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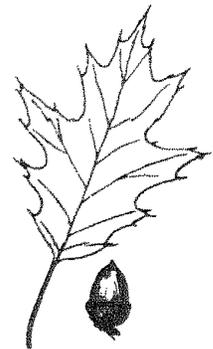
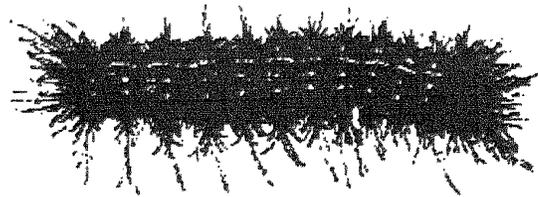
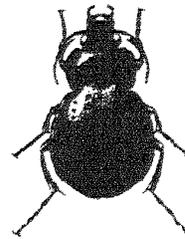
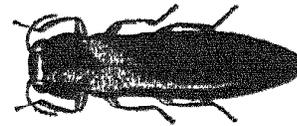
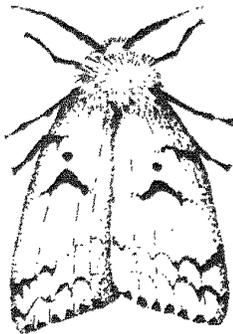
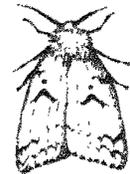
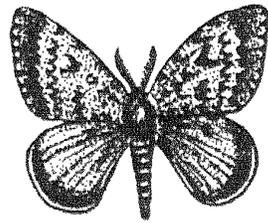
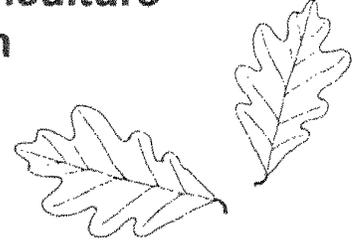
**Northeastern  
Research Station**

General Technical  
Report NE-248



# PROCEEDINGS

## U. S. Department of Agriculture Interagency Gypsy Moth Research Forum 1998



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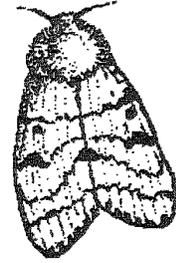
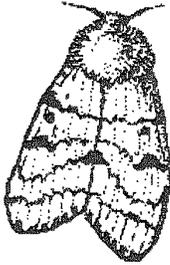
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#### ACKNOWLEDGMENTS

Thanks to Dr. Mark J. Twery for providing the cover and title page design.

Proceedings  
U.S. Department of Agriculture  
Interagency Gypsy Moth Research Forum  
1998



January 20-23, 1998  
Loews Annapolis Hotel  
Annapolis, Maryland

Edited by  
Sandra L. C. Fosbroke and Kurt W. Gottschalk

Sponsored by:

Forest Service Research

Forest Service State and Private Forestry

Agricultural Research Service

Animal and Plant Health Inspection Service

Cooperative State Research, Education and Extension Service



## FOREWORD

This meeting was the ninth in a series of annual USDA Interagency Gypsy Moth Research Forums that are sponsored by the USDA Gypsy Moth Research and Development Coordinating Group. The Committee's original goal of fostering communication and an overview of ongoing research has been continued and accomplished in this meeting.

The proceedings document the efforts of many individuals: those who made the meeting possible, those who made presentations, and those who compiled and edited the proceedings. But more than that, the proceedings illustrate the depth and breadth of studies being supported by the agencies and it is satisfying, indeed, that all of this can be accomplished in a cooperative spirit.

USDA Gypsy Moth Research and Development Coordinating Group

Ernest S. Delfosse, Agricultural Research Service (ARS)

Al Elder, Animal and Plant Health Inspection Service (APHIS)

Tom Hofacker, Forest Service-State & Private Forestry (FS-S&PF)

Hendrick Meyer, Cooperative State Research, Education and Extension Service (CSREES)

Robert Bridges, Forest Service-Research (FS-R), Chairperson

IN MEMORY OF



Normand R. Dubois  
1938 - 1998

Dedicated to Normand Dubois, a close colleague and friend who, with many of us, was a contributor to the USDA Interagency Gypsy Moth Research Forum from its inception. We surely will miss Norm's untiring dedication to his science, but far greater is the loss of a classy guy whose warmth, wit, and humor made life more enjoyable for those around him. Godspeed, Norm.

USDA Interagency Gypsy Moth Research Forum  
January 20-23, 1998  
Loews Annapolis Hotel  
Annapolis, Maryland

AGENDA

Tuesday Afternoon, January 20

REGISTRATION  
POSTER DISPLAY SESSION I

Wednesday Morning, January 21

PLENARY SESSION . . . . . Moderator: M. McFadden, USDA-FS, Retired

Welcome  
Michael McManus, USDA-FS

Introductory Remarks  
Max McFadden, USDA-FS, Retired

Update on the National Campaign Against Non-Invasive Species  
Randy Westbrooks, USDA-APHIS

Forest Health versus Forest Pests: New Challenges in the State of Hesse, Germany  
Horst Gossenauer-Marohn, Hessian Agency of Forest Management Planning, Forest Research,  
and Forest Ecology, Hannover Münden, Germany

GENERAL SESSION . . . . . Moderator: R. Fuester, USDA-ARS

The Potential Impact of Biocontrol Agents on Non-Target Organisms  
Presenters: G. Boettner, University of Massachusetts; L. Solter, Illinois Natural History  
Survey; R. van Driesche, University of Massachusetts

Wednesday Afternoon, January 21

GENERAL SESSION . . . . . Moderator: M. Keena, USDA-FS

Potpourri of Research Reports  
Presenters: F. Hain, North Carolina State University; N. Dubois, USDA-FS; V. D'Amico,  
USDA-FS; F. Hérard, USDA-ARS, European Biological Control Laboratory, France; A. Schopf,  
Institute of Forest Entomology, Austria; Y. Higashiura, Forest Research Institute, Japan

POSTER DISPLAY SESSION II

Thursday Morning, January 22

GENERAL SESSION . . . . . Moderator: N. Lorimer, USDA-FS

Current and Potential Exotic Pests and Their Impact

Presenters: J. Bain, Forest Research Institute, New Zealand; J. Novotný, Forest Research Institute, Slovak Republic; K. Shields, USDA-FS; H. Evans, Forestry Commission Research Agency, United Kingdom; A. Roques, INRA, France

POSTER DISPLAY SESSION III

GENERAL SESSION . . . . . Moderator: M. McManus, USDA-FS

Slow the Spread Demonstration Project

Presenters: D. Leonard, USDA-FS; A. Sharov, Virginia Polytechnic Institute and State University

Thursday Afternoon, January 22

GENERAL SESSION . . . . . Moderator: A. Liebhold, USDA-FS

Has *Entomophaga maimaiga* Reduced the Gypsy Moth to Non-Pest Status?

Presenters: R. Carruthers, USDA-ARS; J. Elkinton, University of Massachusetts; R. Weseloh, Connecticut Agricultural Experiment Station; A. Hajek, Cornell University

GENERAL SESSION . . . . Moderator: B. Dickerson, N.C. Dept. Agric. & Consumer Serv.

Potpourri of Research Reports (continued)

Presenters: G. Csóka, Forest Research Institute, Hungary; F. Hohn, University of Connecticut; A. Hunter, University of Massachusetts; Y. Baranchikov, Institute of Forest, Russia; J. Hilszczanski, Forest Research Institute, Poland

Friday Morning, January 23

GENERAL SESSION . . . . . Moderator: V. Mastro, USDA-APHIS

Asian Long-Horned Beetle: A New Threat

Presenters: J. Cavey, USDA-APHIS; M. Wright, USDA-APHIS; V. Mastro, USDA-APHIS; T. McNary, USDA-APHIS

GENERAL SESSION . . . . . Moderator: J. Robert Bridges, USDA-FS

Update on the Asian Gypsy Moth

Presenters: V. Mastro, USDA-APHIS; S. Bogdanowicz, Cornell University; S. Munson, USDA-FS

Closing Remarks

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FOREST HEALTH VERSUS FOREST PESTS:  
NEW CHALLENGES IN THE STATE OF HESSE

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ABSTRACT

The forests dominated by hardwood in the hessian reed - with nearly 2.4 million people the most populated region in the south of Hesse - have a very high ecological and therefore recreational value for the public.

Anthropogenous impacts like air pollutants e.g. the deposition of nitrogen and high ozone rates in summer as well as locally lowering of the ground water table in temporal combination with climatic extremes like hurricanes, several years of drought, and increasing average temperatures have triggered the destructive influence of harmful forest pests. The predisposing feeding of oak defoliators (leaf rollers, loopers and /or the gypsy moth) leads to subsequent infestations of bark and wood destroying beetles, which on their part open up the stand canopy by the selective killing of host trees.

Weedage as one consequence of the changed microclimatic conditions causes severe structural problems in the stands, especially by fostering the spreading of the cockchafer (*Melolontha hippocastani F.*). With white grub densities up to 75/m<sup>2</sup> (third instar) on appr. 7500 ha he creates critical root damages in all regenerations, underplantings in scots pine and in hardwood pole stands.

The multifunctional forest decline has accelerated in a way that a silvicultural program with a financial volume of \$58 million for the problematic (37%), reconstructable (17%) or still relatively intact sites (46%) had to be developed in order to maintain or to restore the forest functions and to prevent further losses of forest area.

THE STATUS OF THE WHITE-SPOTTED TUSSOCK MOTH,  
*ORGYIA THYELLINA*, IN NEW ZEALAND

John Bain

Forest Research Institute, Private Bag 3020, Rotorua, New Zealand

ABSTRACT

The white-spotted tussock moth (*Orygia thyellina* Butler) which is native to Japan, China, Taiwan, Korea and the Russian Far East, was first found in New Zealand in April 1996. With the realisation that the tussock moth had the potential to be a very serious threat to our forests and horticultural industry, surveys were immediately undertaken to determine its distribution and host preferences in New Zealand. These revealed that the insect was restricted to an area of approximately 7 km<sup>2</sup> in the eastern suburbs of Auckland and that it was very polyphagous: favoured hosts were *Prunus*, *Rosa*, *Malus* (Rosaceae), *Salix* (Salicaceae), *Acer* (Aceraceae) and *Quercus* (Fagaceae). Subsequent feeding trials have shown that *Nothofagus solandri solandri* (Fagaceae) is a favoured host. This species is an important component of many of our forests. *Pinus radiata* (Pinaceae), our most important commercial tree species, is a marginal host.

On the advice of an interdepartmental and multi-disciplinary advisory panel the government decided to attempt to eradicate the tussock moth in the coming spring and "Operation Ever Green" was created. A massive publicity campaign was launched to keep the public informed and to prepare them for the pending spray operations.

Throughout the winter of 1996 egg masses were monitored, sampled and dissected to predict the onset and duration of egg hatch. This proved to be protracted and between 5 October and 6 December a DC6 aircraft sprayed an area of 40 km<sup>2</sup> (7 km<sup>2</sup> plus a buffer zone) nine times. This was backed up with helicopter and ground spraying of selected sites. The spray used was *Bacillus thuringiensis kurstaki* (Btk) (Foray 48B) at a rate of 5 litres/hectare. Because a synthetic pheromone was not available the residual moth population was monitored using flight incapable females caged in delta traps. The first male moth was caught on 24 December and by 17 January 68 males had been caught at 46 trap sites. This led to a series of 14 helicopter sprays between 10 January and 17 April 1997 over an area of about 3 km<sup>2</sup>. A single male was caught in early March and a further six in April 1997. In July 1997 extensive surveys for egg masses were carried out but none were found. In December 1997 a pheromone monitoring program using about 7000 delta traps was started. The program uses a pheromone that was developed in collaboration with Canadian researchers and which had been field tested in Japan during the summer of 1997. Traps are concentrated in the original zone of infestation but there are also some in out-lying areas. So far this summer no tussock moth males have been trapped.

EFFECT OF *DENDROLIMUS SUPERANS* DEFOLIATION AND SPRAY PROGRAMS ON  
DIVERSITY OF FOREST BIOTA IN SIBERIAN FIR TAIGA

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ABSTRACT

On September 9-12, 1996, three possible control measures were used to treat the Siberian moth *Dendrolimus superans sibiricus* Tschtrk infestation in the fir forests of the Krasnoyarsk Kray (Siberia, Russia). Treatments included spraying with perythroids, a commercial formulation of *Bacillus thuringiensis*, and no treatment, which resulted in 100% fir defoliation. To determine the ecological consequences of the treatments, in the summer of 1997, we studied the density and species diversity of herbaceous plants, litter invertebrates, Lepidoptera in the canopies of deciduous trees, and small mammals. The feeding activity of leaf chewing, mining and galling insects on 4 main species of trees and shrubs was determined also.

Results show that after a year of fir forest defoliation by the Siberian moth, the ecological situation and forest plants and animals biota in damaged ecosystems were significantly changed. For plants, growth of aboveground biomass of herbs and grasses observed in defoliated plots, increased fivefold; however, the composition of species did not change. There were pronounced differences between defoliated and undefoliated areas in the litter invertebrates fauna composition, in number of insects flying to the color traps, and in the relative Lepidopteran density, which was estimated by larval head capsules captured in traps. Differences were also observed in the activity of grazing, mining and gall insects, and in density of small mammals.

We did not find any significant difference between three undefoliated areas (control and perythroid and bacterial preparation treated) in any of the investigated groups. There may be two main reasons for this: (a) late timing of application (the second week of September), which reduced exposure of susceptible nontarget species, and (b) the limited defoliation observed in treated plots compared to the control areas. These two factors would make the ecological consequences of spraying programs in the long run negligible.

POTENTIAL ROLES OF BIOTIC AND ABIOTIC FACTORS IN GYPSY MOTH EGG  
MORTALITY IN 1996-97 IN NORTHERN AND SOUTHERN MICHIGAN COUNTIES

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ABSTRACT

Since 1983, gypsy moth populations and defoliation increased steadily throughout Michigan's lower peninsula, peaking at ca. 700,000 acres in 1992. From 1993 through 1996 gypsy moth populations experienced rapid decline to only 3,200 acres of defoliation in two newly infested southeastern counties. During the 1997 gypsy moth season, a more than ten-fold increase in defoliation was measured throughout the lower peninsula. Similar population collapse of gypsy moth occurred throughout the northeastern states, coinciding with the appearance and rapid spread of a fungal pathogen of gypsy moth from Japan, *Entomophaga maimaiga*, in 1989. Historically, periodic and sporadic population outbreaks occur, even in New England where gypsy moth has been naturalized for almost a century. The reasons for recent populations decline throughout Michigan and other infested areas of North America, however, are poorly understood.

In Michigan, the decline in defoliation observed between 1992 and 1997 is attributable, in part, to the rapid spread of *E. maimaiga* during the unusually wet springs of 1993 and 1996. *Entomophaga maimaiga* research sites were established in three northern lower peninsula counties in 1991 and 1992 to compare introduction methodologies. Although these were unusually dry springs, the fungus became established and spread slowly during the first two years of the study. During the wet springs of 1993 and 1996, *E. maimaiga* spread from the initial three counties, to an additional 34 counties, causing massive epizootics statewide. Studies of fungal spread rates from the release epicenters demonstrated that fungal prevalence and rate of spread were positively correlated with precipitation. The exceptionally dry spring of 1997 resulted in increased defoliation statewide.

In addition to the successful establishment of *E. maimaiga* in Michigan, some researchers have noted that gypsy moth defoliation declines after a colder than average winter, suggesting egg mortality may also reduce larval populations. High egg mortality produced unreliable predictions of spring defoliation from standard egg mass counts. Increasingly, suppression-program managers are finding that more acreage is sprayed than is necessary. A better understanding of abiotic and biotic mortality factors in gypsy moth survival is required for design of improved methods to predict defoliation.

Early in 1997, we began studying gypsy moth egg and young larval mortality in northern and southern counties in Michigan's lower peninsula. Egg mass densities averaged ca. 2,000 and 6,000 masses/acre in northern and southern sites, respectively. Although egg masses contained similar numbers of eggs/mass (ca. 450), eggs from the northern populations were 20% smaller than those from southern populations, suggesting less maternal provisioning of eggs laid in the northern sites. Moreover, we also found that only 58% of the egg masses from the northern site (n=338) were viable, and of those egg masses, an average of 46% of the eggs hatched. In the south, 97% of the egg masses (n=210) were viable, with an average 88% egg hatch. In 1997, egg mass height, aspect, and site within each climate zone did not affect egg hatch. Although the prevalence of viral infection was similar among neonates in both populations (ca. 8%), evidence of *Oencyrtus kuvanae* emergence averaged only 0.6% in eggs from northern populations, whereas in the southern sites emergence averaged 20%. Although the underlying causes of these differences in egg survival and successful egg parasitism have not been experimentally verified, correlations with weather data suggest that eggs became desiccated or exhausted energy stores due to temperature extremes and/or fluctuations, particularly during the winter months. The smaller eggs, with both a greater surface area to volume ratio and less yolk reserves, may be less tolerant of environmental factors. Field and laboratory studies are currently underway to examine these and other possible abiotic and biotic factors.

## GYPSY MOTH PARASITES VS. *ENTOMOPHAGA MAIMAIGA* IN PENNSYLVANIA

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### INTRODUCTION

Without question, the gypsy moth, *Lymantria dispar* L., has been more costly and caused more damage to Pennsylvania forests than any other defoliating insect in recorded history. Since its appearance in 1932, over 23 million acres of forest land have been moderately or heavily defoliated, often resulting in severe economic and aesthetic losses due to mortality, decline, and reduced increment growth. Attempts to control or manage this pest also have been expensive. Over 5.3 million acres have been sprayed by Commonwealth agencies, at a cost exceeding \$55 million, not to mention spraying conducted by local municipalities and private land owners. Pesticide applications, however, generally have been very limited in scope, targeting high-use, high-value, or forested residential areas rather than trying to eradicate the insect. Biological control efforts since 1968 have focused on the rearing and release of some 30 million exotic gypsy moth parasites and predators which were very costly to produce. Although these organisms may have reduced the severity and extent of damage, gypsy moth outbreaks have continued to occur at regular intervals. Population collapse usually has resulted from starvation and stress-induced disease following two to three years of defoliation.

This scenario has changed radically since the appearance of an exotic gypsy moth pathogen in 1989-90. The pathogen, *Entomophaga maimaiga*, is a fungus native to Asia which was released in New England in 1910-11 by early biological control workers. Although these releases reportedly were unsuccessful in establishing the fungus, it serendipitously appeared in 1989 in several northeastern states (Elkinton *et al.* 1991, Hajek *et al.* 1996). *E. maimaiga* was recovered from gypsy moth larvae in Pennsylvania the following year, and subsequent spread has resulted in confirmed reports of the fungus in all but 3 of 67 counties in the Commonwealth as of July 1997. It rapidly has become the pest's dominant mortality agent, causing a general decline in statewide gypsy moth populations, tree defoliation, and pesticide applications.

Concurrent with this decline in gypsy moth numbers has been a reduction in apparent mortality by most of the established species of gypsy moth parasites, particularly the univoltine tachinid flies *Parasetigena silvestris* and *Blepharipa pratensis*. This report documents these findings.

## METHODS

Annual evaluations of parasitism and disease in gypsy moth populations have been conducted in Pennsylvania since 1969. Three formal plot systems were established; the Evaluation Plots and the Establishment Plots in the early 1970's (Ticehurst *et al.* 1978) and the Study Block System (SBS) plots in 1978. The latter plots, 40 permanent and dozens of satellite plots, provided the data for this report. Collections of egg masses, larvae and pupae have been made annually in each plot where a weekly sample of 100 larvae/pupae could be obtained throughout the season. All specimens were reared individually in our Middletown biological control facility, evaluated weekly for 4 weeks, and survival or cause of mortality recorded. Larvae were reared on artificial diet.

## RESULTS

Beginning with the appearance of significant larval mortality by in 1991, there has been a steady reduction both in populations of gypsy moth and apparent parasitism by established gypsy moth parasites (Fig. 1). The number of collectable SBS plots declined from 33 in 1991 to 2 in 1997. Concurrently, peak-week parasitism dropped from 36 to 6 percent during this period. Nearly all larval parasitism from 1995 to 1997 was caused, in descending order of occurrence, by *Cotesia melanoscela*, *Compsilura concinnata* and *Phobocampe uncinata*. Although exceptions were noted in a few plots in 1995 with unusually low levels of *E. maimaiga*, incidence of parasitism by *P. silvestris* and *B. pratensis* declined precipitously during this period.

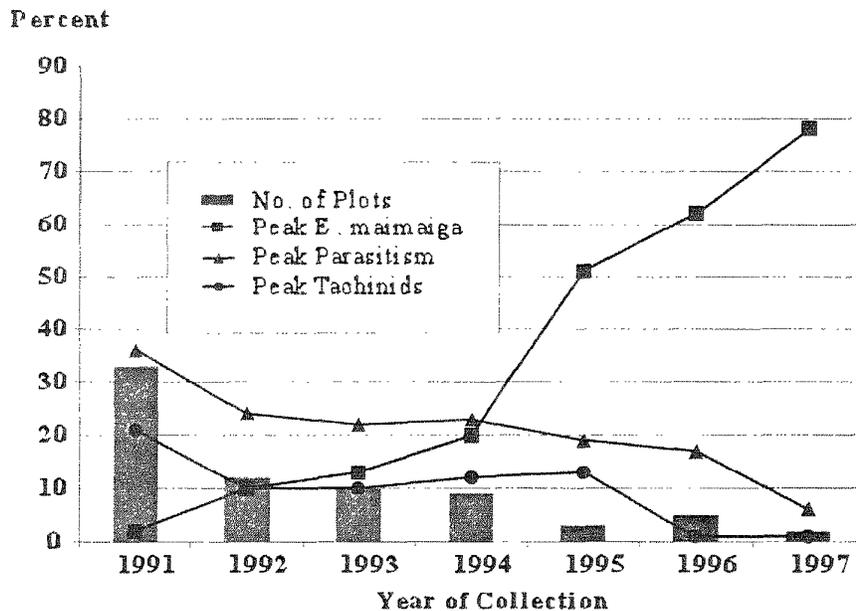


Figure 1. Apparent causes of peak-week mortality in gypsy moth larvae and pupae collected in Pennsylvania plots during 1991-1997.

A closer look at the 1997 data shows the relative importance of parasites and disease in 2 plots with vastly different historical backgrounds. The D11 plot, located near Ransom in Lackawanna County, is in the northeastern area of Pennsylvania first infested in 1932 and subsequently sprayed with lead arsenate, Cryolite, and DDT through 1962. The insect reemerged in 1975 and was attacked periodically with carbaryl, Dylox, diflubenzuron, and BT (*Bacillus thuringiensis*) through 1983. Gypsy moth thereafter remained largely under natural control (predators, parasites, and NPV) until 1990 when the last outbreak occurred. It again has come under natural control, this time due primarily to *E. maimaiga* (Fig. 2), despite state-wide dry weather during most of the larval development period.

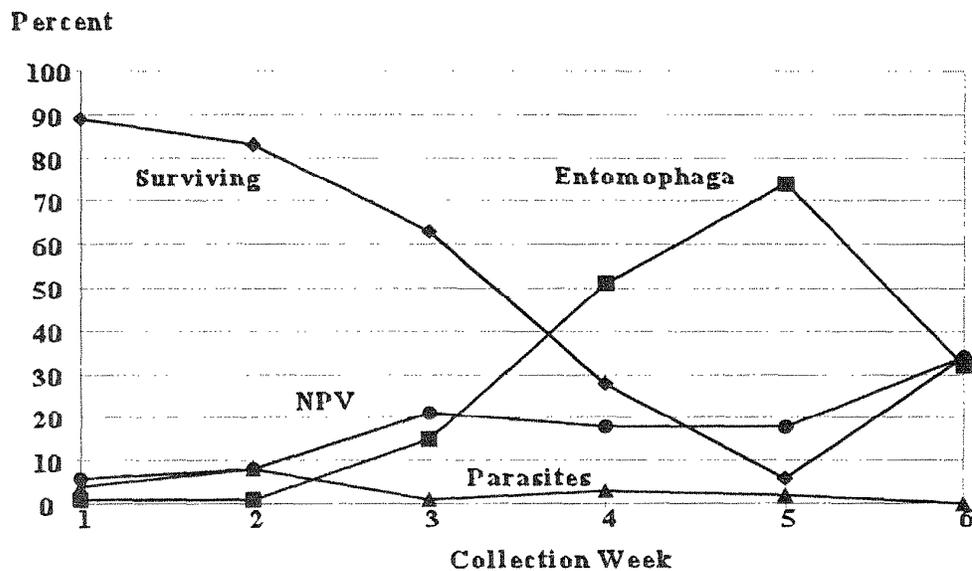


Figure 2. Apparent causes of mortality in gypsy moth collected in Lackawanna County, Pennsylvania in 1997.

In contrast, the D04 plot, located on State Game Lands 223 near Garards Fort in Greene County, first saw defoliation in 1995 and has never been sprayed. Gypsy moth numbers in this plot were drastically reduced in 1997 due to both fungus and virus epizootics (Fig. 3). Introductions of *E. maimaiga* had been made at 17 sites in Greene and surrounding counties in 1991 (Hunter 1996). Larval and pupal parasitism was inconsequential in both plots, never exceeding peak-week rates of 2 and 8 percent in D04 and D11, respectively. Neither *P. silvestris* nor *B. pratensis* were recovered from any hosts evaluated in 1997. Survivorship in these gypsy moth populations was extremely low, suggesting even lower pest densities in 1998.

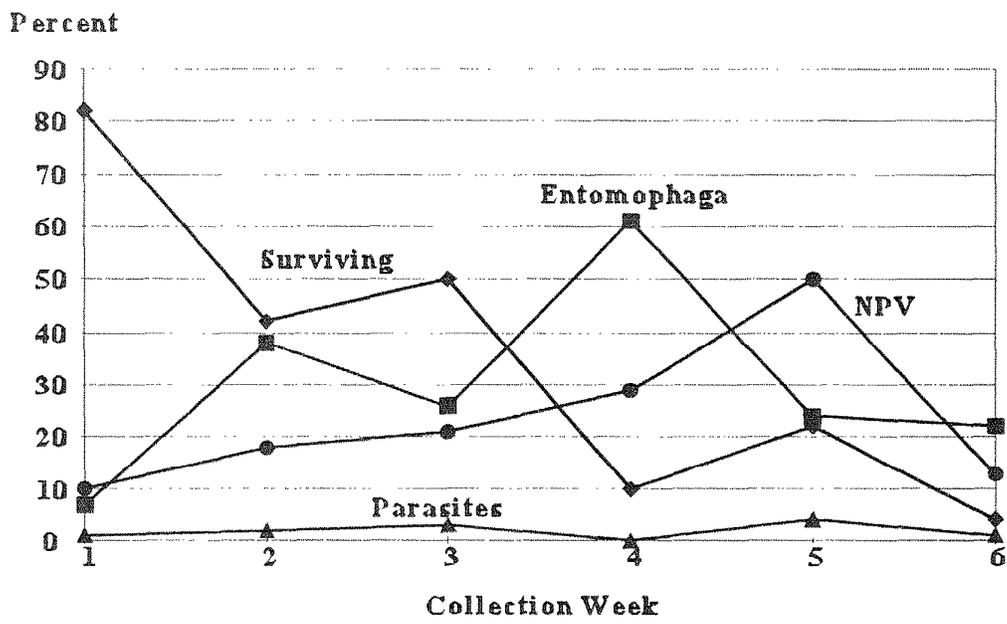


Figure 3. Apparent causes of mortality in gypsy moth collected in Greene County, Pennsylvania in 1997.

### DISCUSSION

It appears from these data that we may be losing, or already have lost, many of the parasites which mitigated gypsy moth damage in the past, and for which so much effort has been expended at state, federal and international levels. Most of the established parasite species require at least some nominal number of gypsy moth hosts in order to maintain their own population levels, and the fungus seems to have encroached severely on this required reservoir for the last several years. As far as gypsy moth biological control is concerned, the health of the northeastern oak resource may now depend on NPV, which usually intercedes too late to prevent defoliation, and our new Asian pathogen.

There are those, however, who are skeptical about the persistence of *E. maimaiga*, citing the climatic and edaphic requirements of the fungus, host resource and resistance issues, and effectiveness in newly infested areas. Others find the evident displacement too severe, too radical, hoping instead for a more gradual integration of the pathogen into the existing natural enemy complex (Schneeberger 1996). Nonetheless, the fungus *seems* to have done more in seven years to reduce gypsy moth defoliation, tree mortality, nuisance problems, and pesticide applications in Pennsylvania than the established natural enemies have accomplished since DDT was banned.

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## HEMLOCK WOOLLY ADELGID DAMAGE ASSESSMENT — WHAT'S THE PROBLEM?

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### ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA) was first found on the eastern hemlock (*Tsuga canadensis* (L.) Carr.) in the early 1950s and has been recognized as a significant pest of eastern hemlock since the mid 1980s. The purpose of the study is to evaluate HWA impacts on trees and stands; identify site and stand characteristics that make hemlock more susceptible to attack, or vulnerable to mortality; identify other pests that may be compounding observed impacts; determine rates of tree mortality; and determine if hemlock are able to survive or recover from a HWA infestation. Data has been collected each year since 1993; 6935 observations have been made from 145 10-tree plots in 29 sites over 4 study areas: Delaware Water Gap National Recreation Area; Northeastern Pennsylvania; South-central Pennsylvania; and West Virginia. A plot-wide assessment of HWA was made using non-destructive sampling of 10 12-inch branch tips per plot, each from a different tree. Where possible, these samples were taken from the permanent sample trees. Samples consisted of (1) the number of terminals present before new growth, (2) the number of terminals producing new growth, and (3) the proportion of terminals infested with HWA.

The spread of HWA into these plots has been much slower than expected. From a regression of the proportion of terminals infested with adelgid against the proportion of terminals that produce new growth, we found a highly significant and rational relationship, but this relationship only explained 1.64% of the total variation. Very few plots have become infested with HWA. The relationship between HWA and the five tree health variables were tested; crown dieback showed a significant slope (Model  $P = 0.0013$ ;  $y = 2.13 + 0.06x$ ) but HWA only explained 2.6% of total variation in plot mean dieback.

We are now considering the best means to revise our sampling plans and our area-site-plot system to optimize our chances to capture the impacts of HWA. We have been reviewing the variability among trees and plots within sites and areas as well as the difficulty of crews to calibrate their eyes to measure and record these data. To capture the effects of adelgid as it attacks new trees, we will expand our sampling to include as many areas as possible that have current adelgid problems, choosing sites that are on the edges of currently infested stands. We

will also utilize our current data to assist in the estimation of sample sizes for capturing the potential range of effects. To date, we have permitted the sampling of HWA at any time during the field season; this requires two separate procedures and counting regimes which, in turn, gives rise to additional variability. Because early season (May-July) sampling for the adelgid can be done with the naked eye, one proposal is to only sample during this time frame each year. Another method would be to use a presence/absence survey of each tip chosen for sampling. These issues and more are currently under review and we plan to complete a revised sampling plan in the next two months.

# A WORLD-WIDE-WEB/JAVA IMPLEMENTATION OF SAMPLING AND ANALYSIS

## TOOLS FOR STAND AND GYPSY MOTH MANAGEMENT:

### THE STAND-DAMAGE MODEL

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#### ABSTRACT

The Stand-Damage Model has been available for several years as a DOS application. It has been available for download from the World-Wide-Web since 1994. We have added the ability to directly enter and save tree data that describes a stand. Besides the ability to mix fixed and variable radius (prism) plots of various sizes, the plot data entry system permits the user to enter and summarize additional data collected on individual sample trees which is useful in a management context. These data will be summarized for the user to the stand level on a per acre basis. Information such as crown position, merchantability, tree health characteristics, or habitat characters can be added for each tree.

We are now working to produce a fully WWW-based version of the model, allowing anyone with a web browser to access and use the model directly. Any new update to a web based model will immediately be available to all users of the web site. The web site provides means for users to submit questions directly to the developers, and a reply can be expected the next working day. The new version is written in Java. Java is a platform neutral language. This means that the computer hardware and operating system will not dictate the form of the executable program that it runs. All the major web browser software packages, like NetScape or Internet Explorer, now come with a JVM installed and ready for use as you browse the WWW. JVMs are also available to download for virtually every hardware/operating system combination. Copies of our models are still available through diskette distribution, thus you can find a JVM for your machine, and run the Java Application version.

The Applet version runs from within a WWW browser while the Application version runs directly on your machine. Java on the WWW is a very secure environment and this means that Java Applets can not access your disk drives or directly print on your machine. One big advantage of the Application over the Applet form is that Java Applications can print as well as save and retrieve files from your local hard disk or floppy drive. We are working to extend the Applet version's printing capabilities.

We still want to provide a method for users to store and retrieve information from the Applet version. A database connected to the server software provides this mechanism. The user of the Applet version can save to and retrieve data from this database located on the server. This will also provide a means to provide a large number of examples of specific forest stands and a tutorial for use of the system. This will be accomplished by integrating the Stand-Damage Model with a database that contains a variety of examples from a diverse set of locations. The user will be able to search the database using either stand characteristics or location to find stands that meet their requirements.

## RECENT TRENDS IN THE HEALTH OF HUNGARIAN FORESTS

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### ABSTRACT

The 1.7 million hectares of Hungarian forest (18 % of the total land area) are dominated by deciduous tree species (84.6 % broadleaved, 15.4 % conifers), and most pest and pathogen problems appear in deciduous forests. But because the majority of coniferous forestry consists of artificial plantations on suboptimal sites (i.e. dry sandy soils), their health status is also far below desirable levels.

To obtain information on the health of Hungarian forests, the Hungarian Forest Research Institute has been operating a Forest Health Reporting System since 1962. All the forest owners are required to report any kind of forest pests or pathogens occurring in their forests. Their reports are frequently monitored and compiled by researchers of the Forest Research Institute. The dataset obtained from these reports enables us to compare the yearly average damage values of different pests and pathogens over the last decade (1988-1997) with damage occurring in earlier years (1962-1987).

The health of Hungarian forests has worsened over the last 10 years, especially in the early nineties. On average, 10 species (or species groups) of the 13 major forest defoliating insects infested a larger area over the last decade than in the preceding period. Two pests - loopers (*Geometridae* spp.) and the oak processionary moth (*Thaumetopoea processionea* L.) - occurred on similar areas in both time periods, and only one species, the lackey moth (*Malacosoma neustria* L.) infested smaller area than earlier. All of the major sap feeders and acorn pests infested much larger areas in the last decade. The majority of xylophagous pests expanded the areas damaged as well, and only the pine shoot boring moth (*Rhyacionia buoliana* Den.& Schiff.) damaged a smaller area between 1988 and 1997 than earlier. All of the major forest pathogens expanded the areas damaged, 2/3 of them to a major degree.

The underlying cause of this increased forest damage has been the unusually long and severe dry period extending from the early eighties through to 1994. The increased frequency and severity of drought undoubtedly played a key role in provoking more serious outbreaks of several forest insects and pathogens, leading to a significant expansion of territory damaged by them.

For many pests, there is a significant positive correlation between areas damaged each year and the drought index for that year (average temperature of the growing season in C°/ the precipitation of the growing season in 100 mms), expressed both as a yearly value and as each of the 2-, 3-, and 4-year moving averages. The correlation was looser for the lepidopteran defoliators, but were highly significant ( $p < 0.01$ ) for spruce bark beetles, *Curculio* acorn weevils, *Kermes quercus* L. and the pine sawfly (*Neodiprion sertifer* Geoff.).

It is worth mentioning that drought is certainly the most important primary factor in the decline of the sessile oak, which has been one of the most significant and spectacular forest health problems of the last two decades in both Hungary and Central Europe. Health indices of sessile oak stands in any given year show a strong negative correlation with the drought indices of the same year, and the previous 1-2 years.

In addition to the unusually long and severe dry period, forestry practice and other human activities can also have a significant impact on damage trends. The steep increase in the area of artificial pine and hybrid poplar plantations in the last couple of decades has also contributed to an increase in damage due to pine and poplar pests.

Extensive draining activities in forested areas have also contributed significantly to increases in the populations of acorn weevils, the browntail moth (*Eurproctis chrysorrhoea* L.) and several other species of pests. In addition to the increasing damage caused by some well-known forest pests and pathogens, a large number of new forest insect and pathogen species have emerged as significant pests over the last 15 years. These include, amongst others, two lepidopteran leafminers, *Parectopa robiniella* Cl. and *Phyllonorycter robiniella* Cl. on black locust, a leaf mining moth *Cameraria ohridella* Deschkla & Dimic on horse chestnut, the defoliating caterpillars of *Ptilophora plumigera* D. & S. on several *Acer* species, a needle fungus, *Diptodia pinea* (Desm.) Kickx. on Austrian pine.

With the end of the prolonged dry period in 1994, a slow improvement in forest health has been observed.

EFFECT OF PARASITISM BY *COTESIA MELANOSCELA* ON  
NPV PATHOGENICITY IN *LYMANTRIA DISPAR*

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ABSTRACT

The braconid wasp *Cotesia melanoscela* parasitizes gypsy moth (*Lymantria dispar*) larvae in the northeastern U.S. (Crossman 1922), where it overlaps with the gypsy moth nuclear polyhedrosis virus (LdNPV) as a cause of early mortality. *C. melanoscela* injects a double-stranded, segmented DNA virus (the polydnavirus CmV) into gypsy moth larvae, along with poison and an egg. This polydnavirus acts to suppress the immune response in the parasitized gypsy moths, so that the wasp egg is not encapsulated. It may also be possible to use the immunosuppressive properties of CmV to improve the efficacy of formulations of LdNPV used for biological control of the gypsy moth.

In a preliminary experiment designed to explore the effects of *C. melanoscela* parasitism and CmV on LdNPV infection in the gypsy moth, second instar gypsy moth larvae were given two treatments; a sting from *C. melanoscela* at 0 h. and an LdNPV dose at 24 h. (Sting + LdNPV), or an LdNPV dose only at 24 h. (LdNPV Only). Stings were administered as follows: three newly molted gypsy moth larvae were placed into an oviposition cup with one naïve female *C. melanoscela* until we saw what appeared to be a successful sting event. We defined this as a clearly observed contact between the ovipositor area of the wasp and the integument of a larva that lasted for approximately 1 second or longer. Larvae were then removed and placed in a sterile petri dish. LdNPV doses were administered to all larvae approximately 24 h. after the stinging procedure; by this time the immunosuppressive action of CmV was considered to be fully expressed. Groups of larvae were fed pieces of virus-contaminated gypsy moth diet *ad libitum* for 48 h., 10 larvae per petri dish. Virus was incorporated into the diet at 0, 5, 50, 500, 5,000, and 50,000 polyhedral occlusion bodies per ml of diet.

The putative LT50 of gypsy moth larvae that were stung by *C. melanoscela* was two days less than the LT50 of unstung larvae. At present, we do not know whether it is poison, CmV, or the developing wasp larva that causes this effect. The Sting + LdNPV treatment slowed the development of gypsy moth larvae at all doses. By Day 12, about 85% of all larvae in the Sting + LdNPV treatment were still 2<sup>nd</sup> instars, compared to ~5% in the LdNPV Only treatment. Although lower doses of LdNPV caused no appreciable gypsy moth mortality, there was an effect of LdNPV dose on time of *C. melanoscela* emergence; higher doses of LdNPV slowed wasp emergence.

DEFOLIATION AND MORTALITY IN MIXED PINE-HARDWOODS:  
FIVE YEAR RESULTS

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ABSTRACT

Recent movement of gypsy moth populations into the southeastern United States has raised some interesting questions concerning the potential for defoliation within mixed pine-hardwood stands and loblolly pine (*Pinus taeda* L.) plantations, and subsequent effects on tree growth and mortality. Will these stands be defoliated? If they are defoliated can tree mortality be expected, and if so at what scale, and which species are likely to be affected? This study was established in the counties of Dorchester, Somerset, Wicomico and Worcester, MD, in the Atlantic Coastal Plain physiographic province. Research plots were established in sixteen mixed pine-hardwood stands in 1991-1993.

The sixteen stands were classified as either pine-oak or pine-sweetgum based on whether oak or sweetgum was the dominant hardwood (on a percentage basal area basis). Between 1992 and 1996 both cover types were subjected to a single gypsy moth defoliation outbreak. Susceptible species (oaks and sweetgum) were consistently defoliated at much greater intensities than the pines. ANOVA revealed significant differences in mean defoliation levels in each of the outbreak years. Pine defoliation was observed in pine-oak stands in 1994. This occurred primarily within three stands where some overstory pines suffered moderate to heavy defoliation, and mean pine defoliation levels rose to 17, 34 and 59%. This increase in pine defoliation appeared to be related to a threshold level of oak defoliation. When oak defoliation exceeded 80%, the intensity of pine defoliation increased dramatically. Following defoliation, tree mortality was observed in both pine-oak and pine-sweetgum cover types. Southern oaks were found to be as vulnerable to mortality as their counterparts in the northeast, but sweetgum appeared to be less vulnerable following defoliation. Where both of these susceptible species comprised a significant percentage of the total initial basal area, they also tended to comprise a greater proportion of the total mortality. Suppressed and intermediate trees in the understory, and trees that were in poor or fair condition, had a much higher probability of dying following defoliation. Results from this study did not clearly implicate gypsy moth defoliation in pine mortality. Pine mortality was observed, however, the majority occurred among suppressed and

intermediate trees. Simple and multiple linear regression were used to formulate a model for use in the prediction of gypsy moth defoliation in mixed pine hardwood stands. Logistic regression was found to be a useful tool in the prediction of tree mortality following defoliation. Two logistic regression equations were derived and validated for use in pine-oak and pine sweetgum cover types. Model 1 predicts the probability of tree mortality in stands where the hardwood component is dominated by oaks; model 2 predicts mortality in stands where sweetgum is the dominant hardwood.

OPTIMIZATION OF *BACILLUS THURINGIENSIS* AGAINST THE BROWNTAIL MOTH,  
*EUPROCTIS CHRYSORRHOEA* L., BY TOXIN AUGMENTATION

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ABSTRACT

The browntail moth, *Euproctis chrysorrhoea* L., was introduced into the United States in 1897 near Summerville, MA. By 1914, it was a major pest in eastern New England and the southern portion of the Canadian Maritime Provinces. Both directed efforts and natural factors, particularly *Entomophaga aulicae*, a fungal entomopathogen, reduced infestations to small residual pockets on some islands in Casco Bay, ME, and the tip of Cape Cod, MA. In the mid-1980's, the population in ME began to expand. Today, *E. chrysorrhoea*'s urticating hairs are causing extensive human discomfort as it defoliates deciduous hardwoods on some islands in Casco Bay as well as the coastal area of Yarmouth and Portland ME. Previous efforts to control this pest with *Bacillus thuringiensis* (Bt), a microbial insecticide routinely used against the Gypsy Moth, were unsatisfactory. Recent studies have shown that augmentation of currently available Bt formulations with the CryIAC Insecticidal Crystal Protein (ICP) can improve their efficacy against this pest. Initial observation of the susceptibility of *E. chrysorrhoea* to CryIAC was made by voltage/current clamp analysis and confirmed by bioassays with pure ICP's and with Bt formulations of known ICP composition. Augmentation of Foray 48F or Condor OF with MVP II (a commercial preparation of CryIAC produced by Mycogen Corp.) resulted in a significant increase in mortality compared to that from either BT formulation alone. A low mixture ratio of Bt:MVP II (0.6:1) increased the efficacy of the Foray formulation significantly whereas a ratio of 5.3:1 increased the efficacy of Condor. In a preliminary field study in which the 5.3:1 mix ratio was used, mortality was significant 4 days after treatment with the Condor mix and 3 days later with the Foray mix. There was no significant difference between Bt formulations. MVP II did not differ from the controls until 2 weeks after treatment. Frequency of Bt infection was equally high for both Bt formulation treatments (>60%). As expected, the MVP II treatment did not differ from the controls.

EXOTIC BARK BEETLES IN THE UNITED KINGDOM: KEEPING THEM OUT AND  
MANAGING THEM IF THEY ESTABLISH

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ABSTRACT

The United Kingdom, by virtue of its relatively small timber industry, imports around 90% of its wood requirements. In addition, the forest industry itself is dominated by the planting of exotic conifer species, mainly from Europe and North America. Overall, UK forests are relatively free of the pests that are associated with these exotic conifers in their native habitats. Among these pests, the bark beetles (Coleoptera: Scolytidae) are known to be the most destructive. The Forestry Commission, under the auspices of the European Union plant health directives, has in place an active program to keep bark beetles out of the country and to develop effective pest management strategies should they eventually establish here.

Pest Risk Analysis (PRA) is used as a primary tool in evaluating the risks from bark beetles and is the first step in developing risk mitigation measures. The PRA approach is based on developing FAO guidelines which have been refined by EPPO (the European and Mediterranean Plant Protection Organization). This has been applied to two of the most destructive bark beetle pests of spruce in Europe: great spruce bark beetle *Dendroctonus micans* (Kug.) and eight toothed European spruce bark beetle *Ips typographus* (L.). In both cases, Protected Zones (PZ) have been set up as part of a EU system to allow trade between member states. The basis of the PZ system is the use of surveys to confirm freedom from the pest and establishment of import rules that require all wood to be free from bark. Surveys for *D. micans* are based on visual assessment for tree damage, while those for *I. typographus* are augmented by use of trap logs combined with pheromone lures. A network of pheromone traps at the ports and selected timber processing plants provides additional information on potential importation of *I. typographus*.

Despite plant health controls on imported timber, *D. micans* has established in Britain (but not in Northern Ireland or the Irish Republic) and is now being managed by a combination of restriction on timber movement to reduce further spread and the rearing and release of the exotic, specific predatory beetle, *Rhizophagus grandis* (Gyll.). *I. typographus*, on the other hand, has not established here, despite interceptions that date back over a hundred years. However, recent findings of the beetle in pheromone traps at a paper mill taking wood only from British forests may indicate the presence of a breeding population. Prospects for management of *I. typographus*, if it has established, will be discussed.

TEMPORAL AND SPATIAL RESPONSES OF *CALOSOMA SYCOPHANTA* L.  
(COLEOPTERA: CARABIDAE) TO THE ABUNDANCE OF GYPSY MOTH  
(LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT

The gypsy moth, *Lymantria dispar* (L.), is the most important defoliator of hardwoods in the northeastern U.S. Native to Eurasia, it was accidentally introduced into North America in 1868. Since the early 1900s, efforts to obtain classical biological control of this pest have resulted in the establishment of over a dozen natural enemies. One of these is the carabid *Calosoma sycophanta* (L.), a large beetle with shiny green elytra. Imported from Europe in 1906, this species is now widely distributed in New England and the Middle Atlantic States. Though reported to feed on a wide range of Lepidoptera, it is usually observed only when the gypsy moth is abundant. Here, we report on the temporal and spatial responses of populations of *C. sycophanta* to those of *L. dispar* over the course of a population gradation.

The abundance of *Calosoma sycophanta* (L.) and the gypsy moth, *Lymantria dispar* (L.), was monitored in oak stands in New Jersey from 1982 to 1996. Time series analysis was used to study temporal responses by *C. sycophanta* to changes in gypsy moth abundance. Regression analysis was used to study spatial responses in predator abundance to gypsy moth abundance.

Over time, the predator population responded positively to changes in prey abundance; the relationship was direct for larvae, but delayed for adults, which remained abundant for four years following the year of peak prey abundance. Adults of *C. sycophanta* were spatially correlated with gypsy moth abundance early in the outbreak, but more strongly correlated over time than spatially. Numbers of *C. sycophanta* larvae were positively correlated with numbers of adults, both in space and time, and the response seemed to be caused by enhanced reproduction where prey were numerous. Ratios of predators to prey were highest late in the outbreak, and lagged 1-3 years behind peak gypsy moth larval counts. The abundance of *C. sycophanta* adults was correlated spatially with the abundance of predator larvae and adults the previous year. Predation of gypsy moth pupae by *C. sycophanta* was density-dependent and correlated with the abundance of predator larvae, but the relationship was stronger with respect to time than spatially. Because the population dynamics of *C. sycophanta* are temporally and spatially associated with gypsy moth, it should be considered a specialized predator of the pest.

## CLONING OF A BT TOXIN RECEPTOR FROM THE GYPSY MOTH

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### ABSTRACT

Brush border membrane aminopeptidase N from gypsy moth (*Lymantria dispar*) midgut has been identified as the receptor for the *Bacillus thuringiensis* CryIAc delta-endotoxin. A complete cDNA corresponding to this receptor has been cloned and sequenced. The cDNA is 3403 basepairs in length and encodes a 1017 amino acid prepro-protein. The sequence contains 5 potential N-linked glycosylation sites. The C-terminal end of the sequence has characteristics of a glycosyl-phosphatidyl-inositol (GPI) anchor signal. The cloned cDNA encodes the N-terminal sequence and several internal peptide sequences from the CryIAc-binding aminopeptidase protein (APN1). During the cloning process, a second cDNA encoding a distinct aminopeptidase N from the gypsy moth was also isolated and characterized. Cloning and analysis of receptor molecules will allow investigation of the mechanisms of toxin binding and pore formation.

INTERACTIONS AMONG GYPSY MOTHS AND ITS PREDATORS AT THE  
SOUTHERN EDGE OF INFESTATION

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ABSTRACT

The potential impact of natural enemies within selected forest ecosystems threatened by gypsy moths was evaluated along the southern edge of infestation. Two study sites were established in each of three geographic areas: the coastal plain, piedmont, and mountains. All plots were mixed hardwood forests with large oak components. Changes in the roles of major predators and parasites commonly associated with this insect should be key to understanding whether forest management might require modification as this pest moves into southern forests.

Because small mammals, in particular white-footed mice, *Peromyscus leucopus* Rafinesque, are major gypsy moth predators in the northeast (Elkinton *et al.* 1996, Jones *et al.* 1998), we surveyed their populations within selected plots from 1992-1995 using Sherman live-traps. One week after each mouse survey, predation of freeze-dried gypsy moth pupae was measured. Pupae were offered at four locations in each of 75 stations within each study site: within the litter and at 0.25, 1.0 and 2.0 m on randomly selected trees. Mast production within each study site was also measured.

The predation data suggested that when mice are at high densities they prey upon more freeze-dried pupae than do the invertebrates. However, invertebrate predation could be partially masked by the manner in which mammals feed on pupae. The contribution made by invertebrates (1-86% of the total predation) is of interest because of the short exposure (3 days) to these pupae and the difference in magnitude of the predation between the northeast and the south. Most of the observed predation attributed to mice was done early in the exposure period, while most of the predation by invertebrates was done late in the exposure period. The mice preyed mainly on those pupae located in the litter and low on the tree bole, while invertebrates tended to prey upon the pupae at the higher locations.

During 1992 predation was predominately by vertebrates and there was an inverse relationship between vertebrate and invertebrate predation. Total predation was almost unchanged from 1992 to 1993, even though mouse populations were low to moderate. The major difference in 1993 was that invertebrates contributed 35-86% of the total predation. For 1994, total predation was much higher in the piedmont and mountains but again most predation was by invertebrates (42-69% of the total predation in the piedmont and mountains). During 1995 the major predators in the piedmont and mountains were vertebrates but invertebrates were the major predators in the coastal plain (64 and 65% of the total predation in the coastal plain) where small mammals remained at sparse densities. The mast measurements, which included both acorns and hickory nuts, suggested that the current years nut crop was a good predictor of next years mouse population (see also Elkinton *et. al.* 1996), and usually a good indicator of pupal predation by mice. However, observations in the mountains in 1994 suggested that if an ample supply of acorns were still present the following spring, the mice would feed less on the freeze-dried pupae. This suggests that when alternative food sources are available, predation by mice on gypsy moth larvae and pupae can be reduced.

The high level of predation within all geographic locations even when small mammal populations were low to moderate suggests that there is a possible difference in richness and impact potential of invertebrates in southern forests. This is unlike information from Northeastern U.S. at Bryant Mountain, VT, (Smith 1989) and Cape Cod and western Mass. (Elkinton and Liebhold 1992) where predation decreased as small mammal populations decreased. Surveys of parasites and pathogens in 1993 and 1994 further confirmed the richness of natural enemies that are influencing the gypsy moth's dynamics in the south.

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EPIZOOTICS CAUSED BY *ENTOMOPHAGA MAIMAIGA*: RISK OF INFECTION  
ASSOCIATED WITH BEHAVIOR OF LATE INSTAR GYPSY MOTH LARVAE

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ABSTRACT

This research was conducted to gain a better understanding of where and when gypsy moth larvae become infected by the fungal pathogen *Entomophaga maimaiga*. *E. maimaiga* produces two types of spores from gypsy moth cadavers: infective conidia are actively discharged from cadavers and overwintering resting spores are produced within cadavers. In general, only conidia are produced from cadavers of early instars while resting spores are produced within cadavers of late instars. In agreement with the locations where early vs. late instars are often found, early instar cadavers of larvae killed by *E. maimaiga* are most often found in the foliage while late instar cadavers are attached to tree trunks. Cadavers on tree trunks fall to the ground and resting spores are leached into the soil at the bases of trees.

Studies were conducted along Piney River, George Washington National Forest, VA by placing fourth instar gypsy moth larvae within cages made of window screening. To compare the relative risk of infection at various locations, cages were either placed on the soil at tree bases, tacked to tree trunks at 2 m height, or tied from branches in the understory vegetation. Cages were in place either during photophase or scotophase to test for any diurnal rhythms in fungal activity. During photophase, larvae placed on the soil were infected at the highest levels with least infection among larvae caged in the vegetation. During scotophase when only soil and vegetation locations were compared, larvae placed on the soil were once again at highest risk of infection. Infection rates did not differ between photophase vs. scotophase for larvae caged in the vegetation but more infections occurred during photophase than scotophase for larvae caged on the ground. We had assumed that larvae caged on the ground would be infected by germ conidia produced from resting spores. However, we found that some infections of ground-caged larvae were caused by airborne conidia that either landed on cages and/or had already been deposited on the soil.

Late instar gypsy moths are known to seek dark locations during photophase, e.g., under burlap bands or bark flaps or in the litter. Studies of burlap band use by WV forest macrolepidoptera have shown that resting in dark locations during the day is relatively uncommon (Butler & Kondo 1993). We hypothesize that the litter dwelling behavior of late instar gypsy moths puts these larvae at high risk of infection by this virulent fungus and lack of this behavior by other species might help to explain the high levels of specificity of *E. maimaiga* for gypsy moth larvae in the field.

INDUCED DEFENSES IN HYBRID POPLAR:  
EFFECTS OF WOUNDING TREATMENT AND CLONAL VARIATION ON  
GYPSY MOTH DEVELOPMENT AND HOST SELECTION

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ABSTRACT

An important component of tree resistance against insect feeding is the rapid induction of defensive chemicals following attack. *Populus* species are a preferred host plant of gypsy moth in North America. The chemical ecology and defenses of native poplars have been well studied. However, few studies have examined the mechanisms of induced pest resistance in hybrid poplar clones. Understanding how genetically distinct clones vary in their extent of foliar induction could be an important component of the silvicultural control of insect pests. The objectives of this study were to evaluate the effects of clonal variation and experimental induction method on gypsy moth host selection and development.

Twelve hybrid poplar clones were evaluated for inducibility using different methods. Trees were established in a shaded glasshouse at the UW-Madison Biotron using dormant hardwood cuttings from clonal sources. Trees were actively growing and approximately 50 days old when used in experiments. The methods of induction were (1) larval gypsy moth feeding, (2) mechanical wounding of leaves, (3) mechanical wounding plus application of gypsy moth regurgitant, and (4) application of jasmonic acid. Jasmonic acid is thought to be an important component in signal transduction in response to tree wounding or stress. Non-induced control trees were left intact. Treatments were performed regularly over 24h. on lower leaves to systemically induce a wounding response. Upper, undamaged leaves were removed and tested for induction using choice and no-choice gypsy moth assays 72h. following initiation of treatments.

Real and simulated herbivory of hybrid poplar trees induced foliar changes that affected subsequent feeding preference and development of gypsy moth larvae. In choice tests, second-instar gypsy moth larvae selected foliage from non-induced trees over induced trees. Similarly, the relative growth rates of larvae fed foliage from induced trees was lower than larvae fed foliage from non-induced trees. The extent to which tree responses to real or simulated herbivory affected insect feeding and development varied significantly among poplar clones and experimental induction treatments. For example, in feeding bioassays the consumption ratio of control to treated foliage ranged among clones from nonsignificant to 9x. However, clone by treatment interactions were weak. Clones that responded to one form of induction were generally responsive to all forms and exhibited an overall stronger response.

GYPSY MOTH PARASITISM IN THE NATIVE RANGE  
DURING YEAR 2 (1997) OF THE LATENCY PHASE

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ABSTRACT

Parasitism of gypsy moth (*Lymantria dispar* (L.)) was studied during the second year (1997) of the current latency phase by exposing laboratory reared larvae on potted 80 cm oak tree seedlings in the forest of Haguenau, Bas-Rhin, northeastern France. The 2 primary parasitoids were the tachinids *Blepharipa schineri* (Mesnil) and *Ceranthia samarensis* (Villeneuve). Dramatic changes in the guild of parasitoids and abundance of the major species were observed during the period from the last year of the outbreak (1995) through the second year (1997) of the latency phase.

A considerable decrease in the rate of parasitism by the tachinid *Parasetigena silvestris* (Robineau-Desvoidy) was observed from the last year of the outbreak (1995) through the second year of the latency phase. The 1997 peak of parasitism was less than 20%. During the first year of the latency phase (1996), *P. silvestris* was still a major species. Although *B. schineri* was present in the host outbreak situation, its abundance was masked by the competition with *P. silvestris*. During the second year (1997) of the latency phase, competition decreased and the peak of parasitism by *B. schineri* exceeded 35 % in recaptured larvae. *B. schineri* impact on the gypsy moth population was noticed more than a month after disappearance of egg laying females, thanks to the durable survival of the eggs on leaves.

*C. samarensis* was present at Haguenau at very low levels in the natural population during the last year of the outbreak (1995), and was not found in exposed larvae during the first year of the latency phase (1996). In contrast, during the second year of the latency phase (1997), it was one of the most abundant parasitoids of exposed gypsy moth larvae (peak, 37%), and its egg laying activity lasted until late July, 1997.

Field studies of egg laying behavior of *B. schineri* showed evidence of a gradient of egg density around spots of contamination after 12 days of exposure. Some leaves at 1.5 m from spots of contamination received a few eggs. Eggs were laid in higher proportion on edges, in the median portion of leaves. When egg laying activity increased in early June, eggs were deposited on a higher proportion of undamaged leaves of contaminated plants, and on a higher proportion of leaves on adjacent, non-contaminated plants. However, the mean number of eggs per leaf, calculated from leaves bearing eggs only, did not exceed 3. The pattern of egg distribution was not significantly different on contaminated and on non-contaminated leaves.

CHANGES IN THE NUMBER OF EGG MASSES  
IN THE JAPANESE GYPSY MOTH IN RELATION TO STAND DENSITY

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ABSTRACT

Stand density is one of the most basic character of forest. I examined changes in number of egg masses in the Japanese gypsy moth, *Lymantria dispar praeterea* Kardakoff, in two Japanese birch (*Betula platyphylla* Sukatchev) plantations with different stand density. The plantations were established in the experimental forest of Hokkaido Forestry Research Institute at Bibai, Hokkaido, in 1961. The sizes of the two stands were the same, 68 m by 34 m, or 0.2312 ha. Stand density was 955 /ha in the dense stand, and was 470 /ha in the sparse stand in 1991. It decreased to 560 /ha in the former and to 280 /ha in the latter after thinning in May, 1992.

Egg mass density in the two stands was varied between 45 /ha in 1994 and 355 /ha in 1995 during seven years, 1991 to 1997. During the years, egg mass density per tree was almost the same in the both stands irrespective of stand densities except in 1994, the year of lowest density of egg masses. Therefore egg mass density per unit area in the sparse stand was about the half of that in the dense stand.

The pattern of egg mass distribution per tree was significantly different from random distribution. The pattern was contagious particularly in the dense stand. Females preferred to oviposit on trees on which egg masses had already been deposited.

There are two hypotheses in animal's aggregation. The first is that ovipositing females attract other females. Whereas the second is that environmental heterogeneity makes aggregation in preferable sites. I tested the second hypothesis using rank correlation coefficient of number of egg masses on trees between two oviposition periods.

In Hokkaido, Japan, females oviposited in the late of August. More than eighty percent egg masses were deposited during 15 - 31 August. I counted the number of ovipositing females at three to seven days intervals in 1992, 1995 and 1996. Kendall's tau-b, rank correlation coefficient, was always minus, and was significantly different from zero. This indicated that females avoided trees having many egg masses. Thus the second hypothesis was rejected. Since the distribution pattern was also contagious in each observation period, ovipositing females attracted other females. But the effect seemed to continue only in a short period.

Since egg parasitism was only few percent, winter predation by birds was unique mortality factor in the egg stage. I examined local density dependence of egg mass predation by birds within years in eighteen grids divided in each stand. Although predation was varied in low density grids, inverse density dependence was observed in higher egg mass density grids. The selection advantage of aggregation in egg stage, or the preference of high tree density stand, is to avoid bird predation. Between years, however, density dependent predation was observed particularly in low tree density stand. Therefore bird predation becomes to regulate gypsy moth population more strictly by thinning practice. The effect of forest practices on pest dynamics should be considered in forestry.

ABNORMAL FEMALE PRODUCING ONLY FEMALE PROGENY  
IN THE JAPANESE GYPSY MOTH IN HOKKAIDO

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ABSTRACT

Although sex ratio of insect is usually 0.5, biased ratio is also observed in many insects. Females that produce only female progeny, so-called son-killer factor, was found in twelve species in Lepidoptera. We found such a female in a natural population of the Japanese gypsy moth, *Lymantria dispar praeterea* Kardakoff, in Bibai, Hokkaido, Japan.

All larvae hatched from one of eight egg masses became female adults in the summer of 1996. The hatchability of the eggs of the egg mass was very low, 54 %. Probably, cytoplasmic factors kill male embryos, and change the sex ratio of the moth.

We crossed females from eight egg masses with males of the egg masses during July in 1996. Hatchability of these egg masses were nearly 100 %, except for egg masses deposited by progeny of the abnormal female and the cases of inbreeding. Hatchability decreased to 40 to 55 % in inbreeding. It was also nearly 50 % in egg masses deposited by progeny of the abnormal female irrespective of males. All larvae of them became females.

Such abnormal females exist widely in natural populations, since two and two egg masses of 42 egg masses and 17 egg masses, respectively, were low hatchability at two Japanese Birch (*Betula platyphylla* Sukatchev) forests in Bibai, Hokkaido. We estimated hatchability of egg masses by counting remaining eggs in July 1996. Number of eggs contained in the mass can be estimated from the product of length times width of egg mass.

We reared about 30 eggs from each of 66 egg masses in a Japanese birch forest in Bibai, Hokkaido, Japan, between May and July 1997. Since only female progeny was observed in six egg masses, abnormal females was 10 %. In the previous generation, it was 11.8 %. The mechanism of persistency in these abnormal females is interesting in Hokkaido population.

## PINE SHOOT BEETLE AND ITS NATURAL ENEMIES IN POLAND

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### ABSTRACT

The pine shoot beetle (*Tomicus piniperda* L.) is one of the most popular of the Palaearctic beetles. In Poland PSB occurs everywhere, where Scots pine (*Pinus silvestris* L.), its main host tree is present. Besides Scots pine, PSB in Poland infests sporadically European larch, Norway spruce and common fir. In addition PSB has been recorded on several pine species introduced from North America. PSB flight begins early i.e. by the end of February and in March. Adults infest trees weakened by defoliators and fires as well as windfalls, fresh logs and stumps.

PSB has a wide complex of natural enemies. Among 150 insect species recorded in Poland and associated with PSB larval galleries, about 110 are considered as potential antagonists of this bark beetle. The most important are predatory beetles from families: *Rhizophagidae* (4 species), *Cleridae* (2 species), *Staphylinidae* (46 species) and parasitoids representing families: *Braconidae* (8 species), *Pteromalidae* (6 species) and *Eupelmidae* (1 species). Natural enemies of PSB are also represented by birds (especially woodpeckers), predatory mites, pathogenic nematodes and fungi. Intraspecific competition of larvae and competition with other phloem-feeding insects like cerambycids *Acanthocinus aedilis*, *Rhagium inquisitor* and scolytid *Hylurgops palliatus* may also be important factors limiting PSB.

In spite of a rich complex of limiting factors, PSB is still a serious threat as secondary pest to pine monocultures and plantations, in which the pest is controlled by sanitation harvesting and with the use of trap logs and pheromone traps. However, it seems that the best solution of the PSB problem in Poland is to enhance its natural enemies by increasing diversity of forest stands.

## CLONING AND CHARACTERIZATION OF THE GYPSY MOTH

### JUVENILE HORMONE ESTERASE mRNA

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#### ABSTRACT

In the gypsy moth a JH-hydrolyzing enzyme, juvenile hormone esterase (JHE), contributes to enzymatic degradation of JH in the hemolymph which is critical for metamorphosis to occur. Since JH removal is requisite for normal development, exploitation of the catabolic activity of JHE is being pursued for the development of environmentally-safe insect control agents.

Immunological and biochemical studies have shown that the gypsy moth JHE differs significantly from the *H. viriscens* enzyme in its primary structure and kinetic properties. The *H. viriscens* enzyme has been cloned and sequenced. Analysis of the nucleotide sequence has shown that it is related to other serine carboxylesterases.

In order to characterize the gypsy moth JHE, a cDNA library was constructed using poly(A) RNA obtained from fat body of 5th instar day6 larvae. Screening of library with JHE antibody resulted in identification of three clones, JHE24, JHE26 and JHE36 which contained cDNA inserts of 1.0, 1.6 and 0.8 kbp, respectively. Nucleotide sequence analysis indicated that the inserts in JHE24 and JHE36 were contained in the JHE26, which represented the 3'-end of JHE mRNA. Northern analysis showed that the JHE mRNA was ~1.7 kb long suggesting that JHE26 did not contain the 5'terminal region of the mRNA. The latter was obtained by 5'-RACE reactions and its nucleotide sequence was determined.

Analysis of the complete nucleotide sequence of JHE mRNA, obtained as described above, indicated that the JHE mRNA was 1768 nt long. Based on the northern analysis, this represented almost full length sequence of JHE mRNA. There was a single open reading frame between nt 51-1646 encoding a protein of 532 amino acids. The 5'- and 3'- untranslated regions were 50 and 119 nt long, respectively. A single polyadenylation site was present at position 1672-1677. The deduced amino acid sequence, with few exceptions, matched well with the sequence determined by direct amino acid sequencing of tryptic peptides.

The nucleotide and amino acid sequence of the gypsy moth JHE mRNA demonstrated that it was significantly different from the *H. viriscens* enzyme. Interestingly, the gypsy moth enzyme showed a strong relatedness to the membrane-bound alkaline phosphatases. Degree of identity

at the amino acid level between phosphatases from *Bombyx mori* and human tissues was more than 90% suggesting that the gypsy moth JHE probably has the phosphatase activity also. This finding was consistent with the observation that trifluoromethyl ketones which inhibit JHE activity also inhibited phosphatase activity of the gypsy moth JHE. In addition, the gypsy moth enzyme was also resistant to classical esterase inhibitors. Whether the gypsy moth enzyme indeed has dual activities can be ascertained by expressing the JHE cDNA in a baculovirus system and testing the in vitro produced protein for the enzyme activities.

## REGULATION OF EXPRESSION OF THE GYPSY MOTH VITELLOGENIN GENE

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### ABSTRACT

Isolation and characterization of a number of vitellogenin (Vg) genes from vertebrate and invertebrate animals has suggested that these genes are derived from a common ancestral gene. We have recently cloned and sequenced the entire Vg gene from the gypsy moth. Analysis indicated that its structure differs significantly from other Vg genes. The most distinctive feature was the arrangement of subunit coding regions in the VgmRNA which was opposite of what has been observed in vertebrate and invertebrate Vg genes, including those of insects. Moreover, while juvenile hormone (JH) generally stimulates Vg synthesis in most insects, the hormone suppresses Vg gene expression in the gypsy moth. It is not clear why the gypsy moth Vg system differs from all other systems.

Comparison of gypsy moth Vg gene promoter sequences to those from other JH-mediated genes from various insects indicated that there were no significant similarities. However, motifs known to be associated with sex-specific and developmentally-regulated genes were present in the promoter region of the gypsy moth Vg gene. DNA foot printing and gel mobility shift analyses were carried out to determine whether any of these elements are involved in the regulation of expression and to identify transcriptional factors present in nuclear extracts that bind to these elements.

The proteins in the crude nuclear extract obtained from fat body of day8-5th instar male and female gypsy moth larvae were isolated by ammonium sulfate precipitation followed by centrifugation. After dialysis, they were used directly in binding experiments. The region corresponding to nucleotides -234 to +90 of the promoter was amplified through PCR, labelled, and used for these analyses. The DNA protein complexes produced were examined by non-denaturing gel electrophoresis in 4% polyacrylamide gels. Both male and female extracts had proteins which bound to the promoter fragment to form several complexes. Most of these complexes were due to specific binding since an excess of non-labelled promoter fragment strongly inhibited binding. In contrast, presence of an excess of non-specific oligonucleotide did not have any effect on the binding. The experiment indicated that both male and female fat body nuclear extracts contain regulatory proteins which specifically bind to the promoter region of the Vg gene. DNA footprinting assays identified at least one putative region where the proteins bound to the promoter fragment. This region did not match to any of the known conserved motif. We are attempting to use the oligonucleotide corresponding to this region to isolate and identify the regulatory protein(s) in the nuclear extract. Characterization of these proteins will be helpful in understanding how juvenile hormone controls expression of the gypsy moth Vg gene by regulating these nuclear proteins.

EFFECTS OF BT ON LITTER DECOMPOSING  
HERMINIINE NOCTUIDS (LEPIDOPTERA)

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ABSTRACT

More than 40 species of herminiine noctuids (Lepidoptera) occur in eastern forests, nearly all of which are macrodecomposers in litter, shredding fallen leaves and accelerating nutrient cycling in forest ecosystems. Nontarget impacts of *Bacillus thuringiensis* (Foray 48b) on herminiine noctuids and leaf litter decomposition were studied. Controlled feeding bioassays demonstrated Bt-susceptibility in all 12 species (8 genera) of herminiines that we assayed: *Idia aemula*, *I. americalis*, *Phalaenophana pyramusalis*, *Zanclognatha cruralis*, *Z. ochreipennis*, *Chytolita morbidalis*, *C. petrealis*, *Bleptina caradrinalis*, *Renia adspersgillus*, *R. sahusalis*, *Lascoria ambigualis*, and *Palthis angulalis*. Sixty two assays (30 larvae per assay) were performed. Mortality over a 1 week period was significant for both second and final instars at concentrations 1% (0.4 BIU/acre) and 10% (4 BIU/acre) of the spray dosages allowed by the EPA for gypsy moth suppression. Considerable mortality was observed 2-4 days after exposure. Larval autopsies indicated that 47% of the larvae which died at the 1% dose and 58% of the larvae which died at the 10% dose were killed directly by Bt infections. A litter decomposition study using the same 2 dosages was also performed. Litter bags of two mesh sizes were placed along transects in oak-dominated deciduous forests in Mansfield, Connecticut and Goshen, Virginia and collected after 6 months. Preliminary analyses suggest that decomposition was significantly greater for the treated leaves in the large mesh bags in Connecticut. No other differences were significant. However, overall decomposition in the litter bags was much lower than expected, and the relevance of nylon bags to oviposition and foraging behaviors of herminiines and other macrodecomposers remains in doubt. Although meaningful differences in rates of decomposition could not be shown, our results demonstrate that herminiines are highly sensitive to Bt, and are among the most sensitive Lepidoptera.