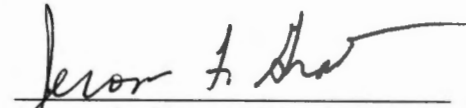


To the Graduate Council:

I am submitting herewith a thesis written by Gary B. Moughler entitled "Biological control agents of the gypsy moth, *Lymantria dispar* L., in Tennessee: Presence, impact, and role in future management programs." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.


Jerome F. Grant, Major Professor

We have read this thesis and
recommend its acceptance:



Accepted for the Council:


Associate Vice Chancellor and
Dean of the Graduate School

**BIOLOGICAL CONTROL AGENTS OF
THE GYPSY MOTH, *LYMANTRIA DISPAR* L., IN TENNESSEE:
PRESENCE, IMPACT, AND ROLE IN FUTURE MANAGEMENT PROGRAMS**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Gary B. Moughler

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ABSTRACT

The gypsy moth, *Lymantria dispar*, is not native to the U.S., but was introduced into Massachusetts in 1869 by Etienne Leopold Trouvelot (Doane and McManus 1981). This insect subsequently escaped into the surrounding forests and is now found throughout the northeastern U.S. The gypsy moth feeds on more than 300 tree species and defoliates an average of 2 million forested hectares per year (Reardon and Hajek 1998). It is estimated that the gypsy moth front will be in Bristol, TN, by 2005 and Knoxville, TN, by 2015 if no efforts are made to slow the gypsy moth (Liebhold 1998). The establishment of the gypsy moth in Tennessee would greatly impact this area. In Tennessee, one-half (5.5 million hectares) of the state is forested, 78% of these forests are composed of oaks and hickories, and 30% are highly susceptible to gypsy moth (Kauffman 1996). In 1995, the forestry industry in Tennessee employed more than 44,000 persons, with an estimated payroll greater than \$827 million (Kauffman 1996). Gypsy moth was first detected in Tennessee in 1972 (Kauffman 1998), and has since been captured in 64 counties throughout the state. However, no studies have been undertaken to examine for the presence of natural enemies of the gypsy moth in Tennessee. Therefore, the specific objectives of this research were to: 1) monitor gypsy moth larval populations and evaluate the presence of *Entomophaga maimaiga*, a fungal pathogen which has caused extensive epizootics of gypsy moth in the northeastern U.S. (Hajek et al. 1995), 2) conduct soil bioassays to assess presence of *E. maimaiga* and other

entomopathogens, and 3) examine for biological control agents (predators and parasitoids of gypsy moth eggs and pupae) in Tennessee.

Although 60 (1998) to 120 (1999) burlaped trees were monitored weekly during 1998 and biweekly during 1999, no gypsy moth larvae were collected at any of the sampling sites. These data are not surprising due to the eradication efforts of the Tennessee Department of Agriculture who quickly treat any infestations found within Tennessee. During this two-year study, 6,600 gypsy moth larvae were exposed to 660 soil and duff samples to assay for the presence of *E. maimaiga*. No *E. maimaiga* was recovered during this study; however, two other fungal pathogens, *Metarhizium anisopliae* and *Beauveria bassiana*, were found to infect gypsy moth larvae (<0.2% of all larvae). Also, more than 30 viable egg masses were collected during winter 1998 from three trees in Cumberland County. No parasitoids emerged from field-collected egg masses. Lastly, between 26 August and 24 September 1999, 123 ants (representing 5 genera) were collected from both pupae and egg masses in the field. Of 330 field-placed, freezer-stored pupae, 79 (24%) were damaged by predators. Of the 330 egg masses placed in the field, 112 (34%) were damaged. The most commonly collected ant genera were *Aphaenogaster* (43%), *Paratrechina* (42%), and *Camponotus* or carpenter ants (11%).

Further effort should be taken to monitor for gypsy moth larvae along with associated parasitoids or pathogens in the field. Testing of soil and duff should be continued to assess for the presence of *E. maimaiga* into Tennessee. If this fungal pathogen is not recovered, release of resting spores should be made in areas where

outbreak populations of gypsy moth occur and efforts should be undertaken to recover *E. maimaiga*. Lastly, monitoring of predators and parasitoids of gypsy moth pupae and eggs is needed to determine seasonality, diversity, and impact of species involved. If permanent gypsy moth populations become established and widespread, eradication programs will no longer be economically feasible. Such research will enable managers to develop appropriate management strategies that may provide long-term reductions of gypsy moth populations and protect Tennessee's forests.

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CHAPTER I

LITERATURE REVIEW

The gypsy moth, *Lymantria dispar* L., is not native to the U.S., but was introduced in 1869 from Europe by a French scientist, Etienne Leopold Trouvelot, who wanted to breed it with silk moths to produce a commercial source of silk. During this time, cotton was unavailable to the Union States during and shortly after the Civil War (Gerardi and Grimm 1979). Unfortunately, these moths escaped Trouvelot's house in Medford, MA, and by 1889, had defoliated more than 550 square kilometers of trees and forests (Nichols 1962). With a wide host range, and no natural predators or diseases to regulate populations, the number of gypsy moths increased quickly throughout the surrounding countryside.

The gypsy moth has since become one of the most serious defoliators of hardwood trees in the northeastern U.S. Since 1980, the gypsy moth has defoliated close to one-half million or more forested hectares annually. In 1981, a record 5.2 million hectares were defoliated, an area larger than Rhode Island, Massachusetts, and Connecticut combined (McManus et al. 1989). This insect feeds on more than 300 tree and shrub species (Doane and McManus 1981). In the eastern U.S., commonly preferred tree species include oaks, apple, sweetgum, speckled alder, basswood, gray and white birch, poplar, willow, and hawthorn; older larvae will feed on several other species including cottonwood, hemlock, and southern white cedar (McManus et al. 1989). Gypsy

moth has not been recorded to feed upon ash, yellow-poplar, sycamore, flowering dogwood, balsam fir, mountain laurel, and rhododendron (McManus et al. 1989).

With many host species in the northeastern U.S., the gypsy moth has spread as far west as Michigan and Indiana and as far south as Virginia (Fig. 1) (Liebhold 1998). In 1997, the edge of the generally infested zone remained north of Roanoke, VA, approximately 240 km from Tennessee, and catches of single male moths were still a common occurrence three counties to the south in the Blacksburg/Christiansburg area, 200 km from Bristol, TN (Kauffman 1996). The spread of the gypsy moth, however, is expected to continue, and if no successful control effort is made, the gypsy moth front is predicted to reach Bristol, TN, by 2005 and Knoxville, TN, by 2015 (Fig. 2) (Liebhold 1998). The gypsy moth front is defined as the boundary between gypsy moth infested and non-infested localities throughout the U.S.

As the gypsy moth moves closer to Tennessee, concern over the establishment of gypsy moth populations in the state has grown. A 1990 Forest Inventory Analysis by the USDA Forest Service classified 30% of Tennessee's hardwoods as highly susceptible and vulnerable to gypsy moth (Kauffman 1996). More than one-half of Tennessee is forested (5.5 million hectares), and 78% of these forests are composed of oaks and hickories, favorite food items of the gypsy moth. West Virginia has already felt the impact of the gypsy moth. With forested land composed of 80.7% oaks, similar to Tennessee (where 3.8 million hectares of total timberland are grown), gypsy moth caused a 17 million dollar loss, not including control costs, to the forestry industry in West Virginia in 1991

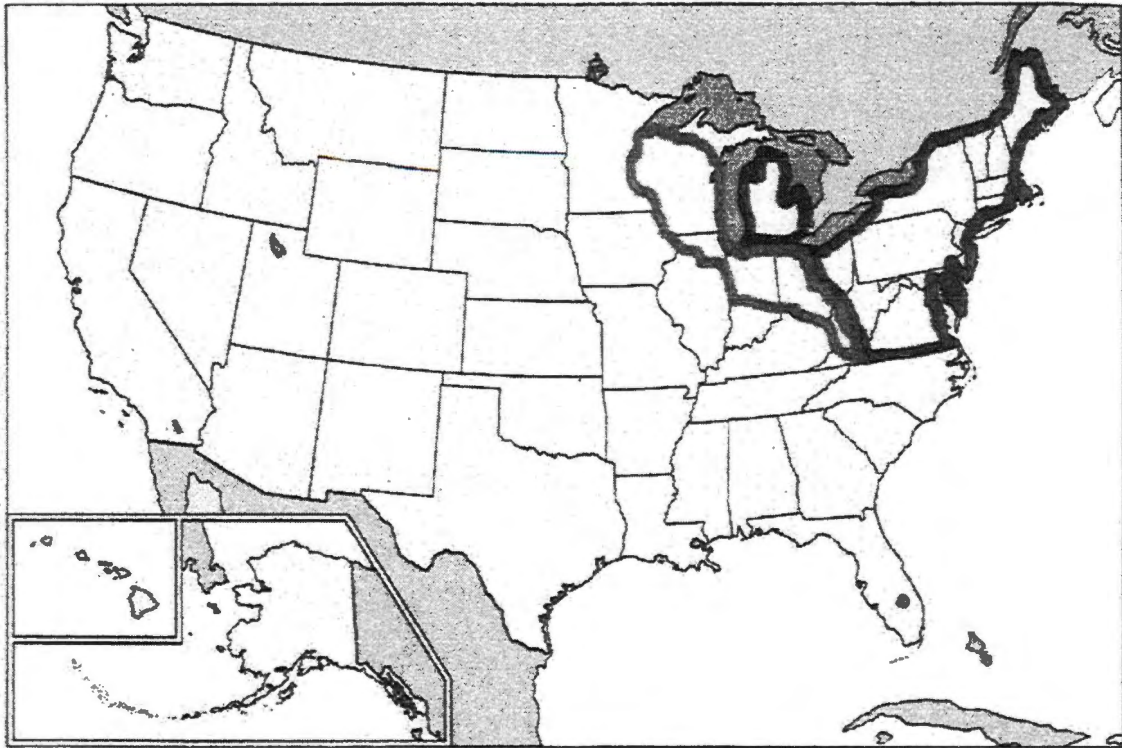


Figure 1. Distribution of gypsy moth in 1997 throughout the U.S. Black borders indicate areas infested with gypsy moth, while gray borders show localities where gypsy moths were commonly captured.

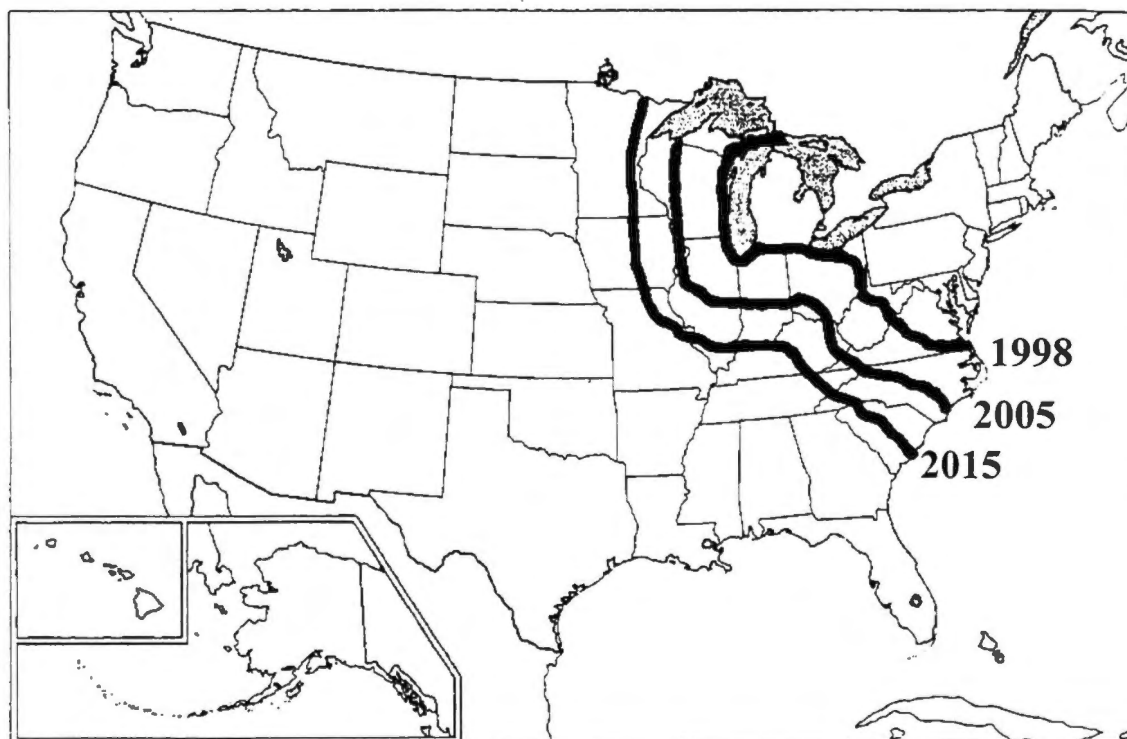


Figure 2. Expected spread without using methods to control gypsy moth. Gypsy moth is expected to reach Bristol, TN, by 2005, and Knoxville, TN, by 2015 (adapted from Liebhold 1998).

(Kauffman 1996). If no control measures against the gypsy moth had been implemented, losses would have reached 2.3 billion dollars during the first infestation.

To prevent similar monetary losses in Tennessee, the protection of its forests are vital to the state's economy. In 1990, Tennessee forests had an industrial value of four billion dollars according to a Tennessee Division of Forestry (TDF) Forestry Industry Survey (Kauffman 1996). Tennessee is among the leaders in the nation in lumber production and manufacturing of secondary wood products, such as cabinetry, furniture, hardwood flooring, and log homes (Kauffman 1996). The forestry industry within Tennessee, as of 1995, employed more than 44,000 persons, with an estimated payroll greater than \$827 million. This industry has generated more than 9,000 new jobs from 1991 to 1995, representing an investment greater than one billion dollars according to the Tennessee Department of Economics and Community Development (Kauffman 1996). The estimated value of standing timber alone within the state is greater than 190 million dollars (Kauffman 1996).

The forestry industry in Tennessee will be threatened once gypsy moth infests Tennessee and defoliates large areas of land. Trees with greater than 50% defoliation are more susceptible to disease and drought, resulting in damage to hardwoods and ultimately death (McManus et al. 1989). A healthy tree can withstand defoliation by the gypsy moth 1 or 2 years in a row by producing new leaves before midsummer (McManus et al. 1989). However, repeated defoliations by gypsy moth populations result in skeletal forests in the summer. These skeletonized areas are not aesthetically pleasing and threaten both the forestry industry and tourism.

Gypsy moths not only threaten Tennessee hardwoods, but they also negatively impact the environment. Plant composition in infested areas may be altered, as tree mortality allows other plant species to replace those trees which died (Gottschalk 1990). Such alterations may negatively impact species within these communities. Invertebrates, such as beetles, flies, wasps, various pollinators, and other species, may experience short term losses; however, diversity of invertebrates may increase due to the addition of various plants (Thomas 1995). Tree mortality and the addition of new plant species can result in fewer acorns causing a reduction of mast, or food material, for vertebrates like the black bear. In the future, the presence of gypsy moth could affect wildlife populations in Tennessee.

Vertebrate populations are not the only organisms that are affected by gypsy moth populations. Heavy gypsy moth defoliation has been shown to increase water temperature due to penetration from sun, which can have a detrimental effect on aquatic plants, animals, and arthropods (Sheath et al. 1986). Defoliation by the gypsy moth also may contribute to alterations in water chemistry and has been shown to decrease a stream's capacity to neutralize acids (Downey 1991). A reduction in acids can lead to increased concentrations of hydrogen and aluminum, which at elevated levels are toxic to fish and other aquatic organisms.

Gypsy moths also can have a negative impact in watershed areas through an increase in organic matter from frass and leaf fragments, which can lead to over-enrichment and excessive growth of algae and other microorganisms. Large amounts of fecal coliform and streptococci densities have been observed in streams where heavy

gypsy moth defoliation has occurred (Corbett and Lynch 1987). Defoliation also is suspected of causing increased nitrate mobility, transferring nutrients from vegetation to the soil surface (Eagle 1993).

Gypsy moths are not only damaging to the environment, but are an annoyance to people. Annoyances such as these are not new, but have occurred since the release of the gypsy moth. In July 1889, the people of West Medford, MA, began to complain about the gypsy moth. In the warm, still summer nights, a sickening odor arose from the masses of larvae and pupae in the woods and orchards, and a constant shower of excrement that fell from the trees (Forbush and Fernald 1896). Larvae also were recorded to fall onto people, sidewalks, and roads, and similar stories can be heard today.

To date, Tennessee has been on the forefront in forest protection against the gypsy moth. Between 1992 and 1996, annual program costs have been as high as \$815,486 and as low as \$235,240 to eradicate known populations of gypsy moth (Kauffman 1996). Program costs should stabilize around \$200,000 to \$300,000 once the current state-wide trapping program becomes established. Currently, such eradication programs are feasible. However, the gypsy moth front will eventually reach Tennessee, due to the ability of the gypsy moth to spread into new areas.

Gypsy moths can spread easily due to their methods of dispersal. Larvae can suspend from trees by strands of silk and be blown for distances of up to 1.5 km (McManus et al. 1989). Gypsy moths can be spread greater distances when egg masses are transported by humans. Eggs (the overwintering stage) are laid in late July to early August, usually in protected areas, such as the underside of branches, or in hollow areas

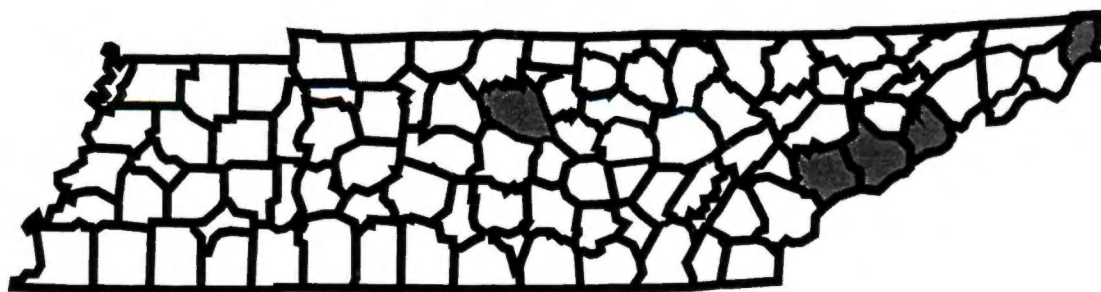
of trees. However, females, which are incapable of flight, also will lay their eggs on any suitable substrate, including vehicles, campers, lawn chairs, toys, etc. Once these infested objects are transported away from their original location, the eggs hatch, and gypsy moth larvae can infest a new area when favorable environmental conditions exist. Eggs usually hatch in late May to early April, and five to six instars feed upon suitable host species until late June, when larvae pupate. The pupal stage usually lasts 2 weeks, and adults emerge during early to mid July (McManus et al. 1989). These adults live only 2 weeks; their only purpose is to mate and oviposit. The life cycle is continued, and if introduced into the right type of area, with suitable host trees to feed upon, the gypsy moth can increase in numbers and spread further into new areas within the U.S.

To prevent or slow the establishment of gypsy moth populations in Tennessee, the Tennessee Department of Agriculture (TDA) and the Animal and Plant Health Inspection Service (APHIS) eradicate any localized infestations of gypsy moth. To detect populations of gypsy moth, pheromone traps are strategically placed throughout Tennessee and monitored regularly for incidence of gypsy moth adult males. Pheromone traps contain a synthetic pheromone called disparlure - a chemical similar to the sexual pheromone exuded by the female. This pheromone attracts males who enter the traps and become entangled on the trap surface. TDA began to survey for gypsy moth adults in the mid 1960s by placing traps on selected properties that were suspect for infestations, such as mobile homes and personal property of those who had moved from the northeastern U.S. In 1967, the first gypsy moth traps used in Tennessee were placed in 10 counties, and Tennessee's battle against the gypsy moth had begun (Kauffman 1998).

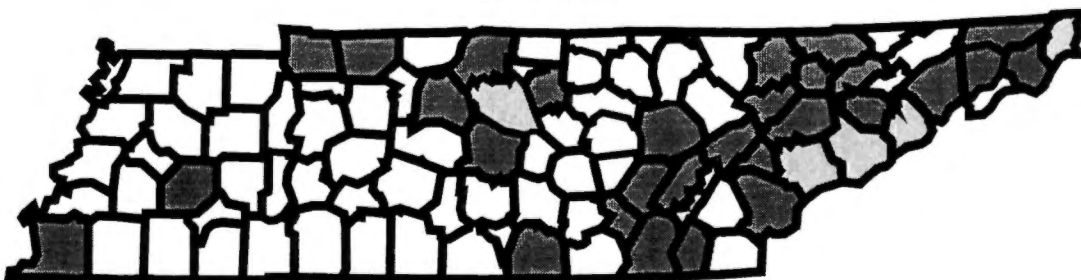
On 27 July 1972, the first gypsy moth was captured in Tennessee in Sevier County, and 2 days later a second moth was captured in Cocke County. During that decade, 12 adult male gypsy moths were found in five counties (Fig. 3). Unfortunately, gypsy moth has become more familiar throughout the state; during the 1980s, gypsy moth was found in 34 counties (29 new counties), and during the 1990s, it was found in 61 counties (30 new counties). Over the years, gypsy moths have been captured in 64 counties throughout the state (Fig. 3), from as far west as Shelby County and as far east as Johnson County. The actual numbers of moths caught per year have varied from 0 in 1975 and 1976 to 6,664 in 1984 (Fig. 4). During 1999, 159 moths were captured statewide.

Gypsy moth trapping is a vital component of Tennessee's eradication program. In areas where many gypsy moths are captured, state and federal officials can examine the area for egg masses to better identify the exact location of populations. These egg mass surveys are usually conducted in areas that have high densities of gypsy moth adults, based on data accumulated during the previous summer's gypsy moth trapping program. In 1983, a highly dense area was discovered when 664 moths were captured, mostly in the southern portion of Johnson County. Egg mass surveys located several dozen egg masses, and plans were developed to initiate treatment of 4,960 hectares the following spring. In 1984, 2,271 traps were placed throughout Johnson County, and 6,633 moths were captured, 66 of these were captured within the treated area, suggesting that the

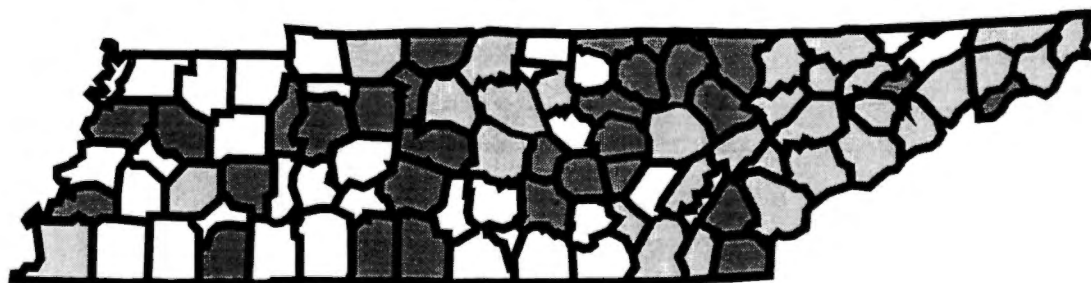
1972-1979



1980-1989



1990-1999



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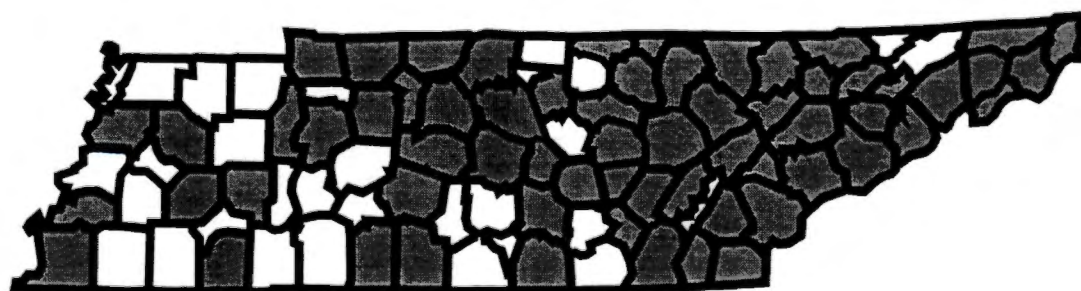


Figure 3. Counties where gypsy moths have been collected in pheromone traps during three decades (1972-1999) in Tennessee [dark shadings within a decade represent new county records].

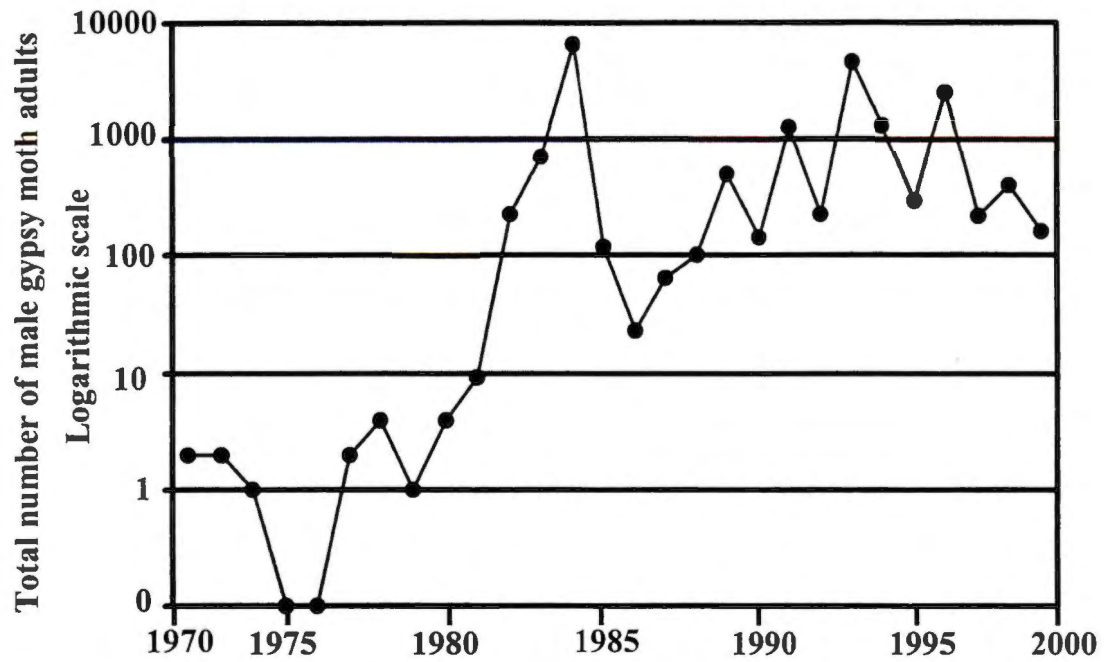


Figure 4. Number of male gypsy moths captured in pheromone traps in Tennessee from 1972-1999 [although trapping began in 1967, the first adult moths were recorded in 1972].

infestation was larger than estimated (31 moths were captured throughout the rest of the state). Officials were then able to treat a larger area (19,200 hectares) to eradicate gypsy moth populations. In 1985, no gypsy moths were captured in the treated area, and only 36 were captured throughout the county in 33 traps. Since 1984, 13 counties (Table 1, Fig. 5) have been targeted for similar eradication programs.

Tennessee has not been alone in the fight against the gypsy moth. State and federal agencies throughout the U.S. have provided monies for gypsy moth management since the late 1800s. In 1890, the legislature of Massachusetts appropriated \$25,000 for control of this pest (Doane and McManus 1981), and today, control programs can cost thousands of dollars. Massachusetts was the first state to treat for gypsy moth, and began by spraying an arsenical, Paris green (Forbush and Fernald 1896). Chemical treatments also were supplemented with other techniques, such as applying creosote to egg masses, burning infested trees and shrubbery and clusters of larvae, and banding trees with burlap and sticky materials to either trap the larvae or prevent their ascent of the trees (Doane and McManus 1981). During the years that Paris green was used, new chemicals were under development.

In 1893, lead arsenate was developed, and for 50 years, it was the standard pesticide for use against gypsy moth (Forbush and Fernald 1896). However, toxicity and application problems led to further research for new chemicals. In 1939, dichloro-diphenyl-trichloro-ethane (DDT) was discovered to have insecticidal properties, and in 1944, the U.S. War Department allotted approximately 45 kg of DDT for testing as a gypsy moth control agent (Doane and McManus 1981). During its years of use, DDT

Table 1. Counties, and representative years, when eradication programs against the gypsy moth have been conducted since 1984.

Counties	Years Treated
Blount	1994
Carter	1994, 1996
Cumberland	1999
Grainger	1994, 1995
Johnson	1984, 1985
Overton	1998
Rhea	1991, 1992, 1995, 1996
Scott	1997, 1999
Sevier	1990
Sequatchie	1990, 1993
Sullivan	1993, 1996, 1997
Union	1994, 1995, 1996
Washington	1993

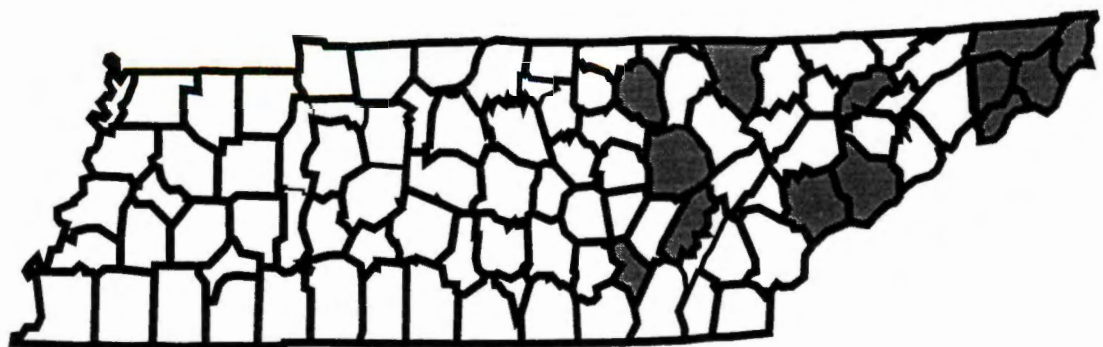


Figure 5. Counties where eradication programs against the gypsy moth have been conducted since 1984.

proved to be effective against the gypsy moth; just 1.13 kg per hectare provided 100% gypsy moth control (Nichols 1962). DDT had a “double action” effect, acting both as a stomach poison and a contact poison.

In the 1960s, Rachel Carson’s (1962) *Silent Spring*, however, gave the public an awareness of the harmful effects of pesticides. In December 1972, the Environmental Protection Agency (EPA) canceled most uses of DDT, due to its negative effects on the environment. Another pesticide, Sevin® (a carbamate), which was introduced in 1958, is still used today in aerial application treatment programs. However, it is highly toxic to honeybees (Doane and McManus 1981), and managers need to exercise caution in using this chemical. Another common pesticide that is used today is the insect growth regulator diflubenzuron (Dimilin®). Dimilin® is nontoxic to mammals, birds, and fish; however, it does affect other invertebrates by acting as a growth regulator, not allowing them to molt properly, eventually resulting in death.

As early as the late 1950s and 1960s, public pressure encouraged the investigation into biological control agents. Biological control is the reduction of pest populations by parasitoids, predators, and pathogens (Metcalf and Luckmann 1994). Biological control of the gypsy moth was not a new idea, but had been studied since the nineteenth century. More than 60 species of natural enemies, including parasitoids, predators, and pathogens, have been introduced into the U.S. from 1906 to present (Hoy 1976). Today, only 11 parasitoids (Table 2), one predator (*Calosoma sycophanta* F.), and two pathogens (nucleopolyhedrosis virus [NPV] and *Entomophaga maimaiga* Humber, Shimazu and

Table 2. European and Asian parasitoids of the gypsy moth that are established in North America, taken from Kenis and Vaamonde (1998).

Gypsy moth life stage attacked	Parasitoid species	Order: Family
Egg	<i>Anastatus japonicus</i> Ashmead (= <i>disparis</i> Rushka)	Hymenoptera: Eupelmidae
	<i>Ooencyrtus kuvanae</i> (Howard)	Hymenoptera: Encyrtidae
Larva	<i>Belepharipa pratensis</i> (Meigen)	Diptera: Tachinidae
	<i>Compsilura concinnata</i> (Meigen)	Diptera: Tachinidae
	<i>Cotesia melanoscelus</i> Ratzeburg	Hymenoptera: Braconidae
	<i>Exorista larvarum</i> (L.)	Diptera: Tachinidae
	<i>Parasetigena silvestris</i> (Rob.-Desv.)	Diptera: Tachinidae
	<i>Phobocampe disparis</i> (Viereck)	Hymenoptera: Ichneumonidae
Pupa	<i>Brachymeria intermedia</i> (Nees)	Hymenoptera: Chalcididae
	<i>Monodontomerus aereus</i> Walker	Hymenoptera: Torymidae
	<i>Pimpla disparis</i> Viereck	Hymenoptera: Ichneumonidae

Soper) of the gypsy moth have become established in North America (Doane and McManus 1981, Schaefer et al. 1989, Hajek et al. 1993).

Numerous studies have been conducted to assess native and introduced parasitoids as potential biological control agents against the gypsy moth (Burgess and Crossman 1929, Dowden 1962, Hoy 1976, Clausen 1978). Parasitoids have been instrumental in control programs directed against the gypsy moth. These insects require another host (such as gypsy moth eggs, larvae and pupae) to complete their life cycle. Research investigating the potential use of gypsy moth parasitoids began in 1905 when L.O. Howard sailed to Europe to evaluate potential natural enemies for introduction into the U.S. (Doane and McManus 1981). Today, 11 parasitoids have become successfully established in the U.S. (Table 2).

Predators also are a major component of the biological control process. Numerous predators, such as birds, mammals, and invertebrates, have been observed to feed upon gypsy moth. Several species of birds, such as red-eyed vireo, *Vireo olivaceus* L., catbird, *Dumetella carolinensis* L., and robin, *Turdus migratorius* L., have been documented to feed upon gypsy moth larvae, pupae, and adults (for a more complete list see Doane and McManus 1981). Blue jays, *Cyanocitta cristata* L., have been observed to feed upon adults (personal observation). Mammals documented to feed upon gypsy moth larvae and pupae include the white-footed mouse, *Peromyscus leucopus* Ranzesque, and the shorttail shrew, *Blarina brevicauda* Say (Doane and McManus 1981).

In the past invertebrate predators have been given little attention (Burgess and Crossman 1929, Campbell 1975, Furuta 1977), but are becoming more recognized as an

important part of natural control of gypsy moth. One of the most successful invertebrate predators is the ground beetle *C. sycophanta* which was imported from Europe in 1906 (Gerardi and Grimm 1979). Native invertebrates, such as carpenter ants, *Camponotus pennsylvanicus* De Geer and *C. ferrugineus* F., and harvestmen, also have been observed to feed upon gypsy moth and may have considerable potential predatory benefit (Doane and McManus 1981).

Another important part of the biological control process involves natural diseases, which can be caused by viruses, bacteria, and fungi. By far the most devastating disease of the gypsy moth is a host specific NPV which affects the larval and pupal stages of gypsy moth (Podgwaite 1979). Larvae feeding on contaminated leaves ingest the NPV occlusion bodies. Rod-shaped virus particles are freed as the polyhedral protein matrix dissolves within the larval gut (Reardon et al. 1996). Once inside the larva, the virus particles penetrate the gut wall and attack internal tissues and organs resulting in death. During the 1950s, the USDA Forest Service initiated research to examine the feasibility of developing this virus into a natural chemical insecticide for use against the gypsy moth. In April 1978, this virus, formulated into a product known as Gypchek®, was registered by the EPA as a general use insecticide for aerial and ground application in management programs against the gypsy moth (Reardon et al. 1996). Gypchek® is currently used in eradication programs.

The only other biological insecticide currently used against gypsy moth is the effective microorganism *Bacillus thuringiensis* Berliner (Bt). Bt normally occurs in the soil, but is applied by helicopter or airplane to induce epizootics within the gypsy moth

population. After aerial application, larvae feed upon Bt-contaminated leaves.

Formulations of Bt contain a crystal parasporal body which contains a toxic protein called the delta-endotoxin (commonly referred to as the crystal) (Doane and McManus 1981).

Once in the gut, these crystals dissolve and break down into smaller toxic structures that cause gut paralysis preventing the larva from eating and ultimately leading to starvation and death (Podgwaite 1979). Bt is popular in management programs today because it is effective in reducing gypsy moth populations and it is nontoxic to humans. However, Bt generally impacts a wide spectrum of Lepidoptera, and research has demonstrated that total abundance and species diversity of non-target lepidopteran species may be reduced for 2-3 years post-treatment (Miller 1990).

A fungal pathogen infecting dead gypsy moth larvae was discovered in Japan in the early 1900s (Hajek et al. 1995). After two diseased larvae were imported into the U.S. from Japan, an inoculum was used to infect large numbers of North American gypsy moth larvae for field release in 1910 (Hajek et al. 1995). Infected larvae were released throughout six sites near Boston, MA. In 1910 and 1911, no fungal infections were recovered from field-collected larvae at these release sites. Research on this fungal pathogen was terminated after an epizootic of the NPV decimated the gypsy moth colonies used for the studies.

In 1984, Soper and Shimazu (Hajek et al. 1995) isolated a similar fungus from Japanese gypsy moth, *Lymantria dispar japonica* (Motschulsky), in Ibaraki Prefecture, and imported it into the U.S., after fungi from 1910 field releases could not be discovered among any gypsy moth populations. This fungus was considered to belong to the same

genus as the fungal pathogen that was released in 1910, but based on host specificity, biogeographic distribution, and allozyme patterns, the Japanese pathogen was described as a new species, *E. maimaiga* (Soper et al. 1988). The fungus released in 1910 was not identified to species prior to release, and because of the loss of host colonies, specific identification was not feasible.

In 1984, a small-scale field release of this fungus occurred in New York and Virginia (Hajek et al. 1995). Collections from these sites were conducted during 1987, and from 1989 to 1991, and no *E. maimaiga* was recovered (Hajek et al. 1995). In 1989, abundant disease mortality among gypsy moths in the northeastern U.S. led to the dissection of larval cadavers by both USDA Forest Service and Connecticut Agricultural Experiment Station researchers who found entomophthoralean spores which were later proven to be *E. maimaiga* (Hajek et al. 1995). During 1989, *E. maimaiga* was identified as responsible for causing fungal epizootics of the gypsy moth populations in Connecticut, Massachusetts, Vermont, New Hampshire, New Jersey, New York, and Pennsylvania (Hajek et al. 1990). *E. maimaiga* also was recovered in Maine, Delaware, Maryland, and southern Ontario in 1990 (Hajek et al. 1995).

In 1991, *E. maimaiga* resting spores also were released in Pennsylvania, West Virginia, Virginia, and Maryland. Infections were recovered in 28 of the 34 release sites (Hajek et al. 1995). In 1992, additional resting spores were released in seven plots in Virginia and West Virginia. High infection levels were found at nearly all release sites for both 1991 and 1992 (Hajek et al. 1995). By July 1992, the fungus had spread extensively

and was documented throughout the release areas, as well as in many additional areas (Hajek et al. 1995).

Entomophaga maimaiga overwinters as an azygospore (resting spore) which is produced within cadavers of the gypsy moth from the previous spring (Hajek et al. 1995). When favorable environmental conditions exist, they can germinate into one to several germ conidia. Germination occurs during spring, usually 1 to 2 days after rainfall (Weseloh and Andreadis 1992a). During germination, conidia are discharged and must contact a suitable host (gypsy moth) for their survival. If they do not land on a suitable host, these asexual spores germinate to actively eject secondary conidia (Hajek et al. 1993). This process can be continued until quaternary conidial production. When conidia land on a suitable host, enzymes enable this fungus to penetrate the cuticle (Reardon and Hajek 1998). Protoplasts are then formed which utilize the nutrients in the body, invade the vital organs, and slowly starve the host. After death of the larva, hyphal bodies form in the hemolymph (Reardon and Hajek 1998), grow through the integument, and form conidiophores which actively discharge more conidia. These short-lived conidia can infect a host immediately. However, if no host is contacted, or if unfavorable environmental conditions exist, resting spores are produced within the cadaver and remain dormant in the soil until the next spring.

A likely scenario for the development of epizootics of this fungus in gypsy moth populations is given by Weseloh and Andreadis (1992b). First, the fungus overwinters as resting spores within dried cadavers or leaf litter. Then the gypsy moth larvae that are in the environment are dispersed by wind and many fall to the ground. This behavior

exposes many of the larvae to existing germinating resting spores that are in the soil and infection soon follows. Resting spores are responsible for production of conidia that cause the primary infections (Hajek et al. 1993) and are usually the cause of death of earlier instars. These infected larvae will climb the tree to feed upon foliage, where after death, they serve as a secondary source to infect other gypsy moth larvae due to ejected conidia. This mode of secondary infection also is the major cause of death of late instars.

Entomophaga maimaiga is spreading throughout the northeastern U.S. (Hajek et al. 1995). This fungal pathogen is believed to be the same species as the one introduced through the biological control efforts in 1910 against the gypsy moth. However, scientists are unsure if the current infestations caused by *E. maimaiga* are the result of its introduction in the early 1900s, or from releases that occurred in the late 1980s and 1990s. Currently, research examining dispersal, spread, and epidemiology of this fungus is under investigation, and recent research has shown that a possible six-year germination period of the resting spores aid in the survival of this fungus (Weseloh and Andreadis 1997). *E. maimaiga* is fairly host specific (Hajek et al. 1996), and many northeastern states have already discovered that *E. maimaiga* is an important biotic factor in regulating gypsy moth populations.

This fungus is usually detected through field collection of gypsy moth larvae. Collected larvae are taken to the laboratory, where they are monitored and bioassayed for *E. maimaiga*. One method for collecting larvae involves the use of burlap, which is placed around tree trunks. In their natural environment, larvae feed on leaves of host trees, but during the warmer parts of the day, they seek shelter from the sun and move down the tree

trunk. Burlap placed around the trunk provides a suitable site for larvae to congregate. Larvae can then be easily removed and collected from underneath the burlap. This fungus can also be detected using soil sampling and analysis. Studies using soil and litter samples have been utilized by Weseloh and Andreadis (1992a) as a tool to study the germination of resting spores of *E. maimaiga*.

Extensive research to assess *E. maimaiga* has been conducted in the northeastern U.S. (Elkinton et al. 1991); however, little information is available on the status of this fungal pathogen in the southeastern U.S. Although gypsy moth populations are not yet established in Tennessee, isolated outbreaks occur periodically. The TDA along with federal agencies have an active eradication program against gypsy moth in Tennessee. The Great Smoky Mountains National Park and other areas in eastern Tennessee are popular tourist destinations and contain suitable hosts, especially oaks, for gypsy moths. Although *E. maimaiga* has not been documented in Tennessee, the high number of tourists visiting this state may have introduced this pathogen into forests through contaminated footwear or transportation of infected gypsy moth larvae.

Because of the lack of information on natural enemies (such as *E. maimaiga*, parasitoids, and predators) of gypsy moth in Tennessee, a two-year research project was initiated to assess their presence in selected areas of the state that had previously experienced gypsy moth populations. Gypsy moth populations are scarce in the state, and eradication efforts are implemented quickly to prevent their spread. Thus, few larvae may be available for evaluation. Testing of Tennessee soils also will be necessary to fully evaluate the presence of *E. maimaiga* in the state. The placement and evaluation of

freezer-stored egg masses and pupae in Tennessee forests could provide vital information as to what insects, if any, may contribute to natural control of gypsy moth. Specific objectives of this research were to: 1) monitor gypsy moth larval populations and evaluate the presence of *E. maimaiga* and other natural enemies, 2) conduct soil bioassays to assess presence of *E. maimaiga* and other entomopathogens, and 3) examine for biological control agents (predators and parasitoids of gypsy moth egg masses and pupae) in Tennessee.

CHAPTER II

MATERIALS AND METHODS

This research was initiated to assess the presence of biological control agents (fungal pathogens, predators, and parasitoids) of the gypsy moth in Tennessee. The fungal pathogen *E. maimaiga* is usually detected through the collection of gypsy moth larvae in the field. However, gypsy moth populations are scarce in Tennessee, and, once infested, TDA quickly implements eradication efforts to prevent their spread. Thus, few field-collected larvae may be available. Therefore, testing of Tennessee soils was necessary to fully evaluate the presence of *E. maimaiga* in the state. Parasitoids and predators also were monitored; however, due to the low number of field-collected, naturally-occurring pupae and egg masses in Tennessee, freezer-stored pupae and egg masses were placed in the field to observe for these biological control agents.

Larval Sampling

To assess the presence of gypsy moth larvae in Tennessee, six sampling areas were chosen in 1998 with the cooperation of TDA (Fig. 6, Table 3). These areas had previously experienced gypsy moth infestations. These localities were chosen on the basis that larval populations existed in the past; therefore, populations may still be present today, or biological control agents may remain from previous infestations. In 1999, three sites were added due to their high gypsy moth catches during the 1998 trapping season (Fig. 6 and 7, Table 4).

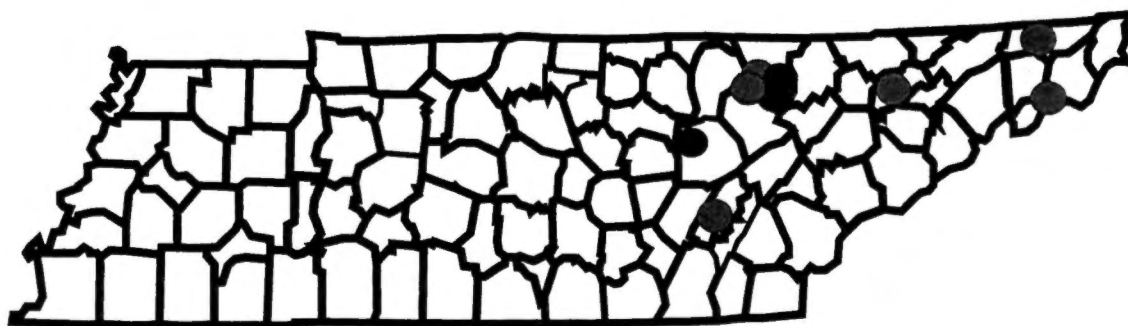


Figure 6. Localities identified as areas of previous gypsy moth activity in Tennessee [● - burlaped in 1998 and 1999; ● - burlaped only in 1999].

Table 3. Locations of sites burlaped and monitored for gypsy moth larvae in Tennessee in 1998 (10 trees were burlaped at each site).

Site	County	GPS	Location	Discovery of infestation	Year treated
1	Grainger	N 36°14.45 W 83°37.83	Private Residence	1993	1994, 1995
2	Rhea	N 35° 30.12 W 85° 06.98	Beside Cemetery	1990	1991, 1992, 1995, 1996
3	Scott	N 36° 22.99 W 84° 29.18	Beside New River Road	1998	1999
4	Scott	N 36° 20.20 W 84° 37.44	Private Residence	1996	1997
5	Sullivan	N 36° 31.93 W 82° 14.76	Avoca Elementary School	1995	1996, 1997
6	Unicoi	N 36° 14.25 W 82° 16.35	Private Residence / Nat. Forest	1993	1994, 1995, 1996

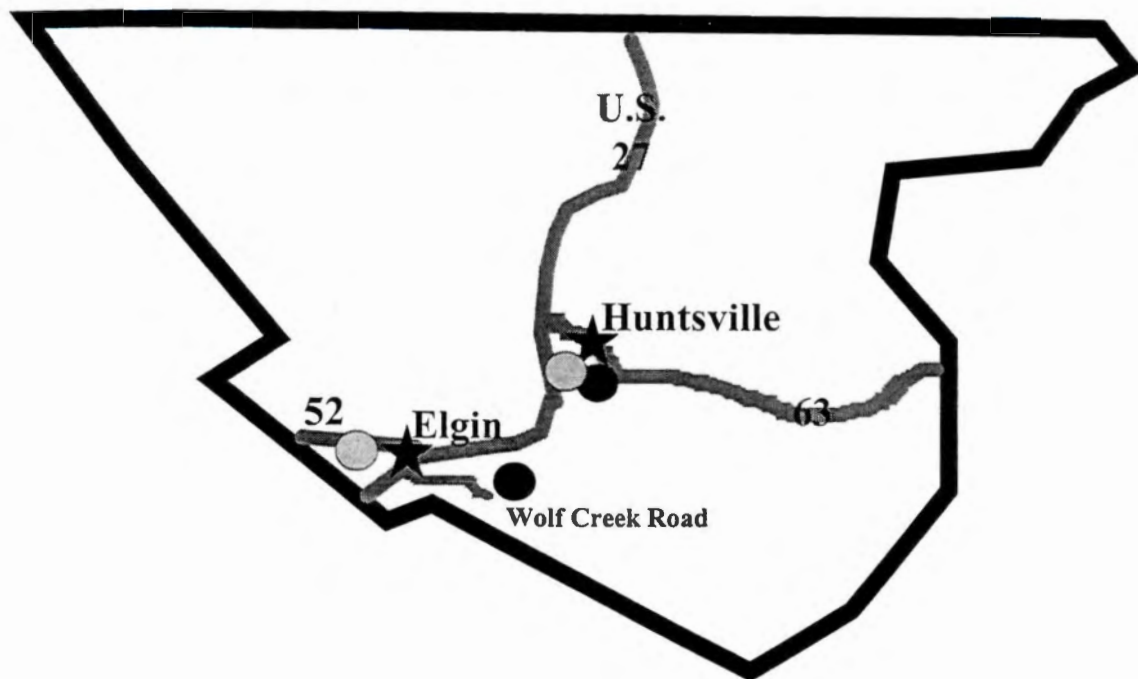


Figure 7. Locations of four research plots in Scott County, TN, 1999. This county was intensively sampled due to high adult gypsy moth catches and multiple life stages found during recent years [● - 1998 and 1999; ● - 1999 only].

Table 4. Locations of sites burlaped and monitored for gypsy moth larvae in Tennessee in 1999 (10 to 25 trees were burlaped at each site).

Site	County	GPS	Location	Discovery of infestation	Year treated
1	Grainger	N 36°14.45 W 83°37.83	Private Residence	1993 ^b	1994, 1995
2	Rhea	N 35° 30.12 W 85° 06.98	Beside Cemetery	1990	1991, 1992, 1995, 1996
3	Scott	N 36° 22.99 W 84° 29.18	Beside New River Road	1998	1999
4	Scott	N 36° 20.20 W 84° 37.44	Private Residence	1996 ^b	1997
5	Sullivan	N 36° 31.93 W 82° 14.76	Avoca Elementary School	1995	1996, 1997
6	Unicoi	N 36° 14.25 W 82° 16.35	Private Residence / Nat. Forest	1993	1994, 1995, 1996
7	Cumberland ^a	N 36° 07.65 W 85° 04.84	Private Residence	1998 ^b	1999
8	Scott ^a	N 36°23.32 W 84° 29.49	Beside New River	1998	1999
9	Scott	N 36° 18.37 W 84° 33.66	Beside Wolf Creek Road	1998	1999

^a 25 trees burlaped within the site; 10 trees were burlaped at all other sites.

^b Sites containing trees with non-viable gypsy moth egg masses from previous infestations.

In 1999, more research sites were located in Scott County than any of the other counties (Fig. 6 and 7). Intensive sampling in Scott County resulted from 2,309 male moths captured at a rural residence in 1996. Examination of the area revealed hundreds of egg masses on one white oak tree. Scott County has since been the focus of gypsy moth detection and eradication efforts using insecticidal treatments which were conducted in 1997 and 1999. Since 1996, gypsy moth adults have been captured every year: 97 moths in 1997, 224 moths in 1998, and 11 moths in 1999 (Kauffman, personal communication). The four research sites that were chosen in Scott County were localities where gypsy moths had been captured during the last four years.

Once a site was chosen, binoculars were used to search for egg masses (from previous gypsy moth populations). After females mate, egg masses are usually laid in areas protected from the elements (wind, rain, etc.), which include the hollowed-out areas of trees and the underside of tree branches. These areas were examined, and any trees discovered with egg masses (Table 4) were burlaped, in the event that any of the egg masses might be viable. Next, any other suitable host species, preferably oaks, were burlaped, until 10 trees were burlaped at each site. In 1999, two sites, in Cumberland (Site 7) and Scott (Site 8) Counties, had 25 trees burlaped per site (Table 4), due to high moth catches in 1998 and upon request by TDA officials.

In spring 1998, burlap was acquired from the University of Tennessee's Department of Ornamental Horticulture and Landscape Design. A burlap strip (0.9 m tall) was wrapped around each tree at a height of 1.4 m, secured in the middle with sisal

binder twine, and folded over the twine. In 1999, burlap from the previous year was reused, and additional burlap was purchased from a local nursery.

Burlap was placed in the field by 9 May 1998 and 11 May 1999. Burlap was monitored weekly in 1998, and biweekly in 1999 until the first week of July, when it was removed during both years. Monitoring included lifting burlap away from the tree and examining all of its surface area for the presence of gypsy moth larvae and/or pupae. Monitoring ended and burlap was removed after gypsy moth flight had begun (as monitored by TDA). Larvae and/or pupae that were collected would be taken to the laboratory in plastic rearing cups (118 ml) and monitored for *E. maimaiga* or associated parasitoids.

Soil and Duff Sampling

Studies using soil and leaf litter samples have been previously utilized as a tool to study *E. maimaiga* (Weseloh and Andreadis 1992a). To assess the presence of *E. maimaiga* in Tennessee soils, nine areas were chosen in 1998 from those identified by Tennessee Department of Agriculture officials as areas previously infested by gypsy moth (Fig. 8, Table 5). By late 1998, three sites (two in Scott County and one in Cumberland County) were added due to gypsy moth catches during the 1998 trapping season (Fig. 8, Table 6). At each respective site, 10 soil and 10 duff samples were collected during spring 1998 (29 May - 8 June), fall 1998 (13 - 22 December), and spring 1999 (29 May - 7 June).

Samples were preferably taken near the base of a tree burlaped the previous spring. If samples were collected from sites that had not been burlaped, 10 trees,

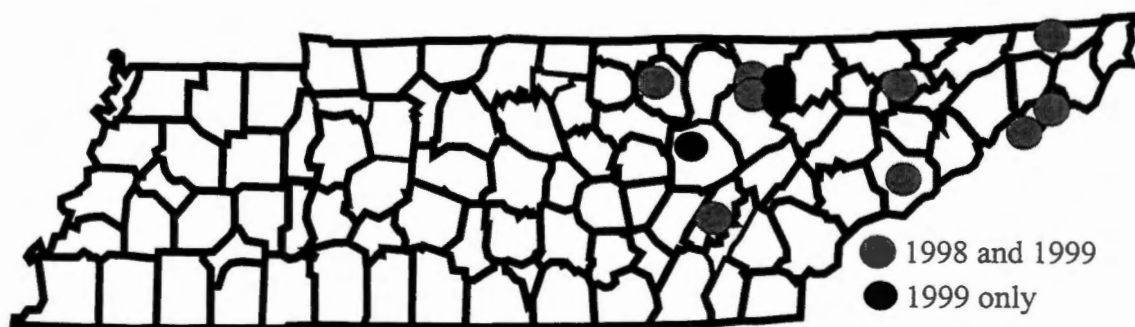


Figure 8. Locations of soil and duff sampling sites in Tennessee during 1998 and 1999.

Table 5. Sites where soil and duff samples were taken in Tennessee to monitor for *Entomophaga maimaiga*, spring 1998.

Site	County	GPS	Location	Discovery of infestation	Year treated
1	Grainger	N 36°14.45 W 83°37.83	Private Residence	1993 ^a	1994, 1995
2	Rhea	N 35° 30.12 W 85° 06.98	Beside Cemetery	1990	1991, 1992, 1995, 1996
3	Scott	N 36° 22.99 W 84° 29.18	Beside New River Road	1998	1999
4	Scott	N 36° 20.20 W 84° 37.44	Private Residence	1996 ^a	1997
5	Sullivan	N 36° 31.93 W 82° 14.76	Avoca Elementary School	1995	1996, 1997
6	Unicoi	N 36° 14.25 W 82° 16.35	Private Residence / Nat. Forest	1993	1994, 1995, 1996
7	Overton	N 36° 18.70 W 85° 08.30	Private Residence	1997	1998
8	Sevier	N 35° 47.21 W 83° 33.01	KOA Campground	----- ^b	----- ^c
9	Unicoi	N 36° 08.31 W 82° 24.26	Erwin Public Utilities	1994	1995

^a Sites containing trees with non-viable gypsy moth egg masses from previous infestations.

^b No infestation has been found at the KOA campground to date; however, single male adult gypsy moth catches are not uncommon each year.

^c No treatment for gypsy moth has been applied at the KOA campground.

Table 6. Sites where soil and duff samples were taken in Tennessee to monitor for *Entomophaga maimaiga*, fall 1998 and spring 1999.

Site	County	GPS	Location	Discovery of infestation	Year treated
1	Grainger	N 36°14.45 W 83°37.83	Private Residence	1993 ^b	1994, 1995
2	Rhea	N 35°30.12 W 85°06.98	Beside Cemetery	1990	1991, 1992, 1995, 1996
3	Scott	N 36°22.99 W 84°29.18	Beside New River Road	1998	1999
4	Scott	N 36°20.20 W 84°37.44	Private Residence	1996 ^b	1997
5	Sullivan	N 36°31.93 W 82°14.76	Avoca Elementary School	1995	1996, 1997
6	Unicoi	N 36°14.25 W 82°16.35	Private Residence / Nat. Forest	1993	1994, 1995, 1996
7	Overton	N 36°18.70 W 85°08.30	Private Residence	1997	1998
8	Sevier	N 35°47.21 W 83°33.01	KOA Campground	----- ^c	----- ^d
9	Unicoi	N 36°08.31 W 82°24.26	Erwin Public Utilities	1994	1995
10	Scott ^a	N 36°23.32 W 84°29.49	Beside New River	1998	1999
11	Scott ^a	N 36°18.37 W 84°33.66	Beside Wolf Creek Road	1998	1999
12	Cumberland ^a	N 36°07.65 W 85°04.84	Private Residence	1998 ^b	1999

^a These sites were sampled only in 1999; all other sites were sampled in 1998 and 1999.

^b Contain trees with non-viable gypsy moth egg masses from previous infestations.

^c No infestation has been found at the KOA campground to date.

^d No treatment for gypsy moth has been applied at the KOA campground.

preferably oaks, were randomly chosen. At the base of chosen trees, leaf litter from a 15x15 cm area was removed and placed into a 3.8 L bag (duff sample). After the leaf litter was removed, soil was collected from the same 15x15 cm area at a depth of 5-10 cm. Ten soil and 10 duff samples were collected at each site. When sampling, attention was given to trees with egg masses; therefore, samples were not always taken at each burlaped tree, because some trees, especially those with egg masses, had multiple soil and duff samples taken. Samples were labeled, placed in a cooler, taken to the laboratory, and stored at 10°C until they could be processed.

Testing of soil and duff samples required live gypsy moth larvae, which were reared from eggs obtained from the Otis Plant Protection Center in Massachusetts. These egg masses were stored in a refrigerator (10°C) for future use. To obtain larvae for bioassays, egg masses were removed from the refrigerator, placed in a petri dish sealed with parafilm, and placed in a growth chamber at 22°C with a photoperiod of 14:10 (L:D). Once larvae hatched, they were transferred to rearing cups (118 ml) and fed a high wheat germ diet (Doane and McManus 1981).

Ten larvae, second or third instar, were placed with either a soil or duff sample into individual plastic containers (18x13x9 cm), the top part of which had its walls smeared with Vaseline® to prevent larval escapes. High wheat germ diet was added to the containers as needed. After 1 week, any live larvae were transferred to diet cups (118 ml) for 2 weeks. Any dead larvae were placed on moistened filter paper within a plastic petri dish (100x15 mm) that was sealed with parafilm. Petri dishes were either wrapped with aluminum foil or placed in cardboard boxes of various sizes to prevent light from

reaching them, and kept in a growth chamber at 22°C. During the 2-week period, any additional larvae that died were placed in petri dishes with moistened filter paper which was sealed with parafilm and monitored for pathogens. Fungal pathogens observed on larvae were identified to species by Dr. Roberto Pereira.

Egg and Pupal Field Studies

To monitor for parasitoids of gypsy moth eggs, viable egg masses were removed from three infested trees in Cumberland County from 30 November to 4 December 1998. These egg masses resulted from high populations of gypsy moth from the previous season. Most (approximately 85%) of the egg masses were located on one oak tree. Initial examination of this tree revealed multiple life stages (pupae, cast exoskeletons, and eggs) of the gypsy moth. Only egg masses were found on the two other trees.

Using a cherry picker (bucket truck) from TDA's Division of Forestry, 30 egg masses were removed using chisels and hammers from the trunks and branches of three trees. Egg masses were placed individually into separate 1.9 L bags, taken to the laboratory, and transferred to individual petri dishes. About one dozen additional egg masses, many of them damaged during removal from the hollowed-out base of one oak tree, were collected, taken to the laboratory, placed randomly in three containers (18x13x9 cm), and monitored for parasitoids.

Roger Fuester (USDA-ARS) provided information on maintaining and rearing these field-collected gypsy moth egg masses, which were stored in a growth chamber at 10°C and held until April 1999. Egg masses were warmed beginning on 28 April, as the

temperature of the growth chamber was raised 2°C every couple of days. Eggs were monitored for larval hatch and parasitoid emergence.

Due to the low number of field-collected, naturally-occurring pupae and egg masses, additional methodologies were developed to better understand the incidence of parasitoids and predators of the gypsy moth. Live pupae and egg masses could not be used in this study because of their possible escape in the field. Because gypsy moth adults may emerge in the field, fly to nearby TDA pheromone traps, and distort trap catch numbers, freezer-stored gypsy moth pupae and egg masses were employed in this research. Also, live field-placed egg masses increase the possibility of gypsy moth becoming established if larvae were to hatch from misplaced or lost egg masses.

Freezer-stored gypsy moth eggs and pupae were obtained as needed from the gypsy moth rearing unit at the Otis Plant Protection Center in Massachusetts (Martin, personal communication). These dead egg masses and pupae were placed in the field and monitored for predation/parasitism at three research sites: Knox (UT Plant Sciences Farm, N 35° 53.72, W 83° 57.27) and Anderson (UT Arboretum and Forestry Experiment Station, N 36° 00.46, W 84° 12.23) Counties (10 oak trees each) and Scott County (south of Huntsville, TN, beside the New River, N 36° 23.32, W 84° 57.27) (10 river birch trees).

Within each site a pupa was fastened to a marked tree at a height of 1.4 m using black sowing thread which was tied around the midsection of the pupa and stapled to a tree. An egg mass was secured to the same tree by either fastening it with a paper clip to the underside of a leaf (within arms reach), under a piece of bark, or by stapling it to the

underside of a branch. At each site, beginning on 25 August 1999, one egg mass and one pupa were placed on each of 10 marked trees for 24 hours. These egg masses and pupae were then removed and replaced for another 24-hour period. This procedure was repeated weekly, terminating on 24 September 1999.

When returning to the field to collect pupae and egg masses, visual observations were made at each site, and damage to pupae and egg masses was noted. Photographs also were taken to document damage. Any predators or parasitoids found on pupae and egg masses were collected with an aspirator, taken to the laboratory, and identified.

Categories (none, minimal, $\leq 1/4$, $>1/4 < 1/2$, $\geq 1/2$) of damage to individual egg masses were developed to measure the impact of predation. Minimal damage to egg masses was defined as the absence of 1-20 individual eggs from the egg mass. All other categories of damage referred to the amount of the egg mass removed. For example, a rating of $\geq 1/2$ was represented by an egg mass where one-half or more of the egg mass was missing. All pupae and egg masses were removed, individually placed in 118 ml cups, taken to the laboratory, rated for damage, placed within a growth chamber (22°C), and monitored for parasitoid emergence.

Statistical analyses of pupal and egg predation were performed using SAS. Sites were considered as complete blocks with 11 dates to compute analyses of variance. Means were separated by Fisher LSD to test significant ($p \leq 0.05$) differences among damaged pupae and egg masses among counties and sampling dates (SAS Institute Incorporated 1989).

CHAPTER III

RESULTS AND DISCUSSION

Larval Sampling

Although 60 (1998) to 120 (1999) burlaped trees were monitored weekly during 1998 and biweekly during 1999, no gypsy moth larvae were collected at any of the sampling sites. These results were not surprising due to the eradication efforts by TDA officials. All 1998 sampling sites (Table 3) had been treated previously for gypsy moth, except for Site 3 (Scott County), which had the greatest potential for collecting larvae. During the 1998 gypsy moth trapping program, 71 moths were captured less than 3.2 km from Site 3. If Site 3 had been located closer to this infestation site, larvae may have been collected during 1998.

During 1999, three sites were added due to high moth catches from the 1998 gypsy moth trapping program. Site 7 in Cumberland County was considered to be an excellent locality to collect gypsy moth larvae, due to multiple gypsy moth egg masses found at the site. However, this site was treated with Dimilin® twice by TDA officials (4 and 11 May 1999), which may account for the lack of larvae collected at this high risk site.

Efforts to locate gypsy moth larvae in Tennessee were unsuccessful. Any areas found to have high populations of gypsy moth in Tennessee are treated immediately by TDA officials, thereby reducing the chances of finding any gypsy moth larvae. Also, it is

difficult to find larvae using burlap in areas where gypsy moths are not abundant. Burlap as a tool for collecting gypsy moth is most successful in areas that have had high populations of moths the previous years and have not been treated for gypsy moth (personal observation). Burlap, however, should still be placed at localities that have been treated for gypsy moth to observe if the treatment has been effective. Also, any localities with high numbers of adult gypsy moths should be burlaped to pinpoint infested areas.

Soil and Duff Sampling

During this two-year study, 6,600 gypsy moth larvae were exposed to 660 soil and duff samples (180 collected spring 1998, and 240 fall 1998 and 240 spring 1999). Each larva was exposed to samples for 1 week and then monitored for 2 weeks for mortality resulting from *E. maimaiga* or from other pathogens. No *E. maimaiga* was recovered during this study; however, two other fungal pathogens, *Metarhizium anisopliae* (Sorok.) Metsch. and *Beauveria bassiana* (Balsamo) Vuillemin, were found to infect gypsy moth larvae at extremely low rates (<0.2% of all larvae) (Table 7).

Fungal pathogens were recovered from five sites in four counties in Tennessee. *M. anisopliae* was recovered in four counties (Grainger, Overton, Scott, and Unicoi Counties), while *B. bassiana* was recovered from only one site (Site 11 in Scott County) (Table 7). At all sites during spring 1998, three larvae (0.17%), all from soil samples, were infected by *M. anisopliae*. Of larvae tested, two (1%) from Grainger County and one (0.5%) from Unicoi County were infected by *M. anisopliae*. During fall 1998, *M. anisopliae* was recovered from one (0.5%) larva from Scott County soil samples and from

Table 7. Fungal pathogens recovered from soil and duff sampled in Tennessee, 1998-1999^a.

Site #	County	Fungal pathogen	No. Larvae infected	Sample area	Collection date
1	Grainger	<i>Metarhizium anisopliae</i> (Sorok.) Metsch.	2	Soil	Spring 1998 ^b
9	Unicoi	<i>Metarhizium anisopliae</i> (Sorok.) Metsch.	1	Soil	Spring 1998
7	Overton	<i>Metarhizium anisopliae</i> (Sorok.) Metsch.	1	Duff	Fall 1998 ^c
3	Scott	<i>Metarhizium anisopliae</i> (Sorok.) Metsch. .	1	Soil	Fall 1998
11	Scott	<i>Beauveria bassiana</i> (Balsamo) Vuillemin	2	Duff	Fall 1998

^a No fungal pathogens were recovered from 240 duff or soil samples from 1999.

^b 180 duff and soil samples were collected and processed from spring 1998.

^c 240 duff and soil samples were collected and processed from fall 1998.

one (0.5%) larva from Overton County duff samples. The other fungal pathogen *B. bassiana* was recovered from two (1%) larvae from Scott County duff samples during fall 1998. Of the two fungal pathogens recovered during this study, *M. anisopliae* was more abundant (71%) than *B. bassiana*. No fungal pathogens were recovered from soil or duff samples collected in spring 1999.

Recovery of these fungal pathogens is not surprising, as both *B. bassiana* and *M. anisopliae* have a wide host range. For example, *M. anisopliae* also has been observed on grubs of Japanese beetle, *Popillia japonica* Newman (personal observation), and both *M. anisopliae* and *B. bassiana* have been reported to cause mortality in isolated populations of gypsy moth (Podgwaite 1979). In laboratory studies, *M. anisopliae* was shown to be fatal to gypsy moth larvae within 95 hours after inoculation (Fuxa et al. 1998). Field studies have demonstrated that the gypsy moth is susceptible to at least three species of pathogenic fungi, *Paecilomyces furinosus* Brown and Smith, *Aspergillus flavus* Link, and *B. bassiana* (less than 1% combined mortality within gypsy moth populations) (Podgwaite 1979).

Fungal pathogens were recovered from gypsy moth in low numbers in Tennessee. Reasons for low incidence is unknown; however, it is suspected that their pathogenicity against gypsy moth larvae may not be great. Laboratory trials of injecting larvae with cultures of *M. anisopliae* were unsuccessful in recovering this fungal pathogen. Also, additional larvae may have died by fungal pathogens, and due to the absence of fruiting bodies of these fungi on the gypsy moths, the cause of death may have been

misidentified. Additional evaluation of soils should be considered to study diversity and incidence of fungal pathogens and their impact on gypsy moth populations in Tennessee.

Entomophaga maimaiga has been introduced into and recovered from several states, such as Michigan, as a management effort against gypsy moth (Bauer and Smitley 1997). *E. maimaiga* could possibly be established within Tennessee forests. Resting spores of *E. maimaiga* have been shown to be carried by footwear of those working in areas containing *E. maimaiga* (Hajek et al. 1995). Persons visiting from the northeastern U.S. could have transported resting spores of *E. maimaiga* into Tennessee. These resting spores, which may remain dormant in the environment for up to six years, could be viable today (Weseloh and Andreadis 1997).

The lack of recovery of *E. maimaiga* in Tennessee, however, is not surprising. If *E. maimaiga* has reached Tennessee, probably no populations of gypsy moths have existed for reproduction and spread of the fungus. The gypsy moth front has not become established in Tennessee as only isolated outbreaks of gypsy moth occur in the state. To date, *E. maimaiga* is easily detected in areas where epizootics occur in gypsy moth populations.

If *E. maimaiga* is not present in Tennessee soils, releases of *E. maimaiga* should be considered in future management programs as a preventative measure when future outbreaks of gypsy moth occur in Tennessee. *E. maimaiga* would be desirable in Tennessee because of its effectiveness in controlling small or large populations of gypsy moth (Reardon and Hajek 1998). Once *E. maimaiga* is released, follow-up studies can be

implemented to monitor its establishment, incidence, spread, and impact on gypsy moth in Tennessee.

Egg and Pupal Field Studies

More than 30 viable egg masses were collected during winter 1998 from three trees in Cumberland County. No parasitoids emerged from the egg masses that were collected and placed in petri dishes or containers. An average of 182.23 ± 126.92 larvae emerged from 23 (77%) of 30 egg masses (Table 8). Doane and McManus (1981) state that the number of eggs per female in each individual mass varies from fewer than 100 to more than 1,000. It is unknown why low numbers of larvae hatched from field-collected egg masses.

Low larval hatch also was observed in the field. Additional egg masses collected by TDA officials were placed individually in cages and allowed to overwinter in the field at localities to be treated for gypsy moth in 1999. These egg masses were monitored and only one larva hatched from a field-placed egg mass in Scott County. Also, at another locality in Scott County, two field-placed egg masses experienced larval numbers lower than usual (Kauffman, personal communication). Reasons for low larval hatch in the field and laboratory are unknown. Due to similar hatch rates in field verses laboratory the viability of egg masses may be responsible for low hatch rates. Future studies should examine the role of biotic (viability of egg masses, parasitoids and predators) and abiotic (climate) factors in regulation of native gypsy moth egg masses in Tennessee.

Table 8. Number of gypsy moth larvae that emerged from individual egg masses collected from Cumberland County, TN, 1998. Egg masses were collected from the canopy (25) or the base of trees (5).

Egg Mass #	# Larvae	Location	Egg Mass #	# Larvae	Location
1	201	Canopy	16	0	Canopy
2	243	Canopy	17	0	Canopy
3	179	Canopy	18	0	Canopy
4	176	Canopy	19	0	Canopy
5	88	Canopy	20	276	Base
6	254	Canopy	21	315	Canopy
7	279	Canopy	22	284	Canopy
8	103	Canopy	23	415	Canopy
9	166	Canopy	24	336	Canopy
10	0	Canopy	25	178	Canopy
11	269	Canopy	26	213	Base
12	243	Canopy	27	247	Canopy
13	112	Canopy	28	372	Canopy
14	0	Base	29	351	Canopy
15	0	Base	30	167	Base

Of 330 field-placed, freezer stored pupae, 79 (25%) were damaged by predators (Fig. 9 and 10). Significant ($p \leq 0.05$) differences occurred daily in pupal predation, with greater amounts of predation occurring at the beginning of the study and decreasing towards 24 September (Table 9). Pupal predation was significantly ($p \leq 0.05$) greater (almost two times greater) in Anderson and Knox Counties than in Scott County (Fig. 10). In both Anderson and Knox Counties, 70% of the pupae were preyed upon, and predation was documented on 10 (91%) of 11 sampling dates in Anderson County and 10 (91%) of 11 sampling dates in Knox County (Fig. 9). However, in Scott County, predation was observed on only six (55%) of the 11 sampling dates. Overall, the highest levels of predation [33 of 110 pupae (30%)] were found in Anderson County, followed by Knox County [32 pupae (29%)] and Scott County [14 pupae (12.7%)] (Fig. 10). Pupal predation in late August and early September was significantly ($p \leq 0.05$) greater when this study was initiated than in late September (Table 9).

The reasons for the decline in pupal predation during this study are unclear and need to be examined further. Initially, predation rates in August may have been higher than normal (as high as 70%) due to summer droughts which may have reduced food supplies. Also, seasonal behavior, species life cycles, and climatic changes may have affected predator activity and caused decreased predation in September.

Predation on egg masses also was observed. Of the 330 egg masses placed in the field, 112 (34%) showed some sign of damage, ranging from only a few individual eggs missing to a large portion of the egg mass missing (Fig. 11 and 12). Significant ($p \leq 0.05$) differences in egg predation occurred daily; however, no significant trends were observed

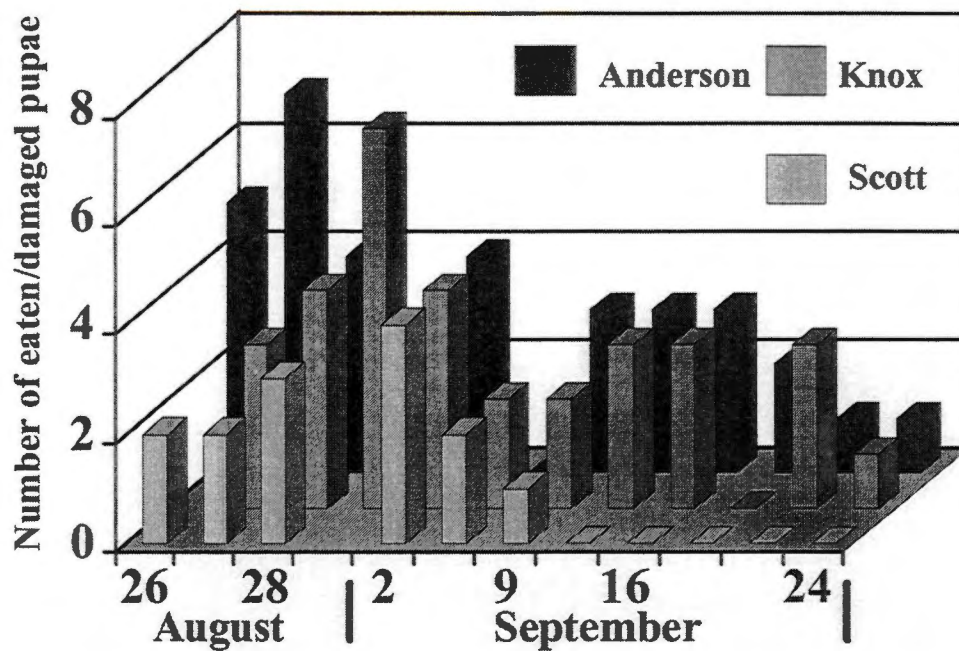


Figure 9. Predation of freezer-stored gypsy moth pupae from 26 August to 24 September 1999 in Tennessee [no data were collected on 26 August in Knox County or on 29 August in Anderson and Scott Counties].

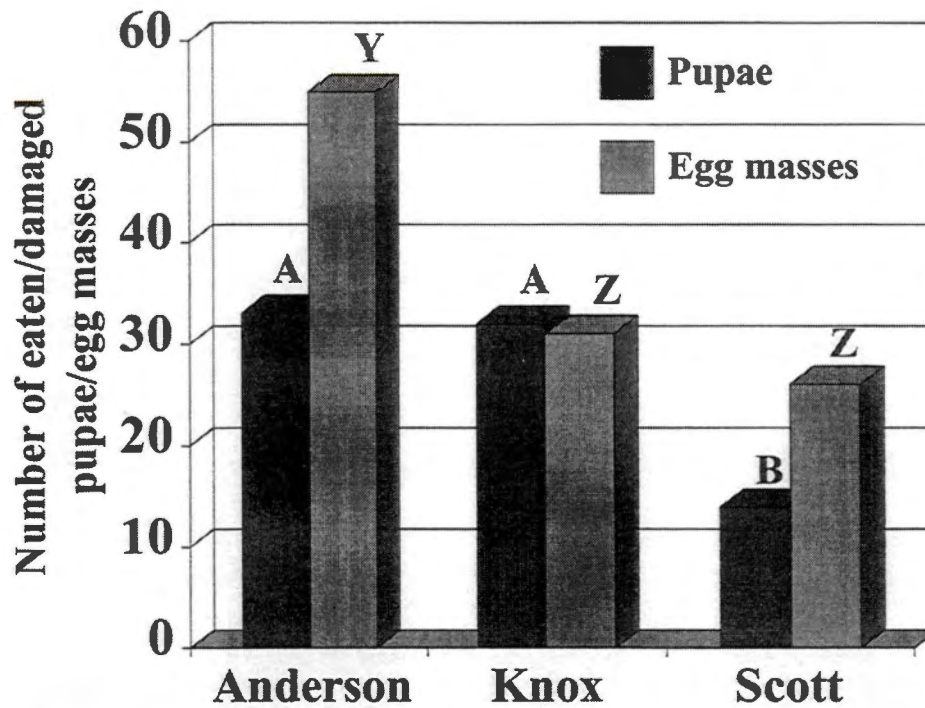


Figure 10. Number of pupae and egg masses preyed upon per county from 26 August to 24 September 1999 in Tennessee [Bars within a life stage with the same letters are not significantly different, LSD Test ($p \leq 0.05$)].

Table 9. Predation of freezer-stored gypsy moth pupae and egg masses, averaged across all sampling sites, in Tennessee, 1999.

Date	Average number pupae eaten/day	Average number egg masses eaten/day
Aug. 26	3.5 ± 2.1 A ^a	2.0 ± 2.8 CD
27	4.0 ± 2.6 A	2.3 ± 3.2 CD
28	3.7 ± 0.6 A	3.0 ± 1.7 BCD
Sept. 2	4.0 ± 0 A	1.7 ± 0.6 D
3	1.3 ± 1.2 A	2.0 ± 1.0 CD
9	2.0 ± 1.0 AB	6.3 ± 4.1 A
10	2.0 ± 1.7 AB	4.7 ± 1.5 ABC
16	2.0 ± 1.7 AB	5.7 ± 2.1 AB
17	0.7 ± 1.2 B	4.0 ± 0.0 ABCD
23	1.3 ± 1.5 B	2.0 ± 1.7 CD
24	0.7 ± 0.6 B	2.7 ± 2.5 CD

^a Means within a column followed by the same letter are not statistically different, LSD test ($p \leq 0.05$), $\bar{x} \pm$ SD.

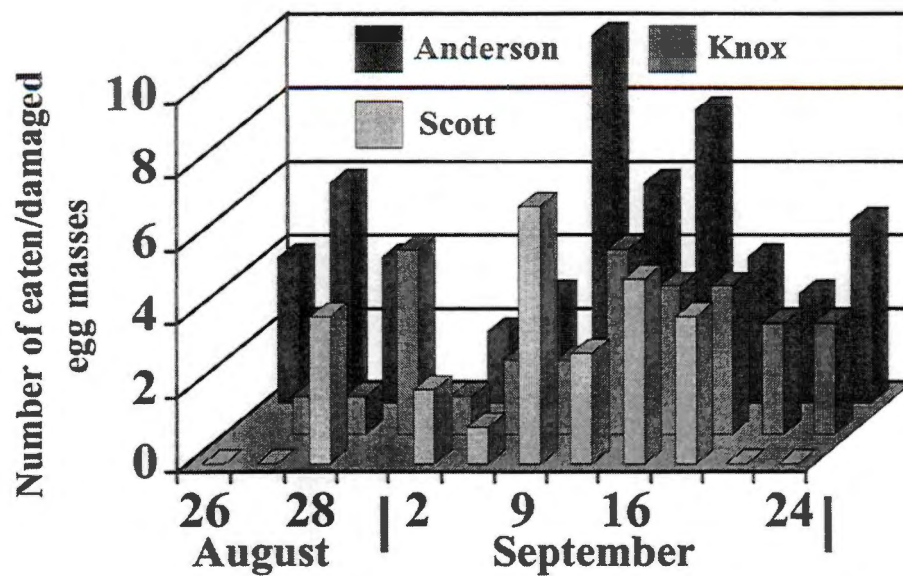


Figure 11. Predation of freezer-stored gypsy moth eggs from 26 August to 24 September 1999 in Tennessee [no data were collected on 26 August in Knox County or on 29 August in Anderson and Scott Counties].

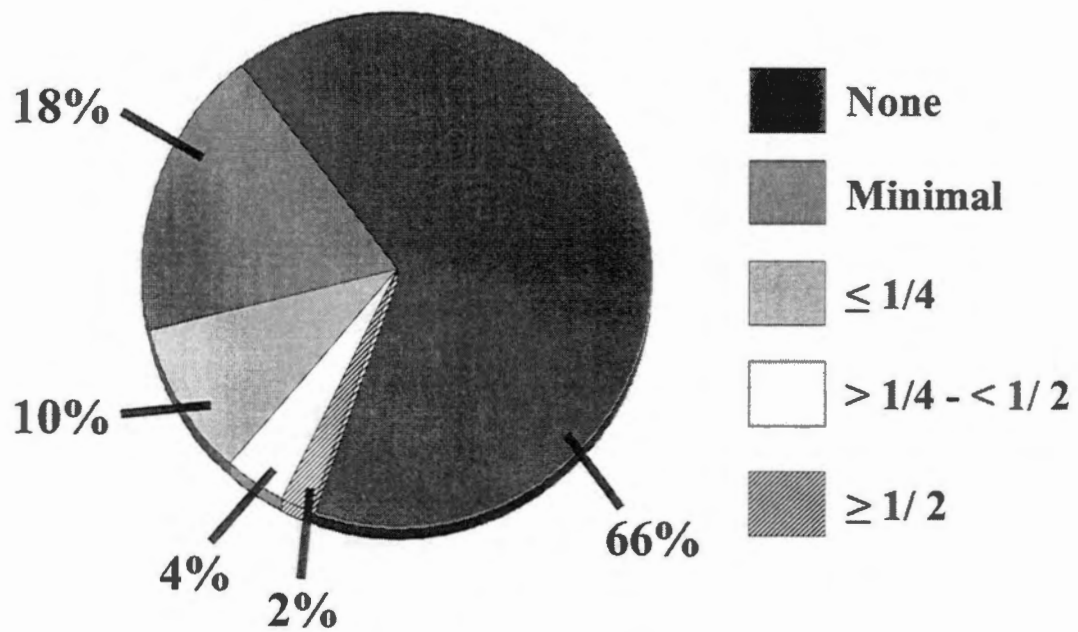


Figure 12. Assessment of impact of predators on freezer-stored gypsy moth egg masses (n=330) in Tennessee, 1999.

(Table 9). Egg predation was significantly ($p \leq 0.05$) greater in Anderson County than Knox or Scott Counties (Fig. 10). Reasons for low egg predation in Knox County are unknown (Knox had significantly [$p \leq 0.05$] higher pupal predation than Scott County). In Anderson County, egg predation was documented on 11 (100%) sampling dates (Fig. 11). However, in Scott County, egg predation reached only 60% and was documented on only seven (64%) sampling dates.

No egg masses were completely devoured by predators. Of all egg masses, 66% ($n=218$) had no damage and 18% ($n=61$) had minimal damage (1 to 20 individual eggs removed from the mass) (Fig. 12). This category was the most difficult to judge, based on the varying conditions of egg masses shipped in the mail, and damage that occurred when placing egg masses in the field with paper clips or staples. Approximately 10% ($n=32$) of the egg masses had $\leq 1/4$ damage, while 4% ($n=13$) had $>1/4 - <1/2$ damage, and 2% ($n=6$) had $\geq 1/2$ of the egg mass damaged. Egg masses were placed in the field for only 24 hours, and during this time, most (94%) did not have greater than one-half of the egg mass damaged. Further study on field-placed egg masses is needed to assess seasonality impact of predators on gypsy moth.

Between 26 August and 24 September 1999, 123 specimens of Formicidae (Fig. 13) (representing 5 genera [Fig. 14]) were collected from both pupae and egg masses in the field. The most commonly collected ant genera from field-placed, freezer-stored pupae and egg masses exposed in the field include *Aphaenogaster* (43%), *Paratrechina* (42%), and *Camponotus* or carpenter ants (11%) (Fig 14). During this study, *Aphaenogaster* (35.8%) and *Camponotus* (9.8%) were the most common genera collected

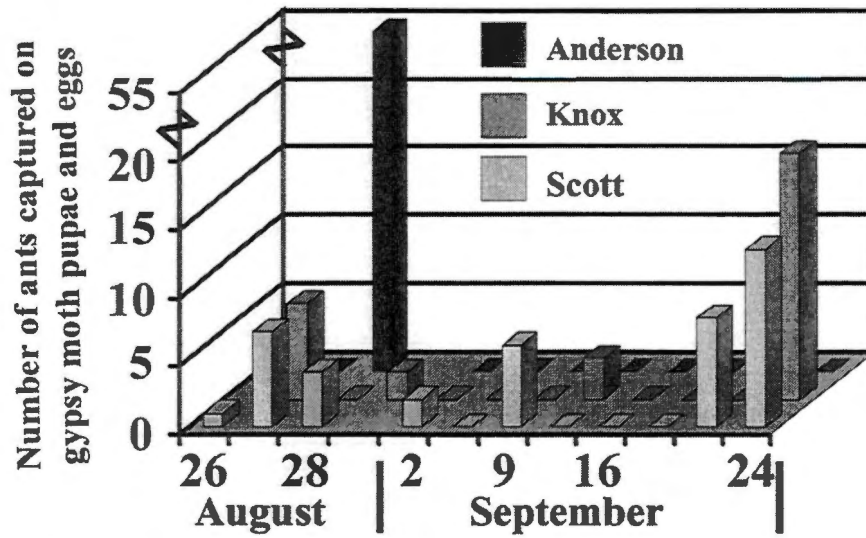


Figure 13. Number of ants collected from freezer-stored gypsy moth pupae and egg masses in Tennessee, 1999 [no data were collected on 26 August in Knox County or on 29 August in Anderson and Scott Counties].

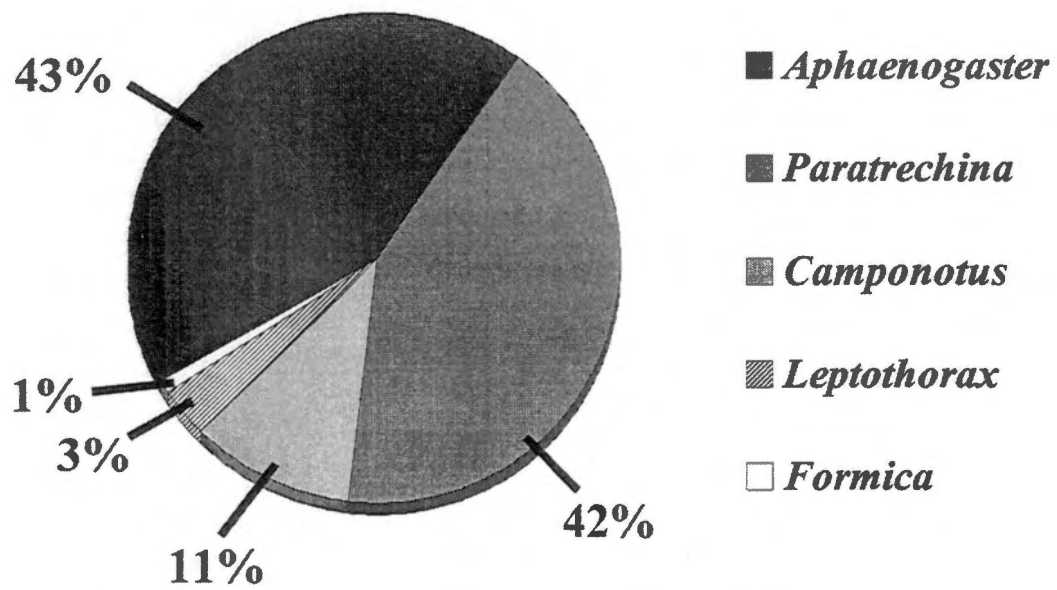


Figure 14. Composition of five ant genera collected from freezer-stored gypsy moth pupae and egg masses in Knox, Anderson, and Scott Counties in Tennessee, 1999.

from pupae, while *Paratrechina* (42.3%) was the most common genus collected from egg masses.

The predatory status of *Paratrechina* spp. is uncertain. All individuals were collected from one egg mass on 28 August 1999 in Scott County (Fig. 13). Individuals were not observed to cause direct damage to this egg mass; however, when the egg mass was removed from the bark, 52 individuals were observed between the egg mass and the bark. Further studies are needed to assess the predatory status of selected species, such as *Paratrechina* spp. and other ant species, against the gypsy moth.

Ants were not the only predators collected from egg masses. Eight spiders were collected during this study from egg masses with minimal to no damage. These spiders were likely waiting for predators to visit the egg masses. One mite was collected from a pupa; however, no damage was observed.

During monitoring of pupae in the laboratory, 15 of 330 (4.5%) pupae were parasitized, and 69 adult dipterans (Family: Phoridae) emerged from parasitized pupae ($\bar{x}$ = 4.6 per pupa). These dipterans may have been attracted to decaying gypsy moth pupae, since the larval habitats of these flies vary from decaying animal matter to internal parasitoids of various insects (Borror and Johnson 1989). During this study, some of the phorids escaped in the laboratory, and completed their life cycle on gypsy moth high wheat germ diet. These phorids remain unidentified at this time.

As the gypsy moth front approaches Tennessee during the next decade it is crucial to continue assessing biological control agents within the state. During this research two fungal pathogens and five genera of ants were identified as natural enemies of gypsy

moth in laboratory and field settings. Continual monitoring of soil and duff is needed to assess for fungal pathogens, especially *E. maimaiga*, in Tennessee. Even though *E. maimaiga* was not found in Tennessee, tourists from the northeastern U.S. and new infestations of gypsy moth increase the possibility of *E. maimaiga* becoming established within the state. In Tennessee, predators may have an important role in regulating future gypsy moth populations (as 79 [24%] pupae were damaged and 112 [34%] egg masses were damaged during 24 hr periods). Because this research was conducted only in the fall (pupae are present in the spring, and eggs throughout the winter), further seasonality studies on pupal and egg predators are needed. No parasitoids were recovered during this study; however, they have been known to affect gypsy moth where populations are known to exist. Therefore, further studies are needed to assess parasitoids and their potential impact on gypsy moth populations in Tennessee.

Biological control agents will be necessary to provide long-term management of the gypsy moth. To date, TDA eradicates any populations through the use of Bt or Dimilin®. Once gypsy moth becomes established in Tennessee's forests, such treatments may not be economically feasible. Therefore, continual research is needed to identify biological control agents that will assist in reducing and maintaining gypsy moth populations below economically damaging levels.

CHAPTER IV

CONCLUSIONS

The gypsy moth is not native to the U.S., but was introduced into Massachusetts in 1869 by Etienne Leopold Trouvelot for use in silkworm breeding. This insect subsequently escaped into the surrounding forests and is now found throughout the northeastern U.S. As of 1997, the edge of the gypsy moth front remained north of Roanoke, VA, approximately 240 km from Tennessee.

The economic and environmental impact of gypsy moth in heavily-infested areas is tremendous, primarily due to its wide host range. The gypsy moth has been reported to feed on more than 300 tree species, with oaks as a preferred host. As a result, gypsy moth defoliates 2 million forested hectares per year annually in the U.S. (Reardon and Hajek 1998). In Tennessee, more than one-half of the state is forested (5.5 million hectares), and 78% of these forests are composed of oaks and hickories, 30% of which is highly susceptible to gypsy moth (Kauffman 1996). The establishment and resulting damage from gypsy moth would greatly impact this area. Thus, protection of Tennessee's forests are vital to the state's economy, which in 1995 employed more than 44,000 persons, with an estimated payroll greater than \$827 million. Today, Tennessee leads the nation in lumber manufacturing and secondary wood products (Kauffman 1996).

Gypsy moth was first detected in Tennessee in 1972, and has since been captured in 64 counties throughout the state. However, no studies have been undertaken to examine for the presence of natural enemies of the gypsy moth in Tennessee. Therefore, the specific objectives of this research were to: 1) monitor gypsy moth larval populations and evaluate the presence of *E. maimaiga* and other natural enemies, 2) conduct soil bioassays to assess presence of *E. maimaiga* and other entomopathogens, and 3) examine for biological control agents (predators and parasitoids of eggs and pupae) in Tennessee.

Although 60 (1998) to 120 (1999) burlaped trees were monitored weekly during 1998 and biweekly during 1999, no gypsy moth larvae were collected at any of the sampling sites. Site 7, in Cumberland County, was considered to be an excellent locality to collect gypsy moth larvae, due to multiple gypsy moth egg masses found at the site during winter 1999. However, this site was treated with Dimilin® twice by TDA officials (4 and 11 May 1999), which may account for the lack of larvae collected at this high risk site. These data are not surprising due to the eradication efforts of TDA who quickly treat any infestations found within Tennessee.

Soil and duff samples also were collected and assayed for the presence of *E. maimaiga*, a fungal pathogen responsible for epizootics in gypsy moth populations in the northeastern U.S. During this two-year study, 6,600 gypsy moth larvae were exposed to 660 soil and duff samples (180 collected spring 1998, 240 fall 1998 and 240 spring 1999). No *E. maimaiga* was recovered during this study; however, two other fungal pathogens, *M. anisopliae* and *B. bassiana*, were found to infect gypsy moth larvae at extremely low rates (<0.2% of all larvae).

More than 30 viable egg masses were collected during winter 1998 from three trees in Cumberland County. No parasitoids emerged from field-collected egg masses. Due to low numbers of naturally-occurring egg masses and the inability to recover naturally-occurring pupae, freezer-stored pupae and egg masses were acquired from Otis Air Force Base in Massachusetts and placed on trees at three localities in Tennessee.

Between 26 August and 24 September 1999, 123 ants (representing 5 genera) were collected from both pupae and egg masses in the field. Of 330 field-placed, freezer-stored pupae, 79 (24%) were damaged by predators. Pupal predation was significantly ($p \leq 0.05$) greater in Knox and Anderson Counties (where 70% pupal predation occurred one day in both counties) than in Scott County.

Of the 330 freezer-stored egg masses placed in the field, 112 (33.9%) were damaged. Egg predation was significantly ($p \leq 0.05$) greater in Anderson County (100% predation on egg masses on one day) than in Knox or Scott Counties. The most commonly collected ant genera from freezer-stored pupae and egg masses were *Aphaenogaster* (43%), *Paratrechina* (42%), and *Camponotus* or carpenter ants (11%). Fifteen (4.5%) pupae were parasitized, and 69 adult dipterans (Family: Phoridae) emerged from parasitized pupae ($\bar{x}=4.6$ per pupa). However, these dipterans may have simply been attracted to decaying gypsy moth pupae.

Research to assess biological control agents of the gypsy moth prior to its establishment in Tennessee is vital to enable managers to develop appropriate management strategies. Therefore, research should be continued to assess presence of fungal pathogens, such as *E. maimaiga*, along with predators and parasitoids that may

assist in regulating populations of gypsy moth in Tennessee. If permanent gypsy moth populations become established and widespread, eradication programs will not be feasible. Management programs, utilizing biological control agents, will need to be developed to provide long-term reductions of gypsy moth populations. If desired biological control agents, such as *E. maimaiga*, are not recovered, release of these agents could occur in isolated populations of gypsy moth in Tennessee, and their impact on gypsy moth populations should be monitored.

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VITA

Gary B. Moughler was born in Hicksville, Ohio, on April 24, 1975. As a child, he lived and grew up on a hog farm near Butler, Indiana, where he received his high school diploma from Eastside High School. In 1993, he enrolled in Manchester College in North Manchester, Indiana, where he received a Bachelor of Science in Environmental Studies and Biology in May 1997. While at Manchester College, he spent four summers working as a gypsy moth trap tender under the direction of the Indiana Department of Natural Resources, Division of Entomology and Plant Pathology. In August 1997, he entered into the Department of Entomology and Plant Pathology at The University of Tennessee, Knoxville. Under the direction of Dr. Jerome F. Grant, he completed the requirements for a Masters of Science degree in Entomology and Plant Pathology in December 1999. He is a member of the Entomological Society of America, Tennessee Entomological Society, Gamma Sigma Delta Agricultural Honor Society, and the Christian Student Fellowship.

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