GENERAL SESSION (CONTINUED) . . . . . Moderator: R. Fuester, USDA-ARS

Entomophaga maimaiga
Presenters: R. Webb, USDA-ARS; L. Bauer, USDA-FS; A. Hajek, Cornell University

CONTENTS

PLENARY PRESENTATIONS

ABSTRACTS AND PAPERS OF PRESENTATIONS AND POSTERS

THE EUROPEAN GYPSY MOTH IN COASTAL PLAIN PINE-HARDWOOD STANDS: OVERSTORY DEFOLIATION AND TREE MORTALITY

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ABSTRACT

As gypsy moth (*Lymantria dispar* L.) populations have expanded their range into the southeastern United States, outbreaks in previously undefoliated forest types have become more frequent. Recent laboratory studies have revealed that sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.) are potential hosts for the gypsy moth. Both of these species occur in pure and mixed stands throughout the Southeast, therefore the potential for defoliation by advancing populations appears to be great. Unfortunately, we cannot extrapolate the results of laboratory feeding studies to the field. Designed field experiments are needed to adequately address forest management issues. Thus, in 1992 we initiated a study to determine the effects of gypsy moth defoliation on the growth and mortality of loblolly pines and hardwoods growing in mixed stands.

Between 1992 and 1993, 141 research plots were established in 47 mixed stands in the Coastal Plain of Virginia and Maryland. Two stand types were selected for study: mixtures of loblolly pines and oaks, and mixtures of loblolly pines and sweetgum. Individual stands were selected in order to achieve a representative sample of a range of stand conditions, from relatively pure pine stands to relatively pure hardwood stands.

In both stand types studied, the proportion of susceptible species present at the time of plot establishment appeared to influence the intensity of defoliation. Stands with a larger proportion of hardwoods had greater annual defoliation levels. Oaks and sweetgum were both heavily defoliated on a wide scale. Individual pines were observed to experience some defoliation, however, even in areas where the susceptible hardwoods had been completely stripped, large-scale defoliation of pines did not occur. Pine-oak stands lost more total basal area than pine-sweetgum stands. The majority of this mortality consisted of susceptible hardwoods, mainly oaks. Conversely, while the defoliation of sweetgum was intense, mortality was negligible indicating that this species may be less vulnerable to mortality than some of the common oak species. Mortality in both stand types was also influenced by factors unrelated to defoliation. The combined effects of defoliation and a wildfire in 1993 produced some of the highest levels of mortality that were observed among pine-oak stands. Also, although some stands did suffer mortality of individual pine trees it was not clear that this mortality was a result of defoliation. In one of the stands examined, pine mortality occurred prior to any defoliation as a direct result of a southern pine beetle (*Dendroctonus frontalis* Zimmerman) outbreak.

ON UNDERSTANDING THE MECHANISM OF *BACILLUS THURINGIENSIS* INSECTICIDAL ACTIVITY

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ABSTRACT

Bacillus thuringiensis (*Bt*) strains are entomopathogenic to species within Lepidoptera, Diptera, or Coleoptera depending on the type of delta-endotoxins (also referred to as the Insecticidal Crystal Proteins or ICP) they produce. They are classified on the basis of the DNA homology of their toxin genes and the insecticidal activity spectrum of the ICP. Briefly, the CryI class is specific for Lepidoptera and the crystal is bipyramidal. The CryII class is Lepidoptera-Diptera specific and the crystal is cuboidal. The CryIII class is specific for the Coleoptera and the crystal is rhomboidal. The CryIV class is specific to Diptera and the crystal of this group can be bipyramidal, ovoid, or bar shaped. The site of action of these Cry classes of toxins is the membrane of the midgut cells of susceptible insects. A fifth class is the Cyt class; its crystal is amorphous and is cytolytic to susceptible Diptera.

Although there is only about a 25% amino acid sequence similarity between the different Cry ICPs, their three-dimensional structure is similar and organized into three domains. Domain I, a bundle of six amphipathic and one hydrophobic alpha-helices, is involved in membrane insertion and pore formation. Domain II is three antiparallel beta-sheets terminating in three loops at the apex of the molecule; they are probably responsible for recognizing and binding to receptors on the midgut columnar cells. The Domain III is a tightly packed beta-sandwich in which the conserved C-terminus is buried within its center. This domain is resistant to further proteolysis and maintains the structural integrity of the molecule. The structural similarity between ICPs may be due to five identical conserved regions; two are located near the C-terminus of Domain I and the N-terminus of Domain II and three are located in Domain III.

After ingestion, the ICP is solubilized by high pH to a protoxin in the midgut of the larva. The protoxin is activated by midgut protease, reducing it to a protease-resistant toxin. The active toxin binds to the receptor (identified as Aminopeptidase N), and inserts the helices in the apical membrane of the columnar cell, resulting in pore formation and disruption of the channel functions (potassium (K^+) transport). Exchange of alanine and serine with glycine and leucine in the alpha-8 loop or asparagine with either glycine or alanine in loop 2 significantly increased the toxicity of the CryIAb against gypsy moth larvae. Combining both mutations in a single CryIAb

further increased the toxicity of the ICP. These increases in toxicity correlated with increases in binding efficiency and with disruption of the K^+ transport (measured by increased short circuit inhibition across the midgut membrane). Deletion of the amino acids in loop 2 resulted in complete loss of toxicity, decreased binding, and K^+ transport inhibition. None of these alterations had an effect on the structural stability of the ICP. The disruption of the K^+ transport causes depolarization of the columnar cell apical membrane; this is followed by closure of the junction gap between the goblet and columnar cells and collapse of the transepithelial potential. Osmotic events (Donnan Effect) occur whereby water is taken up by the columnar cell, causing it to swell and lyse. This final event leads to loss of midgut integrity. These reactions occur within 30-40 minutes after ingestion followed by death 48-72 hours later from starvation or septicemia. This sequence of events generally is common in most susceptible larvae, especially in lepidopteran larvae.

THE OCCURRENCE OF GENETIC MARKERS IN THE CANADIAN GYPSY MOTH POPULATION

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ABSTRACT

To distinguish among varieties of gypsy moth, two genetic markers are presently used: (a) mitochondrial: based on the amplification of a segment of mitochondrial DNA followed by restriction digests (*Nla*III and *Bam*HI) (Bogdanowicz *et al.* 1993), and (b) FS1: based on the amplification of a segment of the genomic DNA (Garner and Slavicek 1996). In 1993, 618 specimens trapped around Eastern Canadian ports were analyzed using the genomic FS1 marker and 58 specimens were heterozygous (N+A) for both the Asian and North American bands while 560 were homozygous (N) for the North American band (P. Covello, Agriculture Canada). In 1994, we analyzed 198 specimens using the mitochondrial test and 6 were N+/B- (mostly seen in Europe and part of Asia) while 192 were N-/B- (mostly seen in North America and sometimes in Europe). This suggests that the genetic background of North American gypsy moth is not homogenous. In 1995, we initiated a survey within the area of Canada generally infested with gypsy moth to: (a) determine the occurrence and distribution of the FS1 and mitochondrial markers in the Canadian gypsy moth population, (b) determine if the genetic make up is different around ports and military bases (high risk of introduction of exotic gypsy moths) than in other areas (low risk of introduction), and © establish a reference collection to evaluate the diagnostic potential of new markers. Traps were placed in 18 chosen sites with a grid of 40 x 30 km. The distance between traps was 5 km for a total of 63 traps per site. More than 14,000 specimens were collected from the traps. A subset of ~2,100 insects (equivalent to 15%) was dissected and DNA was extracted. The DNA extracts were then analyzed using both the mitochondrial and FS1 genomic markers.

Results showed no significant differences between low and high probability sites within the same province or area. The differences were more pronounced between different parts of the infested area. Ontario had a mitochondrial N+/B- average of 3.62% and FS1 heterozygous (N+A) average of 6.59% and the Eastern part of the Canada (Nova Scotia, New Brunswick, and Québec) had a mitochondrial N+/B- average of 0.200% and FS1 heterozygous (N+A) average of 1.67%. The FS1 and mitochondrial markers are able to detect most cases of introduction of exotic gypsy moths. However, more markers are needed to distinguish the North American subpopulation that

displays either heterozygous FS1 (N+A) or N+/B- markers from exotic moths with similar genetic typing.

LITERATURE CITED

Bogdanowicz, S.; Wallner, W.E.; Bell, J.; ODell, T.M.; Harrison, R.G. 1993. Asian gypsy moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. Ann. Entomological Society of America 86:710-715.

Garner, K.J.; Slavicek, J.M. 1996. Identification and characterization of a RAPD-PCR marker for distinguishing Asian and North American gypsy moths. Insect Molecular Biology 5(2):81-91.

BLEPHARIPA SCHINERI (DIPTERA: TACHINIDAE), A PROMISING NATURAL ENEMY OF GYPSY MOTH

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ABSTRACT

The tachinid fly *Blepharipa schineri* (Mesnil) is a Palearctic species that holds promise as a natural enemy of gypsy moth. Significant levels of gypsy moth parasitism have been reported from different temperate regions of Eurasia. It has a number of biological characteristics that are of interest: (1) it is univoltine, having an obligate diapause, and does not require an alternate host; (2) it has a high biotic potential (≈5,000 eggs per female); (3) parasitism is likely to be biased toward female gypsy moths; and (4) it is well adapted to xeric habitats favored by gypsy moth. Initial indications are that host specificity is high, but more information is needed. *Blepharipa schineri* appears to be fairly host specific; only two other hosts, *Endromis versicolora* and *Dendrolimus sibiricus*, are mentioned in literature. While this information is encouraging, we need to challenge *B. schineri* with some representative North American Lepidoptera to acquire more information on the risk it presents to non-target species.

Oviposition cues were tested by offering females of *B. schineri* bouquets of red oak with (1) intact leaves, (2) simulated feeding, leaf edges notched with a cork borer, (3) leaves previously fed upon by larvae of *L. dispar*, and (4) tethered feeding caterpillars of *L. dispar*. After 24 h, bouquets were examined for eggs of *B. schineri* and the numbers tabulated. The results suggested that oviposition by females was biased with 7% of the eggs on intact leaves, 14% on leaves with simulated feeding, 39% on leaves with feeding by *L. dispar*, and 40% on leaves with tethered larvae of *L. dispar*. The data suggested that host feeding was the important stimulus, whether or not a host larva was present.

Gypsy moth populations in Alsace (France) had collapsed in 1995, so it was not possible to collect large numbers of parasitized hosts in 1996. Nevertheless, it was possible to collect adults of *B. schineri* at some sites. Females placed in sleeve cages over branches of trees infested with lab reared hosts oviposited on the leaves, and parasitism was obtained when the leaves were shipped to Newark, DE, and offered to gypsy moth larvae in quarantine.

GYPSES: A DECISION SUPPORT SYSTEM FOR GYPSY MOTH MANAGEMENT

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ABSTRACT

GypsES is a computerized decision support system for gypsy moth management being developed by the USDA Forest Service Northeastern Forest Experiment Station and State & Private Forestry (both the Northeastern Area and Region 8). GypsES provides decision support to gypsy moth managers by identifying areas of concern; recommending areas to monitor; recommending areas to treat using either silvicultural alternatives, direct suppression for established populations, or eradication of localized spot infestations; providing treatment support options for modeling losses with and without treatment; uploading and downloading of spray block and spray line information through Global Positioning System files; and spray deposition modeling. The system is based on GRASS, a public domain set of GIS routines. It can handle all geographic data and features spatially referenced databases as well as a full-featured map creation and edit facility using topographic backgrounds. The system was designed and created with a user-friendly interface programmed in C under Unix X-windows/Motif. Rule-based logic and independent models are also integrated to support users' management decisions. The system can produce reports, create maps, and export graphics files for use in other programs. The basic objectives of GypsES are to model the sequence of evaluations necessary for gypsy moth management decisions and to provide active managers of a gypsy moth problem (or other pest) with useful tools to make their work more efficient and enable better decisions. Version 1.0 is now being finalized.

MANAGING FORESTS FOR GYPSY MOTH USING SILVICULTURE:

INTRODUCTION AND OVERVIEW

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ABSTRACT

A large-scale study was established at the West Virginia University Forest in 1989-90 with the following objectives: (1) to evaluate the effectiveness of two silvicultural treatments (presalvage and sanitation thinnings) in minimizing gypsy moth effects on forests, and (2) to determine the mechanisms involved in silviculture-gypsy moth interactions. Four replicates of each thinning and adjacent unthinned treatment stands were installed prior to gypsy moth defoliation. The study areas were large enough that the dynamics of gypsy moth populations would be only partially affected by dynamics outside of the plot. The mechanisms included in the study were: vegetation dynamics (both overstory and understory), secondary mortality agent dynamics, seed production, litter fall and leaf area index, dendrochronology/growth analysis, gypsy moth population dynamics, small mammal predation on gypsy moth, ground-dwelling invertebrate predator dynamics, bird community predation, gray squirrel habitat, hazard-rating evaluation, and economic feasibility of harvesting gypsy moth silvicultural treatments. Stands were thinned during the winter of 1989-90. Six of the 16 study stands were defoliated in 1990 and 1991. Six other stands were accidentally sprayed with Dimilin during 1990 which prevented further defoliation from occurring. Other factors influencing the study were: droughts in 1988 and 1991; defoliation of yellow-poplar by the yellow-poplar weevil, *Odontopus calceatus*, in 1992-94; defoliation of red maple by the lesser maple spanworm, *Itame pustularia*, in 1995-96; and an ice storm in 1995.

The two silvicultural treatments, presalvage and sanitation thinning, are used in different types of stands and have different objectives. Presalvage thinning's objective is to reduce damage (mortality primarily) by removing trees with higher probabilities of dying before they are defoliated and die and leaving trees with lower probabilities of dying, i.e., to reduce stand vulnerability. It is applied to stands that contain predominately susceptible species (greater than 50 percent of basal area), have sufficient stocking to be thinned, are greater than 15 years from maturity, and are 1 to 3 years or more before gypsy moth defoliation. Sanitation thinning's objective is to prevent the spread and establishment of gypsy moth by reducing gypsy moth habitat via removing preferred food (host) trees, removing structural features or refuges for larvae, and by promoting predator and parasite habitat. It is applied to mixed stands (less than 50 percent basal area in susceptible species) that have sufficient stocking to be thinned, are greater than 15 years from maturity, and are 1 to 3 years or more before gypsy moth arrival.

MANAGING FORESTS FOR GYPSY MOTH USING SILVICULTURE: OVERSTORY SEED PRODUCTION AS INFLUENCED BY DEFOLIATION

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ABSTRACT

As part of the evaluation of the effectiveness of silvicultural treatments to minimize gypsy moth effects on forests at the West Virginia University Forest, two treatments, sanitation thinning and presalvage thinning, are being tested. Estimates of defoliation, growth, and mortality were measured on permanent plots. As part of the evaluation of defoliation patterns and silvicultural treatments, seed traps were used to measure the annual seed fall. Seed fall was analyzed for the influence of silvicultural treatment, defoliation, stand composition, and year effects. Year had a highly significant effect for total, oak, and yellow-poplar seed falls. Seed fall was highly variable from year to year with good and poor years occurring for total, oak, and yellow-poplar seed fall, and year was generally the most important variable affecting seed fall. Oak seed fall was the major component of total seed fall, so they had similar patterns. Defoliation had a highly significant negative effect on total and oak seed falls, but not on yellow-poplar seed fall. Stand composition had a highly significant effect on yellow-poplar seed and was the most important variable for yellow-poplar seed fall where defoliation was not important. It was also significant for oak seed fall, but not for total seed fall. Silvicultural treatment had no effect on total, oak, or yellow-poplar seed fall. Significant interactions between year and defoliation, year and composition, and composition and defoliation are all plausible and can be explained by the relationships between gypsy moth feeding preference and stand seed source and contribute to understanding the seed fall produced in these stands.

MANAGING FORESTS FOR GYPSY MOTH USING SILVICULTURE: TESTING THE EFFECTIVENESS OF SILVICULTURAL TREATMENTS IN REDUCING OVERSTORY DEFOLIATION AND MORTALITY

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ABSTRACT

Invasion of eastern forests by the exotic insect, gypsy moth (*Lymantria dispar* L.), has resulted in defoliation and subsequent tree mortality. Disturbance from these factors varies widely across the landscape. In our study of the management of gypsy moth using silviculture, this presentation focused on the first objective: to evaluate the effectiveness of two silvicultural treatments (presalvage and sanitation thinning) in minimizing gypsy moth effects on forests. Six of 16 stands were defoliated for 2 years by gypsy moth. Three years after defoliation ended, mortality was evaluated. Host preference class had a significant effect on defoliation patterns but thinning did not. Mortality was strongly influenced by defoliation patterns and by thinning. Thinning and defoliation had a significant interaction: in undefoliated stands, thinning had no effect on mortality, but in defoliated stands, it reduced mortality. Defoliated sanitation thinnings did not have a significant effect on mortality, but thinned stands did have lower mortality rates. Defoliated presalvage thinnings had significantly lower mortality rates than unthinned stands.

This study was the worst case test of these treatments as there was no time between the completion of the thinning treatments (April 1990) and defoliation (May-June 1990) for the residual trees to adjust to the treatments and increase in vigor. The sanitation thinning treatment was not effective in reducing defoliation, but did reduce mortality some (not statistically). The presalvage thinning treatment was effective in reducing mortality and has potential for even better results if given more time between treatment and defoliation. These results support the use of silvicultural treatments prior to gypsy moth defoliation to minimize gypsy moth effects on tree mortality.

GYPSY MOTH POPULATIONS OF DIFFERENT ORIGINS CAN BE CLASSIFIED USING HEAD-CAPSULE COLORATION

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ABSTRACT

Spectral frequencies from digitized color images of gypsy moth head capsules were obtained using Imagepro software. The gypsy moths were from 28 populations of larvae collected from field locations in Russia and the United States and from laboratory colonies. Discriminations between populations were done in sequential fashion to derive 26 variables. The usefulness of these 26 variables for distinguishing individuals according to population was verified for the original 28 populations.

A study was designed to assess the reliability of these variables in classifying populations by geographic origin. Gypsy moths were collected from 23 locations in Europe, the United States, and Russia. The 26 variables were used to discriminate among these 23 populations and the classification matrix was used as a measure of similarity. A 2-dimensional projection was produced. When populations from the same country were grouped on this projection, their relative position was consistent with their corresponding location on a map of Eurasia. The position of populations on the projection also reflects similarities in their biological and physical characteristics assessed in separate studies.

A second study examined head capsules from 16 laboratory-reared crosses to determine the reliability of the method to classify populations by genetic origin. The projection for the 16 crosses between Russian and North American strains resulted in a distribution that conformed to genetic relatedness. Backcrosses were located closest to the corresponding parental strain, while the F_1 and F_2 hybrids were located between the backcrosses.

With the introduction of flighted females into the United States from Europe and Asia, a method of distinguishing populations is needed to plan and evaluate suppression and eradication programs. The procedure described is inexpensive and could prove useful in formulating management decisions.

EFFECTS OF FOREST THINNING ON SMALL MAMMAL ABUNDANCE AND PREDATION ON GYPSY MOTH LARVAE AND PUPAE

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ABSTRACT

Silvicultural manipulations may be used to reduce forest susceptibility or vulnerability to defoliation by the gypsy moth. The effects of this management strategy on small mammal abundance were determined by pitfall-trapping small mammals one year prior to silvicultural thinnings and for three years following logging on the West Virginia University Forest. Survival rates of gypsy moth larvae and pupae were monitored in three types of exclosures placed at three heights in the forest strata. The small mammal community sampled on our study site varied in their response to silvicultural manipulation. We found little change in total small mammal, *Sorex cinereus* and *Clethrionomys gapperi* abundance as a result of thinning. *Peromyscus* spp. abundance increased significantly after thinning. They may have responded to increasing amounts of understory vegetation which could provide cover, as well as an increased invertebrate food supply. Thirty-seven percent of larvae and 25 percent of pupae were killed by predators over the 3 days of observations each year. Thinning did not influence the proportion of larvae or pupae killed. Survival of larvae and pupae on the ground was significantly lower than that found on tree boles or in the foliage. Differences in survival among exclosure types indicated that invertebrates were the major predators of larvae and small mammals were the major predators of pupae.

ENTOMOPHAGA MAIMAIGA: WHERE DO WE GO NOW?

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ABSTRACT

During 1996, larval mortality due to the entomophthoralean pathogen *Entomophaga maimaiga* was abundant in many gypsy moth populations across the northeastern distribution of this pest as well as in Michigan. The repeated epizootics caused by this fungal pathogen in North America since 1989, when it was first found in North America, provide convincing evidence that *E. maimaiga* is well established. However, we have much yet to learn about this Asian endemic; I envision two major types of information needed: (1) information about the ecology of *E. maimaiga*, and (2) the use of this pathogen for gypsy moth control.

Knowledge of the interactions of *E. maimaiga* with the biotic and abiotic environment will allow a better understanding of this fungus and hopefully will lead to some ability to predict *E. maimaiga* activity. In considering the biotic environment, we could use more information about the impact of this obligate pathogen on gypsy moth populations and the extent of resulting host population decline under differing conditions. Recently, there has also been interest voiced in the impact of this fungus on other natural enemies of gypsy moth. Fungal entomopathogens can potentially impact other natural enemies directly (within the same host) and/or indirectly (by decreasing host density). We know little about either direct or indirect interactions of *E. maimaiga* with the suite of other gypsy moth natural enemies although recent data suggest that both *E. maimaiga* and the gypsy moth nuclear polyhedrosis virus (LdNPV) can co-occur within cadavers and can both be active in populations at the same time. Extensive studies have been conducted investigating the impact of *E. maimaiga* on non-target Lepidoptera. In field studies of the effect of *E. maimaiga* on non-targets (Hajek *et al.* 1996a), field populations of the most potentially susceptible group, the lymantriids, were extremely low; while no infection was found, due to the small sample size the impact of *E. maimaiga* on non-target lymantriids in the field should be investigated further. Since epizootics of *E. maimaiga* first occurred in North America in 1989, during an exceptionally rainy spring, there has been an assumption that extreme levels of rainfall are necessary for this fungus to cause high levels of infection. Data since 1989 have demonstrated conflicting results regarding whether rainfall in May or June is associated with levels of *E. maimaiga* infection (Weseloh and Andreadis 1990, Hajek *et al.* 1996b), while additional studies have provided further data on the positive association between *E. maimaiga* activity and rainfall (Weseloh and Andreadis 1992, Smitley *et al.* 1995). However, we have seen that *E. maimaiga* can also be active during relatively dry years, e.g., in 1991 (a dry spring) *E. maimaiga* epizootics occurred at one site in Virginia where populations subsequently collapsed and sites in Connecticut (Weseloh and Andreadis 1992) and central New York. It is clear that we have more to learn about the associations between *E. maimaiga* and moisture in the field. In

actuality, the interactions among host, pathogen, and the environment (both biotic and abiotic) determine disease transmission and research programs should attempt to evaluate all three using a holistic approach.

Studies of the use of *E. maimaiga* for gypsy moth control have almost entirely been directed toward introduction of this fungus to areas where it does not occur. We know that this fungus can spread on its own but, at present, we are unable to predict how quickly it will independently move into leading edge populations. Thus, land managers in leading edge populations are generally eager to introduce *E. maimaiga* to these areas. Studies have also begun on the use of this fungus for inoculative augmentation in areas where it already exists, in order to initiate epizootics earlier than they would naturally occur. One stumbling block to use of *E. maimaiga* for gypsy moth control is acquisition of this fungus for release. The potential for in vitro production of hyphal bodies by *E. maimaiga* exists but production using this technology is not presently being investigated and it is not known whether hyphal bodies produced in this manner will be able to survive and initiate disease cycles in the environment after release (this stage occurs within infected insects). *E. maimaiga* can be grown in laboratory colony insects but, at present, this methodology is only suitable on a small scale. At present, almost all control-based studies have been conducted using field-collected resting spores. To acquire resting spores, one can either collect cadavers of late instars from trees during or shortly after an epizootic or one can collect soil contaminated with resting spores. While cadaver collection is preferable to moving soil, this can only be undertaken shortly after epizootics and care must be taken regarding storage of cadavers containing resting spores prior to their release at a new location. In order to collect soil bearing resting spores, one must know the exact locations where epizootics have occurred in the recent past, permissions for collecting and moving soil must be obtained from both state and federal agencies, soil must be collected from locations with the greatest possibility of having high titers of resting spores and, optimally, resting spore content of the soil should be confirmed and quantified (Hajek and Wheeler 1994).

LITERATURE CITED

Hajek, A.E.; Wheeler, M.M. 1994. Application of techniques for quantification of soil-borne entomophthoralean resting spores. J. Invertebr. Pathol. 64:71-73.

Hajek, A.E.; Butler, L.; Walsh, S.R.A.; Silver, J.C.; Hain, F.P.; Hastings, F.L.; ODell, T.M.; Smitley, D.R. 1996a. Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus laboratory. Environ. Entomol. 25:709-721.

Hajek, A.E.; Elkinton, J.S.; Witcosky, J.J. 1996b. Introduction and spread of the fungal pathogen *Entomophaga maimaiga* along the leading edge of gypsy moth spread. Environ. Entomol. 25:1235-1247.

Smitley, D.R.; Bauer, L.S.; Hajek, A.E.; Sapio, F.J.; Humber, R.A. 1995. Introduction and establishment of *Entomophaga maimaiga*, a fungal pathogen of gypsy moth (Lepidoptera: Lymantriidae) in Michigan. Environ. Entomol. 24:1685-1645.

Weseloh, R.G.; Andreadis, T.G. 1990. Epizootiology of the fungus *Entomophaga maimaiga*, and its impact on gypsy moth populations. J. Invertebr. Pathol. 59:133-141.

Weseloh, R.G.; Andreadis, T.G. 1992. Mechanisms of transmission of the gypsy moth (Lepidoptera: Lymantriidae) fungus, *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) and effects of site conditions on its prevalence. Environ. Entomol. 21:901-906.

DO CLIMATE AND TIMING DURING THE OUTBREAK-LATENCY SEQUENCE AFFECT GYPSY MOTH PARASITISM IN THE NATIVE RANGE ?

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ABSTRACT

Gypsy moth (*Lymantria dispar* (L.)) parasitism was studied between 1994 and 1996, during the last year of the outbreak phase in two areas climatically very distinct, Corsica and Alsace (Northeastern France), and during the first year of the latency phase in Alsace.

During the last year of the outbreak phase, young larvae were mainly attacked by the braconid *Glyptapanteles porthetriae* Muesebeck in both regions. Collapse of populations during the second half of gypsy moth larval development was due to the tachinid *Blepharipa pratensis* (Meigen) alone in the warm climate, and to the combined actions of NPV and the tachinid *Parasetigena silvestris* (Robineau-Desvoidy) in the cool climate. *Blepharipa schineri* (Mesnil) attacked a good proportion of gypsy moth larvae during the last year of outbreak in the cool climate site, but its parasitism was masked by multiparasitism with *P. silvestris,* and competition that was very favorable to *P. silvestris*.

During the first year of the latency phase at the cool climate site, *Phobocampe disparis* (Viereck) replaced *Glyptapanteles* as the most abundant parasitoid of young larvae, but *P. silvestris* was still the most abundant parasitoid of old larvae. Its persistence and efficacy in finding targets in low host densities is noteworthy. Predominance of *P. silvestris* was repeated during the first year of the latency phase, although presence and evidence of egg laying by *Blepharipa* was shown.

THE NUN MOTH, *LYMANTRIA MONACHA* L.

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ABSTRACT

The nun moth is a species that is widely distributed in the Palearctic region. In Europe, it occurs in the area located between Sweden and Finland and northern Spain, Italy, Greece, and Bulgaria. In the east, the nun moth can be found in isolated locations as far as Japan. The region of nun moth outbreaks in Europe comprises Poland, Germany, the Czech Republic, and Austria. During the last 100 years, more than 60 outbreaks were recorded in Scots pine and Norway spruce stands characteristic of that area (Bejer 1988).

Host Plants

The nun moth is a polyphagous species. Scots pine, Norway spruce, and European larch are the most preferred coniferous host plants, while among broadleaved species, it prefers European beech, European birch, and species of oak. During the outbreak from 1978-1984 in Poland, the nun moth also attacked exotic American species, such as Jack pine, blue spruce, and Douglas fir.

Biology

The nun moth is closely related to the gypsy moth, so the biology of both species is very similar. Adults of the nun moth appear at the beginning of July and can be observed until mid August. Unlike the gypsy moth, the adults of the nun moth are active at night and they are often attracted to light. The adults of both sexes fly long distances; the males may even fly over a distance of more than 100 kilometers (Sliwa 1987). The males are attracted by a sex pheromone which many be advantageous in monitoring nun moth populations. The similarity of the nun moth and gypsy moth sex pheromones is such that in the traps used for monitoring nun moth males in Poland, 10-20% of the individuals trapped are male gypsy moths.

After mating, the females lay 70-300 eggs that are hidden in bark crevices or under lichens (Sliwa 1987). After embryonation, the larvae overwinter within the eggs, and during that time, they exhibit high tolerance to low temperatures (to -40◦C). Hatching takes place at the end of April and at the beginning of May. After having emerged, the larvae remain for a few days on the bark surface grouped in so-called "little mirrors." This unexplained behavior is important for the survival of the larvae. Young larvae begin their feeding on new foliage or on the male inflorescences of coniferous host plants. It is very important for further population success that there is synchrony in the time of larval hatch and the time of development of young leaves or flower shoots; the older larvae feed on old foliage. The larvae have 5-6 moults in their life cycle

and during that time may damage about 300 pine needles or 1,000 spruce needles. They pupate in June and July, usually on stems and branches of host trees.

Factors Limiting Nun Moth Populations

Spring frosts are an important abiotic factor affecting nun moth populations because the newly hatched larvae are very susceptible to low temperatures. Heavy rains also wash away the youngest larvae from the trees, and thus also may cause high mortality. Winds facilitate the dispersal of first instars, but may sometimes be an unfavorable agent when it deposits them on arable lands. The flying adults are sometimes drifted to the sea or large water reservoirs where they may perish (Sliwa 1987).

Microorganisms, mainly viruses, that cause so-called "tree-top disease," are the major biotic factors that limit nun moth populations. There are many insect predators that attack nun moth life stages. Insect predators include the carabids, coccinelids, raphidids, pentatomids, formicids, and forficulids. The most numerous insect parasitoids include parasitic wasps (about 100 species) and dipterans (sarcophagids and tachinids) of which *Parasetigena silvestris* is the most important species. Other natural enemies of the nun moth include representatives of spiders and birds, such as the genera *Parus* and *Sturnus*, as well as mammals, such as bats and mice.

Potential Risk to North America

The polyphagous nature of the nun moth, its dispersal capabilities, and its concealed placement of eggs on bark suggest that it is a potentially dangerous species to American forests. West coast forests dominated by coniferous stands may be most favorable for the establishment and development of the nun moth. Taking into consideration the success of the related gypsy moth in North America, it may be assumed that the nun moth could also become a serious forest pest.

LITERATURE CITED

Bejer, B. 1988. The nun moth in European spruce forests. In: Berryman, A. A., ed. Dynamics of forest insect population: patterns, causes, implications. New York: Plenum Press: 211-231.

Sliwa, E. 1987. Nun moth. PWRiL, Warsaw (in Polish). 220 p.

CLONING AND SEQUENCING OF ACTIN cDNA FROM THE GYPSY MOTH MIDGUT CELLS

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ABSTRACT

Actins are highly conserved proteins which are found ubiquitously in all eukaryotic cells, from fungi and protists to mammals. They form a major component of the cytoskeleton, and are involved in many forms of cellular and organismal motility. Amino acid sequencing of several actins has revealed the presence of several distinct actin isotypes in vertebrates which can generally be classified as either "cytoplasmic" or "muscle" actins. Cytoplasmic actins are found in non-muscle cells where they are utilized to form cellular microfilaments which function in cell motility and mitosis. The variation among actin isotypes (proteins) is limited to only a few amino acid substitutions. However, at the nucleic acid level, there are significant differences with respect to size of genes, location and number of introns, and length of 5'- and 3'-untranslated regions in the mRNA.

Isolation of actin genes has revealed that in most species these proteins are encoded by multigene families. The high degree of sequence conservation between actin proteins from diverse group of organisms argues strongly that this multigene family arose by duplication and subsequent divergence from a common ancestral gene. Due to their ubiquity and evolutionary conservation, actin genes seem ideal for comparative studies.

Our interest to clone and characterize actin gene sequences from the gypsy moth was based on the following reasons: (1) We had identified a protein in the brain of gypsy moth embryos whose expression was diapause related. Partial peptide sequence analyses indicated that the protein was actin. We wanted to confirm this by northern analyses using cloned actin sequences as probe. (2) Cloned actin sequences are very useful as positive controls in monitoring and standardizing conditions for Southern and northern analyses.

We used oligonucleotide primers (based on peptide sequences) in rTh polymerase-mediated polymerase chain reactions to obtain a 930 bp cDNA fragment complementary to gypsy moth midgut actin mRNA. This fragment was cloned into pNotA vector (5'-3 Prime Corp., Inc., CO) and its nucleotide sequence was determined. Since this cDNA did not contain sequences corresponding to the terminal regions of actin mRNA, 3'- and 5'-RACE reactions were performed and products were sequenced. The gypsy moth actin mRNA was 1,387 nt long which included a 36 nt 5'-untranslated region and a 223 nt 3'-untranslated region. There was a single open reading

frame coding for a protein of 376 amino acids. The conserved polyadenylation signal was present at position 1397-1402. Comparison to actin protein sequences of *Manduca sexta*, *Bombyx mori*, *Aedes aegypti*, *Anophales gambiae*, and *Drosophila melanogaster* (Genbank) indicated that the gypsy moth actin was almost identical to other insect actins. The only significant differences were at positions 140 (a threonine instead of an alanine) and at 199 (a serine instead of a glycine). However, the high degree of conserved sequence indicates that the gypsy moth actin and actins from other insects are derived from a common ancestral gene.

DEVELOPMENT OF GENETICALLY ENGINEERED ECTOMYCORRHIZAL FUNGAL DELIVERY SYSTEM FOR BIOLOGICAL CONTROL

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ABSTRACT

We are pursuing a novel strategy of utilizing genetically engineered ectomycorrhizal fungi as (1) biological control agents against insects and other plant pests, and (2) for providing beneficial factors essential for plant health and vigor.

Nearly all vascular plants establish mutually beneficial interaction with specific soil-borne, root colonizing fungi such as ectomycorrhizal fungi. Although naturally occurring ectomycorrhizae have been used for a long time, very little effort has been made to genetically engineer them for expanded use in agriculture and forestry. Since mycorrhizal fungi are closely associated with the roots and form a mantle around the roots, foreign gene expression in these fungi could have a profound effect on the root system and on the microenvironment around roots.

Our long-term goal is to develop genetically altered ectomycorrhizal fungi as a novel delivery system for: (1) biological control of root damaging insect pests by expressing insect toxins; (2) improving tree growth by increasing the efficiency of mycorrhizal synthesis of phytohormones; (3) changing adverse microenvironments around the root system due to acidity or other pollutants; and (4) biological control of bacterial and fungal diseases of trees through expression of specific genes engaged in reducing/purging the virulence of the pathogens. Initially we plan to develop and test the system mentioned in goal (1). For this, we have selected two plant/insect systems, one involving coleopteran pests (white grubs) and the other a lepidopteran (*K. gracilis*).

We have strains of *L. bicolor* and *P. involutus* that have been isolated from red pine plantations and shown to form effective mycorrhizal association with several conifer species. We have developed a particle gun-mediated transformation system for these fungi and used it successfully to insert genes for hygromycin resistance (selectable marker), beta-glucuronidase (GUS, reporter gene), and a DNA fragment encoding BtCryIIIA, a coleopteran specific insecticidal protein. The genes were stably integrated and functioning properly in transformed fungi. The presence of

introduced genes did not have any adverse effect on the ability of these fungi to form mycorrhizae on pine roots.

While BtCryIIIA is not very effective against scarabs such as white grubs, bioassays with transformed fungi expressing this toxin on susceptible insects will allow us to standardize conditions such as determining the efficiency of expression of toxin, etc. Once these experiments are successful, we plan to introduce other Bt genes such as Bt buibui gene (known to work on several scarab pests) and BtCryIA (lepidopteran-specific).

In order to increase the environmental safety of these genetically engineered fungi, we plan to use symbiosis-specific promoters to drive the insecticidal gene expression in the transformed fungi. In this situation, the engineered fungi will not express insecticidal proteins except when they are in association with host roots. We have identified five such genes from *L. bicolor* that are induced by host plant signals. A cDNA clone of one of them has been sequenced and gene encoding it has been isolated. Experiments to identify and characterize the promoter of this gene are currently in progress.

FACTORS CONTRIBUTING TO REDUCED SPECIES DIVERSITY IN THE THIRD-GROWTH ALLEGHENY HARDWOOD FOREST

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ABSTRACT

The cherry-maple or Allegheny hardwood forest is located on the Allegheny Plateau in northwestern Pennsylvania, southwestern New York, and adjacent areas of Maryland and West Virginia. The type is composed primarily of black cherry (*Prunus serotina* Ehrh.), red (*Acer rubrum* L.) and sugar maple (*Acer saccharum* Marsh.), and American beech (*Fagus grandifolia* Ehrh.). Important associates include white ash (*Fraxinus americana* L.), yellow-poplar (*Liriodendron tulipifera* L.), black (*Betula lenta* L.) and yellow birch (*Betula allegheniensis* Britton), cucumber (*Magnolia acuminata* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carr.). During the past 15 to 20 years the northern Pennsylvania portion of the type has experienced unusual levels of overstory mortality. Some of this mortality has occurred rapidly and can be attributed to a succession of native and exotic defoliating insect/disease outbreaks of unusual intensity and duration combined with unusual or untimely climatic events. Sugar maple has been the most severely affected species; unusual levels of sugar maple mortality have been documented across northern Pennsylvania since the early-mid 1980's. Much of it has been characterized by slow deterioration of radial growth and crown vigor, particularly on unglaciated sites above 2000 feet in elevation, and fits the definition of a decline. Recent research has shown that declining sugar maple had significantly lower foliar concentrations of calcium and magnesium and significantly higher concentrations of potentially toxic aluminum and manganese than healthy trees and that crown vigor, health, and foliar nutrition of sugar maple were significantly altered by addition of dolomitic limestone. Slope position and glaciation also influenced sugar maple health and foliar nutrition. Trees on glaciated sites and on the lower slopes of unglaciated sites were healthier and had higher foliar calcium and magnesium concentrations than those on unglaciated, upper slope sites. Work to evaluate the effects of *Armillaria*, changes in soil and soil leachate chemistry, aluminum and manganese toxicity, and accelerated base cation leaching is underway.

The situation is made worse by the fact that sites where mortality has occurred often lack advance seedlings of the species that were present in the overstory. Long-term herbivory by white-tailed deer (*Odocoileus virginianus* Boddaert) has been one of the most important factors influencing vegetation development in the hardwood forests of Pennsylvania in this century. Not only have deer affected the abundance and rate of growth of forest vegetation, but due to the selective nature of browsing, the species composition as well. Browsing has affected species

composition directly through species removals and indirectly by setting in motion a series of plant-plant interactions which over time result in altered species composition and regeneration failure. Deer density interacts with lighting and seed supply to determine whether established advance seedlings are present on the forest floor.

The species composition of the Allegheny hardwood forest has changed from domination by beech and hemlock in the pre-settlement forest to domination by black cherry, red and sugar maple, and other less shade tolerant species in the second-growth forest. The third-growth forest is likely to be less diverse than those which preceded it. Present research suggests that defoliating insects and insect/disease complexes, drought and other climatic events, species and site-related nutritional factors, herbivory by white-tailed deer, lighting, and seed supply are the principal factors influencing health, regeneration, and diversity of the Allegheny hardwood forest.

COMPARISON OF FEMALE FLIGHT CAPABILITY OF *LYMANTRIA DISPAR* L. REARED ON ARTIFICIAL DIET VERSUS FOLIAGE

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ABSTRACT

We compared the flight capability of female gypsy moths from Far East Russian (RM) and North American (CT) colonies and from reciprocal F_1 hybrids (RM x CT or CT x RM) reared on two larval food sources (high wheat germ artificial diet and black oak foliage). Two laboratory techniques, a free-flight test and a flip test, were used to assess flight capability of < 2 day old, mated females. The free-flight test allows the female to initiate flight at will in a dimly lit room and the flip test evaluates the ability of the female to right herself.

In the free-flight test, all of the RM females reared on foliage exhibited sustained flight compared with only 77% of those reared on artificial diet. Of the North American females reared on foliage, 13% were able to glide for a short distance, while none of those reared on artificial diet exhibited gliding behavior. A greater percentage of reciprocal F_1 hybrid females reared on foliage exhibited gliding behavior than siblings reared on artificial diet. Additionally, 8% of the CT x RM cross females reared on foliage exhibited sustained flight; none of the reciprocal F_1 hybrid females reared on artificial diet demonstrated sustained flight.

The slopes of the linear regression of female front wing length on female weight were similar for all strains when reared on artificial diet, and for the RM and CT strains reared on foliage. However, the slope of the linear regression of wing length on weight was steeper for F_1 hybrids reared on foliage than for those reared on artificial diet. A greater percentage of females reared on foliage were able to right themselves in the flip test than siblings reared on artificial diet. A female that can right herself easily with one quick wing beat generally is able to glide or sustain flight. This suggests that the greater flight capability of foliage-reared females than females reared on artificial diet may be due to more than a change in the ratio of wing length to weight.

Our data suggest that the female flight capability of a gypsy moth strain may be underestimated when reared on artificial diet. Thus, if flighted females are introduced and become established in the generally infested area of North America, more flight capability than originally predicted may be retained with hybridization. These results also emphasize the need for caution when using laboratory data for gypsy moths reared on artificial diet to predict behavior in their natural habitat.

ACTIN GENE EXPRESSION DURING DIAPAUSE IN THE GYPSY MOTH

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ABSTRACT

Diapause of the gypsy moth, *Lymantria dispar*, is obligatory as a pharate first instar larvae, and a long period of chilling is essential for its termination. Like most late-embryonic diapauses, the mechanism of gypsy moth diapause is poorly understood, largely due to the experimental challenges posed by working with the small early stage larvae. Knowledge of changes in gene expression regulated specifically by diapause is useful not only for understanding the physiological processes of diapause, but also for identifying developmental markers that can be used to probe regulatory mechanisms of diapause. In our search for such markers, we focused on changes in gene expression in the gypsy moth brain.

Newly deposited gypsy moth eggs were kept at 25°C for up to 34 days to allow completion of embryogenesis and the entry into diapause as pharate first instar larvae. The brain and subesophageal ganglion (CNS) were dissected from dechorionated pharate larvae at different developmental stages and subjected to *in vitro* protein synthesis experiments. The reactions were pulse-labeled with ^{35}S-methionine for 1 h at 25°C to label newly synthesized proteins. SDS-PAGE analysis of labeled proteins revealed significant variations during development among which a 45 kDa protein appeared to be specifically diapause-related. Expression of this protein decreased gradually from day 10 to day 16, which coincides with the time of diapause initiation in the gypsy moth. After chilling for 60 or more days and transferring to 25°C, conditions required for termination of the diapause, expression of the 45 kDa protein was stimulated again. Amino acid sequences of two peptides derived from this protein suggested that the 45 kDa protein was actin. This was confirmed by using anti-actin monoclonal antibody (Sigma) in immunoprecipitation analyses. In order to test whether regulation of actin gene expression was at the transcriptional level, we attempted northern analyses using gypsy moth actin cDNA clone as a probe. However, northern analysis was not sensitive enough to detect actin mRNA in small amount of brain samples that were available. Therefore, we used rTh polymerase-mediated polymerase chain reaction (PCR) to reverse transcribe and amplify actin mRNA sequences present in RNA isolated from brain at different developmental stages. Results obtained were consistent with the protein data described above, indicating that regulation of actin gene expression was at the transcriptional level. Our work has identified actin as one of the diapause-regulated proteins in the central nervous system of the gypsy moth. Furthermore, it demonstrates that actin can serve as a reliable marker to monitor the diapause status.