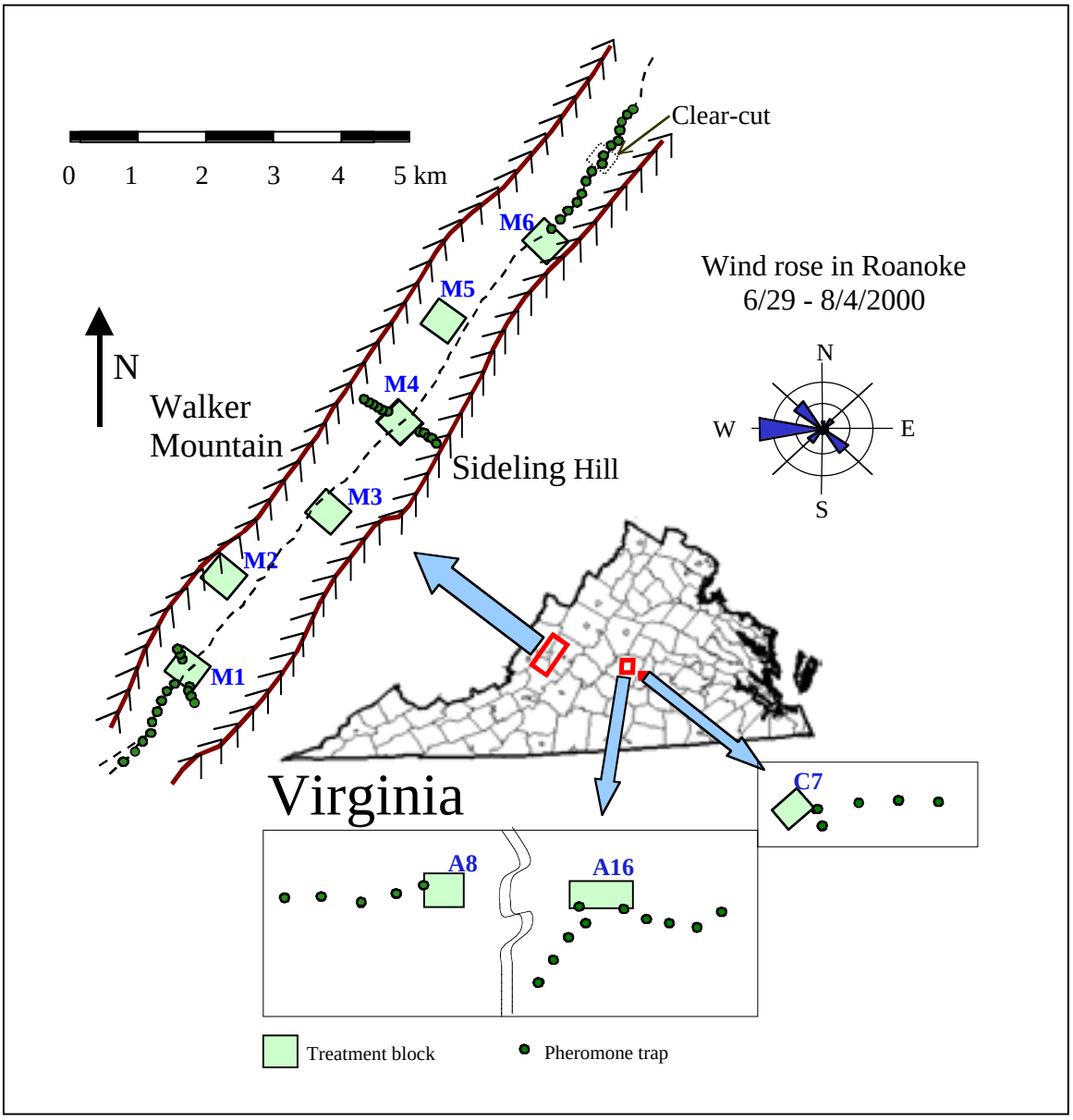

Figure 5.1: **Map of experimental plots and pheromone traps in VA, 2000-2001**

5.2.1. Mating Disruption Beyond Treated Area: 2000

An experiment was conducted in the Appalachian Mountains, near Millboro Springs, Virginia to determine the distant effect of pheromone as measured by the mating success of females and male moth capture in pheromone traps. Six 25-ha square plots separated by a distance of 1 km were located along a road between the ridges of Walker Mountain and Sideling

Hill. Two of the plots (M1 and M6) were treated with pheromone flakes at 37.5 g a.i./ha, two others (M2 and M4) were treated with pheromone flakes at 75 g a.i./ha, and the remaining two (M3 and M5) were treated with 3M microcapsules at 75 g a.i./ha. Pheromone traps were placed at 200 m apart along the road to the southwest of plot M1 and also northeast of plot M6. Transects of traps also were established on both sides of plots M1 and M4 between mountain ridges. Rows of 10 tethered females were also placed between pheromone traps in transects between plots (Figure 5.2, Figure 5.3). Females were placed on tree trunks and a band of tanglefoot glue was used to protect them from insect predators. The distance between traps in transects was 200 meters, the distance between traps and tethered females in the row was 100 m, and the distance between females in a row was 15–20 m.

Statistical Analysis. The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to test for differences in proportion of fertilized females at various distances from the treated area. The proportion of fertilized females was modeled as a function of distance class from the treated area. The 10 distance classes used in the analysis were: –100 m (inside the plot, 100 m from the boundary), 0 m (at the boundary), 100 m, 300 m, 500 m, 700 m, 900 m, 1100 m, and 1300 m.

The relationship between moth counts in traps (N) and distance from the nearest treated plot (x) was modeled as

$$\ln(N+1) = a \cdot [1 - \exp(-3x/b)] \qquad \textbf{(5.1)}$$

where a is the maximum of log moth count and parameter b is the range of disparlure effect. Both a and b were determined using non-linear regression (least square method). Traps located within the treated area ($x < 0$) were not used in the non-linear regression. The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values(SAS 1996, Proc GLM) was used to test the difference in moth counts between groups of traps located at various distances from area treated. Log-transformed total moth counts in a trap for the entire flight period, $\ln(N+1)$, were modeled as a function of distance class from the treated area and line of traps without interactions of factors. Six distance classes were used: <50 m, 50–150 m, 150–250 m, 250–600 m, 600–1200 m, and >1200 m from the boundary of area treated.

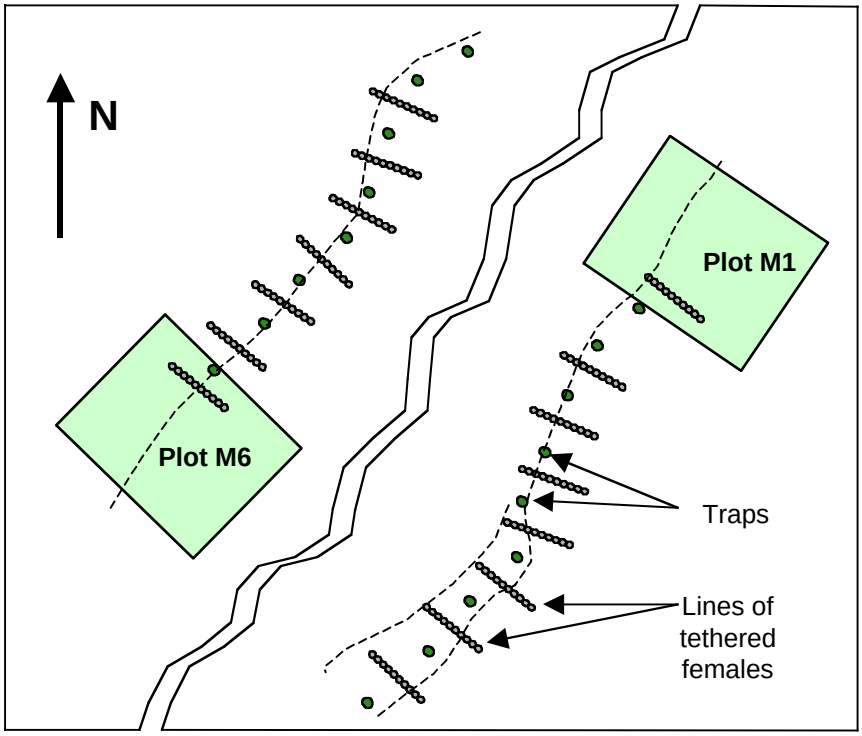

Figure 5.2: Layout of pheromone traps and tethered females in Millboro Springs, VA, 2000

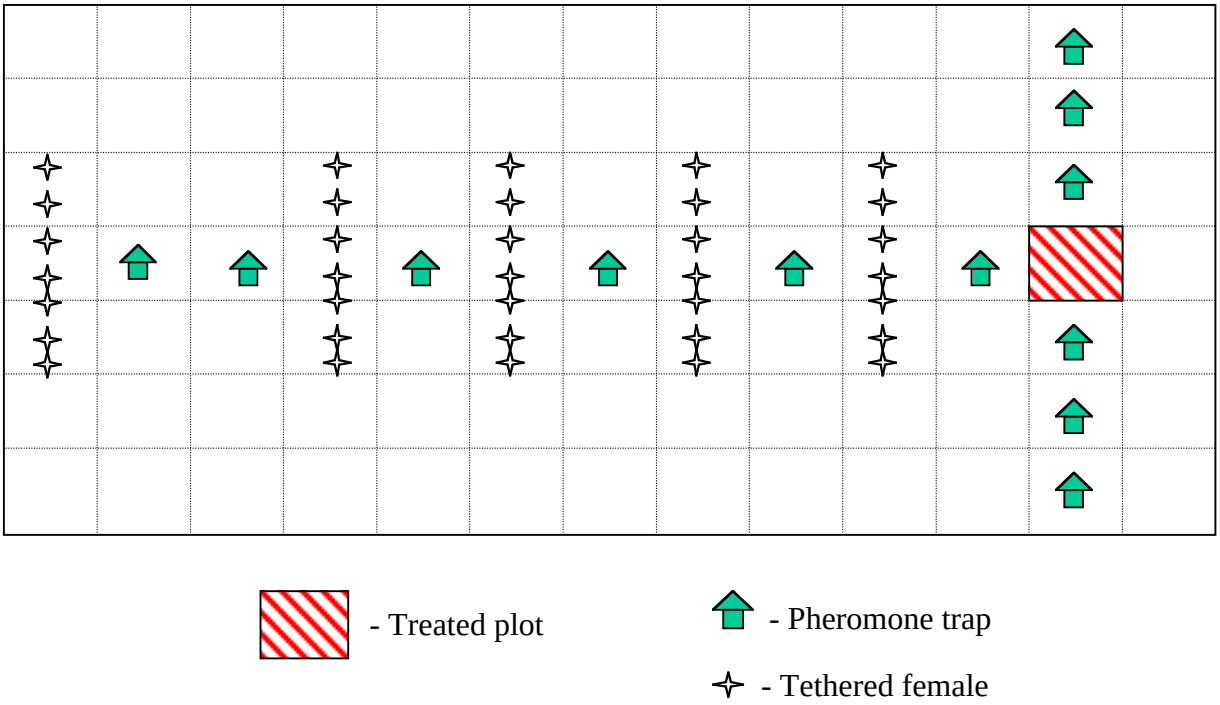

Figure 5.3: Layout of the pheromone traps and females in Millboro Springs, VA, 2000

5.2.2. Mating Disruption Beyond Treated Area: 2001

Experiments were conducted in Buckingham-Appomattox SF, Virginia, to determine the effect of distance on efficacy of pheromone measured by male moth capture in pheromone traps. Four lines of release-recapture sites were used to evaluate the effect of distance on the efficacy of the applied pheromone. Two lines were set up near plot A16 and one line near each of plots A8 and C7 (Figure 5.1). Each line consisted of five release-recapture sites, which had a release point surrounded by four pheromone-baited traps placed at 25 m from the release point. The release-recapture sites were placed at distances of 0, 100, 200, 500 and 1000 m from treated plots A16 and A8, and at distances of 100, 400, 500, and 1000 m from the treated plot C7.

One 50 ha-plot (1 by 0.5 km) was treated on June 11, 2001 with racemic disparlure at a dose of 37.5 g a.i./ha. The plot was divided into 30 m wide strips with every fourth strip sprayed twice and three stripes left unsprayed (

Figure 5.4). Thus, a 90 m gap was left between each pair of sprayed stripes. Pheromone was applied from a fixed-wing airplane equipped with a differential global positioning system (DGPS). A transect of 32 gypsy moth male release points located 30 m apart from each other was placed perpendicular to the aircraft flight path across the plot. Two lines of pheromone-baited traps were located 30 m to the east and west parallel to the release points transect. Traps were checked and new male moths were released weekly in July and twice a week in August.

Male gypsy moths were shipped as pupae from USDA APHIS Otis Methods Development Center, Massachusetts. Pupae were kept in laminated paper cups with plastic lids and then transferred to the release cups in the field. The release cups were the same types of cups used for the rearing stapled to the trunks of trees. Several openings were cut at mid-height of the release cups for emerging males to get out. Tanglefoot (The Tanglefoot Co., Grand Rapids, Michigan) glue was applied in circles around the tree trunk. Fluorescent powder dye was added to cups to mark emerging male moths. The same number of males was released at each release point each week (~100). Male moths were removed from the pheromone traps and stored in the freezer. Later they were examined under the microscope with UV light to distinguish between released and natural moth by looking at the presence of fluorescent powder on wings, antennae or body.

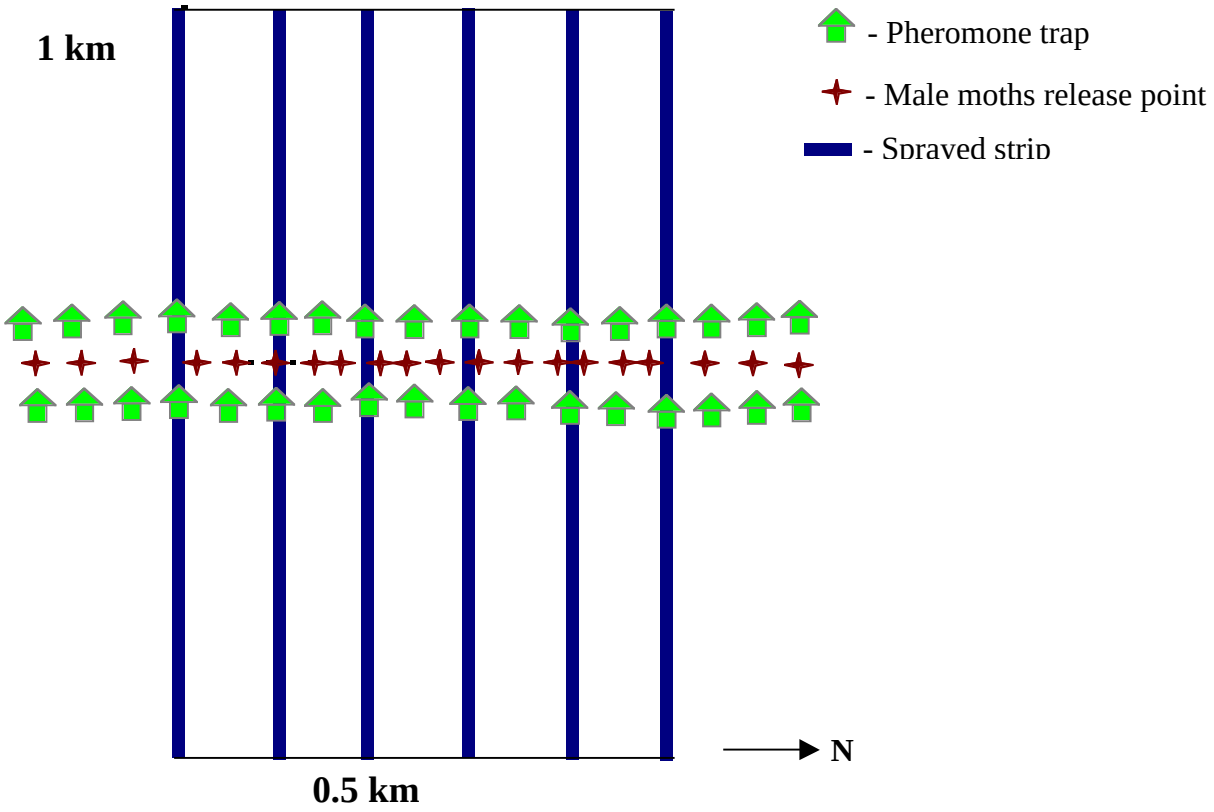

Figure 5.4: Layout of the plot for "Skipped Swaths" experiment in Appomattox-Buckingham State Forest, VA, 2001

Statistical Analysis. The relationship between moth counts in traps (N) and distance from the nearest treated plot (x) was modeled by equation 5.2. Parameters a and b were estimated using non-linear regression (least squares method). Traps located in the treated area ($x < 0$) were not used in the non-linear regression. The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to test the difference in moth counts between groups of traps located at various distances from treated area. Log-transformed total moth counts in a trap for each week, $\ln(N+1)$, were modeled as a function of distance class from the treated area, line of traps, and time without interactions of factors. The distance classes were: <50 m, 50–150 m, 150–250 m, 250–600 m, 600–1200 m, and >1200 m from the boundary of the treated area. The analogous procedure (SAS 1996, Proc GLM) was also used to analyze male moth counts in pheromone traps placed across the plot treated with

skipped-swaths. Log-transformed total moth counts in a trap for each week, ln(N+1), were modeled as a function of a trap number, line of traps, and time without interactions of factors.

5.3. Results

5.3.1. Mating Disruption Beyond Treated Area: 2000

The proportion of fertilized females increased with the distance from treated areas (Figure 5.5). Mating success of females was significantly reduced at distances up to 700 m from the boundary of the treated plot compared with larger distances (F = 22.8, P < 0.001).

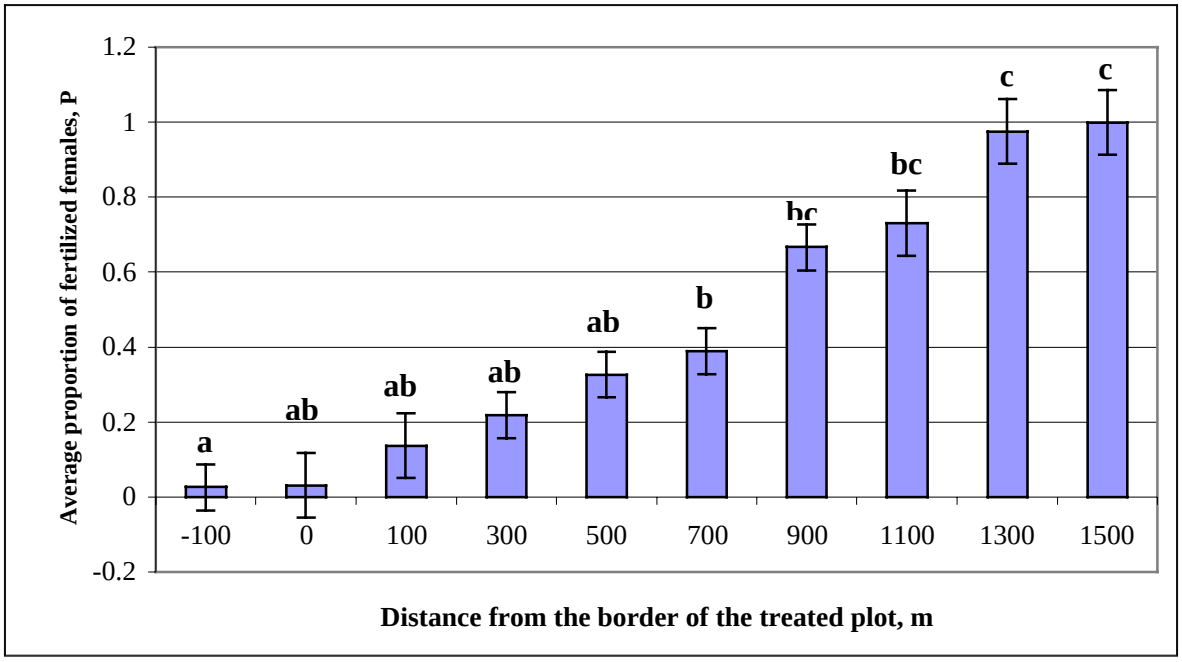

Figure 5.5: Mating success of gypsy moth females (P ± SD) at various distances from the treated area in Millboro Springs, VA, 2000. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$)

Male moth catches in traps placed along the valley were much lower at distances of <600 m from the treated area compared with traps at larger distances (Figure 5.6). Traps placed across the valley caught fewer males at <150 m from the treated area than at distances larger than 150 m (Figure 5.7). The increase in male moth catches in pheromone traps with distance from the treated area was shown to be significant for lines of traps placed both along (R^2 = 0.70, F = 45, P < 0.001; Figure 5.8) and across (R^2 = 0.61, F = 26, P < 0.001; Figure 5.9) the valley. The low moth counts in two of the traps in

Figure 5.8 were from those placed in a clear-cut; these two points were not used in the statistical analysis. From the equation 5.2, the range of disparlure effect was b = 1899 ± 189 m along the valley and b = 312 ± 66 m across the valley. Male moth catches decreased significantly at distances of 633 ± 63 m along the valley and 104 ± 22 m across the valley. The GLM analysis confirmed the significance in the reduction of moth catches in traps placed along the valley ($F = 9.38$, $P < 0.001$) and across the valley ($F = 25.41$, $P < 0.001$) (Table 5.1). In lines of traps placed along the valley, mean moth counts at 250-600 m from treated plots were significantly lower ($P < 0.05$) than at distances >1200 m (Figure 5.10). In lines of traps across the valley, the mean moth counts at 50–150 m from treated plots was significantly lower ($P < 0.05$) than at distances >150 m (Figure 5.11).

Table 5.1: General Linear Model (GLM) analysis of log-transformed male moths counts in pheromone traps placed at various distances from the treated area in Millboro Springs, VA, 2000

Location of traps	Source	DF	Sum of Squares	Adjusted Mean Squares	F	P
Along the valley	Transect	1	3.51	0.13	0.1	0.76
	Distance	4	47.28	11.82	9.38	< 0.0001
	Error	20	25.20	1.26		
	Total	25	76.00			
Across the valley	Transect	1	0.48	1	1.35	0.26
	Distance	3	54.07	18.02	25.41	< 0.0001
	Error	16	11.35	0.71		
	Total	20	65.90			

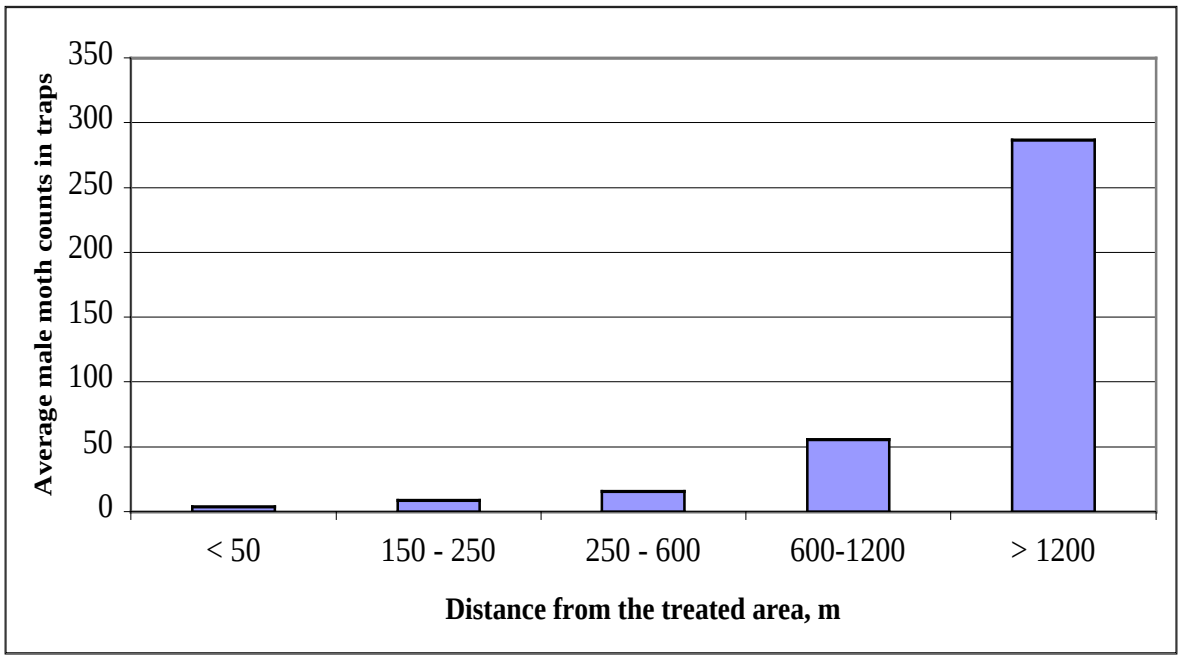

Figure 5.6: Average male moth counts in traps placed along the valley at various distances from the treated area in Millboro Springs, VA, 2000

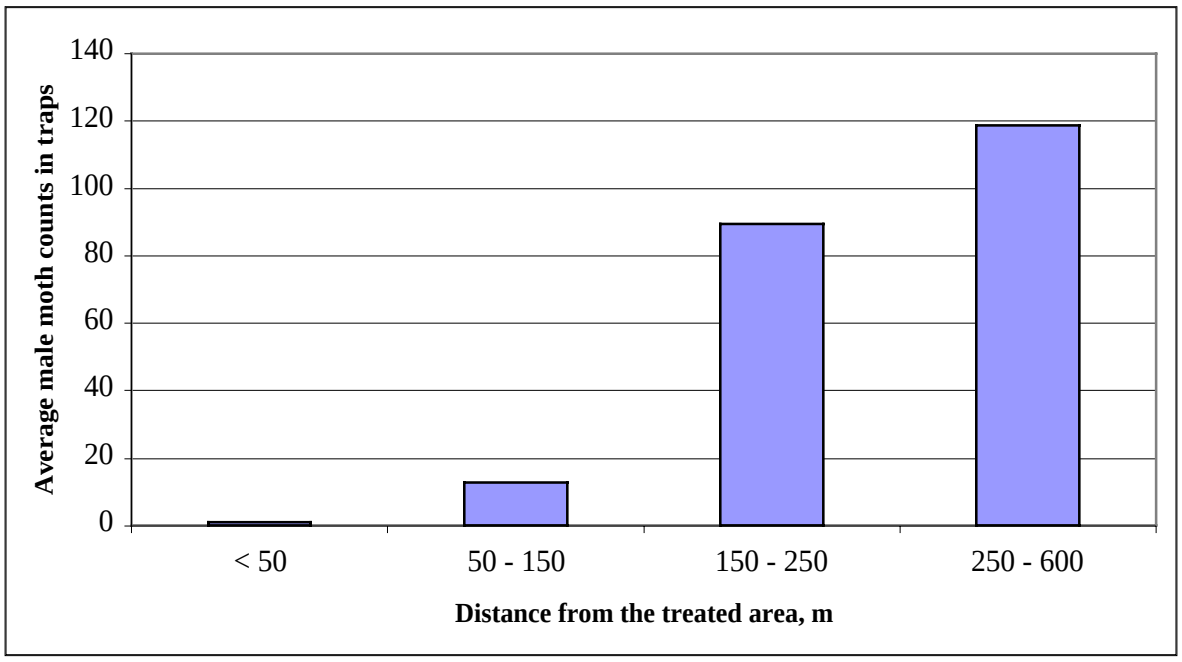

Figure 5.7: Average male moth counts in traps placed across the valley at various distances from the treated area in Millboro Springs, VA, 2000

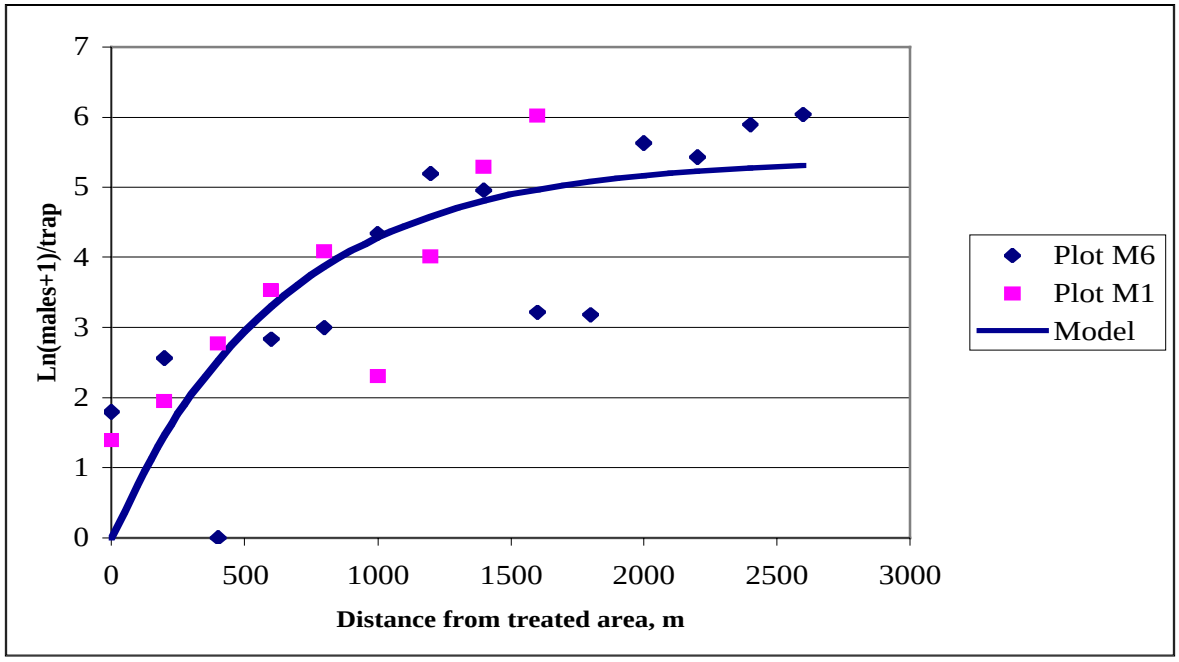

Figure 5.8: Log male moth counts in traps placed along the valley at various distances from the treated area in Millboro Springs, VA, 2000

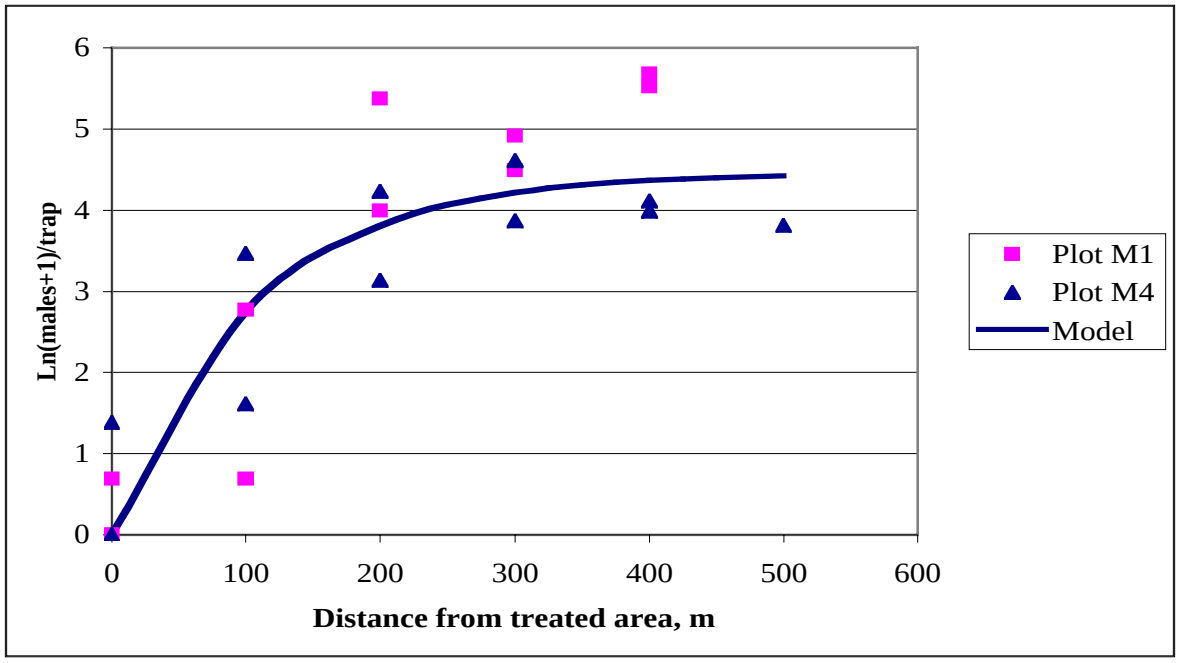

Figure 5.9: Log male moth counts in traps placed across the valley at various distances from the treated area in Millboro Springs, VA, 2000

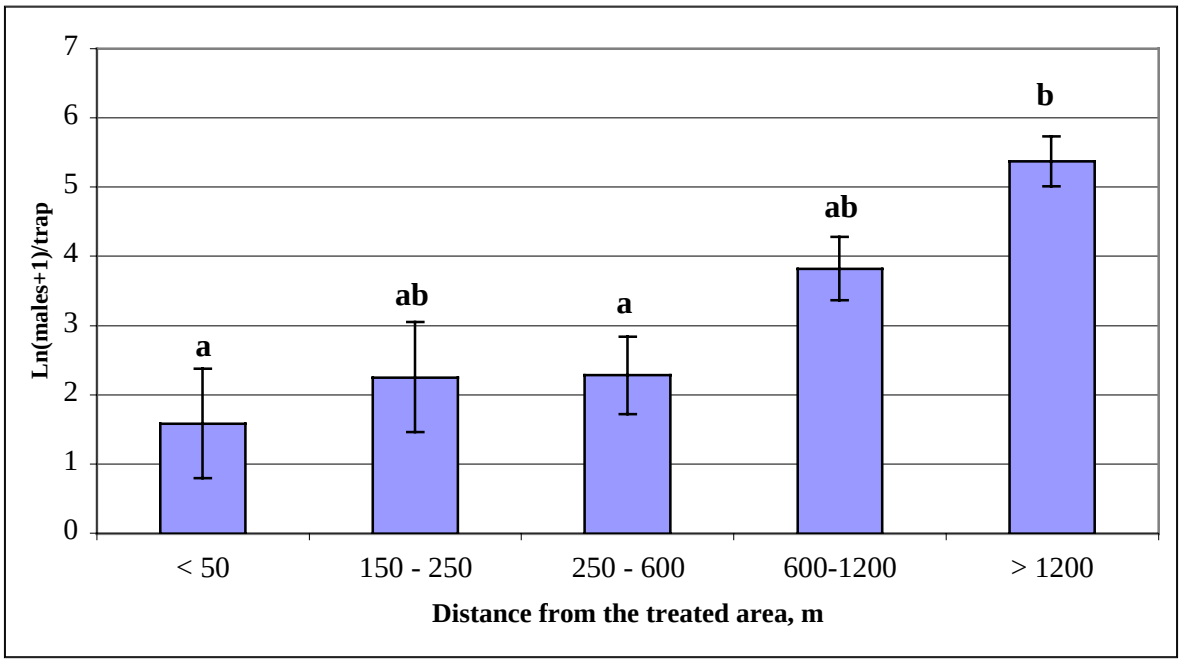

Figure 5.10: Log male moth counts in traps (ln(N+1) ± SD) placed along the valley at various distances from the treated area in Millboro Springs, VA, 2000. Bars with the same letters are not significantly different, Tukey's HSD (α < 0.005)

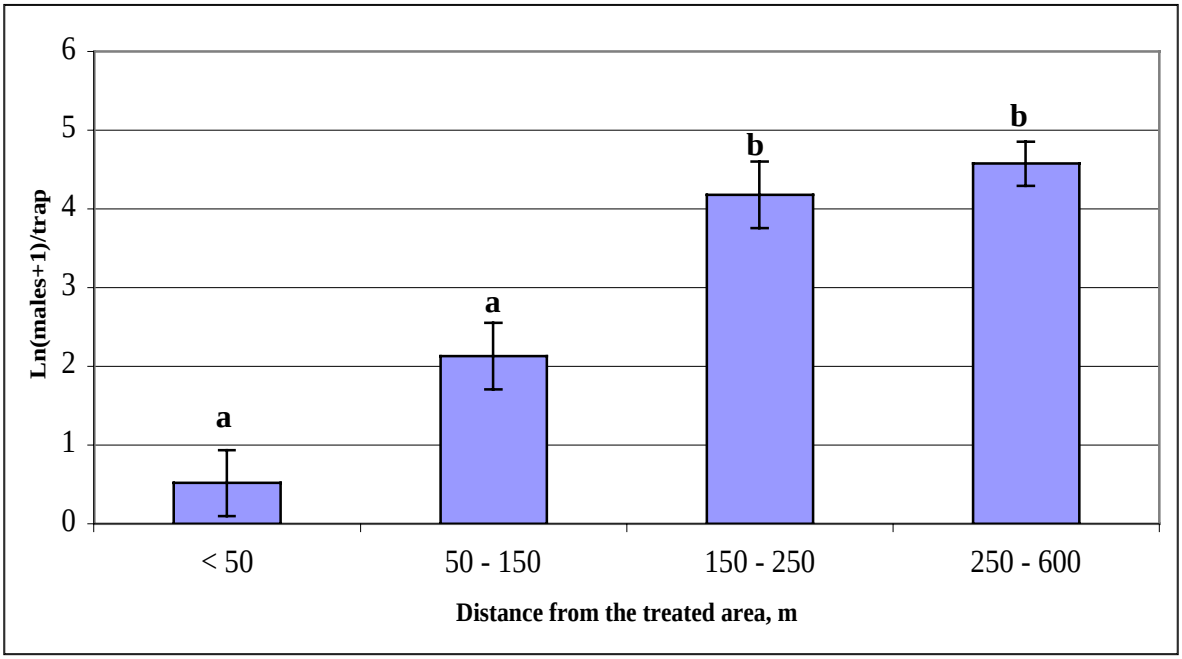

Figure 5.11: Log male moth counts in traps (ln(N+1) ± SD) placed across the valley at various distances from the treated area in Millboro Springs, VA, 2000. Bars with the same letters are not significantly different, Tukey's HSD (α < 0.005)

5.3.2. Mating Disruption Beyond Treated Area: 2001

The rate of male moths recapture in pheromone-baited traps increased with the distance from treated plots (Figure 5.12). The relationship between rate of male catches in traps and the distance from the treated area was statistically significant (R^2 = 0.38, F = 23.6, P < 0.001). According to the equation 5.2 the range of disparlure effect was b = 200 ± 51 m, however a strong effect of disparlure was observed at the distance of 67±17 m (Figure 5.13). The GLM analysis also confirmed the significance of the effect of pheromone outside treated areas on the male moth catches in pheromone traps (F = 36.4, P < 0.001; Table 5.2, Figure 5.14). The moth recapture rate was significantly lower at distances between 0-150 m from the treated plot than at distance >250 m.

Table 5.2 General Linear Model (GLM) analysis of log-transformed male moths counts in pheromone traps placed at various distances from the treated area in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

Source	DF	Sum of Squares	Adjusted Mean Squares	F	P
Week	7	29.43	4.17	7.59	< 0.0001
Transect	3	13.68	4.43	8.06	< 0.0001
Distance	3	59.96	19.97	36.39	< 0.0001
Error	104	57.11	0.55		
Total	117	160.19			

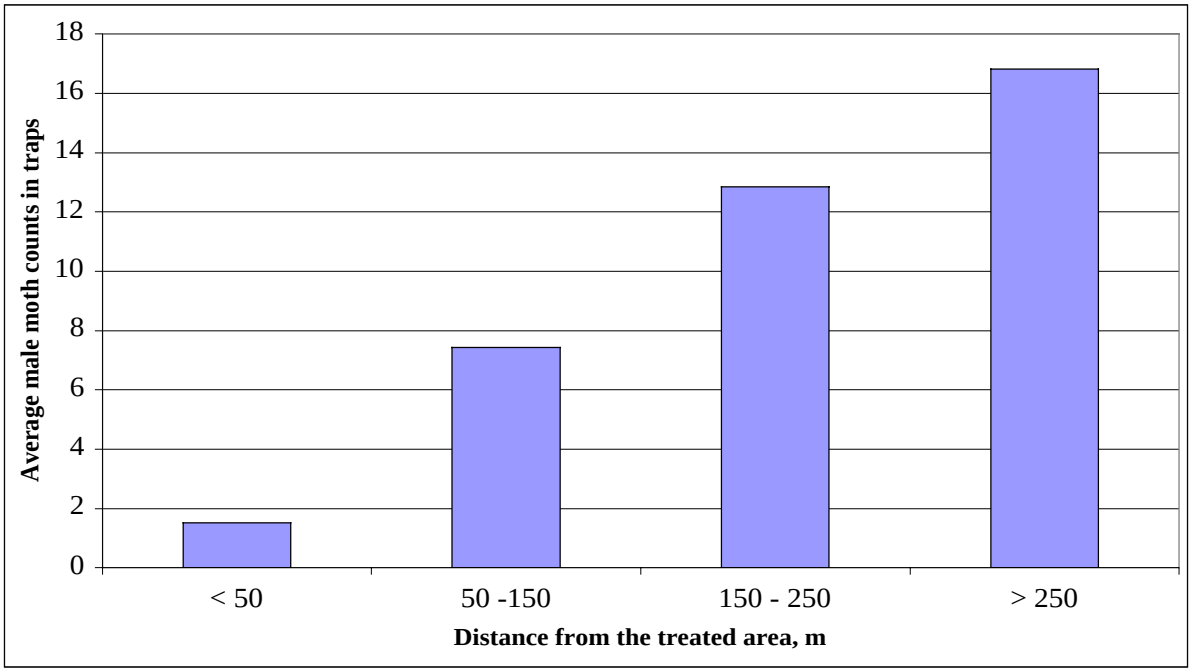

Figure 5.12: Average male moth counts in traps placed at various distances from the treated area in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

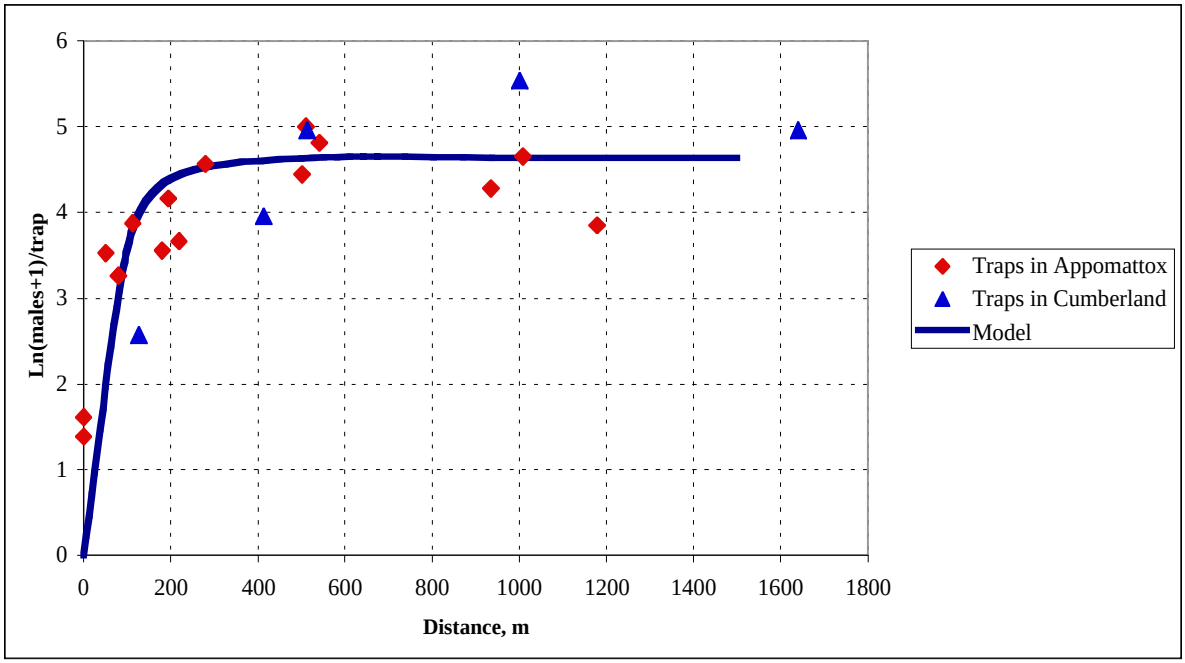

Figure 5.13: Log counts of male moths recaptured in traps at various distances from the treated area in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

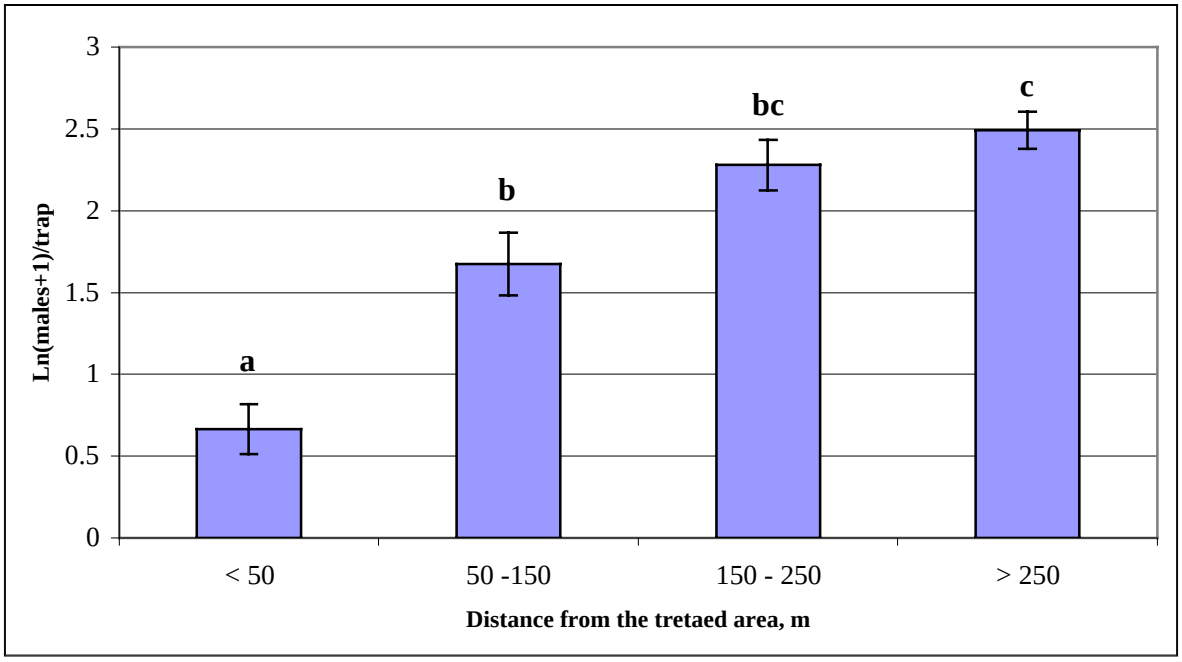

Figure 5.14: Log counts of male moths (ln(N+1) ± SD) recaptured in traps at various distances from the treated area in Cumberland and Appomattox-Buckingham State Forests, VA, 2001. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$)

Traps 1 to 11 were located in a non-treated area in the skipped-swaths plot, where wide non-treated swaths alternated with treated stripes. Traps 12, 13, 16, 17, 20, 21, 25, and 29 were located in sprayed stripes (Figure 5.15). The GLM analysis indicated that the differences between trap catches were significant (Table 5.3). Higher catches in traps 12, 16 and 20 can be explained by the fact that these traps were located on the border of the treated and untreated swaths. Traps 14, 15, 19, 23 and 24 were located in non-treated swaths and the catches in these traps were lower than catches in traps located outside treated area but the difference was not statistically significant. Traps 22, 26, 27, 28, and 30 were also located in the non-treated swaths; however, male moth counts in these traps were significantly lower than counts in the traps placed outside the plot. Catches in traps located inside the treated swaths (13, 17, 21, 25 and 29) were lower than in most of the other traps (Figure 5.15). However, the male moth catches were reduced in all traps to the north of trap #12, which was located in the first sprayed stripe.

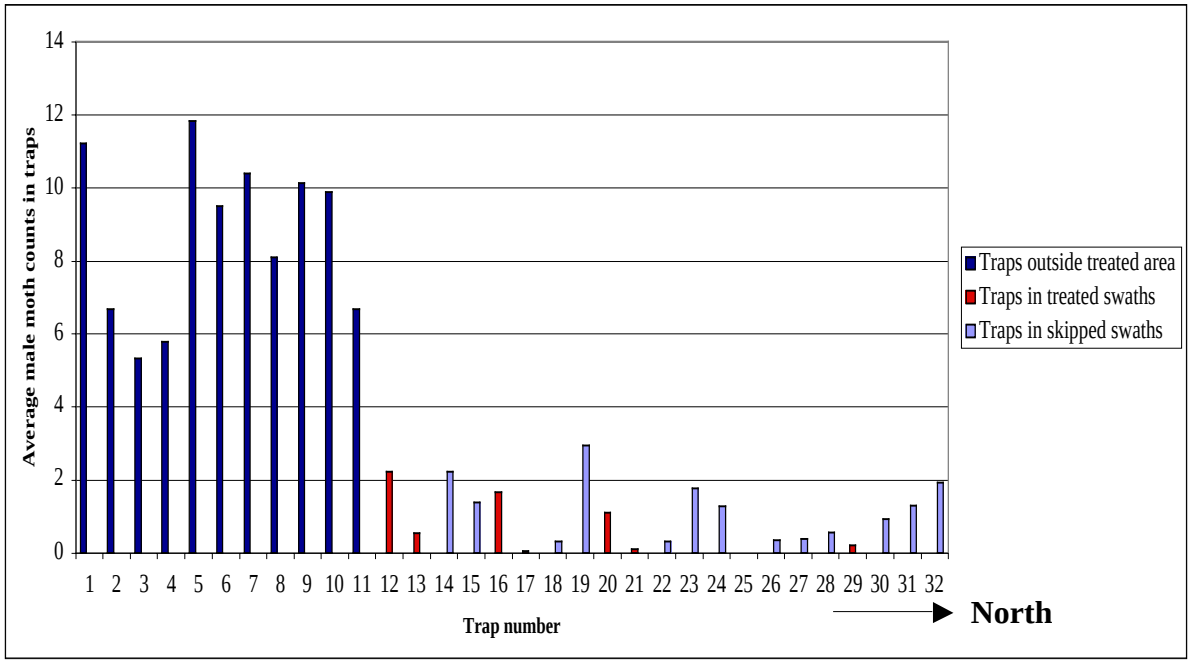

Figure 5.15: Average male moth counts in the plot treated using wide swaths in Appomattox-Buckingham State Forest, VA, 2001

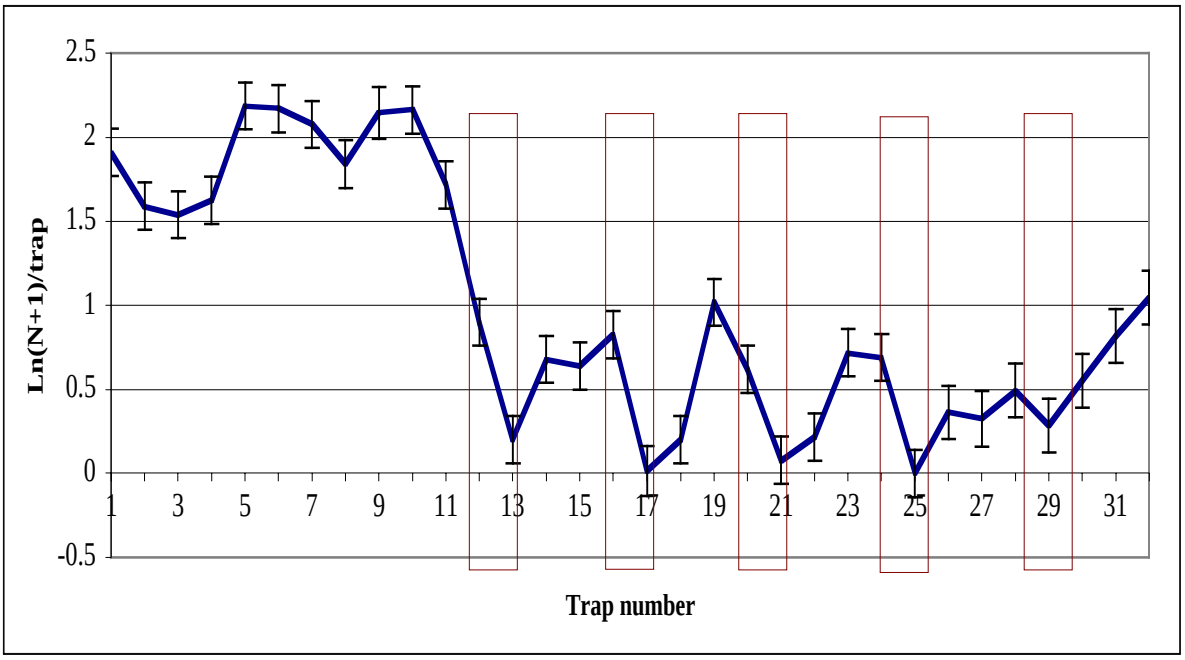

Figure 5.16: Log male moth counts(ln(N+1) ± SD) in traps in the plot treated using wide swaths in Appomattox-Buckingham State Forest, VA, 2001. Red bars represent sprayed strips. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.005$).

Table 5.3: General Linear Model (GLM) analysis of log-transformed male moths counts in pheromone traps placed across the plot treated using wide swaths in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

Source	DF	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Trap	31	296.14	9.55	20.94	<0.0001
East/West	1	4.57	4.57	10.0.2	0.0016
Week	1	3.76	3.76	8.24	0.0043
Error	508	231.74	0.46		
Total	541	539.6			

5.4. Discussion

This study was conducted in forested areas in Virginia to examine the dispersion and spatial effects of dispalure on gypsy moth populations. Mating success of females and male moth catches in pheromone-baited traps are used routinely to measure the effects of pheromone treatments in gypsy moth populations (Reardon et al. 1998). The study showed that both of these indicators of the level of mating communication were lower near plots treated with the pheromone than at larger distances away from the treated areas. The study, therefore, clearly indicated that disparlure disperses from treated areas and can disrupt mating communication beyond these areas.

The structure of the landscapes at the study sites where the pheromone was applied and its effects on wind patterns at these sites appear to have affected the dispersion of the pheromone and the level of its effect on gypsy moth populations beyond areas that were treated. The study sites in Millboro Springs, for example, were located in a valley between Walker Mountain and Sideling Hill. Sites in Cumberland and Appomattox-Buckingham State Forests were located in areas that are relatively flat (Figure 5.1). These differences in the topographic relief among the study areas seem to have some effect on the extents at which the pheromone was effective. In general, the studies showed that in relatively flat and open areas the effect of the pheromone on male moth catches in pheromone-baited traps was observed at relatively short distances (104 ± 22m across the valley in Millboro Springs and 67 ± 17m at sites in Cumberland and Appomattox-Buckingham state forests). Also, male moth catches were found to be lower at these sites at distances <150 m than at larger distances. In contrast, the effects of the pheromone on male moth catches were observed at larger distances (633 ± 63 m) along the valley in Millboro Springs.

One explanation for the differences in the effect of the pheromone among the study sites is based on the wind and airflow patterns that result from differences in topographic relief among study sites. Wind patterns are influenced by the landscape, and, therefore, the distance at which disparlure disperses also is affected by characteristics of the landscape. Large wind eddies are common in mountainous topography, such as in Millboro Springs (Kimmins 1987). Specific wind systems resulting from differential heating are produced in valleys. As such, cross-valley wind speeds at lower levels tend to be relatively low, but increase at higher elevations. Cross-valley wind speeds also tend to be maximal on the leeward side near the ridge or above the ridge

(Tang and Peng 1977). The narrowing of the valley in mountainous terrain creates a venture (tunneling) effect that increases turbulence and accelerates wind speeds along the valley. At the same time, wind speeds across the valley remain low (Tang and Peng 1977, Kimmins 1987). This has the effect of moving chemical molecules (such as the gypsy moth pheromone) in the air further along than across the valley.

Based on the results of the initial studies conducted in Millsboro Springs, VA wide-swath experiments were conducted in Appomattox-Buckingham State Forest, VA to determine whether the effect of pheromone beyond treated areas could be used operationally in mating disruption treatments against gypsy moth. These experiments showed that there was a significant reduction of male moth catches over the entire study site, including strips that were not treated with the pheromone. The reduction of male catches in traps was noted at the distances up to 60 m from the boundary of the treated swath. This agrees with the results of studies of the effect of pheromone beyond treated areas conducted in the same area. In similar studies conducted in Millboro Springs, VA in 2001 and 2002, no definite pattern in male moth catches was observed that reflected the distribution of pheromone in swaths that were treated (Thorpe et al., unpublished). Trap catches in the study conducted in 2001 were significantly reduced over the entire plot compared with traps outside the plot. A significant reduction in male moth catches also was observed at distances between 165–195 m in 2001 and between 75–225 m in 2002. In 2002, the effect of the pheromone still could be observed at the distance of 345 m from the treated area.

The difference in pheromone effects between Millboro Springs and Appomattox-Buckingham State Forest with respect to skipped-swaths applications of pheromone also can be explained by the differences in relief of the landscapes in these areas. In Buckingham-Appomattox State Forest, the effect of pheromone was sufficient to disrupt mating completely at distances up to 30 m from the treated area. Taking into an account that each gap was surrounded by two sprayed strips, it was recommended that the pheromone should be applied using plots with 30–60 m gaps of unsprayed strips between sprayed strips.

Milli et al. (1997) noted that pheromone treatments for mating disruption might sometimes cause concerns about edge leakage and depletion, which might increase the pest attack rates. They referred to the area where the leakage occurs as a transition zone. A transition zone could occur because as air flows across a treated plot, as a result of wind currents, the

pheromone cloud is likely to move into the treated plot thus depleting the border. Wind blowing from treated to untreated areas pushes the pheromone cloud beyond the border of the treated plot thereby creating the edge leakage. Therefore, a transition zone appears around the border of the plot where the pheromone concentration is unstable and is affected by wind (Milli et al. 1997). The pattern of male moth catches that was observed in pheromone-baited traps across the plot treated using skipped-swaths (Figure 5.16) does not support the idea of a transition zone. In all experiments, including those of the effect of pheromone beyond treated areas conducted in 2000 and 2001, mating communication was disrupted at and beyond the boundaries of the treated area. This might have been due to the differences in the environment or pheromone formulations used for mating disruption. Both 3M microcapsules and plastic flakes stick to the foliage by electrostatic forces and sticking agent, respectively. A deposition study demonstrated that in the case of plastic flakes, deposition of the pheromone occurs throughout all layers of the canopy, including the understory foliage and only 16% of flakes penetrated all levels of the foliage and reached the ground during six weeks after application (Reardon et al. 1998). This suggests that the flakes tended to stay attached to the foliage while constantly emitting pheromone, and, therefore, the edge depletion is not an issue.

5.5. Conclusions

In all experiments moth counts in pheromone-baited traps located outside treated area were lower near treated plots and increased with increasing distance. This, together with the increase of proportion of fertilized females, indicates that disparlure dispersed from the area where it was applied and affected gypsy moth population beyond the boundary of the treated area. Although normally the effect of disparlure is limited to 100-150 m, in narrow valleys (as in Millboro Springs) the effect of disparlure can be observed at distances up to 600 m from treated areas.

In all skipped-swath experiments mating success and male moth catches in traps were significantly reduced in the entire treated area. This indicates that the method of skipped-swath pheromone application is effective and can be recommended for operational mating disruption treatments.

6. Evaluation of a Portable EAG Device for Detecting Gypsy Moth Pheromone in the Field

6.1. Introduction

The sex pheromone emitted by virgin gypsy moth females to attract males consists of a single compound, disparlure, identified as (7R8S)-*cis*-7,8-epoxy-2-methyloctadecane (Bierl et al. 1970). The olfactory system of gypsy moth males is highly sensitive to disparlure (Schneider et al. 1977). Detection of the pheromone by males ranges from a lower threshold of ~1 pm/s (cited by Kowcun et al. 2001) to a saturation level of 1 μm/s (Kaissling 1977). The behavioral threshold is ~900 molecules/antenna (cited by Kowcun et al. 2001). Gypsy moth antennae are covered with sensilla trichodea, which are hollow sensory hairs. Olfactory neurons send dendrites into the hollow space in a sensillum (Kaissling and Torson 1980). The dendrites in the sensillum are surrounded by lymph containing pheromone-binding proteins (PBPs) (Vogt 1987). The pheromone enters the sensillum through pores in the cuticle and is transported through the lymph to the dendrite membrane with the help of highly abundant PBPs. Contact of the pheromone molecule with the dendrite membrane evokes an electrical response. The sum of electrical responses of all receptors on the tested antennae can be measured and recorded simultaneously with the help of an electroantennogram (EAG) device (Schneider 1963). The amplitude and shape of the EAG depend on the concentration and chemical structure of the stimulus and therefore can be used to evaluate the concentration of the pheromone detected by the antennae.

Volatile trapping and solvent extraction are two techniques that are well established and used widely for sampling purposes. A method for quantifying disparlure in the air by trapping it in hexane and determining its content using flame ionization gas chromatography was reported in 1975 (cited by Kowcun et al. 2001). This method was suitable for laboratory analyses but was not sensitive enough for use in the field. In 1977, another method was developed and used successfully in field experiments (Caro et al. 1977, Caro et al. 1978). In this method, a bed of molecular sieves (a solid adsorbent) was used instead of the liquid trap. Solid adsorbents were shown to be more efficient because they allow a higher flow rate through the adsorbent (Seiber and Woodrow 1976). However, the trapping and extraction method is not cost effective and takes a long time to perform.

The goal of this study was to test a portable electroantennogram (EAG) device (van der Pers and Minks 1998) for its ability to detect disparlure sprayed for mating disruption in gypsy moth population. The operation of the portable electroantennogram device is the same as that of a laboratory set-up (van der Pers and Minks 1998, van der Pers 2001). The portable EAG device could be used to evaluate pheromone treatments by measuring pheromone concentration in the field. The ability to measure pheromone concentration in the field will help to improve our understanding of pheromone diffusion and our predictions of mating probability in the treated area.

6.2. Materials and Methods

Electroantennogram Device

A portable Electroantennogram (EAG) device (Figure 6.1) was used to measure gypsy moth sex pheromone concentration in the field. The device works as follows: an antenna of male gypsy moth is fixed between two silver contact pads dipped into the plastic holder using electrically conductive gel (Spectra 360 Electrode Gel, Parker Laboratories, Orange, NJ). The electrical contact between the antenna and amplifier is achieved by a pair of springs. The device contains three pumps. The first pump (1) provides the constant airflow over the antenna. A small amount of air is sucked in from the outside through the narrow opening and is filtered through the activated charcoal filter. Constant flow of the filtered air maintains the non-stimulation condition of the antenna (van der Pers and Minks 1998). The air is then blown out through the ambient air inlet tube to prevent this tube from becoming contaminated and to get rid of the remains of the previous sample. The second pump (2) is combined with a solenoid valve. Activation of the valve changes the airflow and unfiltered ambient air is sucked in through the sample inlet. The third pump (3) is also combined with a micro solenoid valve and, when activated, mixes a sample from the reference capsule with the air that flows over the antenna. The responses of the antenna to the ambient air are recorded by the EAG together with the responses to the stable reference. Each reading, therefore, consists of three measurements: a first reference, an ambient air sample, and a second reference. Hexyl acetate can be used as a reference because as it elicits EAG depolarizations in male moths similar to pheromone (Dickens et al. 1997).

Readings taken with the EAG can be downloaded to the computer and analyzed using the AuotoSpike32 program (Syntech, Hilversum, the Netherlands). Voltage differences between the spikes and the baseline are measured in the program (Figure 6.2).

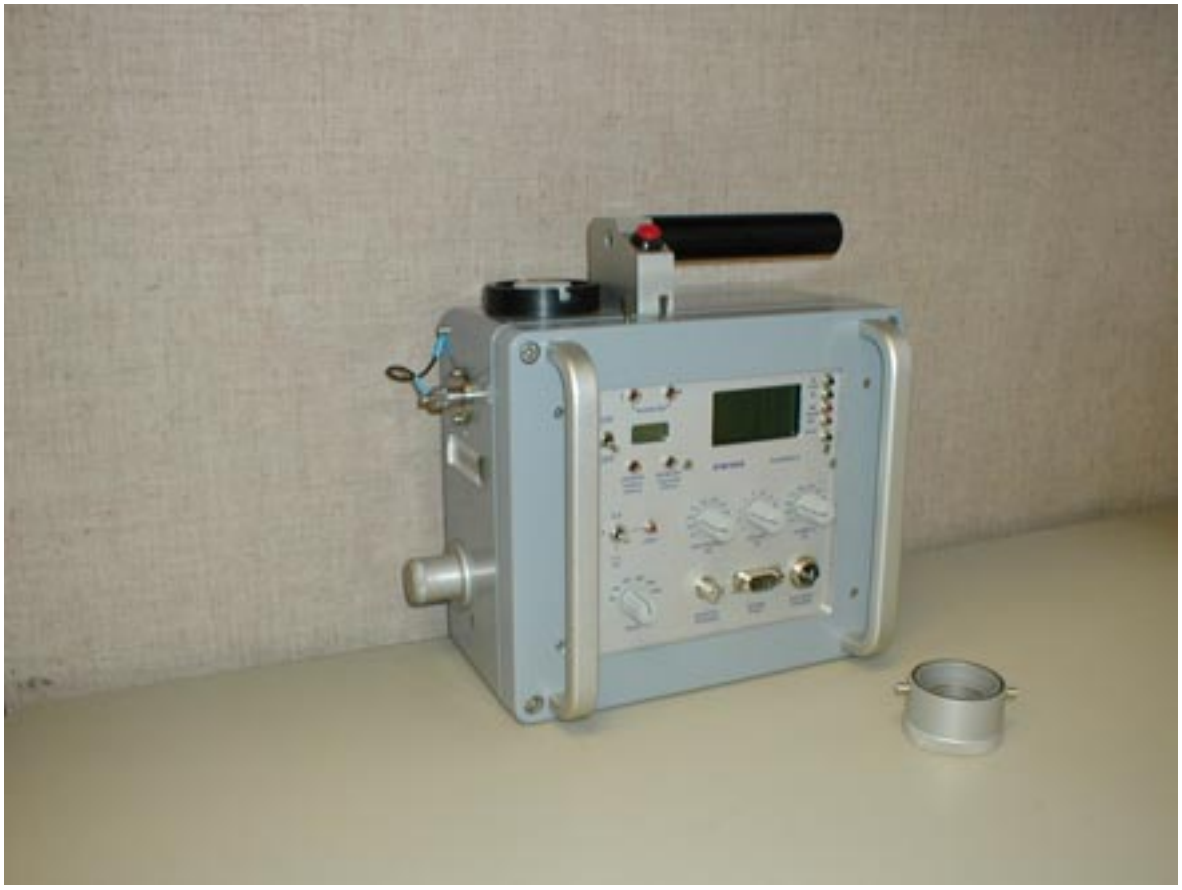

Figure 6.1: Portable EAG unit

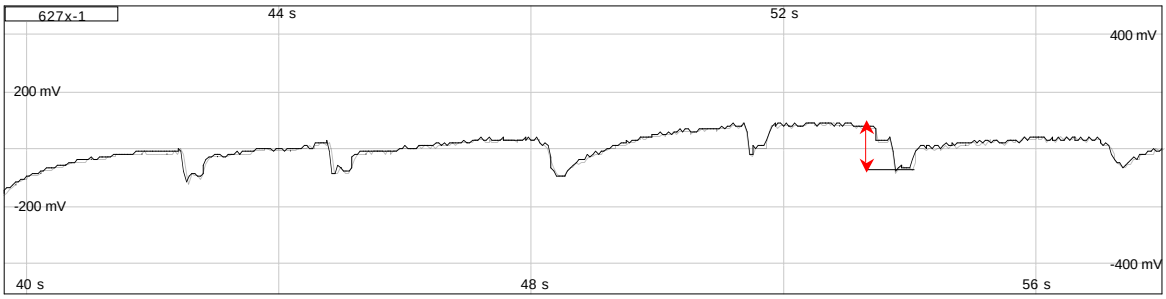

Figure 6.2: An example of the EAG recording. Red arrow shows a measurement taken automatically by the AutoSpike32 program in response to disparlure.

Experiments were conducted in summer of 2001 and 2002 in Appomattox-Buckingham (Buckingham and Appomattox Co., VA) and Cumberland (Cumberland Co., VA) State Forests in plots treated with synthetic disparlure in plastic flake and 3M microcapsule formulations for mating disruption experiments.

6.2.1. EAG Experiments: 2001

In 2001, EAG readings were taken in the plots treated with disparlure for gypsy moth mating disruption experiments. Racemic disparlure was applied aerially in two formulations, 3M Microcapsules (3M Canada Co., London, Ontario, Canada) and plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA). The doses of pheromone ranged from 0.15 to 75 g a.i./ha. Each plot was treated either with 3M microcapsules at 0.15, 0.75, 3, 15, 37.5, or 75 g a.i./ha or with plastic flakes at 15 g a.i./ha. All doses were replicated twice, except for doses of the 3M formulation at 15 and 37.5 g a.i./ha, which were unreplicated. Two plots were used as a control

EAG readings were taken weekly during 4 weeks (July 7 – August 1) in the centers of treated plots. In control plots, measurements were taken over a 6-week period (June 23 – August 1). Five to seven readings with three antennae were taken in each plot resulting in a minimum 15 readings per plot each time a plot was visited.

Antennae were taken from male gypsy moths, which were shipped as pupae from USDA APHIS Otis Methods Development Center, Massachusetts and reared in the lab. The sensitivity of the antennae was measured using the hexyl acetate as a reference compound (Dickens et al. 1997). Internal reference was used to monitor gradual decline in sensitivity of antennae (van der

Pers and Minks 1998). Therefore, only antennae that exhibited strong reaction to hexyl acetate were used in the experiments.

Statistical Analysis. The responses of the antennae to the airborne pheromone were compared to the responses to the reference compound by calculating voltage ratios of the responses. Voltage ratio was calculated by dividing the voltage difference obtained for the response of the antenna to airborne pheromone by the average of voltage differences obtained for two responses to the standard compound. The General Linear Model Nested ANOVA procedure (Ott 1993, Sokal and Rohlf 1981) was used to test the differences in voltage ratios between plots treated with various doses of pheromone. Voltage ratio was modeled as a function of dose, week within dose, and antenna within dose and week.

Readings from control plots were also analyzed separately by week. The General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to test the differences in voltage ratios obtained for control plots between weeks. Voltage ratio was modeled as a function of week and antenna without interaction of factors.

6.2.2. EAG Experiments: 2002

In 2002, EAG readings were taken in plots treated with disparlure for gypsy moth mating disruption experiments. Disparlure was applied aerially in plastic flake formulation (Disrupt® II, Hercon Environmental, Emigsville, PA) 15 and 37.5 g a.i./ha. Doses were replicated four times. Readings were taken once every two weeks (June 26, July 09, and July 23) in the centers of control and treated plots.

Five to six antennae were used to measure the response to the airborne pheromone in each plot; five to seven readings were taken using each antennal preparation. The same preparation was used in two plots if the antenna exhibited a strong response to the reference compound.

Male gypsy moths were shipped as pupae from USDA APHIS Otis Methods Development Center, Massachusetts and reared in the lab. The sensitivity of their antennae was measured using the hexyl acetate as a reference compound (Dickens et al. 1997). Only antennae that exhibited strong reaction to hexyl acetate were used in the experiments.

Statistical Analysis. The response of the antennae to the airborne pheromone was compared to the response to the reference compound by calculating voltage ratios of the responses. The voltage ratio was calculated by dividing the voltage difference obtained for the response to the airborne pheromone by the average of voltage differences obtained for two responses to the standard compound. A General Linear Model Nested ANOVA procedure (Ott 1993, Sokal and Rohlf 1981) was used to test the differences in voltage ratios between plots treated with various doses of pheromone. Voltage ratio was modeled as a function of dose, week within dose, and antenna within dose and week.

Readings from control plots were also analyzed separately by week. The General Linear Model procedure with Tukey's adjustment for multiple comparisons of mean values (SAS 1996, Proc GLM) was used to test the differences in voltage ratios obtained for control plots between weeks. Voltage ratio was modeled as a function of week and antenna without interaction of factors.

6.3. Results

6.3.1. EAG Experiments: 2001

The analysis showed that there was a significant effect of the antenna on the voltage ratio. However, the effects of dose and time were not significant (Table 6.1). Readings obtained in plots treated with high doses of pheromone (15, 37.5, 75 g a.i./ha) were not significantly different from any of the lower doses, including 3 g a.i./ha (Figure 6.3).

To check for contamination of the device, the readings from control plots were analyzed separately by week. The GLM indicated a significant effect of time and antenna on voltage ratio (Table 6.2). The highest readings were obtained during the third and the fifth weeks. However, during the fourth and sixth weeks the readings were as low as the readings obtained during the first and the second weeks (Figure 6.4).

Table 6.1: General Linear Model (GLM) analysis of voltage ratios between the responses to the airborne pheromone and to the standard compound measured with the portable EAG unit in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

Source	DF	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Dose	7	6.81	0.97	0.78	>0.50
Week(Dose)	24	29.75	1.24	0.67	>0.50
Antenna(Dose*Week)	64	118.17	1.85	6.33	<0.0001
Error	724	211.31	0.29		
Total	819	391.16			

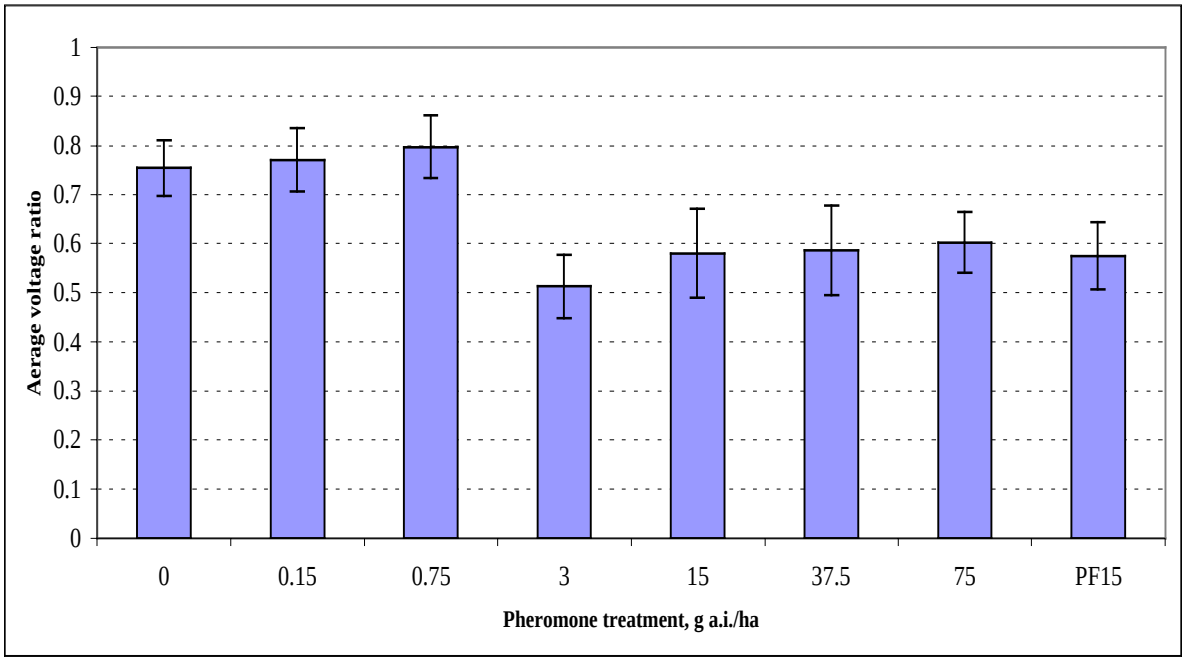

Figure 6.3: Average voltage ratios (average voltage ratio ± SD) measured in the plots treated with various doses of disparlure in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

Table 6.2: General Linear Model (GLM) analysis of voltage ratios between the responses to measured with the portable EAG unit in the control plots in Cumberland and Appomattox-Buckingham State Forests, VA, 2001

Source	DF	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Antenna	5	16.09	4.87	7.24	<0.0001
Week	5	24.36	3.22	10.96	<0.0001
Error	184	81.76	0.44		
Total	194	112.45			

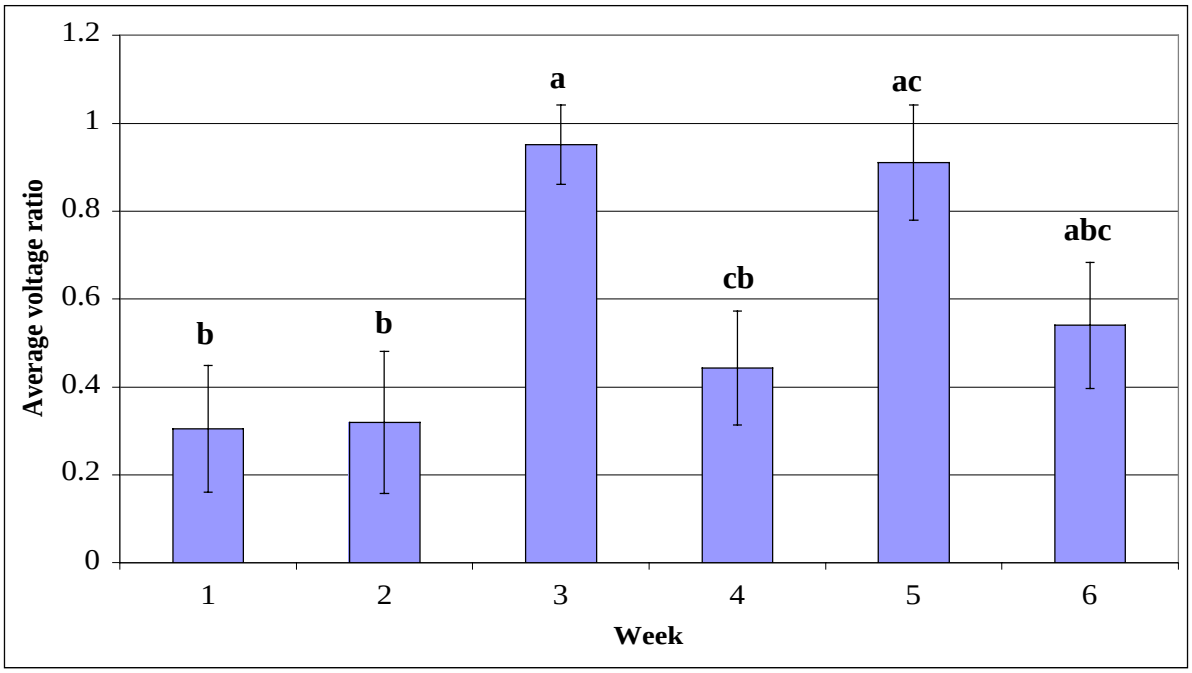

Figure 6.4: Average voltage ratios (average voltage ratio ± SD) measured weekly in control plots in Cumberland and Appomattox-Buckingham State Forests, VA, 2001. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.005$)

6.3.2. EAG Experiments: 2002

The results of the analysis showed that there was a significant effect of time and antennae on voltage ratio (Table 6.3). The readings in control plots were lower than in plots treated with pheromone at 15 and 37.5 g a.i./ha (Figure 6.5). Readings obtained in plots treated with 15 g a.i./ha were significantly higher than readings in control plots and plots treated with 37.5 g a.i./ha.

To check for contamination of the device, the readings from control plots were analyzed separately by week. The GLM indicated significant effect of time on voltage ratio (Table 6.4). Readings in control plots were higher during the first week compared with the second and third weeks (

Figure **6.6**).

Table 6.3: General Linear Model (GLM) analysis of voltage ratios between the responses to the airborne pheromone and to the standard compound measured with the portable EAG unit in Cumberland and Appomattox-Buckingham State Forests, VA, 2002

Source	DF	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Dose	2	2.32	1.16	0.49	<0.5
Week(Dose)	5	11.94	2.39	5.53	<0.01
Antenna(Dose*Week)	33	14.17	0.43	2.04	<0.005
Error	472	98.67	0.21		
Total	512	131.08			

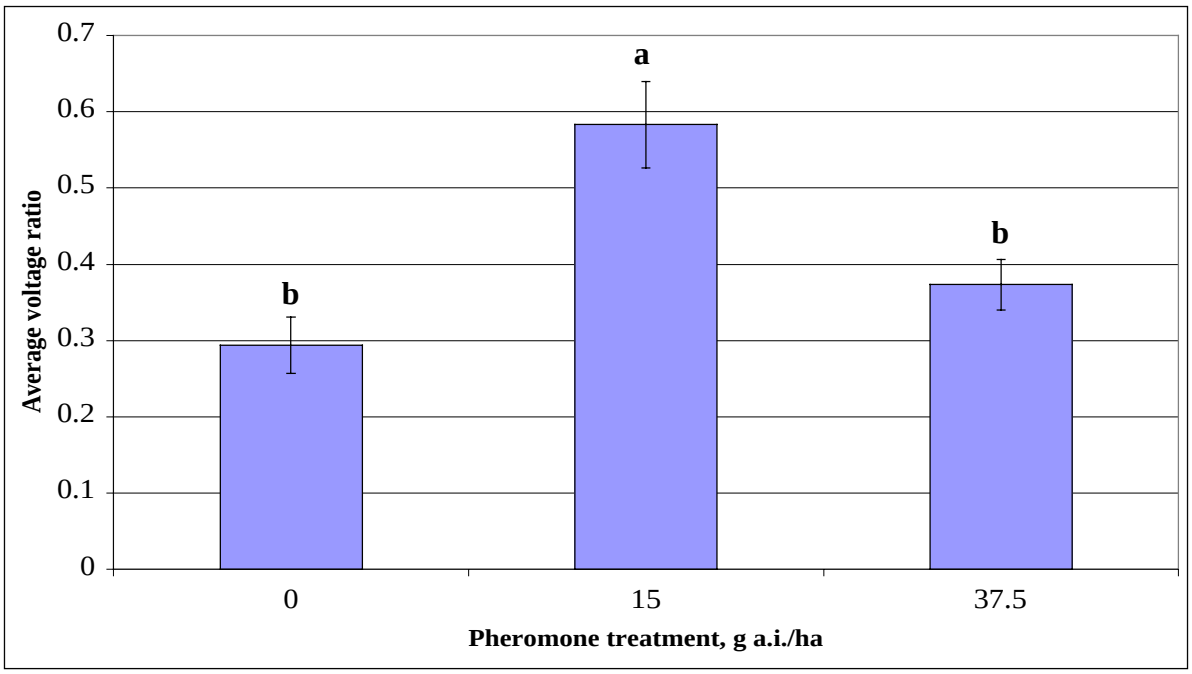

Figure 6.5: Average voltage ratios (average voltage ratio ± SD) measured in the plots treated with various doses of disparlure in Cumberland and Appomattox-Buckingham State Forests, VA, 2002. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$)

Table 6.4: General Linear Model (GLM) analysis of voltage ratios between the responses to measured with the portable EAG unit in the control plots in Cumberland and Appomattox-Buckingham State Forests, VA, 2002

Source	DF	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Antenna	4	0.74	0.19	1.70	<0.50
Week	2	1.19	0.6	5.46	<0.01
Error	192	20.97	0.11		
Total	198	22.83			

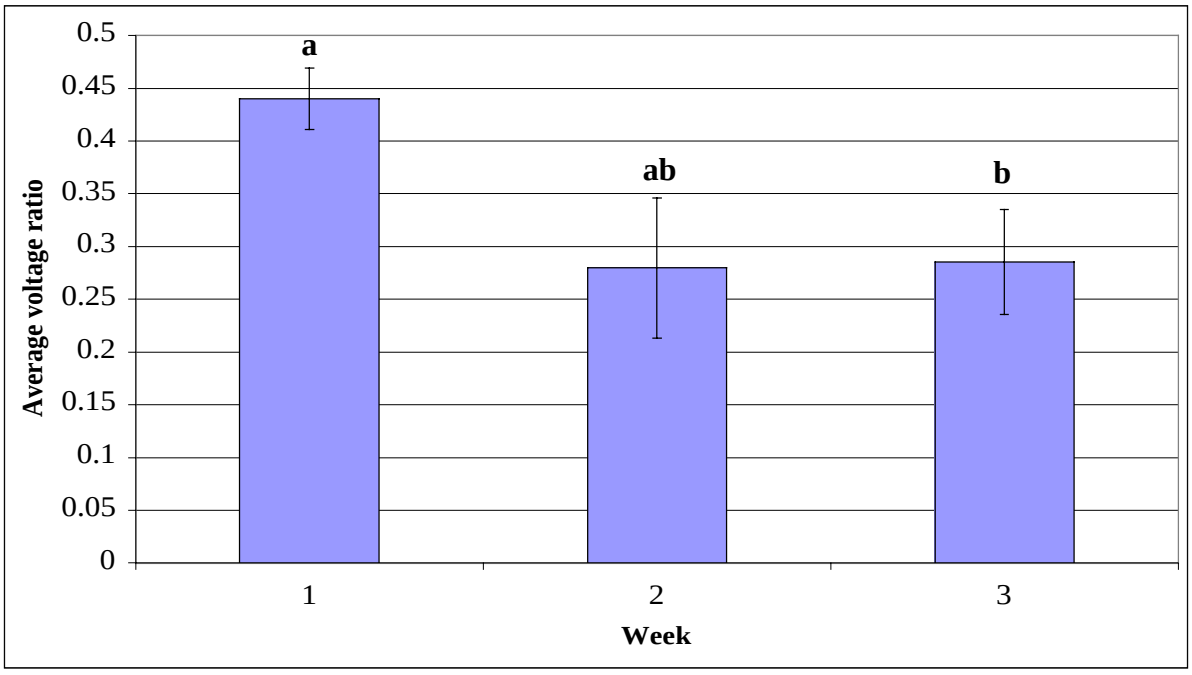

Figure 6.6: Average voltage ratios (average voltage ratio ± SD) measured weekly in control plots in Cumberland and Appomattox-Buckingham State Forests, VA, 2002. Bars with the same letters are not significantly different, Tukey's HSD ($\alpha < 0.05$)

6.4 Discussion

The use of bioassays in studies of insect pheromones has been mainly to allow selection of responses that could be recorded easily and which could also serve as good predictors of final behavior (Howse et al. 1998). Although many bioassay methods directly involve recording of behavioral changes, some methods (such as electrophysiological bioassays) record instead changes in neurophysiological activity. The value of electrophysiological bioassays in studies of pheromone communication is in its ability to identify communication channels and redirect the focus away from those aspects of the signal that the animal is unable to detect (Howse et al. 1998). By far the most practical approach in electrophysiological bioassays has been the use of the Electroantennogram (EAG), which was first developed by Schneider (1957). Traditionally, because of the cost and size of EAG devices, their use was restricted to the laboratory. Some early attempts were made to move EAG set-ups from the lab to an open area (Baker and Hayne 1989). However, these authors reported problems with the detection of plumes from pheromone sources, which were due mainly to the stationary position of the device.

Färbert et al. (1996) described the first portable EAG system for measuring pheromone concentrations in the field. Their device performed reliable well and was able to record detailed measurements of concentration gradients and the spatial distribution of pheromone in various crops (Sauer et al. 1992, Suckling et al. 1994, Milli et al. 1997, Färbert et al. 1997). Experiments conducted to measure the concentration of pheromone sprayed for mating disruption of gypsy moth at various heights in the canopy showed that the portable EAG system (Färbert et al. 1996) was reliable and sensitive to differences in disparlure concentration at the different heights tested in the canopy (Thorpe et al. 2002).

The portable EAG device used in this study was first tested in greenhouses using antennae of male moths of the tomato lopper, *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) (van der Pers and Minks 1998). Relative differences in pheromone concentrations between untreated greenhouses and greenhouses treated with 54 g a.i./ha were measured and changes in pheromone concentrations were clearly demonstrated. Greenhouses provide the environment with constant temperature and humidity, and lack the air turbulence, which is characteristic of open systems (e.g., orchard and forest). Outdoors tests of the EAG device also were conducted using *Cydia pomonella* (van der Pers and Minks 1998). The EAG readings showed that the sensor was able to measure differences between treated and untreated plots. However, the

authors suggested that because of greater intensity of air turbulence compared with the greenhouses, more measurements were required in order to get reliable results.

The studies reported here, which were conducted in 2001 and 2002, showed that there was no consistent effect of pheromone dose on the response of male gypsy moth antenna as measured with a portable EAG device developed by van der Pers and Minks (1998). It was suggested that the device might have become contaminated when measurements were taken in plots, which were treated with high doses of pheromone. If this happened, then, it would have eliminated the possibility of taking correct readings in untreated areas and plots treated with low doses of the pheromone. The results of data analysis do not provide strong enough evidence of contamination. In 2001, the voltage readings taken in control plots were significantly higher during the third and the fifth weeks after pheromone application compared with the first and second weeks. However, voltage readings obtained during the fourth and sixth weeks were as low as readings obtained during the first two weeks. In 2002, the voltage ratios in the first week were significantly higher than during the rest of the study. If contamination of the EAG device occurred, then the readings in control plots would have increased with time. Because no relationship between time and antennal response was observed, contamination of device was unlikely.

One may speculate that differences in the sensitivity between the EAG device used in this study and that of Färbert et al. (1996) could be due to the differences in the way these devices operate. The portable EAG device described by Färbert et al. (1996) uses three calibration stimuli of different concentrations. In the portable EAG used in this study, an internal reference is used to monitor gradual decline in the sensitivity of antennae rather than for calibration (van der Pers and Minks 1998). It was shown that sensitivity and concentration threshold of the antenna vary independently (Färbert et al. 1996). Response amplitude to the same stimulus may be affected by a change in threshold or by change in sensitivity, or both; therefore, Färbert et al. (1996) concluded that the sensitivity and the concentration threshold should be determined by stimulating the antenna with at least two different calibration sources in two successions.

There are still many questions left unanswered about the use of the portable EAG device. Further studies are needed to determine the utility of the device for measuring airborn pheromone in the field. The sensitivity of the device needs to be studied in the lab to estimate signal-to-noise ratio and to obtain a dose-response relationship. In the field, a large number of

readings could be taken to address the issue of the greater air turbulence in open areas compared with greenhouse environemnets, where the device was tested previously. Also, a high variability in antennal response was shown. However, the source of the variability is unknown. Comparison of the antennal responses of males at various ages, and the use of antennae from males at optimum age collected from natural populations (which would tend to have the strongest response to the pheromone), might improve the technique. An additional calibration of antennal preparation also might help to eliminate insensitive antennae and increase the chances of detecting differences between various doses of airborne pheromone.

6.5. Conclusions

In all experiments, no relationship between dose of artificial airborne pheromone and response of gypsy moth antenna as measured with the portable EAG device was found. The inability of the portable EAG device to detect differences between airborne pheromone concentrations in the plots treated for mating disruption could be explained by the high variability among antennae and a low concentration of the airborne pheromone used operationally for mating disruption.

7. Summary

Gypsy moth, *Lymantria dispar* (L), an insect pest of hardwood forests, was introduced accidentally into the United States in late 1860s and since then has expanded its range significantly in North America (Liebhold et al. 1989). Today, *L. dispar* is one of the most important forest pests in the eastern United States (Doane and McManus 1981). Gypsy moth populations normally occur in low densities, but for reasons, which are unknown, their numbers can increase rapidly resulting in extremely high population densities (Liebhold and Elkinton 1988, Elkinton and Liebhold 1990, Liebhold et al. 2000) and serious damage to the forest (Schoëner 1988).

Many attempts have been made to control and manage populations of the gypsy moth. These attempts have included strategies such as burning of forest stands (Kirkland 1905, Burgess 1930), insecticide applications using chemicals such as carbaryl (Sevin®), acephate (Orthene®), and diflubenzuron (Dimilin®), and the use natural enemies and pathogens. Problems with these management strategies led to the search for new and innovative approaches to managing populations of the gypsy moth. The use of pesticides as a management strategy, for example, was found to be limited by both the cost of treatment and negative community reaction against pesticide usage (Plimmer et al. 1982).

In 1970s the sex pheromone produced by gypsy moth females to attract males was identified and synthesized (Bierl et al. 1970, Iwaki et al. 1974). The discovery of the pheromone represented a new era in the control and management of gypsy moth population. The pheromone was found to be useful for sampling and monitoring the spread of gypsy moth populations (Talerico, 1981, Ravlin et al. 1987) and in the development of the new method of control by disrupting mating communication (Knipling 1971, Beroza and Knipling 1972, Beroza et al. 1974). The use of the pheromones in trapping was helpful for locating isolated gypsy moth populations, delineating areas for egg mass sampling (Kolodny-Hirsch and Schwalbe 1990), predicting subsequent population density (Thorpe et al. 1993), and for measuring the rate of spread of the population (Sharov et al. 1995). Monitoring of the spread of the gypsy moth has been necessary for planning preventive methods, identification of areas of domestic quarantine, and for evaluating the effects of management on the rate of gypsy moth spread (Sharov et al. 1997).

Pheromone traps have several advantages compared with the other sampling methods such as the use of aerial maps of forest defoliation and counts of overwintering egg masses (Kolodny-Hirsch 1986). Pheromone traps are inexpensive, easy to use, standardized, catch mostly the target species, and are effective in extremely low population densities (Elkinton and Cardé 1981). The advantages of pheromone traps usually outweigh problems with their use such as the decline in the number of male moth captured with time as the traps fill up and as odors associated with decompositing moths increases (Elkinton 1987). There is also the problem with the use of the pheromone in traps for monitoring gypsy moth populations, which is related to the relative attractiveness of the trap to males compared with the attractiveness of female moths. Pheromone traps compete with females and tend to catch fewer males if the pheromone bait is not strong enough (Webb 1982). The proper use of the pheromone for trapping, therefore, requires knowledge of the phenology of flight of male gypsy moth populations, which can be used to determine the correct time for deployment of traps before the beginning of moth flight and retrieval after flight termination (Régnière and Sharov 1998).

The technique of mating disruption is used widely in agricultural and forest ecosystems for managing pest populations (Reardon et al. 1998). The method requires the use of a synthetic pheromone to modify the behavior of males in their search for females and involves application of the pheromone in sufficient quantities to be present throughout the entire mating season. The use of the synthetic pheromones also tends to be ecologically safe due to the low toxicity of the pheromone to mammals and the rapid degradability (Bierl et al. 1976, Reardon et al. 1998). However, a lot is still unknown about the method of mating disruption particularly with respects to the gypsy moth. In the gypsy moth, for example, there may be several possible effects of synthetic pheromone on male moth populations (Granett 1976). The synthetic pheromone may compete with the female source of the pheromone, males may become habituated to the high levels of pheromone in the air and do not respond to it, an artificially high level of pheromone may cause abnormal behavioral responses in males so that they become overstimulated and are not able to orient and find females (Granett and Doane 1975, Richerson et al. 1976a), or the presence of artificial pheromone in the environment may change the copulatory behavior of the gypsy moths. There is evidence which suggests that high airborne levels of the pheromone cause males to leave treated areas (Granett and Doane 1975), that the density of male moths is slightly higher in treated areas than in control plots, and that the duration of precopulatory and copulatory

periods are similar for all females regardless of disruptant treatment (Schwalbe and Mastro 1988).

It is clear that for successful disruption, the artificial pheromone must be present in the air in sufficient concentration throughout the mating period (Reardon et al. 1998). It is also now known that for the gypsy moth, the use of mating disruption treatments would be most powerful against low-density populations (Plimmer et al. 1982), where males are guided to females primarily by the pheromone. As such, the strategy of mating disruption is being used for the eradication of isolated infestations and to control the spread of low-density populations that become established beyond the main population front (Schwalbe et al. 1983, Webb et al. 1990).

Currently, several products are being used for mating disruption. These include Disrupt® II (Hercon) and Luretape Gypsy Moth, which are registered for "general use", and Luretape Plus, which does not require EPA registration. Two controlled-release formulations of disparlure that have been registered with the EPA are the three-layered plastic laminated tape, Hercon Luretape GM and granulated flakes, Hercon Disrupt® II (Kolodny-Hirsch and Schwalbe 1990, Reardon et al. 1998). Hercon Luretape GM (Health-Chem Corporation, New York, NY) is usually used for ground application. Hercon Disrupt® II is a formulation of polymeric 3-layer laminated flakes 3 mm by 3 mm, which is usually applied aerially with special equipment (Plimmer et al. 1982). The 3M Corporation of Canada has also developed a liquid microencapsulated pheromone product "Sprayable Pheromone", which contains 20.0% of racemic disparlure (Report by Cowan and DeVilbiss 2001). This formulation is still under development and has not been registered by the EPA and, therefore, is not available commercially.

Several approaches also are being used to assess the biological effectiveness of mating disruption. Counting life stages is used to detect changes in the abundance of pupae, larvae and egg masses. By this method, the effectiveness of mating disruption in the previous year is evident by a decrease in numbers of larvae and pupae under the burlap bands placed on tree boles, while the decrease in the abundance of egg masses shows the effectiveness of applied pheromone in the year of application. Pheromone-baited traps are used usually for monitoring male flight. Low catches or no catches, as well as the inability of males to find females in delta traps placed for monitoring, indicate successful disruption of pheromone communication. Also, the absence of embryos in the eggs collected from females can be used as an indication of successful mating disruption. Finally, low numbers or the absence of egg masses found during a

survey also can be used to determine the effectiveness of mating disruption (Reardon et al. 1998).

The overall goal of the project was to evaluate the use of mating disruption on the mating success of the gypsy moth in low-density populations. Studies were designed and carried out specifically to develop an understanding of the mechanism of mating success, to evaluate existing techniques of mating disruption, and to improve methods for application of pheromone used for mating disruption so as to reduce the costs associated with the use of this management tactic. The studies were aimed at improving the efficiency of pheromone treatments by determining the lowest dose of pheromone that could be used and by defining the maximum distance between sprayed strips that will disrupt mating effectively. It is believed that knowledge of the spatial and temporal dynamics of pheromone that is applied aerially will allow for a more rational use of the pheromone. The project had several specific objectives.

The first objective of the project was to understand mating success of gypsy moth in low-density populations. Few studies have been devoted to understanding the population dynamics mechanisms specific to low-density gypsy moth populations. Sharov et al. (1995) found that mating success of female was a critical inverse density-dependent factor, which depended mainly on the local abundance of adult males and was correlated highly with the rate of male moth capture in pheromone traps. Because of this, they believed that mating success could be predicted from male moth capture in pheromone-baited traps. Sharov et al. (1995) used this approach to study the mating success of gypsy moths in the Appalachian Mountains, VA. They also found that predation by birds and insects such as ants tended to decrease the mating probability of female gypsy moths by decreasing their longevity.

Based on the findings of Sharov et al. (1995), a study was conducted under this project to examine mating success of gypsy moth populations in Wisconsin for comparison with the mating success observed for the insect in the Appalachian Mountains, VA. The study also compared mortality of gypsy moth females in low-density populations in Virginia and Wisconsin. No significant difference was found in relationships between mating success of gypsy moth females and male moth counts in traps in Virginia and Wisconsin. However, mortality of females from predation was significantly higher in Virginia than in Wisconsin ($P < 0.001$). Gypsy moth females, therefore, appear to live longer and have a higher chance of mating in Wisconsin than in Virginia. The results suggests that the higher rate of population spread in Wisconsin may be due

to the increased mating success of females, which is probably caused by increased long-distance dispersal of males and increased longevity of females.

In the second objective of the project, attempts were made to improve the methods of pheromone application and to obtain dose-response relationships for the wider range of pheromone doses than had been studied previously. The use of mating disruption for gypsy moth population control has been attempted since 1971 (Stevens and Beroza 1972, Schwalbe et al. 1974, Granett and Doane 1975) with research conducted to find an appropriate formulation and concentration of pheromone for use in mating disruption. Because most dose-response studies have been conducted using high doses of pheromone, little is known about the effects of low doses of the pheromone on gypsy moth populations. In addition, the densities of gypsy moth populations that have been tested were usually high and the range of pheromone doses used was limited. Therefore, this study used dose-response experiments to examine the effects of a wider range of pheromone doses on the mating success of females and male moth catches in pheromone-baited traps in low-density gypsy moth populations. The study also compared different formulations of the pheromone with Hercon Disrupt® II, which is the only formulation of disparlure currently available for aerial applications.

Dose-response relationships were obtained for the pheromone for doses ranging from 0.15 to 75 g a.i./ha. Doses of 37.5 and 15 g a.i./ha were compared with the dose of 75 g a.i./ha (standard operational dose) for the effects on mating success. In all experiments, the dose of 37.5 g a.i./ha of the pheromone was shown to effectively disrupt mating and, therefore, was recommended for operational use. The dose of 15 g a.i./ha was also very effective and, therefore, could be also used operationally.

Three formulations of disparlure, plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA), microcapsules (3M Canada Co., London, Ontario, Canada) and liquid pheromone formulation (Shin-Etsu Chemical Co. Ltd, Tokyo, Japan), were compared for their effect on mating success of females and male moth catches in pheromone-baited traps. The effect of pheromone in the plastic flakes (Disrupt® II, Hercon Environmental, Emigsville, PA) formulation was found to be stronger and lasted longer than the effect of pheromone in both microcapsule (3M Canada Co., London, Ontario, Canada) and liquid (Shin-Etsu Chemical Co. Ltd, Tokyo, Japan) formulations. These results suggest that much lower doses of pheromone than were used previously could effectively disrupt mating in gypsy moth populations.

The third objective of the project focused on improving the use and efficacy of the pheromone for mating disruption by reducing the amount of pheromone that is sprayed and also by reducing the flight distance during aerial application. In the past, pheromone was distributed evenly over the entire infestation area in a manner similar to pesticides. In 2000, the distance effect of the pheromone was discovered accidentally and the first attempts were made to study the effect of the pheromone beyond treated plots in areas with different landscape architectures. In a mountainous area, the effect of disparlure along the valley between the mountains was noted at a larger distance (633 ± 63 m) from the treated area than across the valley (104 ± 22m). In a relatively flat area, the effective distance for disparlure was similar to the effective distance across the valley in a mountainous area (67 ± 17m). The studies also showed that moth counts in pheromone-baited traps were low near treated plots, but increased with increasing distance. The pheromone appeared to be most effective at distances of up to 150 m from treated area ($F = 36.39$, d.f. = 3, $P < 0.0001$), and in narrow valleys the effective distance may extend up to 600 m ($F = 9.38$, d.f. = 4, $P < 0.0001$). These findings, together with the increase in the proportion of fertilized females near treated plots, indicated that the pheromone (disparlure) disperses from treated areas to affect gypsy moth populations beyond the boundary of the treated area. A new method of pheromone application that involves using an unsprayed strip that is 30 m wide between sprayed stripes also was tested and shown to be effective in disrupting mating in gypsy moth populations. These results also help to understand the mechanism of mating disruption ruling out the possibility of pheromone treatments causing abnormal behavior in males (Richerson et al. 1976a, Granett and Doane 1975).

The mechanism of mating disruption is still unknown; several hypotheses were suggested to explain it (Granett 1976). One of the hypotheses suggests that artificially high level of airborne pheromone causes abnormal behavioral responses in males. Males may become overstimulated and thus are unable to find the females (Richerson et al. 1976a), or the behavioral response may not elicit proper orientation in the male (Granett and Doane 1975). A second possibility is that the males become habituated to the high level of airborne pheromone and fail to respond to it (Granett 1976). The results of this study disagree with both of the hypotheses. If aerial applications of pheromone were causing abnormal behavior in gypsy moth males, they would not be able to find pheromone-baited traps in treated areas. However, even though trap catches are reduced in the treated plots and in the vicinity, males are still able to find them. This

also contradicts the hypothesis of habituation of gypsy moth males to the high doses of pheromone in the air. Based on the results of this study, the hypothesis of competition of pheromone sources with females (Granett 1976, Reardon et al. 1998) seems to be the most convincing one.

The aim of the final objective of the project was to test a method for measuring pheromone concentration in the field, which can be used to improve our understanding of pheromone diffusion and our predictions of mating probability in treated areas. For this study, a portable Electroantennogram (EAG) device (van der Pers and Minks 1998) was evaluated for its ability to detect disparlure sprayed for mating disruption in gypsy moth population. The device operated in a manner that was similar to EAG devices in the laboratory (van der Pers and Minks 1998, van der Pers 2001).

In all experiments, no relationship between dose of artificial airborne pheromone and response of gypsy moth antenna was found. The inability to detect differences between airborne pheromone concentrations in the plots treated for mating disruption could be explained by the high variability among antennae and a low concentration of the airborne pheromone used operationally for mating disruption. High variability in antennal response was shown, but, the source of the variability is unknown. Therefore, additional experiemnts need to be conducted to improve the sensitivity of the portable EAG device.

In conclusion, this project supports the need for better understanding of the population dynamics mechanisms specific to low-density gypsy moth populations. It also highlighted the important properties of pheromone sprayed for mating disruption, such as the relationship between the dose of sprayed pheromone and the mating success in low-density gypsy moth population and the effect of distance from the treated area on efficacy of pheromone treatments.

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9. Vita

Ksenia Sergeyevna Tcheslavskaia, daughter of Natalia Borisovna Rosanova and Sergey Borisovich Milman, was born on March 27, 1977 in Moscow, Russia. She attended English specialized school named after V.V. Mayakovsky from 1984 to 1994. In May of 1999, she graduated with honors from Lomonosov Moscow State University with a Master's degree in Zoology (Entomology). While in Moscow State University Ksenia Tcheslavskaia worked as a volunteer in the Genetic lab, Koltzov Institute of Developmental Biology, Russian Academy of Sciences, where she continued working as a lab technician after graduation from Moscow State University. In January 2000, Ksenia moved to Blacksburg, VA to pursue a PhD in Entomology at Virginia Polytechnic Institute and State University.