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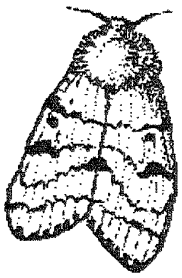
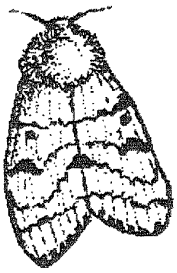
Interagency Gypsy Moth Research Forum
1995





January 17-20, 1995
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 Service

FOREWORD

This meeting was the sixth 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

R. Faust, Agricultural Research Service (ARS)
N. Leppla, Animal and Plant Health Inspection Service (APHIS)
R. Riley, Cooperative State Research Service (CSRS)
T. Hofacker, Forest Service-State and Private Forestry (FS-S&PF)
M. McFadden, Forest Service-Research (FS-R), Chairperson

USDA Interagency Gypsy Moth Research Forum
January 17-20, 1995
Loews Annapolis Hotel
Annapolis, Maryland

AGENDA

Tuesday Afternoon, January 17

REGISTRATION
POSTER DISPLAY SESSION I
WELCOME MIXER

Wednesday Morning, January 18

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

Changes in Funding and Direction of Science

Welcome
Michael McManus, USDA-FS

Research and Service Programs of the USDA-ARS European Biological Control Laboratory in Montpellier, France
Lloyd Knutson, USDA-ARS

Agricultural Research Service Program Strategies and Priorities
Edward Knipling, USDA-ARS

Inside the Animal and Plant Health Inspection Service
Charles Schwalbe, USDA-APHIS

Changes in Forest Service Research: What Lies Ahead?
Robert Lewis, Jr., USDA-FS

Wednesday Afternoon, January 18

CONCURRENT SESSION A . . . . . Moderator: E. Delfosse, USDA-APHIS

Concerns About Biological Control Agents and Non-Target Lepidoptera
Presenters: A. Hajek, Cornell University; L. Solter, Illinois Natural History Survey; J. M. Scriber, Michigan State University; J. Miller, Oregon State University; J. Maddox, Illinois Natural History Survey

CONCURRENT SESSION B . . . . . Moderator: R.M. Muzika, USDA-FS

Potpourri
Presenters: D. Leonard, USDA-FS and A. Sharov, Virginia Polytechnic Institute & State University; R. Hicks, Jr., West Virginia University; D. Gray, Virginia Polytechnic Institute & State University; A. Liebhold, USDA-FS; R. Whitmore, West Virginia University; R. A. Smith, Abbott Laboratories

POSTER DISPLAY SESSION II

Thursday Morning, January 19

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

Asian Gypsy Moth
Presenters: K. Garner, D. Schreiber and J. Slavicek, USDA-FS; D. Prasher, USDA-APHIS; M. Keena, USDA-FS; R. Cardé, University of Massachusetts; W. Wallner, USDA-FS; P. Schaefer, USDA-ARS; T. McGovern, USDA-APHIS

Thursday Afternoon, January 19

GENERAL SESSION . . . . . Moderator: K. Thorpe, USDA-ARS

Entomophaga maimaiga: A Fungus Among Us
Presenters: A. Hajek, Cornell University; R. Weseloh, Connecticut Agricultural Experiment Station; J. Elkinton, University of Massachusetts; L. Bauer, USDA-FS; S. Walsh, University of Toronto

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

Gypsy Moth Biological Control Activities in Europe
Presenters: E. A. Cameron, Pennsylvania State University; J. Novotný, Forest Research Institute, Slovak Republic; M. McManus, USDA-FS

Friday Morning, January 20

GENERAL SESSION . . . . . Moderator: N. Leppla, USDA-APHIS

The Increasing Significance of Biological Control and an Overview of Regulations Governing Biological Control Organisms
Presenters: E. Delfosse, USDA-APHIS; K. Lakin, USDA-APHIS; L. Turner, US-EPA; J. Brooks, USFWS

GENERAL SESSION . . . . . Moderator: R. Reardon, USDA-FS

Future Directions in Virus Research
Presenters: V. D'Amico, University of Massachusetts; J. Podgwaite, USDA-FS; J. Slavicek, USDA-FS; M. McFadden, USDA-FS

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THE RUSSIAN AND UKRAINIAN LITERATURE ON THE GYPSY MOTH

Yuri N. Baranchikov[1], Galina N. Nikitenko[2], and Michael E. Montgomery[3]

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ABSTRACT

The bibliography on the gypsy moth in the former territory of the USSR covers the period from 1837 to 1991. It contains publications on gypsy moth distribution, ecology, biocenotic relations, physiology, and biochemistry, as well as prognosis and pest control, both chemical and biological. Most of the 1,154 references are followed by an abstract, keywords, and the region of the former USSR where the study was conducted. The full scientific name of natural enemies is listed in a separate field. An index of keywords and natural enemies is cross-referenced. The references have been entered into Papyrus, a bibliographic database. Both a hard copy of output and the database will be available to users.

The introduction to the bibliography gives information needed to locate an original in Russian, including the main libraries and catalogues. The bibliographic description of literature in the former USSR was regulated by State Standards, which remain in effect in Russia. These standards have several peculiarities that make it difficult for the Western reader to recognize the different parts of the bibliographic description. The uniform style and the fact that all published material was deposited in central libraries and catalogued means that all the literature, theoretically, is retrievable. The source names of the citation records are in Russian or Ukrainian to facilitate location and retrieval of the publication.

SUITABILITY OF FOREIGN TREE SPECIES FOR *LYMANTRIA MATHURA* MOORE

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ABSTRACT

The pink gypsy moth (*Lymantria mathura* Moore) is widely distributed over southeastern Asia from northwestern India to the Russian Far East. Only one publication documents outbreaks of this species in the Primorie region of the Russian Far East where hundreds of hectares of forests with *Quercus mongolica, Juglans mandjurica, Malus mandjurica* and species of *Ulmus, Betula*, and *Populus* were defoliated in different years. The biology of *L. mathura* is reported to be similar to that of gypsy moth (*Lymantria dispar*), but no special investigations were made. Since *L. mathura* has been found on ships from the Far East entering ports on the West Coast of North America, there is concern about its introduction to the North American continent. To assess its danger to North American forests, we examined its host plant range.

The study was done at a field laboratory in the arboretum of the Institute of Forest near the city of Krasnoyarsk located in Central Siberia, far from the natural distribution of *L. mathura*. *L. mathura* from the Russian Far East, the Asian race of *L. dispar* from south-central Siberia, and the North American race from Michigan (1993) and West Virginia (1994) were reared on the same hosts at the same time. This allowed comparisons to *L. dispar*, which is more familiar to forest pest workers on both continents. All tests were done in the laboratory with excised foliage fed to larvae in petri dishes.

When newly hatched larvae of each species were reared for 10 days, mortality was high for both species on *Fraxinus pennsylvanica, Lonicera edulis, Pinus sylvestris*, and *Parthenocissus quinquefolia*, but *L. mathura* also had high mortality on *Tilia cordata, Sorbus aucuparia*, and *Shepherdia argentea*. Hosts that were good for both species were: *Hippophae rhamnoides, Malus pruniflora, Quercus mongolica*, and *Rosa rugosa*. *Salix fragilis* was a good host for *L. mathura*, but only moderately good for *L. dispar*. *Larix siberica* was good for gypsy moth, but *L. mathura* grew poorly on it. Development during the entire larval period was about the same in terms of weight and head capsule width, until the last instar, which is longer in *L. mathura*. *L. mathura* pupae were 10 to 35 percent heavier than *L. dispar* pupae.

L. mathura grew well on several species of host plants that are foreign to it. Growth on species in the plant families of Betulaceae, Fagacea, Rosaceae, and Salicaceae was comparable to that of the gypsy moth. On Pinaceae, though, *L. mathura* did not do as well as the gypsy moth.

STUDIES ON THE TRANSMISSION OF AN EXOTIC MICROSPORIDIUM THAT INFECTS THE GYPSY MOTH

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ABSTRACT

Microsporidia are a diverse group of obligate, intracellular pathogens that are commonly found infecting invertebrates, but that are conspicuously absent from North American gypsy moth populations. They cause chronic and often debilitating disease in their hosts, characterized by egg, larval, and pupal mortality, and reductions in larval vigor, growth, adult fecundity and longevity, and egg fertility. Infected larvae release spores into the environment via cadavers, frass, silk, or regurgitate, transmitting the disease to other larvae within the same generation (horizontal transmission). Infected individuals that survive to the adult stage may transmit the pathogen to the next generation in or on eggs (vertical transmission). Thus, the prevalence of microsporidian disease increases throughout the season, between generations, and as host density increases. We are conducting studies on a microsporidium isolate from gypsy moth populations in Portugal in order to estimate the degree of transmission within this species; this microsporidium has a high degree of specificity to gypsy moth and is a good candidate for release as a classical biological control agent.

We determined that fecundity of infected females (mean = 507 eggs) was almost half that of healthy females (mean = 902 eggs). In addition, only 52% of the eggs from infected females hatched, whereas 69% of eggs from healthy females hatched. Infected females transmitted this microsporidium in the egg (transovarially) to F_1 progeny. Unhatched embryonated eggs, as well as successfully eclosing neonates, contained large numbers of mature spores. There was considerable variation in transmission efficiency, ranging from 9 to 100% infection from 23 different egg masses from infected females. This variation was correlated with maternal dose and level of disease. Significantly fewer progeny from infected females (68%) survived to adult stage, when compared to progeny from control females (97%). Infected males transferred microsporidian spores to female reproductive tracts during mating; however, transmission to F_1 progeny was not detected.

Estimates of horizontal transmission were ascertained by placing an equal number (25) of transovarially-infected neonates along with healthy neonates on 1-meter poplar saplings enclosed in a large mesh bag. Larvae were removed over a period of 20 days and placed on artificial diet for at least two weeks; larvae removed at day 15 and day 20 were reared to the adult stage. We detected a steady increase in prevalence of infection over the 20-day period, beginning with 44% at day 5, and increasing to 63, 86, and 92% infection at days 10, 15, and 20, respectively. The early initiation of horizontal transmission from transovarially-infected larvae to uninfected larvae results from the early dissemination of mature spores from infected neonates, particularly in the silk. The survival of individuals to adult stage reared from days 15 and 20 was similar (69 and 67%, respectively). The high survival of infected individuals to the adult stage supports this microsporidium's ability to successfully maintain itself in the population, even at low host density.

DYNAMICS AND IMPACT OF *ENTOMOPHAGA MAIMAIGA* INTRODUCED INTO GYPSY MOTH POPULATIONS IN MICHIGAN

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ABSTRACT

In 1989, researchers made the surprising discovery that gypsy moth larvae in the Northeast were succumbing in large numbers to the fungal pathogen *Entomophaga maimaiga*, which is native to Japan. Overt symptomology is similar to that of nuclear polyhedrosis virus (NPV), the only known causal agent of widespread epizootics throughout the range of gypsy moth in North America before 1989. There remains much uncertainty regarding the sudden and relatively widespread appearance of *E. maimaiga*, although early speculation presumed that the 1910-1911 efforts to introduce this pathogen in eastern Massachusetts were actually successful (Hajek *et al.* 1995, Amer. Entomol. 41:31-42).

By the fall of 1990, further survey of gypsy moth cadavers revealed that *E. maimaiga* was well established in North America, and that its distribution was expanding. In Michigan, there was considerable interest in this pathogen as a natural enemy of gypsy moth. Gypsy moth populations in Michigan were not surveyed in 1989-90 for *E. maimaiga* because they are disjunct from the northeastern populations, the epicenter of the fungal epizootic. Therefore, in the fall of 1990, we developed an *E. maimaiga* research project with the following objectives: 1) to survey the gypsy moth populations throughout Michigan for presence of *E. maimaiga* infections in 1991-1992 (10 counties); 2) to compare the establishment of *E. maimaiga* after introduction by either the application of soil contaminated with resting spores or the release of infected larvae (Crawford, Lake, and Grand Traverse Counties); 3) to monitor establishment and rate of spread over time from these release sites in relation to weather, NPV, population density; and lastly, 4) to determine the impact of *E. maimaiga* on gypsy moth infestations.

The pathogen survey did not detect larvae infected with *E. maimaiga* in over 1500 larvae sampled in 1991 from 50 sites, some of which have been infested with gypsy moths since 1984. In 1991, the two methods for introducing *E. maimaiga* showed low-level establishment only in plots that received the infected larvae. However, by 1992 establishment of *E. maimaiga* was confirmed for both inoculation methods, and there were no significant differences between the level of infection achieved by the two release methods, ranging from 9.3 to 11.7%. In addition, by 1992, infection was detected at low levels in the control plots (0.5 to 2.4%) which were located ca. 100 m away from the treatment plots. In 1992, we added a third research site to the study, using the method of relocating soil containing resting spores. Post-treatment sampling of that site in 1992 detected low levels of establishment at the epicenter only (5.5% infection).

Continued monitoring of fungal establishment during the 1993 season revealed that epizootics of *E. maimaiga* occurred at all three research sites, with the incidence of infection ranging from 29 to 99% in the plots. Differences in infection levels between sites were highly correlated with the precipitation totals or the relative humidity ≥90% for the two weeks or 10 days before larval sampling, respectively. In 1993, the egg mass densities at the three research sites averaged 3- to 10-fold lower than in control plots. Our data also supports that *E. maimaiga* epizootics are less density dependent than gypsy moth NPV. In 1993, we also monitored the establishment and rate of spread of *E. maimaiga* at 20 release sites set up by the Michigan Department of Natural Resources in 1991 by the relocation of soil containing resting spores. *E. maimaiga* establishment was confirmed in six of the 20 release sites, and the rate of spread was estimated at ca. 1.0 km per year.

In 1994, sampling of live larvae and cadavers at the study sites in the three counties revealed 1) few larvae at the plot epicenters; 2) infection distributed at least 2000 m from original epicenters; and 3) seasonal amplification of fungal infection. In addition, egg mass counts at the center plot were also lower than in surrounding areas. The slow rate of spread of *E. maimaiga* in Michigan, when compared to that observed in the eastern states during the same time period, may be attributed to low rainfall during late May and early June when the fungus releases airborne conidia from cadavers of young caterpillars dying from *E. maimaiga* infection. As the season progresses, *E. maimaiga* switches to propagation of the large, overwintering resting spores that are eventually washed into the soil from cadavers adhering to tree boles. Two independent studies established research release sites in Ingham and Kalamazoo Counties using contaminated soil relocated from our 1991 study site in Lake Co. However, establishment of *E. maimaiga* was not confirmed during the 1994 season, and we speculate that the low numbers of resting spores present in the soil from Lake Co. were inadequate for establishment.

STATUS OF MASS-REARED GYPSY MOTHS: PROTOCOL

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ABSTRACT

A laboratory strain of the gypsy moth, referred to as the New Jersey Standard Strain (NJSS), has been maintained at the USDA-APHIS Otis Methods Development Center for over 40 generations. Although the strain was originally developed for APHIS programs, it is now used for a variety of projects. APHIS provides this resource, primarily as diapaused egg masses, to any research group that might contribute to an overall integrated pest management program for the gypsy moth. In 1994, over 100,000 egg masses were provided to 50 individuals or groups working on the gypsy moth. An additional 6,000,000 larvae were mass-reared and inoculated with the gypsy moth nucleopolyhedrosis virus. This cooperative project with the Forest Service was the only source of the virus in 1994.

The NJSS is often cited as a resource in the literature but recent developments in rearing methodology remain unpublished. Each week, 6,000 larvae are reared, yielding approximately 2,500 egg masses after larval development (35 days) and oviposition/embryonation (42 days). Each weekly cohort of egg masses is then placed in chill mediated diapause for 170 days. The egg masses are stored and shipped in quart containers with egg masses attached to strips of paper removed from the oviposition containers.

Egg masses used to maintain each subcolony are surface sterilized in 3 percent formaldehyde, "dehaired" and seeded onto the artificial diet using a mechanical egging machine. For production projects, the larvae are reared to pupation from eggs seeded directly into containers. However, to optimize colony, neonate larvae are counted and placed with a brush onto fresh diet. The lineage of each subcolony is maintained with 200 egg masses selected at random. Details of rearing procedures are available upon request (1994 Gypsy Moth Rearing Protocol).

The laboratory life cycle is 35 weeks. However, preliminary results from diapause experiments indicated that the chill mediated diapause can vary from 150 - 180 days with no significant differences in the next generation; this may allow reducing subcolonies to as few as 12 per year.

A problem of differential larval growth has been absent from the colony since 1989, thus allowing for a consistent and dependable supply of insects for both APHIS projects and cooperator needs. The APHIS gypsy moth mass-rearing program now plays an integral role in the entire spectrum of on-going research to better manage this pest.

IDENTIFICATION OF THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS *25K* GENE

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ABSTRACT

The baculovirus life cycle has two morphologically distinct forms: a non-occluded budded virus that infects cells within the same larva, and an occluded form termed a polyhedron that ensures transmission from one larva to another. Passage of multinucleocapsid nuclear polyhedrosis viruses (MNPV) in cell culture results in the rapid generation of few polyhedra (FP) mutants with increased budded virus titre and reduced numbers of polyhedra. Polyhedra produced from FP mutants are virtually devoid of nucleocapsids. The phenotype associated with FP mutants of *Autographa californica* MNPV (AcMNPV) and *Galleria mellonella* MNPV (GmMNPV) have been shown to be due to the inactivation of a 25 kDa protein. Often these mutants have deletions or large (0.8 to 2.8 kB) insertions of host DNA into the *25K* gene. This loci appears to be a "hot spot" for DNA mutation as these insertions and deletions occur at a higher frequency than mutations in other regions of the viral genome. The function of the 25K protein is unknown. It is not required for viral replication but is required for the production of wild type numbers of polyhedra. One possibility is that the 25K protein is a nucleocapsid envelope protein that interacts with the polyhedrin and initiates nucleation. FP mutants are also generated upon serial transfer of the *Lymantria dispar* MNPV (LdMNPV) in cell culture. Unlike AcMNPV and GmMNPV FP mutants, LdMNPV mutants do not appear to contain large deletions or insertions as determined by DNA restriction analysis. While mapping the genomic location of a group of FP mutations in LdMNPV, we have identified, sequenced, and characterized a gene encoding the LdMNPV 25K homologue.

SEQUENCE CHARACTERIZATION AND TEMPORAL EXPRESSION OF AN EARLY GENE IN THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS

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ABSTRACT

Baculovirus expression studies indicate that viral genes are expressed in a temporal cascade in which early genes encode regulatory proteins that control the transcription of all other viral genes. Identification of these regulatory proteins is an important step in understanding the molecular aspects that govern viral potency. Many of the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus (AcMNPV) early genes have been identified and sequenced. These genes are transcribed by the host RNA polymerase and do not require other viral proteins for their expression, although transcription of these genes may be enhanced by *trans*-activation with other viral factors.

Temporal analysis of transcripts in the *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (LdMNPV) had identified three putative early genes which were localized between 6.0 and 13.7 map units on the viral genome. One of the genes, designated *G22* because it is located within the *Eco*RV-G fragment and is predicted to encode a 22 kDa protein, was chosen for further study. This region has been cloned and sequenced. A 24 kDa protein is seen when *G22* is expressed in an *in vitro* translation and transcription system, which is in close agreement to the 22 kDa protein predicted from the DNA sequence. Comparison of the predicted amino acid sequence of G22 with other proteins in GenBank reveals no strong homology to any other known protein. Temporal expression studies indicate that *G22* codes for a 0.85 kB transcript that can be detected immediately following the adsorption period of the virus even in the presence of the protein synthesis inhibitor cycloheximide. This transcript continues to be expressed throughout the time-course of infection with an increase in transcription from 4 to 8 hr post-infection. This enhanced transcription was not seen when cycloheximide was present, indicating that G22 can be *trans*-activated by itself or other viral proteins.

CORSICA, GYPSY MOTH, AND PARASITOIDS: CHALLENGES AND OPPORTUNITIES

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ABSTRACT

During the 1994 field season, twice-monthly trips to gypsy moth infestations on the island of Corsica were made to investigate the natural enemy complex associated with the current gypsy moth outbreak. Three plots were established in the southeastern portion of the island, one each chosen to represent sparse, intermediate, and high density populations based on preseason egg mass counts. Primary hosts were cork oak, *Quercus suber*, and holly oak, *Q. ilex*. Corsica has a Mediterranean climate, with dry and hot summers; it has some climatic similarities to areas in the southern United States that may become infested with gypsy moth within the next decade. Parasitoids and/or predators from Corsica thus might be well adapted to and a priority for consideration for introduction into southern states as the gypsy moth continues to expand its North American range.

At each site, four trees (3-4 m in height) were selected. A 20-cm high sticky barrier was placed on the ground outside the canopy projection of each tree in an effort to minimize tree-to-tree movement of larvae across the ground. All larvae were individually picked off each tree (foliage and trunk); lab-reared larvae were placed on the foliage for 5-day exposure periods. At each site, two of the four trees received 200 L_1-L_2 larvae, plus 40 L_4-L_5 larvae ('high' density), and two received 40 L_1-L_2 larvae plus 10 L_4-L_5 larvae ('low' density); up to 10 pupae (as available) were also exposed per tree. Five days later, as many exposed larvae and pupae as possible were recovered, and returned to the laboratory to be reared for parasitoid emergence or until adult emergence (or earlier death). While we hoped to recover only those which we had placed, there was a lot of immigration to the trees from natural populations. Recoveries were supplemented by targeted and mass collections (up to 2,000 larvae per site per trip) which were reared for natural enemies.

Natural enemies of eggs included a suspected dermestid predator (evidence observed; no individuals were collected) and unidentified avian predation. *Calosoma sycophanta* was common as a larval predator. Large numbers of *Blepharipa* sp., and small numbers of *Parasetigena sylvestris*, will be forwarded to USDA quarantine facilities later. Other species recovered, some in considerable numbers, included the egg parasitoid *Ooencyrtus kuvanae*, and larval parasitoids

Glyptapanteles porthetria, *G. liparidis*, *Cotesia melanoscelus*, and *Meteorus pulchricornis*. Evidence of the fungus, *Entomophaga maimaiga*, was not seen on the island.

Corsica is an area from which a number of species could be recovered for introduction into the United States. However, the logistical problems and costs associated with operations on the island impose constraints on easy utilization of the species available unless there is evidence that biotypes from Corsica possess special attributes desired for introductions to targeted areas.

A FIELD ASSESSMENT OF THE EFFECTS OF *BACILLUS THURINGIENSIS* ON NON-TARGET LEPIDOPTERA: LIGHT TRAP SAMPLING

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ABSTRACT

Bacillus thuringiensis Berliner var. *kurstaki* (*Btk*) is one of the most commonly used pesticides against lepidopteran forest pests. Previously, we reported on the results of foliage and burlap sampling from a 3-year field study examining the impacts of a single application of *Btk* on non-target Lepidoptera. In addition to foliage and burlap sampling, our field study included light trap sampling and results from that sampling are reported here.

The study was conducted on 10 plots in the Goshen Wildlife Management Area, Rockbridge Co., Virginia. Five replications were obtained by pairing adjacent plots. One member of the pair was randomly selected to be treated with a single aerial application of *Btk* (Foray 48B, 14.4 BIU's/ha) and the other served as the control plot. A single light trap (modified BioQuip 2851A with 8-watt BL tube) was deployed in the approximate center of each plot at a height of 2 meters and set to operate for 1/2 hour every two hours. Trapping was conducted one to two times weekly from about 1 March to the end of September in 1991-93. Statistical analysis was conducted only on the trap samples from June 1992 through September 1993. The samples were divided into three groups: summer 1992, captures from June to September 1992; spring 1993, captures from March to April 1993 (adults that would have been larvae during summer of treatment); and summer 1993, captures from May to September 1993. Data were log transformed and a t-test performed on selected families (Arctiidae, Geometridae, Lasiocampidae, Limacodidae, Lymantriidae, collective group of Microlepidoptera, Noctuidae, Notodontidae, Saturniide, and Sphingidae).

Thirty-seven families representing 353 genera and 621 species were collected and identified. No significant differences were found between control and treatment areas for the total number of adults collected for the majority of the families and years analyzed. There was a significant difference between control and treatment areas in spring 1993 for the noctuids.

GYPSY MOTH MANAGEMENT IN NON-FOREST SETTINGS:

1994 FIELD AND LABORATORY STUDIES

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ABSTRACT

Two of the microbial agents currently being investigated for use in the management of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), populations in non-forest environments are a bacterium, *Bacillus thuringiensis* (*Bt*), and a baculovirus, the *L. dispar* nuclear polyhedrosis virus (NPV). During 1994, ground application of *Bt* in combination with sticky barrier bands was examined and compared with similar treatments of the chemical insecticide, cyfluthrin. Both insecticide treatments resulted in significant reductions in within-canopy gypsy moth populations and tree defoliation. The sticky barrier bands were also a significant population reduction factor, but had no influence on defoliation levels. No insecticide or barrier band treatment had a significant influence on egg mass replacement rate.

The gypsy moth NPV was examined using aerial applications. Several aspects of viral treatment efficacy testing were examined. Following treatment, no significant differences in overall larval mortality between understory and canopy collected larvae were detected. However, the data suggest that higher rates of parasitism may have occurred in the understory sample. Sample sizes were small and more work is planned for 1995.

The substrate on which the gypsy moth NPV is consumed can significantly impact viral efficacy. During 1994, we compared sweetgum and white oak as host substrate. Initial data analyses indicate no significant differences in larval infection rates between the two species, suggesting that sweetgum and white oak behave similarly as a host substrate for the NPV. Additional samples are currently being processed.

During 1994, our laboratory studies focused on examining potential viral enhancing agents for use in non-forest environments. The stilbene disulfonic acid Blankophor BBH was examined at various concentrations in formulations with the gypsy moth NPV. There appears to be an upper threshold beyond which the addition of more stilbene reduces the enhancement activity. This threshold varies with droplet size and possibly substrate. To better delineate this threshold and other properties of the adjuvants, additional tests are being conducted with this and other potential viral enhancing agents.

A FIELD TEST OF GENETICALLY ENGINEERED GYPSY MOTH NPV

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ABSTRACT

The gypsy moth (*Lymantria dispar* L.) nuclear polyhedrosis virus (LdNPV) was genetically engineered for non-persistence by removal of the gene coding for polyhedra production. A beta-galactosidase marker gene was inserted into this virus, so that larvae infected with the engineered virus could be easily tested for its presence using a chemical assay. In May 1993, field tests were established in two 4-ha plots in Otis Air Force Base on Cape Cod. Gypsy moth larvae were released in both plots to serve as test populations. The virus was released in the center of the test plot by confining infected gypsy moth eggs on oak foliage in mesh bags. These bags were not removed until larvae hatching from infected eggs were 2nd instar. Most of these larvae became infected with the engineered virus. No virus was released in the control plot. Weekly collections were made in both plots, beginning one week prior to virus release, and continuing for six weeks thereafter. Each week, several hundred larvae were collected at random from the control plot and 25 larvae were collected from each of the 33 sectors in the test plot. These larvae were reared individually and checked for mortality every second day. Parasitism by the wasp *Cotesia melanoscela* was high on both plots. Because we discovered that gypsy moth larvae infected with immature of *C. melanoscela* respond positively (turn blue) to the beta-galactosidase assay, the gypsy moth cadavers were tested for the presence of the engineered virus using both the chemical assay and restriction enzyme analysis. Throughout the seven collection weeks, the engineered virus was recovered in both early and late instars, but only from collection points within 50 m of the release point. No engineered virus was seen in the test plot in weeks 1 or 7. No virus was seen in the control plot at any time. In 1994, 18 million gypsy moth larvae were released in each of the plots to test for persistence of the engineered virus. Weekly collections were made as above. Larval mortality due to *C. melanoscela* was far less than in 1993, possibly due to the larger number of larvae released or the timing of the release. Some samples of virus-killed larvae have tentatively been identified as containing the genetically engineered virus.

GYPSY MOTH DEFOLIATION IN COASTAL PLAIN PINE-HARDWOOD STANDS

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ABSTRACT

Despite numerous attempts at control, the European gypsy moth (*Lymantria dispar* L.) has continued to expand its range within the United States. Extensive investigations have been conducted in hardwood forests of the northeastern and north-central United States. However, the effect of defoliation on the growth of individual species in southeastern mixed pine-hardwood stands, and their vulnerability to mortality remains to be proven. We designed a study to examine the impact of gypsy moth defoliation on tree growth and mortality in mixed pine-hardwood stands. Two stand types were selected for study: loblolly pine (*Pinus taeda*) mixed with oak (*Quercus* sp.) and loblolly pine mixed with sweetgum (*Liquidambar styraciflua*). In 1992 and 1993, research plots were established in 47 stands in the Atlantic Coastal Plain physiographic province of Virginia and Maryland. The study has three objectives: 1) determine whether the pine component in mixed stands reduces stand susceptibility, or conversely, whether the presence of pines contributes to defoliation; 2) determine whether defoliation of the preferred hardwoods serves to promote pine growth, or only leads to subsequent pine defoliation; and 3) determine whether the proportion of pine (or hardwoods) in the stand is a useful predictor of growth loss and/or mortality of the hardwood species. In 1994 gypsy moth populations were active in 18 stands. These populations were substantially larger than in previous years which resulted in considerable defoliation. Mean total stand defoliation in 1994 ranged from 5.9% to 66.2%, and averaged almost 30% for all stands. In 1993, defoliation of all stands averaged only 15%. Susceptible species in both stand types suffered extensive defoliation in 1994. In pine-oak stands defoliation averaged 71%; in pine-sweetgum stands defoliation averaged 56%. Complete defoliation (100%) of numerous oak and sweetgum within the plots was not uncommon; these trees then refoliated later in the growing season. Refoliation has a negative effect on the physiological condition of individual trees, and when combined with other factors may increase vulnerability to mortality. For the first time, extensive defoliation of resistant species (mainly pines) was observed. The majority occurred in pine-oak stands, and was most likely due to the larger gypsy moth populations within these stands. Defoliation of resistant species in pine-oak stands averaged 19%, and 13% in pine-sweetgum stands. In previous studies, crown position has been related to defoliation intensity and subsequent mortality. In 1994, mean defoliation of susceptible species was relatively consistent across crown classes (≈66.7%). Among resistant species, suppressed and intermediate trees were defoliated at a higher rate (38.9% and 25.0%) than dominants and codominants (13.1% and 16.5%). Prior research indicates that tree mortality rapidly increases two years after a severe defoliation episode. If mixed stands in the southeast respond similarly, we should begin to observe mortality in 1995 and 1996.

LITTERFALL DYNAMICS IN GYPSY MOTH DEFOLIATED PINE-HARDWOOD STANDS

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ABSTRACT

While phytophagous insects are ubiquitous in forest ecosystems, the extensive defoliation that accompanies epidemic outbreaks results in dramatic changes in the normal cycle of litter fall. We designed a study to examine these changes in mixed pine-hardwood stands defoliated by *Lymantria dispar* (L.), the European gypsy moth. The study has two objectives: 1) determine the relationship between stand composition and the quantity of litter fall deposited in defoliated mixed stands, and 2) determine the relationship between stand composition and nutrient content of litter fall in defoliated mixed stands.

Sixteen mixed loblolly pine-hardwood stands were selected for study. Selection was based on stand composition (similarity in species composition and basal area) and defoliation activity. Each stand contains three 400 m^2 plots; these plots are part of an ongoing study to determine the effect of defoliation on tree growth and mortality. In May of 1994, three 1 m^2 litter traps were randomly located within each stand, each trap falling within the boundary of a single 400 m^2 plot. Litter was collected weekly during periods of gypsy moth larval activity. When larval activity ceased, collections were reduced to a monthly basis until the completion of leaf fall in the autumn. A single collection was taken during winter. Subsequent to collection, samples were sorted into six litter fall components, and dry weight of individual components and total sample dry weight were determined. Each litter fall component will also be tested to determine the concentrations of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (M). Prior to nutrient analysis, the samples from each stand will be assigned to one of seven seasonal periods based on date of collection: 1) winter, 2) gypsy moth activity periods (a total of four), 3) remainder of growing season, and 4) autumn. Within each period all samples for a single stand will be bulked by litter fall component.

Stands with active populations experienced heavy to severe defoliation in 1994. This was reflected in the dry weights of litter fall components collected during this period. Due to the quantity of litter collected, sorting and weighing of samples is incomplete. It is anticipated that this portion of the analysis will be completed in early 1995, prior to the resumption of gypsy moth larval activity and concurrent litter fall collection. Nutrient analysis of the 1994 data will occur during the summer of 1995. This study was designed as a complement to an existing investigation and will continue through 1995 with the completion of sample collections in February, 1996.

EFFECT OF REPEATED TREATMENTS OF *BACILLUS THURINGIENSIS* AGAINST GYPSY MOTH POPULATIONS: INITIAL SURVEY

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ABSTRACT

Gypsy moth populations located in the George Washington National Forest (GWNF) in Virginia (i.e. Sherando Lake, Trout Pond, Hawk, Little Fort, Powell's Fort and Elizabeth Furnace) and in the Allegheny National Forest (ANF) in Pennsylvania (i.e. PA #3708, #2430, #3724, and #5234) that had been treated with *Bacillus thuringiensis* (*Bt*) three to five times in the last three to six years, were analyzed for their susceptibility to *Bt*. Untreated populations adjacent to and associated with these sprayed populations were analyzed also for their susceptibility to *Bt*. Response to the graded doses of Foray 48B (BBN 6325) by the different untreated populations were parallel (no significant difference between their respective regression coefficients (slopes)). Based on the LC_{50}, there were three levels of susceptibility. The populations from untreated areas near Sherando Lake and Little Fort were the least susceptible and were not significantly different from the F_{40} N.J. gypsy moth standard routinely used in the laboratory. Populations from Hawk, Trout Pond, and the PA #3708 were equally the most susceptible. The differences between these two extreme groups appeared to be significant. In between and not significantly different from either group were the populations from Elizabeth Furnace and Powell's Fort. With the treated populations, regression coefficients indicated that dose response relationships were similar (i.e. parallel slopes), and except for the population from Little Fort, the LC_{50} estimates were not significantly different from each other nor from the most susceptible untreated populations (Hawk, Trout Pond, and PA #3708). Neither the response to graded doses (slope) nor susceptibility (LC_{50}) to *Bt* by population in PA #3708 after having been treated three times between 1987 and 1992 indicated that there was any treatment effect when compared to its associated untreated population. Response of the population in PA #3724 did not appear unusual and its susceptibility (LC_{50}) was not significantly different from most of the other populations that were analyzed. Response to graded doses of *Bt* in population PA #2430 was very variable resulting in unacceptably large confidence limits about the LC_{50}. Therefore, *Bt* treatment effects, if any, could not be assessed. Based on this preliminary analysis, it does not appear that gypsy moth populations treated up to five times within a six-year period show any indication of having developed any resistance to *Bacillus thuringiensis*.

IMPACT OF *ENTOMOPHAGA MAIMAIGA* ON GYPSY MOTH POPULATION DYNAMICS

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ABSTRACT

There was a massive epizootic caused by *E. maimaiga* among gypsy moth populations in 1989 and 1990 throughout the northeastern United States. In subsequent years, however, we noted a steady decline in the mortality caused by this agent and we wondered if its impact would dwindle to the insignificant levels that existed prior to 1989. In 1994, however, we recorded high levels of mortality in several high and low density populations in western Massachusetts. These mortalities were nearly as great as those we observed in 1989 and were presumably caused by the higher levels of rainfall we had in May and June compared to 1992 and 1993. These findings support the view that *E. maimaiga* has caused a major shift in gypsy moth population dynamics and is not an ephemeral phenomenon.

We have also studied the interaction between *E. maimaiga* and nuclear polyhedrosis virus LdNPV. In previous laboratory studies we have shown that when larvae are inoculated simultaneously with LdNPV and *E. maimaiga,* both agents will develop, but that larvae usually die from *E. maimaiga* because of the shorter incubation time compared to LdNPV. When the larvae are inoculated with LdNPV first and several days later with *E. maimaiga,* they are equally likely to die of either agent. Our conclusion from these studies was that there is no evidence for direct antagonistic or synergistic interaction between the two pathogens within the same host individual. However, it is still likely that the two pathogens may influence one another at the population level by way of their mutual effects on population density. In particular, since LdNPV epizootics reach peak levels just before pupation, we hypothesized that mortality from *E. maimaiga* among early instars may suppress densities of larvae sufficiently to have an adverse impact on mortality from LdNPV. In 1994 we explored these population level interactions by collecting 100 gypsy moth larvae weekly during the larval stage from four plots in a high density population. We fit a host-pathogen model to the data and compared the levels of LdNPV and overall survival with and without *E. maimaiga.* The model fit the observed mortality in our 1994 field collections quite well. However, removal of *E. maimaiga* from the model had an insignificant effect on mortality from LdNPV. The biological explanation for this result is that both pathogens attain peak levels of mortality during the last larval instar. Incubation of LdNPV in gypsy moth is relatively long. Larvae dying at peak LdNPV mortality acquire their inoculum as fourth instars. Prior to the fourth instar, there have been insufficient declines in density caused by *E. maimaiga* to have any measurable effect on LdNPV transmission rates or resulting mortality from LdNPV.

FATE OF F_1-STERILE GYPSY MOTHS RELEASED IN FLORIDA IN 1994

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ABSTRACT

Analysis of gypsy moth detection and delimitation data collected since 1981 indicates that the males trapped each year come from newly introduced eggs and pupae. This information suggests that Florida is beyond the southern limit for gypsy moth establishment in the United States. To learn more about factors affecting hatch-to-adult survival, we placed ready-to-hatch F_1-sterile eggs on eight plots in mid February, mid March, and mid April. Initial neonates/plot were ca. 175,000, 222,000, and 202,000, respectively. Weekly observations and pheromone traps provided information on cohort development and survival.

The presence/absence of newly flushed foliage of acceptable hosts appeared to be a key factor affecting neonate establishment. Water, swamp chestnut, and overcup oaks (*Quercus nigra*, *Q. michauxii*, and *Q. lyrata*) were present on the bottomland hardwood plot having the two highest survival rates (0.098, 0.089%). Water oak was also the principal hardwood on the two plots having the next three highest survival rates (0.074, 0.067, and 0.043%). Turkey oak (*Q. laevis*) on one plot was readily colonized by neonates of the March cohort, although overall survival fell to just 0.016%. The remaining species, including laurel, live, and sand live oaks (*Q. laurifolia, Q. virginiana, Q. geminata*), river birch (*Betula nigra*), and sweetgum (*Liquidambar styraciflua*), never showed signs of supporting neonate establishment.

Laboratory rearing of field collected larvae yielded low numbers of two tachinid parasitoids. Otherwise, we were unable to quantify or assess factors producing the obvious decline in numbers as each cohort matured. Pheromone trap collections for the 24 cohorts ranged from 0 to 73 male moths and survival rates ranged from 0 to 0.098%. Four of the February cohorts and five of the April cohorts produced no adults. The best survival occurred in three of the February cohorts and two of the March cohorts. For 1994 the "best" hatch period for neonates ranged from late February to the end of March. The ensuing flights of males began in mid April and were completed by early June.

Historical data and the results of this and other research indicate there is little likelihood of the North American strain of the gypsy moth becoming established in Central Florida, and probably North Florida as well. Detection and delimitation surveys can be safely reduced, and traps need be operated for only the April to June flight period.

STATUS OF THE INTRODUCED GYPSY MOTH PUPAL PARASITE *COCCYGOMIMUS DISPARIS* (VIERECK) ON THE DEL-MAR-VA PENINSULA

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ABSTRACT

Collections of gypsy moth pupae were made on the Del-Mar-Va Peninsula during 1989-93, for the purpose of recovering the recently introduced pupal parasite *Coccygomimus disparis* (Viereck) [Hymenoptera: Ichneumonidae]. This species was recovered in all regions of the peninsula. Parasitism was usually low, less than 1%, but averaged 4.5% in 1990. Parasitism by this species did not seem to be affected by host density, habitat, or latitude. Discriminant analysis suggested that sample size, minimum temperature the previous December, and June rainfall were the most important factors affecting recoveries of this species.

A field experiment was conducted in which females of *C. disparis* were allowed to sting pupae of *L. dispar* developing in the field. The fate of stung pupae was compared with those not known to be stung. Even though only a few of the stung pupae yielded parasite progeny, overall mortality was much higher in stung hosts, with enhanced incidence of disease, desiccation, and attacks by another introduced parasite *Brachymeria intermedia*.

TRENDS IN PARASITISM AND HOST DENSITY AFFINITIES IN PENNSYLVANIA POPULATIONS OF *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT

Parasitism of gypsy moth, *Lymantria dispar* (L.), was monitored in 40 study plots in Pennsylvania during 1984-92. Parasitism of eggs by *Ooencyrtus kuvanae* (Howard) varied between regions, averaging ca. 30%. Parasitism by the larval parasite *Cotesia melanoscela* (Ratzeburg) was lowest in building populations. Parasitism by *Phobocampe unicincta* (Gravenhorst) was low during the outbreak, but rose during the host decline. Parasitism by *Compsilura concinnata* (Meigen) was highest in low declining and stable populations. *Parasetigena silvestris* (R.-D.) was the dominant larval parasite, and exerted highest parasitism in low stable host populations. Parasitism by *Blepharipa pratensis* (Meigen) and *Brachymeria intermedia* (Nees) remained low over most of the host population cycle, rising during the outbreak. *Blepharipa pratensis* and *B. intermedia* showed direct density dependence, whereas *C. melanoscela, P. unicincta, C. concinnata*, and *P. silvestris* showed inverse density dependence. Only *O. kuvanae, C. melanoscela*, and *B. pratensis* exhibited delayed density dependence. Usually, density dependent responses were strongest in declining populations, weakest in building populations, and intermediate in stable populations. The only exception, *B. pratensis*, showed a statistically significant response only in stable populations. This suggests that a breakdown in the density dependent responses of larval parasites could contribute to increases in gypsy moth density.

Parasitism by some species differed between physiographic regions, suggesting influences of climate and habitat suitability. Density dependent responses were strongest in those regions where mean parasitism was highest. Most species exhibited weak responses in the Unglaciated Allegheny Plateau, the region most recently invaded by the gypsy moth.

The relationship of parasitism to changes in host population was examined through stepwise multiple regression analysis. The most important parasites were *C. melanoscela, P. silvestris,* and *B. intermedia.*

STATUS OF NUCLEAR DNA MARKERS

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ABSTRACT

Because of the recent introductions of Asian gypsy moths into North America, in some regions it has become necessary to determine the origin of trapped male moths in order to plan control strategies. The identification of the trapped moths by morphology alone can be difficult, so methods of identification based on analysis of the specimen's DNA have been sought. Mitochondrial DNA markers for Asian and North American moths are presently in use but have the disadvantage of identifying only the maternal parent's origin when hybrid moths are analyzed.

Four nuclear DNA markers have now been found using the DNA amplification method known as random amplification of polymorphic DNA by the polymerase chain reaction (RAPD PCR). Results obtained with RAPD PCR can be difficult to reproduce, so further analyses of diagnostic DNA fragments have been done so that locus-specific primer sequences can be determined. Amplification using locus-specific primers should be much more reliable and sensitive when used on a variety of DNA samples, including those from small or poorly-preserved specimens.

The FS1 marker has been previously described (Garner and Slavicek, USDA Interagency Gypsy Moth Research Forum, 1994). This DNA region contains a size polymorphism diagnostic for the sample origin. Locus-specific primers are available, and these primers amplify a region which is 110 basepairs longer in Asian than in North American moths. The FS2 marker is an 800 basepair fragment which is amplified only in North American moths. Locus-specific primers are not yet available for this marker. The FS3 marker is a 600 basepair fragment also limited to North American specimens. Locus-specific primers are now being tested for this marker. The FS4 marker is a 700 basepair fragment seen only in Asian moths, and sequence information is now available and locus-specific primers will be designed and tested in the near future.

The success rate of these markers has been tested using known Asian samples from eastern Russia (Minneralni) and ships intercepted in the Pacific Northwest in 1991. Known North American samples came from Massachusetts, Michigan, North Carolina, Ohio, Pennsylvania, and West Virginia. At least 50 samples of each strain have been tested. FS1 correctly identified 100% of Asian and 96% of North American samples; FS2 correctly identified 96% of Asian and 100% of North American samples; FS3 correctly identified 91% of Asian and 94% of North American samples; and FS4 correctly identified 91% of Asian and 98% of North American samples.

GEOGRAPHIC ROBUSTNESS OF A THREE-PHASE MODEL OF GYPSY MOTH EGG PHENOLOGY

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ABSTRACT

A model of a biological process is described as geographically robust if it accurately simulates the process regardless of the geographic location in which it is applied, without the need to alter parameter values. Geographic location can have a profound effect on temperature, the predominant driving variable in insect development. Thus, geographic location can have a profound effect on gypsy moth egg phenology.

A geographically robust model of gypsy moth egg phenology is of interest for various reasons. Complex landscapes on a county or state scale will result in significant differences in the timing of egg hatch. A robust model can accurately predict the timing across such a landscape, and can thereby assist in planning pest management activities. Climatic limits to the potential range of gypsy moth in North America are currently unknown. A geographically robust model can be used in an investigation of potential limits. The expected timing of egg hatch can be completely unknown in situations where egg masses are transported great distances to their final hatch site. Such situations can occur in North America with intra-continental transportation of North American egg masses, or with the inter-continental transportation of various strains of Asian egg masses (Figure 1). Existing models of gypsy moth egg phenology have failed to demonstrate geographic robustness.

Figure 1.

We are developing a unique model of gypsy moth egg development that encompasses three distinguishable phases of egg development: prediapause, diapause, and postdiapause. Phase-specific developmental responses to temperature have been empirically estimated for the prediapause (Gray *et al.* 1991) and postdiapause (Gray *et al.* 1995) phases (Figure 2).

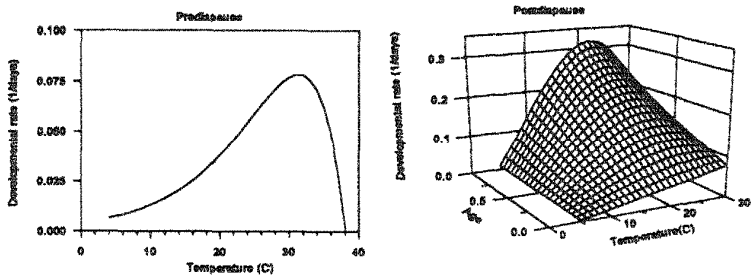

Figure 2.

A "best-guess" estimate of temperature-dependent, age-independent, diapause development is being used in the model until an ongoing experiment examining diapause is completed.

Our three-phase model has demonstrated good geographic robustness in simulations of temperature conditions in eastern North America from Quebec to Florida, and in British Columbia. Robustness is conferred on the model through the use of phase-specific, temperature- and age-dependent development responses. Warm winters in areas south of Georgia limit simulated egg hatch by failing to satisfy diapause requirements for a large proportion of the population. Simulated egg hatch can be limited in northern Canada where cool summers can be expected to retard larval development and therefore delay oviposition. Late oviposition results in limited simulated egg hatch the following year if prediapause cannot be completed before the onset of winter conditions. In locations between Maine and South Carolina, egg hatch is completely successful. Timing of simulated egg hatch in these locations is strongly dependent on the time of diapause completion, and the prevailing temperature conditions at the time of diapause completion. Final validation of the three-phase model will be undertaken upon completion of an ongoing diapause experiment.

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EFFECTS OF SILVICULTURAL MANAGEMENT ON RATES OF PREDATION ON GYPSY MOTH LARVAE AND PUPAE

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ABSTRACT

The gypsy moth, *Lymantria dispar,* has perhaps become the most important forest pest in North America. As an alternative to the application of pesticides, and as a long-term management strategy, silvicultural techniques have the potential of decreasing forest susceptibility or vulnerability to gypsy moth damage. Silvicultural thinnings may also benefit natural enemies of the gypsy moth, and thus indirectly increase predation rates on larvae and pupae. The effects of silvicultural management on predation rates of gypsy moth larvae and pupae were studied on the West Virginia University Forest from 1989-1992. Rates of predation on larvae and pupae were monitored in eight thinned and eight un-cut stands with the use of three types of exclosures placed at three heights in the forest strata. Small mammals were pitfall trapped within these stands so that any relationships between their abundance and predation rates could be ascertained. In the years following thinning, 37 percent of larvae and 25 percent of pupae were destroyed by predators. Silvicultural thinning did not influence the proportion of larvae or pupae killed; however, survival of larvae and pupae on the ground was significantly lower than that found on tree boles or in the foliage. It was found that invertebrates may have been the most important predators of larvae, whereas small mammals may have been the most important predators of pupae during the study period. Since silvicultural manipulations increased small mammal predator populations, but did not affect rates of predation on the gypsy moth, the vulnerability of larvae or pupae likely was not influenced. Vulnerability of gypsy moth larvae or pupae to predation may have declined due to an increase in the structural complexity of ground level vegetation and debris cover, or from an increase in alternate foods.

PERSISTENCE OF *ENTOMOPHAGA MAIMAIGA* IN THE ENVIRONMENT

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ABSTRACT

Since the gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper was first found in North America in 1989, the potential for use of this fungus as a biological control agent has been discussed. This pathogen was introduced as resting spores (RSP) in small plots in central New York in 1990 and in Maryland, Pennsylvania, Virginia, and West Virginia in 1991 and 1992. These releases resulted in establishment in most plots and epizootics in many. We have investigated the persistence of this fungus in 1991-92 release plots. At seven plots in George Washington National Forest, VA, where *E. maimaiga* was released in 1991-92, fungal infection levels found in 1994 ranged from 40.8-97.5% (mean $\pm$ SE; 72.9 $\pm$ 7.7). This constituted an increase in infection from the year of release for five of these seven plots. In plots in central New York, *E. maimaiga* caused epizootics in 1991 and 1992, leading to a collapse in the gypsy moth population. During 1993 and 1994, gypsy moth larvae were extremely difficult to find yet during experiments, the majority of gypsy moth larvae caged over the ground became infected throughout the season. These infections were due to the presence of germinating RSP in the soil. Presumably, these RSP had been formed during the 1991-92 epizootics.

Therefore, the RSP produced by this fungus are responsible for persistence of this disease in gypsy moth populations, as well as being the stage successfully used for introductions. RSP are formed by many species of insect-pathogenic fungi in the Entomophthorales, yet this stage is poorly understood. Thus, we have been studying the RSP stage produced by *E. maimaiga*. For *E. maimaiga*, RSP are predominantly formed within cadavers of later instars that become infected by the relatively short-lived, asexual conidial spores. When RSP germinate and infect larvae, the resulting cadavers produce only conidia. After a late instar dies, RSP require at least 2 d to mature, with the majority of RSP appearing mature by 4 d. RSP are produced in abundance, with approximately 1-2 x 10^6 within each late instar. Larvae frequently die from this disease while on tree boles where cadavers hang for some period but then fall to the ground and RSP are leached into the soil. We have found 1202-3895 RSP/g dry soil at the bases of trees that were covered with cadavers the previous spring. After being produced, RSP are constitutively dormant and will not germinate. Germination trials on RSP maintained in the field and brought to the lab on a monthly basis detected germination beginning only in April, with increased germination at 14:10 L:D compared with 13:11 or 12:12.

Preliminary studies demonstrated that counts of RSP overwintering in soil directly at the bases of five central trees did not predict levels of *E. maimaiga* infection in small plots the following spring. Unfortunately, our lack of knowledge regarding the distribution of RSP in the forest soil may have influenced the predictive ability of this technique. Experimentation using a simulation model of the *E. maimaiga/L. dispar* system suggests that conidial infections, as well as infections initiated by RSP, make significant contributions to the development of epizootics. The fact that infections initiated by RSP and conidia work in concert to produce epizootics may also explain why simple counts of RSP alone did not predict subsequent infection levels.

EFFECT OF *ENTOMOPHAGA MAIMAIGA* ON NON-TARGET LEPIDOPTERA

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ABSTRACT

The gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper was first reported in North America in 1989. Since then, this pathogen has repeatedly caused epizootics and has spread extensively. Further development of this fungus for biological control necessitates knowledge of its impact on species other than gypsy moth. Laboratory bioassays have never demonstrated infection in any organisms other than larval Lepidoptera. During 1992 and 1993, laboratory bioassays were conducted to test whether *E. maimaiga* could infect species of Lepidoptera native to West Virginia. Approximately one-third of the 78 species tested became infected although, in general, infection levels were very low. Infection in >50% of individuals tested was found only in three species of lymantriid and one species of sphingid. As a caveat, in the field of insect pathology, laboratory bioassays testing host specificity are well known to be poor indicators of naturally occurring host range.

During 1994, seven sites in the George Washington National Forest, VA, where *E. maimaiga* was released in 1991 and 1992, were chosen to sample non-target Lepidoptera (NTL). Throughout the field season, NTL were collected weekly under burlaps and on foliage, identified, and reared on foliage for 14 d. Simultaneously, gypsy moth larvae were sampled and reared to document the activity of *E. maimaiga*. For any NTL that died, cadavers were diagnosed microscopically and then DNA probes were used to identify infections by *E. maimaiga*. *E. maimaiga* was active in gypsy moth populations at all sites, causing epizootics at several. Of the 1421 NTL belonging to 53 species that were reared, *E. maimaiga* was found in only two individuals: *Malacosoma disstria* Hbn. and *Catocala ilia* Cramer. Comparisons of laboratory bioassay results with field sampling for 24 species demonstrated that all species not infected in the laboratory were not infected in the field. Of the six species infected in the laboratory, only one, *M. disstria*, was infected in the field but at a much lower level (lab 61.0%, field 0.4%). Our results confirm the disparity between host specificity data from the laboratory and field and suggest that evaluation of the potential impact of fungal pathogens in the field should take this discrepancy into account and should not be based solely on laboratory results, if at all possible.

NTL were also sampled in eastern Virginia, Michigan, and central New York at plots where *E. maimaiga* was active in gypsy moth populations. For the 300 larvae reared, no NTL were infected with *E. maimaiga*. In summary, sampling of NTL during 1994 in plots where *E. maimaiga* was simultaneously infecting gypsy moth yielded only two larvae (of two different species) that were infected with *E. maimaiga* out of 1721 larvae collected and reared from 62 different species.

Samples of NTL collected when opportunities occurred between 1989-1994 were tested for *E. maimaiga* or *Entomophaga aulicae* (Reich. in Bail) Humber. *E. aulicae* is a North American native fungal pathogen infecting Lepidoptera that is morphologically identical to *E. maimaiga* as well as sympatric but cannot infect gypsy moth. Results demonstrated *E. maimaiga* infection only in two species of the lymantriid genus *Dasychira* while *E. aulicae* was present in a notodontid, a geometrid, and two species of arctiid.

NATURAL ENEMIES OF THE GYPSY MOTH AT THE LEADING EDGE OF ITS INVASION INTO THE SOUTHERN U.S.

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ABSTRACT

In a continuing effort to evaluate the impact of an exotic herbivore, the gypsy moth, on selected forest ecosystems of the South, small mammal live trap surveys were conducted in six sites, two each in the coastal plain (Currituck Co., NC and Northampton Co., VA), piedmont (Lake Anna State Park in Orange Co., VA) and mountains (George Washington National Forest in Amherst Co., VA) during 1992, 1993 and 1994. The predation of gypsy moth pupae by small mammals was examined between the live-trap surveys by placing freeze-dried pupae at trap stations, at the litter layer and at 0.25, 1.0, and 2.0 m heights on the bole. During 1992 total predation was high at all of the study sites, even where the mouse population was low. The lower predation by mice was at least partially compensated for by increased predation by invertebrates. In fact, our 1992 and 1993 data appeared to show an inverse relationship between vertebrate and invertebrate predation. During both years total predation remained about the same, even where low mouse density occurred. However, in 1994 predation was directly related to mouse density but again, invertebrates continued to contribute significantly to total predation. This constancy of total predation in these southern sites, despite radical reductions in small mammal populations, would be consistent with an hypothesis of greater biodiversity in these sites. This is unlike information from the northeastern U.S. (Bryant Mountain, VT, Cape Cod and Western MA) where there was a direct decrease in predation as small mammal populations decreased.

Trap-host populations using F_1-sterile gypsy moth eggs were created in three of six plots established in Virginia in 1993, one each in Northampton, Orange and Amherst Counties. An additional plot was established in Camden County, NC. Three collections of gypsy moth were made at each site; collections corresponded to instars 1-3, 4-5, and 5-pupa. Larvae were reared individually in plastic containers with artificial diet. Parasites were removed weekly. Dead larvae were autopsied for *Entomophaga maimaiga* and nuclear polyhedrosis virus (NPV). Significant habitat differences in parasitoid species diversity, within and between years, indicate the difficulty of using short-term data collection for making management decisions, and support the need for long-term permanent plot surveys to detect and quantify habitat specific trends in parasitoid diversity. The addition of a survey for *E. maimaiga* and NPV increased our knowledge of the diversity of compensatory natural biological control agents in the three habitat types.

PREDICTING SUSCEPTIBILITY OF FOREST STANDS TO GYPSY MOTH DEFOLIATION

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ABSTRACT

Gypsy moth hazard rating is based on the accurate prediction of forest stand susceptibility to defoliation. Herrick and Gansner (1979) have developed a model that predicts susceptibility of forests based on tree species and crown vigor. We utilized the data from approximately 400 0.1-acre plots in Pennsylvania and Maryland to test the accuracy of the Herrick and Gansner model. We measured the variables needed to input into the prediction model and monitored actual defoliation by gypsy moth over a three-year period. For the plots located in the Appalachian Plateau physiographic province, the model tended to predict a somewhat lower defoliation rate than actually occurred. The reverse was true for the Ridge and Valley province.

The prediction model was reasonably effective at predicting relative defoliation, but did not accurately predict the exact percent defoliation for a given stand. Additional testing of this model is recommended to delineate the useful range of the defoliation susceptibility model.

COMPLETE NUCLEOTIDE SEQUENCING OF A GENOMIC CLONE ENCODING THE LARGE SUBUNIT OF VITELLOGENIN FROM THE GYPSY MOTH

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ABSTRACT

In the gypsy moth, vitellogenin (Vg) is synthesized and secreted by the fat body of female larvae during the last larval stadium. Expression of gene(s) coding for the Vg is developmentally regulated and is sex-specific. Furthermore, the Vg gene expression is suppressed by the juvenile hormone in the gypsy moth. This is in contrast to other insect systems, where the juvenile hormone either induces Vg gene expression or has no effect at all. Also, the oligomeric protein structure of gypsy moth Vg appears to be different from those of Vg's from other insects including lepidopterans. In order to understand more about the gypsy moth Vg and regulation of Vg gene expression by the juvenile hormone, we have attempted to isolate and characterize gene(s) coding for Vg.

A gypsy moth EcoRI subgenomic library was constructed in bacteriophage vector EMBL4 and screened using a cDNA clone encoding the large subunit of Vg (Vg190). One of the clones isolated, E4VgL, had an insert of ~12.5 kbp and appeared to contain the entire gene for Vg190. Initial characterization using Southern analyses and partial nucleotide sequencing identified the region corresponding to the N-terminus of the processed Vg190 protein.

In order to further characterize this clone and the gene, we have determined the nucleotide sequence of the entire clone in both orientations. At least one big intron was present just upstream of the region corresponding to the N-terminus of Vg190. Isolation and cloning (through 5'-RACE reactions), and sequencing of a cDNA clone representing the 5'- region of Vg mRNA indicated that the intron, 1335 nucleotides long, was present within the region encoding the signal peptide. The length (15 amino acids) of the signal peptide was consistent with those reported for Vg's from other insects and invertebrates. Partial sequence of Vg mRNA has indicated that the gene contains other introns. However, identification of the number and locations of these introns will have to wait until studies in progress to determine the complete sequence of Vg mRNA are completed.

THE PARASITOID COMPLEX OF THE GYPSY MOTH IN HIGH AND LOW LEVEL POPULATIONS IN EASTERN AUSTRIA AND SLOVAKIA

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ABSTRACT

Investigations on differences between the parasitoid complexes of the gypsy moth were carried out in eastern Austria and Slovakia in 1993 and 1994. In both countries two mixed oak stands with *Quercus cerris* and *Q. petraea* as the main tree species were chosen as latency and gradation sites. Gypsy moth egg masses, larvae and pupae were collected stage specific. The larvae were reared on oak foliage at the laboratory and checked daily for parasitoid emergence. At the latency site egg masses were exposed by attaching them to the stems of a group of small isolated oak trees.

The following egg, larval and pupal parasitoids were recovered in 1993 and 1994: *Glyptapanteles liparidis* (Bouché), *G. porthetriae* (Muesebeck) and *Cotesia melanoscelus* (Ratzeburg) (Braconidae), *Phobocampe unicincta* (Gravenhorst) (Ichneumonidae), *Para-setigena silvestris* (Robineau-Desvoidy), *Blepharipa pratensis* (Meigen) and *Zenillia libatrix* (Panzer) (Tachinidae) in Austria and Slovakia; *Cotesia ocneriae* Ivanov (Braconidae), *Brachymeria intermedia* (Nees) (Chalcididae), *Lymantrichneumon disparis* (Poda), *Theronia atalantae* (Poda) and *Pimpla hypochondriaca* (Ratzeburg) (Ichneumonidae) and *Para-sarcophaga uliginosa* (Kramer) (Sarcophagidae) in Austria only; and *Anastatus disparis* Ruschka (Eupelmidae), *Ooencyrtus kuvanae* (Howard) (Encyrtidae), *Monodontomerus sp.* (Chalcididae), *Compsilura concinnata* (Meigen), *Drino incospicua* (Meigen) and *Pales pavida* (Meigen) (Tachinidae) in Slovakia only.

The laboratory rearings of field collected larvae showed that *P. silvestris* was the dominant parasitoid at the gradation sites. There, the rates of total parasitism were rather low and did not differ significantly between Austria and Slovakia.

At the latency site the exposed hosts were parasitized frequently, mainly by the specialized braconids. Parasitism was higher than at the outbreak sites, with *G. liparidis* as the dominant species in Austria and *C. melanoscelus* in Slovakia. The rate of total parasitism in Austria was significantly higher than in Slovakia.

ASIAN GYPSY MOTH GENETICS:

BIOLOGICAL CONSEQUENCES OF HYBRIDIZATION

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ABSTRACT

The following summarizes the results of research to determine the biological consequences of hybridization between gypsy moths of Asian and European origins. The research was conducted in the USDA Forest Service's Quarantine Laboratory in Ansonia, Connecticut.

Hybridization between a Far East Russian strain and a strain from North Carolina (collected in 1992) had the following biological consequences. Lower diapause chill requirements of Russian eggs were retained in their hybrids. The hybrids (F_1 and F_2) had similar chill requirements to that of the Russians and significantly less chill than those from North America. Faster larval growth was retained and accentuated with hybridization. The Far East Russian strain grew slower than the North Carolina strain. The growth of F_1 hybrid larvae was as fast or faster than that of the respective strain of the female parent. F_2 hybrid larvae grew as fast or faster than the original Far East Russian and North Carolina strains. The full range of larval color variation was present in hybrids. The Far East Russian strain had all color forms present (black, gray, yellow-gray, and yellow), but yellow predominated. The larvae of the North Carolina strain were predominantly the gray form and a few yellow-gray larvae were present. All larval color forms were present in the hybrids (F_1 and F_2), but yellow-gray and gray accounted for approximately 80 percent of the population. Some female flight capability is retained with hybridization, but the proportion of the population with strong directed flight is reduced. No females from the North Carolina strain attempted to fly; approximately 90 percent of the females from the Far East Russian strain were capable of strong directed flight. Virtually all of the F_1 hybrids were unable to gain altitude or sustain flight, but about half glided for a few meters while vigorously flapping their wings. In the F_2 generation, approximately 10 to 15 percent of the females were capable of strong directed flight. This suggests that the presence of hybrids in North America could substantially increase the ability of the gypsy moth to spread and could alter the timing of the presence of various life stages.

Gypsy moths collected from German populations in September 1993, where hybridization was suspected, were evaluated and compared to the hybrids produced in the laboratory. These populations had egg diapause chill requirements, larval color and growth rate, and adult female flight characteristics consistent with that of a population where hybridization between Asian and European gypsy moths has occurred for more than one generation.

MIXING EXPERIMENTS BETWEEN CRYIAA AND CRYIAC INSECTICIDAL CRYSTAL PROTEINS SUGGEST OLIGOMERIZATION OF TOXINS

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ABSTRACT

Force-feeding bioassays were performed with CryIAa and CryIAc insecticidal crystal proteins (ICP) to gypsy moth larvae to test synergism among *Bacillus thuringiensis* (*Bt*) toxins. CryIAa ICP is about seven times more toxic than CryIAc ICP to gypsy moth larvae. From mixing experiments with these two ICPs, expected LD_{50} values were calculated from individual toxins as described by Tabashnik (Appl. Environ. Microbiol. 58:3343, 1992), and observed LD_{50} values were calculated by Probit analysis. Positive synergism was observed at all ratios tested. Observed LD_{50} values were shown to be four to eight times lower than expected over the range from 1:1 to 1:8 ratio of CryIAa to CryIAc toxins. Synergism effects start getting lower at 1:12 ratio. These bioassay data support the model for oligomerization of *Bt* toxins.

SLOW THE SPREAD PROJECT UPDATE: DEVELOPING A PROCESS FOR EVALUATION

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ABSTRACT

The Slow the Spread (STS) pilot project was initiated by the USDA Forest Service in 1993 in order to determine the feasibility of using IPM strategies to slow the spread of gypsy moth over large geographical areas. Three project areas have been established in the transition zone immediately ahead of the advancing front of gypsy moth populations. One is located in the upper peninsula of Michigan, one in the Central Appalachians in Virginia and West Virginia, and one in northeastern North Carolina.

Project objectives are to:

1. Demonstrate that new and current technology can slow the rate of gypsy moth spread.
2. Assess the technological, economic, ecological, and environmental viability of implementing an operational STS program.
3. Implement a plan for integration of STS technology into a national strategy for suppression of the gypsy moth, assuming the STS project is successful.

The project strategy is to detect and manage isolated gypsy moth populations located immediately ahead of the leading edge of gypsy moth spread (Fig. 1). Management of newly established low-level infestations can prevent their growth, coalescence, and subsequent contribution to gypsy moth spread. The previous Appalachian Integrated Pest Management (AIPM) program (1988-1992) conducted in Virginia and West Virginia was targeted both at high- and low-density populations. Reduction of gypsy moth spread rate was one of the AIPM objectives which was inherited by STS. However, STS is designed to slow gypsy moth spread with reduced pesticide use and management costs as compared to AIPM.

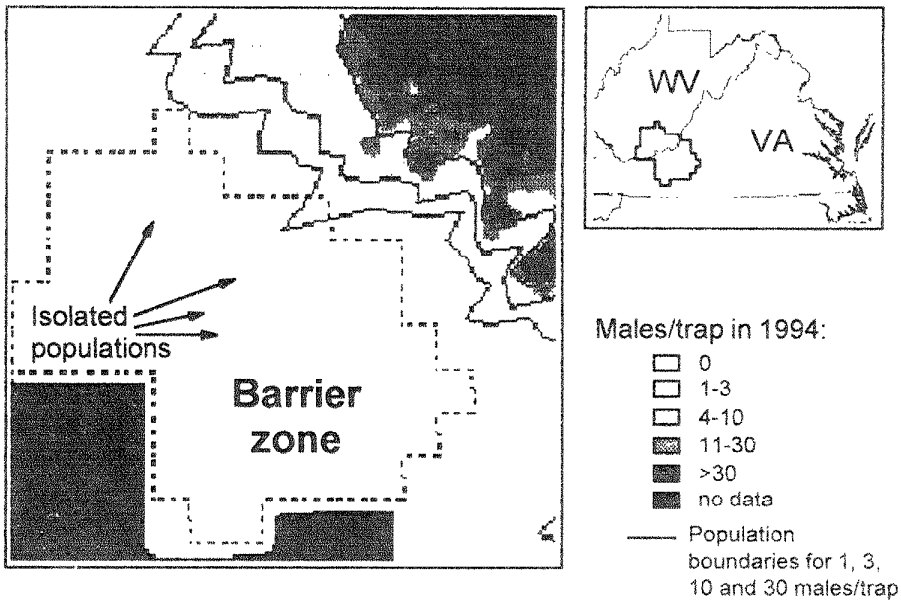

Figure 1. Barrier zone for slowing gypsy moth spread in the Central Appalachians.

Project components include:

1. Monitoring System. There are two monitoring zones within each STS area: the action zone where isolated populations are detected and managed, and the evaluation zone where the advance of the population front is estimated. Pheromone-baited traps are deployed at 1 km spacing in the action zone (805 m in Michigan), and at 3 km spacing in the evaluation zone. Monitoring results are used as the basis for most decision making in STS.

2. Data Management. The two databases (at Virginia Polytechnic Institute and State University and Michigan State University) are integrated with geographic information systems and are used for quality control in data collection as well as analysis of results.

3. Control Tactics. Emphasis is placed on the most environmentally safe tactics that will meet management objectives. Traditional methods for managing high density populations (diflubenzuron, *Bt* and Gypchek) are employed, as well as tactics designed to manage low-density populations such as mass trapping and mating disruption.

4. Environmental Documentation. All documents required for full compliance with the National Environmental Policy Act are produced for the project each year.

5. Public Information and Education.

The efficiency of the project will be evaluated using two criteria: 1) rate of population spread, and 2) boundary compression. The criteria will be compared among the STS area, historical data and non-managed areas. The rate of spread can be measured as a distance between population boundaries in two consecutive years. We developed several methods for estimation of regular population boundaries (a regular boundary has no "islands", "lakes", or folds) which are most convenient for estimation of both criteria. The Best Cell Classification method was shown to be one of the most accurate and robust methods. It minimizes the number of misclassified grid cells (e.g., occupied cells considered as non-occupied). Gypsy moth spread rate in 1988-1994 in the Central Appalachians ranged from 2.3 to 19.7 km/year, with the average of 10 km/year (Fig. 2). It was significantly lower than the average historical rate of spread in 1960-1990 of 20.78 km/year (Liebhold *et al.* 1992). However, no trend in spread rate change was detected within the period of 1988-1994.

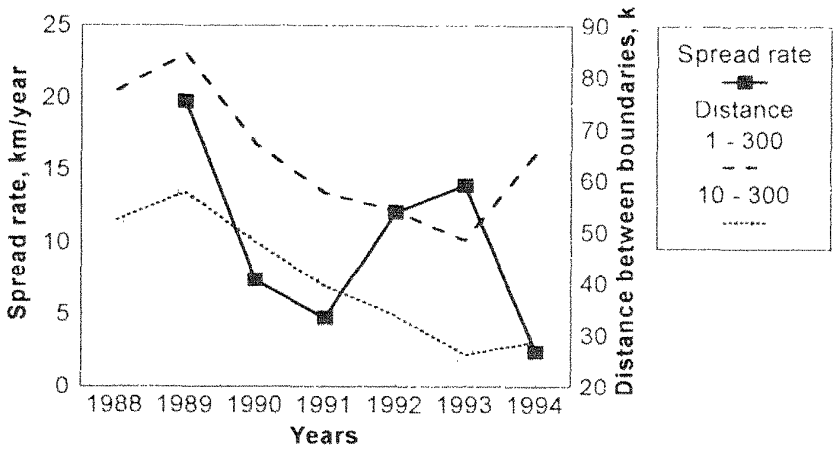

Figure 2. Gypsy moth spread rate and boundary "compression" (distance between population boundaries for 1, 10 and 300 moths/trap) in the Central Appalachians.

A simple model indicates that the distance between population boundaries for different male moth thresholds decreases as gypsy moth spread rate is reduced (boundary compression). If the intrinsic growth rate of established populations is constant, then the distance between boundaries is proportional to the rate of population spread. The distance between boundaries for 1 and 300 moths/trap, and between boundaries for 10 and 300 moths/trap in 1990-1994 in the Central Appalachians was significantly lower (by 31%) than in 1988-1989 (Fig. 2).

Thus, significant changes in the rate of population spread and in boundary compression have already been detected in the Central Appalachians. We expect that additional historical and future data which will be obtained for the STS project will increase the accuracy of the evaluation criteria. In Michigan and North Carolina, the evaluation of STS results may be problematic. In Michigan, migration of male moths has complicated the yearly interpretation of monitoring data; to date no control projects have been initiated. Therefore, there is no reason to expect any change in the spread rate. The North Carolina STS project area is located a greater distance ahead of the leading edge of gypsy moth populations (measured by moth boundaries). Thus, the changes in the rate of spread may be very limited. Also, the historical data in southern Virginia (evaluation zone for the NC STS) that can be used for comparison of spread rates and boundary compression are very limited.

Liebhold, A.M., J.A. Halverson, and G.A. Elmes. 1992. Gypsy moth invasion in North America: a quantitative analysis. J. of Biogeography 19: 513-520.

GYPSY MOTH POPULATION SUPPRESSION WITH PESTICIDES:

HOW OFTEN IS SUPPRESSION REALIZED?

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ABSTRACT

The effectiveness of aerial applications of *Bacillus thuringiensis* (*Bt*) and diflubenzuron (Dimilin©) in the AIPM gypsy moth management program was evaluated using data compiled in a geographical information system. These data included counts of overwintering egg mass densities, defoliation maps, and treatment block boundaries collected by the Appalachian Integrated Pest Management Program in VA and WV from 1989-1992. Results indicated that Dimilin treatments resulted in a greater level of foliage protection and population reduction than did applications of *Bt* except when pretreatment egg mass densities were less than 1,000 egg masses per ha. Applications of both materials often did not provide foliage protection in the year following treatment, especially when treatment blocks were small and/or near to defoliating populations. Only application of Dimilin in large blocks appeared to provide population suppression adequate to protect foliage for several years. However, this strategy is undesirable because it probably causes the greatest impacts on non-target insects. Evaluation of increasing expenditures by the USDA Forest Service on both suppression and eradication suggests that eradication may be the most important focus of gypsy moth management in the future.

FOREST TYPE AFFECTS PREDATION ON GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) PUPAE IN JAPAN

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ABSTRACT

A compilation of historical defoliation data from Hokkaido, Japan confirmed previous reports that plantations of *Larix leptolepsis* are more susceptible to defoliation by the gypsy moth, *Lymantria dispar* (L.), than are natural forests dominated by *Quercus mongolica*. In this study, we compared levels of predation on laboratory-reared pupae deployed at a natural *Quercus* stand, a plantation of *Betula platyphylla*, and a plantation of *L. leptolepsis*. Predation was highest in the natural *Quercus* stand, lowest in the *Larix* plantation and intermediate in the *Betula* plantation. Counts of small mammals in snap-traps indicated that populations of *Apodemus argenteus*, *Apodemus speciousus ainu*, and *Clethriomomys rufocanus bedfordiae* were most abundant in the oak forest, least abundant in the *Larix* plantation, and intermediate in the *Betula* plantation. We hypothesize that variation in the abundance of small mammals and their predation upon gypsy moth pupae during periods of low gypsy moth densities is the partial cause of the variation among stand types in their susceptibility to defoliation by the gypsy moth. We also argue that predation on gypsy moth pupae by small mammals in natural oak forests is more important to gypsy moth dynamics than previously considered and that considerable similarities may exist between gypsy moth dynamics in Japan and North America.

A COMPUTER PROGRAM TO PREDICT GYPSY MOTH LARVAL MORTALITY IN SPRAYED FORESTS

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ABSTRACT

A computer model "Deathguess" was created to aid in the prediction of gypsy moth larval mortality from aerially applied pesticides. The user inputs the material used, sampling results from deposit assessment of four leaves on 20 trees, larval age distribution, and the degree of larval control required. The output gives a spatially referenced map of likely larval mortality in the spray block, highlighting areas where the desired control level may not be achieved. The program integrates larval feeding, deposition and spread factor studies done at Penn State to construct mortality curves for gypsy moth larvae. Feeding studies initially looked at the effect of various droplet sizes and densities of Foray 48B on oak foliage when exposed to second, third and fourth instar caterpillars. The size and densities of Foray droplets were similar to deposits occurring during field spray projects. Subsequent studies were performed with NPV and Diflubenzuron. These last two materials have not yet been incorporated into Deathguess.

Deposition studies used as demonstration examples in the program were obtained from a NEFAAT study held on the George Washington National Forest (GWNF) in 1992. Foliage was sampled with shotguns from 20 oaks/plot with eight leaves/tree sampled. Data from two 50-acre plots were used in the program but due to space requirements, only data from only four leaves/tree were used.

A spread factor is a formula which defines the relationship between deposit stains and droplets. Spread factors used in Deathguess were determined under field conditions with aircraft or by using a 20-foot spray tower. The user enters deposit data containing only stain diameters. Deathguess calculates droplet size data from the stain size data using the spread factor in order to be able to relate the field deposit data to the results obtained with feeding studies.

Deathguess calculates mortality predictions based on larval numbers present at the time of spray. Deposition parameters that are used in this calculation are droplet diameter and leaf area. Data are stored in ASCII format and are compatible with most image analysis software packages. Output from the program is presented by tree by averaging predicted mortalities by leaf. The trees are drawn on a map based on their coordinates, so that it may quickly become apparent if any part of the spray block received an under-dosing of control material. The spatial reference aspect of the program has not yet been implemented at the time of writing. It is hoped that Deathguess can be used in field projects to quickly predict whether adequate deposition exists to ensure desired control.

FUTURE GYPCHEK PRODUCTION

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ABSTRACT

Over the years, production of Gypchek by USDA scientists at the Otis Methods Development Center and the Northeastern Forest Experiment Station has been limited to approximately 2,000 acre equivalents/year. More recently, with support from State & Private Forestry, Forest Pest Management and with increased efficiencies in the purification process that amount has been increased to about 20,000 AE. Nevertheless, this amount does not begin to approach the perceived demand for this product. Cooperative efforts with several private companies to produce Gypchek on a commercial scale has met with repeated failure.

Last year, after the decision by American Cyanimid to drop its efforts to produce Gypchek in-vivo, the USDA Interagency Gypsy Moth Research and Development Coordination Committee met to determine the fate of Gypchek. Three alternatives were discussed: 1) drop all USDA sponsored work on Gypchek production; 2) maintain the current level of production through 1995 and then revert to pre-1988 levels of 2,000 AE; or 3) expand current production at a locality yet to be determined to a level that could be considered as operational (100,000 AE).

With concurrence of the USDA Gypsy Moth Working Group, the policy group for the Department of Agriculture, a decision was made to increment Gypchek production to approximately 30,000 AE. The Working Group also directed the Coordinating Committee to conduct a national needs assessment before deciding a future course of action. The needs assessment questionnaire is currently under development but will be distributed shortly. In the meantime, a new firm has expressed interest in producing Gypchek commercially and we are looking forward to working with them.

APHIS ASIAN GYPSY MOTH POLICY

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ABSTRACT

In response to a request from APHIS, PPQ, a Science Advisory Panel (SAP) was formed to address specific questions related to the Asian gypsy moth (AGM). These questions included what would be the likely consequences of an AGM introduction into the United States, and what would be an appropriate response should an introduction occur. On March 16, 1994 the SAP produced a report, based on current information available, that included the following recommendations:

1) In the event of a confirmed AGM introduction anywhere in the United States, treat the population with the most suitable materials to impart the greatest impact. If practical, the goal should be eradication.

2) Intensify efforts to exclude introductions of AGM into North America by lowering the risks at the point of origin and at the point of arrival.

3) Establish a monitoring program in ports and other high risk sites to rapidly detect introductions.

4) Support research efforts to allow informed decision making and provide the technology to support operational programs. Specifically this work should focus on the identification of precise diagnostic molecular markers and the examination of genetic and behavioral consequences of hybridization.

The SAP's report was reviewed at the April '94 PPQ Management Team/National Plant Board Council meeting. Examination of the recommendations resulted in the decision to develop a clearly defined policy to deal with future introductions of AGM. Of special concern was the identification of the appropriate course of action to take in the event AGM is introduced into an area already infested with North American gypsy moth (NAGM). Since the majority of GM control work is done in a cooperative manner, APHIS proposed the formation of a policy committee to develop the needed guidelines. Membership on this committee included the Forest Service, the National Plant Board, the National Association of State Departments of Agriculture, Agriculture Canada, and APHIS. The AGM Policy Committee (AGMPC) met on June 9, 1994, and developed the following recommendations for dealing with AGM both within and outside of the area considered to be generally infested with NAGM.

AGMPC Recommendations:

1) No major NAGM policy change is recommended at this time. However, the AGMPC concurs with the SAP that AGM possess certain behavioral characteristics that are significantly different from NAGM. Therefore, the AGMPC recommends that AGM should be regarded as a significant economic pest not known to be currently established in the United States.

2) While operational reactions to both NAGM and AGM introductions outside of the generally infested area are similar, the programmatic differences implemented to date are justified and should be continued.

3) Exclusionary efforts should be intensified and a regular detection survey should be carried out at all sites at high risk for introduction.

4) When the source of a new AGM introduction into the NAGM generally infested area is known, such as a specific infested cargo shipment, container, or vessel, aggressive efforts to eradicate the pest should be implemented.

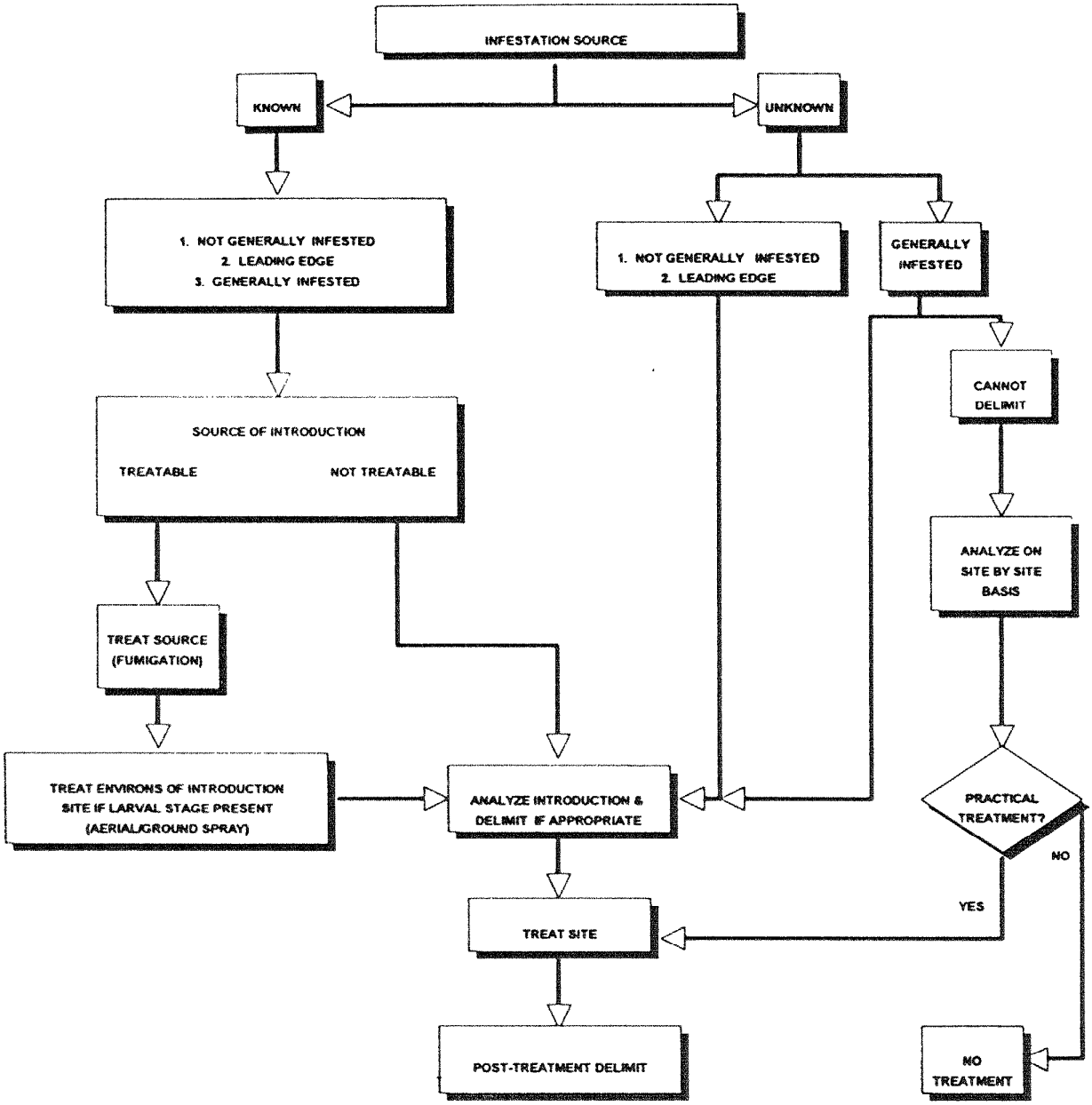
5) Any future change in policy should be "triggered" by advances on the scientific front regarding such issues as DNA identification, population genetics, behavioral traits of hybrids and backcrosses, etc. The AGMPC recommends that work of this nature be supported with all the necessary means and resources required.

Recent interceptions of exotic GM in North Carolina, South Carolina, and Maryland indicate that the threat of introductions from places other than the Russian Far East is very real. In addition to ships, the possible pathways of these introductions has been expanded to include cargo and containers. In response to the pest risk posed by AGM, APHIS has implemented a nationwide port survey program to provide for the early detection of future introductions. The APHIS AGM policy has been developed to provide guidance on how to deal with any introductions, regardless of where they occur.

The APHIS AGM Policy Statement* is attached for reference.

*** UPDATE - Since the date of the Research Forum, the APHIS policy statement has been adopted without change by the Forest Service and is now considered to be Departmental policy. The revised USDA AGM Policy Statement is substituted here for the original APHIS version.**

OPERATIONAL RESPONSE TO ASIAN GYPSY MOTH



USDA ASIAN GYPSY MOTH POLICY

It is well documented that gypsy moths (*Lymantria dispar*) display considerable variation in behavior throughout their range. Most Asian strains of gypsy moth are characterized by females capable of strong directed flight and host ranges broader than that of the gypsy moth strain currently established in North America (narrow genetic range based on isolation of population originally introduced from Europe in 1869; characterized by nonflying female moths). In recognition of these significant behavioral differences, it has been determined that Asian Gypsy Moth (AGM) warrants status as a significant, exotic pest of economic importance. Contrary to USDA's North American gypsy moth (NAGM) policy of not conducting eradication activities within the generally infested area, action will be taken against confirmed AGM infestations in the generally infested area when the source of the introduction is known. Knowledge of the time, location and extent of an Asian introduction will be required to trigger treatment activities. In cases where deductive, circumstantial or investigative information can be developed about an introduction of uncertain origin, appropriate action may also be recommended and taken. The goal of such treatments will be to eliminate all of the gypsy moths that exhibit traits characteristic of AGM.

USDA's current policy of excluding the introduction and preventing the establishment of exotic economic pests will be applied to AGM, regardless of whether an introduction occurs within or outside of the area generally infested with NAGM.

The consequences of an AGM introduction into the United States will be determined by several factors, the most important of which are: 1) source of the introduced AGM, 2) site of introduction, and 3) size of introduction relative to any resident North American population. Operational responses to mitigate these consequences will be based upon the specific circumstances of each occurrence to maximize the effectiveness of treatment strategies.

Recent studies indicate that several Asian strains are sexually compatible with NAGM, resulting in hybrid progeny that possess a mixture of behavioral characteristics and demonstrate observed hybrid vigor. While the exact effects of such hybridization are not well defined, the presence of NAGM in superior numbers is believed to dilute the expression of noxious Asian behaviors (i.e. female flight and a broader host range) in mixed populations. However, the possible retention of these traits at some low level requires mitigative measures where feasible to prevent or reduce the likelihood of introducing Asian genetic material into NAGM populations.

In recognition of the behavioral differences between AGM and NAGM, standard programmatic operations used outside of the generally infested area will be modified. Pretreatment delimiting surveys will not be conducted for AGM due to the potential increase in size and scope an AGM population can achieve in a single year. Control measures will commence as soon as possible after confirmation of an Asian introduction based upon the best information available, followed by extensive post-treatment delimiting surveys.

In order to reduce the likelihood of future introductions, USDA will conduct multifaceted exclusionary activities, supported by effective detection surveys at high risk introduction sites. These sites will include ports of entry, selected military bases, and other locations as needed.

Future policy changes will be determined by scientific advances that provide new information required for informed decision making and improved operational support.

DISTRIBUTION OF MICROSPORIDIA ISOLATED FROM GYPSY MOTH POPULATIONS IN EUROPE

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ABSTRACT

Microsporidia are important entomopathogens of gypsy moth larval populations in Eastern Europe; however, these organisms have never been recovered from populations in North America. In 1985, foreign exploration for these organisms was conducted in Portugal, the former Czechoslovakia and Yugoslavia with assistance from insect pathologists in these countries. Two isolates were recovered from larvae on cork oak in Portugal, and one isolate obtained from a laboratory colony in Czechoslovakia. Over the next several years, extensive laboratory studies were conducted to characterize and identify these isolates and to evaluate their impact on gypsy moth larvae. It was determined that one isolate from Portugal is a new species, the other is a generalist that infects many lepidopteran species, and the isolate from Czechoslovakia had been mistakenly placed in the wrong genus because ultrastructural data were lacking at the time the species was described.

Because of the reported significance of microsporidia as mortality agents in Eurasian gypsy moth populations, we expanded our explorations in 1993-94 to include Siberia, Romania, Austria, Czech Republic, and the Slovak Republic. With assistance from cooperators, we also obtained single isolates from gypsy moth populations in northern Germany and Bulgaria. Preliminary results from laboratory studies indicate that we now have 8 to 10 distinct species of microsporidia that have been isolated from gypsy moth larval populations that occur over a broad geographical range and that occupy diverse habitats. We did not recover microsporidia from three geographically separated populations in Central Siberia, nor from sites in Bohemia and Moravia in the Czech

Republic; however, gypsy moth populations were at very low densities in the Czech Republic. The prevalence of microsporidia is affected greatly by both the density of host populations and the phase of the outbreak encountered.

Many of these isolates vary in their virulence to the gypsy moth, their infectivity to non-target Lepidoptera, and their ability to infect specific tissues of their host. This latter characteristic is a critical determinant to their horizontal and vertical transmission among larval populations in forested environments.

Based on our knowledge about the occurrence of microsporidia among species of forest Lepidoptera, it is unusual for one species, such as the gypsy moth, to serve as host for more than one or two species of microsporidia. After conducting the ultrastructural and life-cycle studies required to identify the isolates in our collection, we will describe these species and then publish a catalogue of microsporidia known to infect gypsy moth populations throughout its known range. In the meantime, we will continue to work with our network of cooperators to obtain additional isolates from gypsy moth populations in countries that have not yet been sampled, but where this pest is known to occur.

ASSESSING THE IMPACT OF *BACILLUS THURINGIENSIS KURSTAKI* ON FIELD POPULATIONS OF NON-TARGET LEPIDOPTERA

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ABSTRACT

Large-scale application of *Bacillus thuringiensis* var. *kurstaki* [*Btk*] is an integral component of current forest pest management programs. The use of *Btk* avoids many of the non-target and environmental contamination problems inherent in the use of synthetic pesticides. However, concerns also exist regarding the large-scale application of *Btk* in woodlands and forests. These concerns focus on the effects of *Btk* on populations of non-target Lepidoptera. This is relevant to ecosystem processes because Lepidoptera are important components of certain food webs for other insects, vertebrates, and key to plant growth/competition relationships.

The impact of *Btk* may be assessed by sampling treated and untreated sites for: 1) the immature Lepidoptera (caterpillars) occurring on certain plants, or 2) the adult moths and butterflies in the general area. The technique, frequency and intensity of sampling is in part dependent upon whether caterpillars or adults are being collected. Also, as will be discussed, the interpretation of results is in part dependent upon which life stages are sampled. Ideally, both adults and immatures would be sampled during a non-target assessment project. Comments on monitoring protocol are presented to provide an outline for conducting a project focusing on the sampling of immatures and/or adults for an assessment of non-target impacts on populations of Lepidoptera. This discussion compares sampling with a beating sheet versus bagging foliage along transects. Samples should be assessed to determine species richness, abundance of individuals, and biomass of all Lepidoptera. The analysis of data should involve a comparison of values before and after the *Btk* application for each site and between each site by sample date. Typically, the data do not conform to the criteria of parametric statistical analysis. Thus, nonparametric tests might be most appropriate.

COMPARISON OF PERFORMANCE ON SEVERAL TREE SPECIES OF GYPSY MOTH FROM CENTRAL ASIA, NORTH AMERICA, AND THEIR HYBRIDS

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ABSTRACT

Differences in the utilization of host plants by gypsy moth, *Lymantria dispar*, from Asia (Siberia, Russia) and North America (Massachusetts, West Virginia) have been examined during the past two years. These studies are being conducted at the USDA Forest Service Quarantine Laboratory in Ansonia, Connecticut, and at the arboretum of the V.N. Sukachev Institute of Forest, Krasnoyarsk, Russia. The trees selected for testing include species native to Asia and North America and species susceptible and resistant to the gypsy moth.

A comparison of newly hatched larvae found that the Asian larvae weighed more after a 10-day period for 39 of the 40 species where both biotypes survived. The Asian larvae also developed faster with more larvae in the second instar at the end of the 10 days. If survival of one race on a particular host was high (>85%), the other race also had high survival. Conversely, if one race had poor survival (<10%), the other race also had poor survival. The greatest differences in survival between the races occurred on intermediate hosts such as *Pseudotsugae menziesii, Robinia pseudoacacia, Acer rubrum*, and *Prunus serotina*. Tests on a more limited number of species were done with the pure strains and their hybrids reared from hatch to pupation. Performance of hybrids was more similar to the Asian than the North American race. At times, the hybrids outperformed the pure Asian race, but no general pattern of inheritance of faster growth or better survival was evident. Measurements made during instar IV larvae found no clear generalizations about instar IV growth being better for any group. The Asian race and hybrids produced heavier pupae and had shorter larval development times than the North American race. It seems that the differences among the races and their hybrids in pupal weight and duration of the larval period are primarily a reflection of patterns observed for the first 10 days of growth.

In summary, Asian (Siberian) populations of gypsy moth can be expected to survive and grow better than the gypsy moth generally established in North America, particularly on hosts such as Douglas-fir that are of intermediate suitability to gypsy moth. Hybrids between the Asian and North American gypsy moth can be expected to perform better than North American gypsy moth.

EFFECTS OF DEFOLIATION AND THINNING ON HERBACEOUS GROUND FLORA

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ABSTRACT

Herbaceous vegetation was inventoried in 1992 and 1993 in eight Appalachian mixed hardwood stands (< 50% basal area/acre in oak species) and eight oak stands (> 50% basal area/acre in oak species) in north central West Virginia. Vegetation was sampled on 20 6-foot radius plots per stand twice each growing season (once during late spring to sample spring ephemeral species, and later in mid summer). Stand disturbance prior to herbaceous sampling included 1) a silvicultural thinning (eight stands) in the winter/spring of 1990, and/or 2) moderate to heavy gypsy moth defoliation (six stands) in 1990 and 1991. Most overstory mortality occurred within three years of the first defoliation. By the fall of 1993, residual overstory basal area ranged from 30 - 74 ft^2 / acre for three thinned, defoliated stands; from 38 - 66 ft^2 / acre for three unthinned, defoliated stands; from 78 - 104 ft^2 / acre for five thinned, undefoliated stands; and from 117 - 132 ft^2 / acre for five unthinned, undefoliated stands.

In 1992, species richness ranged from 22 - 65 in mixed hardwood stands and from 19 - 38 in oak stands. In 1993, species richness ranged from 24 - 70 in mixed hardwood stands and from 23 - 42 in oak stands. Regardless of thinning or defoliation disturbance, the dominant herbaceous species (i.e. the species that had the highest average percent cover) consisted of common greenbrier (*Smilax rotundifolia*), Hayscented Fern (*Dennstaedtia punctilobula*), or New York Fern (*Thelypteris noveboracensis*); the one exception was stand #6, a mixed hardwood stand with the highest species richness values recorded in the study. Detrended Correspondence Analysis (DCA) was used to further examine relationships among herbaceous species and stand/site characteristics.

SEEDLING DYNAMICS IN OAK FORESTS MANAGED FOR GYPSY MOTH

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ABSTRACT

In advance of defoliation by the gypsy moth (*Lymantria dispar* L.), eight stands (10-12 ha each) were thinned in 1989, with the objectives of reducing susceptibility and vulnerability. Eight comparable, nearby stands remained unthinned. Six stands (three unthinned and three thinned) were defoliated severely in 1990 and 1991. The composition and size structure of the woody species understory was assessed from 1989 to 1992. Total number of seedlings declined in 1990 and 1991 in all disturbed stands, but thinning appeared to moderate the defoliation effect. By 1992 the number of seedlings exceeded or equaled the 1989 values, except in the control stands. The previous dominant overstory species in the defoliated stands, primarily oak (*Quercus* spp.), accounted for a minor amount of regeneration. By 1992, red maple (*Acer rubrum* L.) was numerically dominant in defoliated stands, regardless of thinning. In terms of size structure, however, black cherry (*Prunus serotina*) dominated, for this species increased in all treatments from 1989 to 1992, in stems greater than 1.5 m tall.

MATERNAL EFFECTS REVISITED

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ABSTRACT

Previous researchers have suggested that population density experienced by the female gypsy moth, *Lymantria dispar*, influences the development rate, body size, fecundity or sex ratio of her offspring. To test this, we collected egg masses from three high density and three low density populations from western Massachusetts. These egg masses were disinfected and then allowed to hatch. The larvae were reared individually on artificial diet. We recorded time to second instar, time to pupation, pupal weight, survival and sex ratio. Female pupal weight, time from egg to pupa and egg mass weight from the emerged adults did not vary between high and low density populations. However, more males were observed from high density populations. We concluded that on artificial diet, there is no maternal effect on growth, size or survivals. Therefore, we explored another mechanism - whether sublethal viral infection influences fecundity.

We inoculated 5-day old fifth instars with three different doses of LdNPV (nuclear polyhedrosis virus) @ $5x10^3$, $5x10^4$ and $5x10^5$ occlusion bodies/larva and reared them on artificial diet at 28°C. We observed that female pupal weight of the survivors of virus challenge was significantly lower than that of control pupae. The weight of pupae decreased as the viral dose increased. Egg mass weight from the virus exposed group was lower than from the control groups. Therefore, we concluded that the sublethal effects of the virus are more likely than the maternal effect to reduce fecundity or body weight in the following generations.

THE USE OF *BT* FORAY 48 FC *(NOVO NORDISK)* FOR CONTROL OF GYPSY MOTH IN THE FORESTS OF THE SLOVAK REPUBLIC

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ABSTRACT

Leaf-eating insects cause significant damage in forests of the Slovak Republic and it is often necessary to reduce their abundance below an economic threshold. The gypsy moth (*Lymantria dispar* L.) is a dominant pest with an outbreak period in oak forests every 5-7 years. Regulating populations of gypsy moth using broad spectrum chemical insecticides such as pyrethroids, carbamates and organophosphates is ecologically unacceptable. Therefore, attention has been focused on seeking ecologically acceptable, effective methods of insect pest control. One of the potential avenues is the use of biological agents. During the last decade, forest protection in Slovakia has focused on using entomopathogenic microorganisms for the bioregulation of defoliating caterpillars. Significant scientific resources concentrated on the application of several kinds of *Bt* preparations in field experiments of gypsy moth biocontrol. The best results were achieved with an ultra low volume (ULV) application of FORAY 48 FC.

Three doses of FORAY 48 FC (2 l/ha, 3 l/ha and 4 l/ha) were used in field trials, with two different types of application technologies - ultra low volume (2-4 l per ha) and high volume (HV) (100 l water suspension per ha). MICRONAIR 40000 atomizers were used in the ULV application; water nozzles were used in the HV application. The experiments were conducted in oak forests in southwestern Slovakia naturally infested with an outbreak of gypsy moth. Mortality of caterpillars was checked on the 2nd, 5th, 7th and 14th days post-spray, and was compared with mortality in an untreated check area. The efficacy of the applications was calculated using Abbott's formula.

In the field trials, there was no significant difference in the efficacy between the 3 and 4 liters per hectare application doses of FORAY 48 FC. However, the efficacy of the 2 l/ha dose was considerably lower. At 14 days post-spray, population reduction from the 2 l/ha dose was 82% compared to 95% for the 3 l/ha dose and 99% for the 4 l/ha dose of FORAY 48 FC. Therefore, the dose of 3 liters per hectare (not significantly different from 4 liters per hectare) was confirmed as the optimum dose for undiluted FORAY 48 FC for gypsy moth control in oak forests of Slovakia. At the high dose of 4 l/ha, post-spray mortality on day 2 after application was 75% and increased significantly to 85% by day 5 (i.e., 72 hours later); by day 7, post-spray efficacy was 98% mortality and, 7 days later, by day 14 post-spray, mortality continued to increase slightly to 98.8%. Generally, it appears that the crucial time for the most rapid increase in gypsy moth larval mortality from the *Bt* product, FORAY 48 FC, is between the second and fifth day post-spray.

MORTALITY AGENTS AFFECTING GYPSY MOTH POPULATIONS IN SLOVAKIA

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ABSTRACT

Gypsy moth, *(Lymantria dispar* L.), is the most important pest of oak forests in the Slovak Republic. There have been six outbreaks in the Slovak region since the end of the second World War, usually 5-8 years apart. The largest outbreak occurred during the period 1946-49 and 1993-95. In much of Central Europe, entomopathogenic organisms and parasitoids are very important in the bioregulation of gypsy moth populations.

Gypsy moth defoliation severely weakens trees in Slovak forests, which are then attacked by decay organisms. Therefore, it is often necessary to reduce the density of gypsy moth populations to below an economic threshold by using ecologically acceptable and effective methods of insect pest control. Biological control agents such as parasitoids and pathogens are promising candidates for our consideration.

Laboratory and field observations during two recent outbreak periods have confirmed that the natural enemy complex has been very important in reducing the abundance of gypsy moths; however, the intensity of this effect varies with the gradation of the pest. Mortality caused by natural enemies varied from 79.1 - 97.3% during the progression phase, 86.2-99.7% during the culmination, and 83.6-85.1% during the period of regression.

Entomopathogenic microorganisms and undetermined causes afflicted pest abundance most significantly. These factors caused 70.6% mortality during latency, 52.7% during progression, 66.8% during culmination, and 68.3% during the regression phase. Highest mortality was caused by the gypsy moth nucleopolyhedrosis virus. Several species of an unidentified entomopathogenic bacteria caused mortality in early larval stages of gypsy moth; however, spores of fungi were rarely observed. Microsporidia, specifically of the genera *Nosema* and *Vairimorpha,* were very common causes of mortality and these occurred frequently in mixed infections with the nucleopolyhedrosis virus. The effect of parasitoids was substantially lower especially during advanced phases of the gradation. Parasitoids caused 29.4% mortality during latency, 47.3% during progression, 33.2% during culmination, and 31.7% during regression. Parasitoids in the families *Braconidae* and *Tachinidae* were most important, though the braconids (*Cotesia melanoscelus* (Ratz.), *Glyptapanteles liparidis* (Bouché)) were most abundant during the latency and culmination phases. Tachinids, specifically *Parasetigena silvestris* (R-D) and *Blepharipa pratensis* (Meigen) were most abundant during the progression and regression phases of gypsy moth outbreaks.

DEVELOPMENT OF THE ENDOPHAGOUS PARASITOID *GLYPTAPANTELES PORTHETRIAE* (HYM., BRACONIDAE) IN ITS HOST *LYMANTRIA DISPAR*

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ABSTRACT

The solitary endoparasitic braconid *Glyptapanteles porthetriae* (Muesebeck) is an important natural controlling agent of the gypsy moth throughout its native range which is mainly Europe but also extends into Northern Africa. At the beginning of this century several attempts to establish this species in the USA have been made but they failed apparently due to the parasitoids' inability to find a suitable alternate host. Three native species were found in the USA in which *G. porthetriae* successfully developed but still there is a lack of knowledge on the biology of this wasp in general.

This study examined different developmental parameters of both the host and its parasitoid depending on time of parasitization. Host larvae during the premolt between first and second instar and second and third instar, respectively, were parasitized and reared on artificial diet. Duration of host's and parasitoid's instars, weight gain of host larvae as well as volume gain of parasitoid larvae and total developmental time of *G. porthetriae* were investigated and compared with unparasitized control larvae of the gypsy moth from the same egg clusters. Furthermore, parasitization efficiency and sex ratio were calculated.

The percentage of successfully developing parasitoids was much higher when first instar larvae were parasitized (62% compared to 36% when eggs were laid into second instar larvae). Older host larvae showed a high amount of pseudoparasitized larvae. They didn't release any parasitoids but contained teratocytes, which derive from the parasitoid's egg shell. Delayed larval growth and mortality even occurred in the absence of the parasitoid. Whether the parasitoid itself or other confounding factors like venom, teratocytes and/or calyx fluid are responsible for the developmental stop of the host is still to be investigated.

Glyptapanteles porthetriae who attack early instars of gypsy moth larvae are able to effectively diminish the damage of their host. Since under natural conditions gypsy moth larvae are parasitized mainly in first or late first instar, they die during their third instar before the massive weight gain starts.

GYPSY MOTH NUTRITIONAL ECOLOGY: THE IMPORTANCE OF IRON BIOAVAILABILITY DURING VARIOUS PARENTAL LARVAL GROWTH PERIODS ON THE DEVELOPMENT AND SURVIVAL OF THEIR PROGENY

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ABSTRACT

Knowledge of insect nutritional ecology is central to proper interpretation of the effects of food quality upon physiology and behavior at the population and ecosystem levels. Iron (Fe) is an essential element for all organisms, and plays a central part in life processes as a constituent of oxygen carrier molecules and as a versatile biocatalyst. Laboratory studies have shown that trace amounts of Fe in the diet of gypsy moth parents can significantly affect both parent and progeny development depending on whether the Fe is in a form available (bioavailability) to the insect.

In preliminary tests, larvae (progeny) reared from field-collected eggs on a diet containing reduced Fe bioavailability grew significantly slower than siblings fed diet with available Fe. This suggests that bioavailability of Fe during parent development in the field was inadequate. High plant-fiber levels and dietary phenolics and tannins have been implicated in the reduced bioavailability of Fe. Variations in these plant variables occur due to phenological and ontogenetic changes, and to induced changes that occur following, for example, defoliation. Knowledge of when during the parent-feeding period Fe bioavailability affects gypsy moth maternal provisioning of the egg will increase our understanding of the importance of Fe in this plant/folivore relationship, and provide information that can be used to help predict the viability of the progeny generation. It also will increase our understanding of the effect of insect "physiological quality" on biological control organisms.

The purpose of this research was to evaluate the reduction of Fe bioavailability at various times (coinciding with each instar) during the parent-larval stage on parent and progeny development and survival. Iron was an essential element for development and survival of parent and progeny gypsy moth. When Fe bioavailability was reduced during early instar development of the parent generation, a significant portion of parent-female pupation was delayed. Reduction in the bioavailability of Fe beginning during any period of parent-larval development significantly reduced growth of progeny. The results of this study provide evidence that female deprivation of Fe, beginning at any stage of larval development including the penultimate instar, can significantly influence maternal provisioning of eggs and result in reduction in post-hatch development and survival of progeny.

MOLECULAR MARKERS FOR THE LYMANTRIAE

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ABSTRACT

The genus *Lymantria* contains several species which are common in Asian Pacific countries and throughout Europe and other non Eurasian countries. The Russian/Asian subspecies of *L. dispar* has a number of undesirable characteristics which could hasten spread of an introduction throughout the Pacific Northwest. The accidental introduction of the Russian subspecies of *L. dispar* on both the East and West Coast of North America acutely precipitated the need to distinguish between these nearly identical subspecies in order to monitor introductions.

Two molecular markers are presented here. The first marker, BC-1, was initially developed as a nuclear marker to differentiate between the North American subspecies of *L. dispar*, the Russian Far East subspecies, and other *Lymantria* species. It consists of a PCR product identified by restriction site polymorphism and has been tested on over 100 samples with accurate results. A second marker, BC-2, was developed for the identification of offspring resulting from mixing of the two *L. dispar* subspecies ('hybrids'). This marker has been developed and modified to identify 'hybrids' resulting from a Russian by North American cross and also identifies other *Lymantria* species. This marker is also based on PCR amplification of a known restriction site polymorphism and has been tested with control crosses. It has correctly identified hybrid organisms when using egg, pupae or moths as the testing material. In addition, the genetic characteristics of BC-2 were determined by testing F1 backcrosses and intercrosses. Approximate Mendelian ratios were seen in both instances.

These tests have been developed into rapid diagnostics which contain internal controls and can be applied to field collected samples. Through monitoring and testing of field trapped samples, the presence of Russian/Asian Lymantriae can be confirmed by molecular markers.

PRODUCTION AND FORMULATION OF GYPCHEK

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ABSTRACT

The gypsy moth nucleopolyhedrosis virus (NPV) product Gypchek is produced by infecting laboratory-reared larvae and processing the cadavers to yield a technical powder that contains about 15% (by wt.) viral occlusion bodies (Obs). Though Gypchek is not currently available as a commercial product, 25,000- to 30,000-acre treatments of Gypchek are produced and made available for use each year through the collaborative efforts of the Forest Service (FS) and the Animal and Plant Health Inspection Service (APHIS). Current collaborative FS-APHIS studies are focused on optimizing *in vivo* production through the development of more efficient rearing and processing technology. In 1995, a market survey will be conducted to clearly define the national and international Gypchek markets for 1995 and beyond. Information gleaned from the survey will be used to formulate a Gypchek production strategy that will satisfy user needs for the foreseeable future.

Inconsistencies in Gypchek's performance as an operational pesticide have been addressed through numerous methods improvement projects and the development of a *ready-to-use* spray adjuvant [Carrier 038, Aqueous Flowable Carrier for Gypchek NPV (Novo Nordisk)] for use in operational Gypchek programs. Laboratory and field testing have indicated that, when applied at 1 gallon per acre, Carrier 038 provides UV-protection and foliar deposition superior to that of the standard FS molasses-lignosulfonate tank-mix applied at 2 gallons per acre. Novo Nordisk is continuing to refine the adhesive and flowability characteristics of Carrier 038 to improve performance. The FS will continue to work with any commercial interest toward providing a safe, efficacious, cost-effective Gypchek formulation for use in gypsy moth management programs.

THE ASIAN GENOTYPE IN THE 1994 GYPSY MOTH PORT SURVEY

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ABSTRACT

Plant Protection and Quarantine of APHIS expanded its monitoring activities in 1994 to include a nationwide AGM survey at all major U.S. ports to detect introductions of exotic moths. This followed interceptions at the ports of Baltimore and Charleston during the spring of 1994 and the introduction at the Sunny Point, NC military terminal in July 1993. The trapped specimens were sent to the Molecular Diagnostics Group at the Otis MDC for genetic analysis to distinguish the Asian strain from the North American strain. More than 60,000 specimens have been submitted and only a small fraction have been analyzed to date (~3800).

Two genetic markers, FS1 and mitochondrial DNA (mtDNA), out of the six under development, were used to analyze the port specimens. Two alleles (i.e. variations) of the nuclear marker FS1 can be detected, North American and Asian. FS1 was developed by Jim Slavicek's group of the Forest Service. Three variations (i.e. haplotypes) of the mtDNA were used: North American, Siberian, and Russian far eastern. The mtDNA marker was originally developed by Rick Harrison's group at Cornell. Since results of any port specimens had to be compared to the pre-existing situation in the North American population, a large number of specimens were collected far from U.S. ports. These specimens represent our N.A. 'control' population. Similar specimen collections, representing the Asian 'control' populations, were analyzed from Krasnoyarsk (central Siberia) and Khabarovsk and Vladivostok (Russian far east). Asian forms of FS1 and the mtDNA (Siberian only) were found in the N. American population at frequencies of 3.5% (gene frequency) and 2.6%, respectively. Thus, the N.A. and Asian populations were clearly distinct using the two genetic markers (Nei's genetic distance). However, because the error was so great (0.8) in this value, the level of confidence in this differentiation is very low.

Several sites were identified as potentially having the genes specific to the AGM. The most significant site was Puyallup near Tacoma, WA where a single specimen contained Asian forms of four of the six markers (FS and UBC aided in this analysis). The frequency of the Asian mtDNA was also found to be significantly elevated at the following sites: Sunny Point, NC (22.7%), Cleveland, OH (26%), Andover, OH (22%), and Detroit, MI (18%). Puyallup is adjacent to Fort Lewis which receives regular shipments directly from Germany, known to have flying female gypsy moths. The mtDNA was of the Siberian type, ruling out the possibility it was a descendant of the 1991 introduction from the far east. Andover also showed an elevated level of the Asian form of FS1 (8.3%); the nearest port is Ashtabula, 23 miles to the northwest. The explanation for the elevated levels of the markers at this site is not clear. Absent from the list is Baltimore, a site of known interception in the spring of 1994.

BIOCONTROL PERSPECTIVE OF PARASITOIDS OF THE LYMANTRIIDS, *LYMANTRIA OBFUSCATA* WLK. AND *L. AMPLA* IN INDIA

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ABSTRACT

Lymantria obfuscata Wlk. (Lepidoptera: Lymantriidae), a voracious, polyphagous defoliator, and other lymantriids allied to *Lymantria dispar* (L.), localized in parts of Himachal Pradesh and Kashmir appear to have a good reservoir of promising natural enemies for trials to contain gypsy moth (*L. dispar*) in North America. Following requests for selected parasitoids, collections of *L. obfuscata* and its parasitoids were made in the Kullu Valley from April 22 to July 16, 1994. Ten airfreight shipments containing an ichneumonid, *Hyposoter lymantriae* Cushman; three braconids, *Rogas indiscretus* Reardon, *Glyptapanteles indiensis* Marsh, and *G. flavicoxis* Marsh; and several tachinids, *Compsilura concinnata* Meigen and *Palexorista* spp., were sent to the USA for quarantine handling and eventual release in regions of the South Atlantic and Great Lakes recently infested by the gypsy moth. In addition, *G. indiensis* was obtained because it harbors an unusual virus capable of transforming several insect cell lines.

Observations made earlier indicated that the eupelmid *Anastatus disparis* Ruschka was the dominant egg parasitoid in the Shimla Hills and Kullu Valley, while *A. kashmirensis* was dominant in Kashmir. Numbers of *A. disparis* were positively and significantly correlated with the abundance of eggs of *L. obfuscata*. There was a significant positive correlation between males and females of this parasitoid. Percent parasitism was positively correlated with males of *A. disparis*. Additional parasitoid species newly recorded here include the braconids *Apanteles* sp. A (*ater* group), *Apanteles* sp. B (*ater* group), *Apanteles* sp. A (*ultor* group), *Apanteles* sp. B (*ultor* group), *Cotesia ruficrus* (Haliday), *Dolichogenidea lacteicolor* (Vier.), *D. hyposidrae* Wilkinson, *Glyptapanteles obliquae* Wilkinson; an ichneumonid, *Casinaria* sp. near *elegantula* Gupta & Maheshwary; and a tachinid, *Palexorista subanajama* (Townsend). The need for specific determinations of several Indian *Apanteles* spp. is stressed, warranting further taxonomic studies.

Further search for lymantriid parasitoids suitable for control of gypsy moth is indicated. It is suggested that explorations be conducted in Nepal, a country hitherto unexplored for natural enemies of *L. obfuscata*.

SOME OF THE OTHER SPECIES OF *LYMANTRIA* (LYMANTRIIDAE)

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ABSTRACT

In recent years we have heard much about the Asian gypsy moth, *Lymantria dispar*, as it accidentally penetrated the North American continent (in the Pacific Northwest and more recently in North Carolina). The Asian gypsy moth (AGM) is that form of *L. dispar* in which the females are fully capable of flight, unlike the established North American (NAGM) or European form. It differs also in its overall size, development characteristics and food plant preferences. In addition, there are other species of *Lymantria* that deserve our concern and awareness as these too possess attributes that simulate the AGM and which could become established as new pests in North America. Several of these potential pests were reviewed and illustrated. These include the following which were compared to AGM, particularly in their traits that would make them potential invaders and unwanted pests:

Lymantria xylina: Native to Southern Japan, Taiwan, China and India, *L. xylina* is white in both sexes with a prominent brown band across the forewings (although this is quite variable) and entirely nocturnal. In Taiwan and China they are a pest in windbreak plantings of *Casuarina* and females readily fly to lights where they can oviposit like AGM in a mass covered with hairs on lights or ships. In this way, they could easily be transported into tropical and semi-tropical areas. Their highly polyphagous nature would make them ideal candidates for establishment. In Taiwan in 1994 we successfully trapped large numbers of males at standard gypsy moth milk-carton type traps baited with (+)-disparlure.

Lymantria monacha: The Nun moth of Eurasia is notorious as a serious defoliating pest of conifers and some deciduous trees. The sexes are similar, basically white with peppering of black spots, nocturnal, females flighted, attracted to lights and disparlure. In addition to possible invasion like AGM, in this species females insert their eggs under bark scales and any movement of raw (bark covered) logs could easily transport this pest to new areas.

Lymantria fumida: This exclusively conifer feeder from Japan and China is a brownish, non-distinctive species, in every other way identical to *L. monacha* and also could be unintentionally transported on raw logs.

Lymantria mathura: Found in Japan, eastern Siberia, China, India and Taiwan, this species is more sexually dimorphic like gypsy moth but with the female body pink (male yellow). It feeds mostly on deciduous trees but some conifers also. Nocturnally active, females fly to lights and oviposition is of the insertion under bark scales type and these two attributes make this species likely to be accidentally moved. In fact, viable eggs of this moth have been removed from ship superstructures in the Pacific Northwest and laboratory reared to confirm viability. Unlike all the above mentioned species, *L. mathura* is not readily trapped at (+)-disparlure baited pheromone

traps, although some specimens were captured in China. It appears that in this species, the pheromone is somewhat removed from (+)-disparlure. The potential for invasion by this species appears very real, either via movement of ships or raw logs.

By examining these congeners of the notorious gypsy moth, more particularly the AGM, we note that such traits as female flight, especially to artificial outdoor lighting sources (which might easily be ships at port facilities or shipping container transfer yards), and oviposition in masses, either under abdominal hairs or under tree bark scales, lend themselves well to potential invasion to other regions of the world. These species should, therefore, be watched for by those responsible for detection at port facilities everywhere. Should invasions be suspected anywhere, in most cases, the use of standard (+)-disparlure baited pheromone traps should be the first course of action to confirm an invading moth's presence.

LARVAL GYPSY MOTH DORSAL ABDOMINAL GLANDS: HISTOLOGY, ULTRASTRUCTURE AND PRELIMINARY CHEMICAL IDENTIFICATION OF EXUDATE[1]

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ABSTRACT

Unique to larvae of the Lymantriidae (Lepidoptera) are dorsal abdominal glands (DAG) located on the midline of the 6th and 7th abdominal segments (some Eurasian species have but one gland). The DAG are often brightly colored, mainly reds, orange and yellow, while their function is not yet clear. Using gypsy moth larvae, *Lymantria dispar*, we examined these glands with LM, TEM and

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[1]Presented at the 1994 U.S. Department of Agriculture Interagency Gypsy Moth Research Forum.

SEM and we collected and chemically analyzed the exudate with gas chromatography (GC) and GC-mass spectrometry.

The DAG are composed of an often colored external papilla that is eversible, bears many minute pegs and bears several slits or pores at the apex. Internally, each papilla is fed by two enormous, single-celled secretory glands each lying dorsal and somewhat lateral of the dorsal vessel and each with associated ductule cells forming a channel from the glandular cells to the pores. Internally, the secretory cells show a central ramified nucleus and large elongate secretory vacuoles appear between the nucleus and cell periphery and appear to conduct glandular exudate toward the ductule channel. The exudate exits through the pits.

Chemical analyses reveal that both laboratory reared and field collected larvae produce similar exudate, and the exudate does not contain formic acid as has been previously reported. Instead, our preliminary analysis reveals the presence of the following (in order of appearance in the chromatogram): 2-hydroxy-2-methyl-propanoic acid; phosphoric acid; 4-(dimethylamino)-butanoic acid; succinic acid; 4-(methylamino)-butanoic acid; 4-aminobutanoic acid; 4-hydroxyquinoline; cisaconitic acid; citric acid; isocitric acid; and 4-hydroxy-2-quinoline-carboxylic acid. The function of this exudate with these numerous components remains unclear. We propose that a curious "back-arching" behavior, performed by larvae on occasions, is a concerted effort to spread exudate over greater surface areas (e.g. long setae) and presumably enhance the dissemination of exudate, as a benefit to the caterpillar. (Eversible nature of gypsy moth DAG shown.)

PERITROPHIC MEMBRANE: SITE OF ACTION FOR LDMNPV/OPTICAL BRIGHTENER IN GYPSY MOTH LARVAE

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ABSTRACT

A variety of stilbenedisulfonic acid derivatives, known as optical brighteners, have been shown to increase mortality of gypsy moth, *Lymantria dispar*, larvae when administered *per os* in combination with *L. dispar* nuclear polyhedrosis virus (LdMNPV). We tested the effect of the optical brightener Blankophor BBH (BBH) on potency of LdMNPV in gypsy moth larvae, and determined the consequences of ingesting BBH/LdMNPV combinations on the structural integrity of the larval peritrophic membrane.

Newly molted second instar larvae were fed high wheat germ diet (30 ml) overlaid with 1 ml of either water, LdMNPV (10^2-10^6 OBs/ml), BBH (0.5%), or LdMNPV + BBH. Five larvae from each group were dissected at intervals ranging from 30 minutes to 8 days post-ingestion of inoculum. Intact peritrophic membranes were removed, fixed, dehydrated, and critical-point dried in preparation for examination by scanning electron microscopy. Remaining larvae (50/dilution) were reared for 21 days.

The addition of BBH to LdMNPV resulted in enhanced potency of LdMNPV and decreased lethal times at all dose levels. The LC_{50} of LdMNPV + BBH was 2.7×10^2 OB/ml compared with 1.3×10^4 OB/ml for LdMNPV alone. The LC_{90} of LdMNPV + BBH was 2.7×10^3 OB/ml compared with 1.4×10^5 OB/ml for LdMNPV alone. This represents about a fiftyfold difference in potency at both the LC_{50} and LC_{90} values. The LT_{50} for LdMNPV + BBH was 6.0 days compared with 8.2 days for LdMNPV alone.

Our data indicate that peritrophic membrane is the site of action for BBH in gypsy moth larvae. LdMNPV treatment had little, if any, observable effect on peritrophic membrane, but BBH treatment resulted in aberrations in peritrophic membrane surface structure. BBH + LdMNPV treatment caused similar, but much more rapid and progressive changes, and ultimately resulted in complete disintegration of the peritrophic membrane. We suggest that BBH combined with viral proteins rapidly degrades structural elements of the peritrophic membrane, allowing greater numbers of virions to pass through the damaged peritrophic membrane into the ectoperitrophic space. The greater numbers of infectious particles available to enter and replicate in susceptible cells would account for the observed increase in the potency.

DEVELOPMENT OF ENHANCED VIRAL STRAINS FOR CELL CULTURE PRODUCTION

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ABSTRACT

The *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) is currently being produced in *L. dispar* larvae. Production of virus in cell culture is an alternative to larval production that has the advantages of large scale production in 10,000 or 50,000 liter fermentors, a high degree of process control, and a final product that is free from bacterial, fungal, and viral contaminants. However, wild type LdNPV exhibits a high frequency of mutation to form few polyhedra mutants during propagation in cell culture, and polyhedra generated in cell culture are often significantly less potent compared to polyhedra produced in *L. dispar* larvae. To overcome these problems we have been developing improved LdNPV strains for cell culture production.

LdNPV few polyhedra (FP) mutants exhibit the characteristics of production of very few polyhedra, synthesis of polyhedra that are essentially devoid of viral nucleocapsids, and production of increased amounts of budded virus (BV). Increased BV synthesis results in the conversion of the virus population from one exhibiting a wild type phenotype to one with an FP phenotype during viral production in cell culture. Consequently, the formation of FP mutants during viral propagation in cell culture is a significant impediment to development of cell culture virus production systems that would facilitate availability of these viruses for biocontrol purposes. We have isolated and characterized a strain of LdNPV that exhibits resistance to FP mutant formation. This viral strain was successfully produced in a cell culture pilot production system by a private sector company.

Comparisons of biological activities between polyhedra generated in cell culture and *L. dispar* larvae have shown that polyhedra produced in the Ld652Y cell line are approximately 50 to 100 fold less potent compared to polyhedra generated in *L. dispar* larvae. Polyhedra generated in cell culture were found to contain approximately half the number of viral nucleocapsids compared to polyhedra generated in cell culture. To determine if the decreased potency of polyhedra generated in cell culture is a consequence of decreased virion occlusion, we are attempting to isolate viral strains that occlude the same number of viral nucleocapsids when produced in cell culture as when produced in *L. dispar* larvae. Several LdNPV polyhedra formation mutants have been isolated that exhibit abnormal polyhedra synthesis. These mutants are being studied to gain an understanding of the processes of polyhedra formation and virion occlusion. Once these processes are understood, it may be possible to manipulate polyhedral traits to enhance viral potency.

INTERACTION OF EXOTIC MICROSPORIDIA WITH FOREST LEPIDOPTERA

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ABSTRACT

Microsporidia are common obligate parasites in insects. They are horizontally transmitted when susceptible hosts feed on contaminated feces, silk, or on infected cadavers. Gregarious Lepidoptera such as fall webworms (*Hyphantria cunea*) and tent caterpillars (*Malacosoma* spp.) may be particularly suitable hosts for horizontal transmission, and populations of these species have reportedly been suppressed by microsporidia infections.

Several species of microsporidia have been recovered from gypsy moth populations in Europe but surveys of gypsy moth populations in the U.S. have revealed no microsporidian infections. Our dilemma is to predict the ecological host range of exotic microsporidia using results from laboratory studies before exotic microsporidia are released in the field. Non-target species are often infected by a microsporidium under ideal laboratory conditions. Nevertheless, unusual responses occur that assist in the interpretation of host specificity data. We are currently pursuing three strategies. We tested four species of gypsy moth microsporidia in 47 native non-target Lepidoptera species, all of which occur in areas invaded by the gypsy moth. We found a continuum of responses by non-target hosts to the microsporidia. We grouped the responses into three categories: 1. refractory, 2. light and/or atypical infections, and 3. heavy infections, in which sufficient infective spores were produced to predict possible horizontal transmission. We are also studying host and non-target host responses at the tissue level. Early results show that the hemolymph may be involved in the dissemination of infection to the target tissues. A better understanding of the development of infection in the natural host will help us evaluate abnormal infections in non-target hosts. In a third study, we are using a novel approach by designating the gypsy moth as a non-target host. The gypsy moth occurs sympatrically with many species of native Lepidoptera. Many of these species are infected with microsporidia. If the gypsy moth is susceptible to indigenous microsporidia from indigenous Lepidoptera, we can compare these results to the known ecological data - no known microsporidian infections in U.S. populations of the gypsy moth. Our most promising microsporidium for release as a biological control agent for the gypsy moth, an isolate of *Microsporidium* sp. from Portugal, produced patent infections in very few of the non-target hosts we tested in the laboratory. Laboratory host specificity experiments determine the physiological susceptibility but environmental factors also influence the transmission and dispersal of microsporidia. We predict that the Portugal isolate is very host specific in the field. Studies of tissue-level responses to microsporidian challenge may have value in predicting ecological host specificity, and may be useful in developing experimental guidelines for conducting host specificity studies on exotic biological control agents.

INFECTIVITY OF NON-INDIGENOUS GYPSY MOTH MICROSPORIDIA TO NATIVE NON-TARGET FOREST LEPIDOPTERA

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ABSTRACT

The physiological host specificity of a biological control organism, which is usually determined through laboratory experimentation, is used commonly to estimate the potential ecological host range of that organism. This information is critical to the assessment of any candidate biological control organism prior to its approval for release into the environment. Many non-target hosts that may be infected by an entomopathogen during laboratory challenges may not support infection in the field.

We studied the effects of four species of microsporidia, isolated from gypsy moth (*Lymantria dispar* L.) populations in Europe, on larvae of 40 species of native forest Lepidoptera. Larvae were fed both a high and a low dose of each isolate; observations were recorded over time on the tissues affected and whether typical or abnormal spores were produced. We observed a wide range of responses among the non-target larvae to the four isolates that were tested. *Endoreticulatis schubergi*, from Portugal, produced an "all or nothing" response; larvae were either completely refractive to this isolate or developed characteristic heavy infections in affected tissues. A *Vairimorpha* isolate from the Czech Republic produced infectious spores in several of the non-target species; however, all other species were either refractive to this microsporidium or developed atypical infections. We characterized infections to be atypical when abnormal spores were produced and/or the progression of infection was terminated at an early stage of development. The *Nosema*-like isolate from Portugal and Romania produced typical infections only in gypsy moth larvae and in a few species of non-targets, though the isolate from Portugal was the least infective of all the isolates that we studied.

We believe that horizontal transmission of microsporidia among larval populations can occur only when normal infectious spores are produced in susceptible tissues of hosts in relatively large numbers. When the isolates from Portugal, Romania, and the Czech Republic infected non-target hosts, the development of the microsporidium was altered and small numbers of mainly abnormal spores were produced. Therefore, little or no horizontal transmission should be realized and the ecological host range of these isolates is probably quite narrow. *E. schubergi* produced large numbers of normal infectious spores in many of the non-target species challenged and is, therefore, not a good candidate for release against the gypsy moth in North America.

IDENTIFICATION OF A *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS HOST RANGE GENE

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ABSTRACT

Lymantria dispar nuclear polyhedrosis virus (LdMNPV) is a baculovirus used to control the gypsy moth. It has a narrow host range. Another baculovirus, *Autographa californica* nuclear polyhedrosis (AcMNPV), has a relatively broad host range, infecting approximately 30 lepidopteran species but not the gypsy moth. LdMNPV is propagated in cell culture using Ld652Y cells, a cell line that does not support the replication of AcMNPV. However, if AcMNPV-infected Ld652Y cells are superinfected with LdMNPV, AcMNPV is produced. We determined that transfected LdMNPV DNA was able to provide this "helper function". This allowed us to map the LdMNPV gene that promoted AcMNPV replication in Ld652Y cells. By transfecting AcMNPV DNA and various cloned fragments of LdMNPV DNA into Ld652Y cells, we identified a single 835 bp region of the LdMNPV genome that was sufficient to allow AcMNPV replication in this cell line. We sequenced the DNA fragment and found it to have an open reading frame encoding a polypeptide with a predicted molecular weight of 25.7 kDal. We then constructed recombinant AcMNPV that carried a segment of LdMNPV DNA including this region. The recombinant virus was able to replicate in Ld652Y cells and in gypsy moth larvae.

GypsES: A DECISION SUPPORT SYSTEM FOR GYPSY MOTH MANAGEMENT

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ABSTRACT

GypsES is a decision support system for the management of gypsy moth. It provides tools which enable the user to develop and/or import GIS and database information about a particular location. It also allows the user to extract information and pass it to Forest Service developed models which address such issues as phenology, defoliation prediction, spray deposit, and the long-term effects of treatment decisions.

GypsES provides an interface between a geographic information system, a database, and a rule base interpreter. It utilizes the Unix X-Windows system to provide a user-friendly environment.

The system provides hazard and risk ratings based on site conditions and the user's management objectives. It supports both suppression (egg mass surveys, defoliation prediction) and eradication (trap placement and trap data management) applications. It will also support treatment operations. Users may draw their spray blocks within GypsES utilizing various types of images as a backdrop. The Phenology model helps in determining optimal spray timing, and the Spray Deposit model (FSCBG) will provide information on average deposition.

Plans for additional development include pre-flight analysis of probable spray deposit, analysis of actual flight lines recorded by GPS equipment, rule-based development of environmental risk factors, and expanded use of the stand damage model.

BACILLUS THURINGIENSIS CRYIA INSECTICIDAL TOXINS EFFECT RAPID RELEASE OF GYPSY MOTH MIDGUT EPITHELIUM AMINOPEPTIDASE

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ABSTRACT

The CryIA class of *Bacillus thuringiensis* insecticidal proteins are toxic to the larvae of the gypsy moth (*Lymantria dispar* L.), a major forest pest in the United States. However, members of the CryIA delta endotoxins show variation in their toxicity to the gypsy moth that may be attributed in large part to the presence of specific receptors in the midgut epithelium. The binding of a toxin to its receptor causes disruption of the epithelium membrane, leakage of ions and water into the cell, and eventually, lysis of the epithelial cells. Recently, the receptor for the CryIA(c) toxin in the midgut of the gypsy moth was identified as the brush border membrane aminopeptidase (APN). The receptor for another member of the CryIA delta endotoxins, CryIA(a), is a different binding protein with an apparent size of 210 kDa. In this study, gypsy moth larvae were force-fed various concentrations of CryIA(c) and CryIA(a) toxins to determine what effect the toxins may have on the brush border membrane APN and other gut enzyme activities.

Dose and time-course studies showed that even though CryIA(a) and CryIA(c) bind to different receptors in the brush border membrane of midgut epithelial cells, both induced a rapid release (solubilization) of the glycosyl-phosphatidylinositol (G-PI) anchored APN from the midgut epithelium. There was no change in trypsin and elastase activities in the luminal fluid. Brush border membrane vesicles (BBMV) from the intoxicated larvae treated with a bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) showed that APN associated with the BBMV was depleted. Force feeding AMP and GMP mimicked the effect of the toxins, and cyclic-AMP antagonized the effect of the toxins. These results suggest that one early event elicited by the CryIA toxins is the release of the membrane-bound APN possibly mediated by the activation of endogenous phospholipases. Since APN serves as the receptor for the CryIA(c) toxin, the release of APN from the cell surface offers an explanation as to why CryIA(c) is less toxic than CryIA(a) to the gypsy moth.

IDENTIFICATION OF THE *BACILLUS THURINGIENSIS* CRYIAC TOXIN BINDING PROTEIN IN THE GYPSY MOTH MIDGUT

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ABSTRACT

Aminopeptidase N (APN) was purified from gypsy moth (*Lymantria dispar* L.) brush border membrane vesicle (BBMV) proteins by mono-Q chromatography and Superdex-75 gel filtration in the presence of the zwitterionic detergent, CHAPS, using FPLC. The purified APN, identified by its enzymatic activity, had an apparent size of 100 kDa, and was identified as the unique *Bacillus thuringiensis* insecticidal toxin, CryIAc, binding protein. Protein blots of the BBMV proteins probed with biotin-labeled and ^{125}I-labeled insecticidal proteins revealed that CryIAc binds to a 120 kDa protein. The purified APN exhibited a slightly smaller size in comparison to the binding protein observed in blots of detergent-solubilized BBMV proteins. However, it clearly displayed the characteristic properties expected of the CryIAc receptor, binding strongly to CryIAc, exhibiting little or no binding to CryIAa or CryIAb, and showing no affinity for the coleopteran-specific toxin, CryIIIA. Antibodies raised against the gypsy moth APN demonstrated that the purified APN and the 120 kDa CryIAc binding protein of total BBMV proteins are antigenically identical.

ASIAN/SIBERIAN/EUROPEAN GYPSY MOTH RESEARCH:

ARE FURTHER EFFORTS NECESSARY?

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ABSTRACT

In November 1992, a comprehensive, 3-year research planning mission was completed for the Asian gypsy moth (AGM). At that time, the AGM had been introduced into North America on ships from the Russian Far East in the Pacific Northwest to load grain. While those introductions apparently have declined in numbers, new problems have arisen from a Siberian strain that has been introduced into western Europe and subsequently transported into the U.S. and Canada on military equipment and personal belongings of military personnel. Since the problem has changed in its complexity, the new research plan needed to be reformulated.

In December 1994, a preliminary AGM research outline consisting of seven major groupings was sent to more than 40 individuals at federal, state, and Provincial agencies and universities in the U.S. and Canada for comments and suggestions. This resulted in the following research/development document designed to address the problem of gypsy moth of Asian/Siberian/European origins. A detailed research schedule and priorities have not been ascribed to the various activities. The intent was to provide a comprehensive document for researchers, research administrators, regulatory personnel, and politicians in the U.S. and Canada that identifies needed research. Hopefully, the resources to confront this increasingly difficult situation can be found.

Most components are research tasks; others are regulatory or administrative issues reflecting the complex nature of this international problem. This complexity necessitates coordination, sharing research results, and harmonizing detection, eradication, and regulatory programs at state, federal and provincial levels. Given the modest budgets allocated for Asian gypsy moth research, the achievements both in basic findings and technology development are noteworthy. Clearly, the existence of an active research and development program for gypsy moth provided a substantive basis for focusing on gypsy moth of non-North American origins. However, reduction in program funding coupled with emerging global markets and increased international trade presents a daunting task for regulatory agencies to prevent additional introductions.

Detection and Delineation

Accomplishments

- attractancy of males to disparlure verified
- identified additional pheromone compounds from AGM
- AGM and NAGM male periodicity determined in the field in Russia and in Germany

Current Planned Activities

- observe and document pre-mating and post-mating behavior of adults in the field in Russia and in Germany

Research Needs/Development Activities

- relationship of male AGM captures to female location
- factors influencing female flight distance and direction in Russia and in Germany
- compile worldwide distribution records for hosts and outbreak frequency in proximity to shipping centers
- determine the feasibility of light trap monitoring for predicting population densities in foreign ports
- ascertain AGM male dispersal characteristics *vs* NAGM

Prevent AGM from Entering North America

Accomplishments

- lighting devices less attractive to AGM adults ascertained
- shipping schedules determined by female flight period - an accepted protocol by the industry
- developed pre-clearance inspection procedures for military goods returning from Europe

Current Planned Activities

- monitoring system operational for Russian ports for AGM as well as nun moth and pink gypsy moth

Research Needs/Development Activities

- highly attractive lighting devices for sampling females
- egg mass sampling procedure for containers
- suppression techniques (products, buffer treatments, etc.) for AGM populations around infested ports
- incorporate features imparted by filters that make lighting devices unattractive to adults into prototype lamps in cooperation with the lighting industry and field test

- expand monitoring to other Asian and European ports
- develop an international network to determine risk at port and cargo/container locations
- develop operational tracking system to detect at risk cargo/containers

Eradication of AGM from North America

Accomplishments

- effectiveness of *Bt* and NPV documented in laboratory bioassays for Far East and Russian strains
- utilized NAGM pheromone monitoring system for AGM
- evaluated microsporidia against AGM and pink gypsy moth

Current Planned Activities

- bioassaying *Bt* and NPV on German strains and its hybrids with NA strains
- effectiveness of *Bt* and NPV against hybrid populations
- effectiveness of *Bt* and NPV against central Siberian populations
- development of flying female light trap for delimitation activities
- comparison of electrophysiological characteristics of *Bt* ICP-receptor binding between North American and Asian gypsy moth (and hybrids)

Research Needs/Development Activities

- harmonize eradication/suppression activities with U.S./Canada, states and provinces
- assess impacts of eradication activities on non-target arthropods
- more precise delimitation of AGM infestations (e.g., trap densities over time)
- effectiveness of *Bt* and NPV against other Asian populations
- ascertain the effectiveness of different NPV genotypes against AGM and its NAGM hybrids
- ascertain infectivity of microsporidian isolates against AGM and its NAGM hybrids and related *Lymantria* spp.

Determining Susceptibility of Host Plants and Forests

Accomplishments

- in Russia, growth and survival highest for Siberian larvae followed by Germany and last, U.S.
- in U.S., AGM grew better than NAGM on 50 plant species
- greatest differences in growth rates were on conifers

Current Planned Activities

- susceptibility of host plants to different Asian populations (Far East Russian, Central Siberian, Japanese, Chinese)
- susceptibility of host plants to hybrid populations

Research Needs/Development Activities

- assess AGM and NAGM crosses on host utilization
- test additional U.S. tree and shrub species, especially southern pines and western species, including riparian types
- susceptibility of host plants to different Asian populations (Far East Russian, Central Siberian, Japanese, Chinese)
- susceptibility of host plants to hybrid populations

Consequences of Hybridization

Accomplishments

- AGM and NAGM sexually compatible
- lower diapause chill requirements of Russian eggs are retained in their hybrids
- faster larval growth is retained and accentuated with hybridization
- the full range of larval color variation is present in hybrids
- some female flight capability is retained with hybridization but the proportion of the population with strong directed flight is reduced
- behavioral and physiological traits of German gypsy moth are consistent with that of a population with multiple generations of hybridization

Current Planned Activities

- assess behavioral and biological characteristics of central Siberian x North American hybrids
- assess behavioral and biological characteristics of gypsy moths from other world areas (both Asian and European)
- correlate behavioral and biological characteristics with DNA marker results
- ascertain the behavioral and biological characteristics of individuals from North America whose DNA type is hybrid

Research Needs/Development Activities

- monitor female flight distribution in Europe for spread and effects of hybridization
- ascertain if mating between strains in the field (Germany or France) is random or assortative
- compare adult eclosion patterns for hybrids and their parent strains
- determine amount of outbreeding required to eliminate the capacity for female flight
- ascertain hybridization rates over time in Germany and France
- evaluate the influence of eclosion position within egg masses upon female flight propensity
- pheromone trap males in known AGM regions in Germany or France to determine seasonal phenology and verify by molecular diagnostic tools

Diagnostic Methods to Differentiate between Geographic/Behavioral Strains

Accomplishments

- three mtDNA haplotypes identified
- mtDNA procedure for batch samples developed
- six nuclear gene markers identified
- head capsule color discrimination developed
- selectivity of two nuclear markers determined

Current Planned Activities

- wing venation characterization for separating AGM/NAGM
- cuticular hydrocarbon separation of AGM/NAGM
- examine and describe female flight muscle anatomy and histology for AGM and EGM and their hybrids
- determine if head capsule color discrimination can identify AGM/NAGM hybrids
- correlate marker results with behavioral and biological characteristics

Research Needs/Development Activities

- develop additional nuclear gene markers
- identify nuclear chromosomal location marker for female flight and other phenotypic traits (e.g., host range/preferences)
- develop methodology for processing field samples
- clarify taxonomic relationships
- develop accuracy measurements for developed markers
- construct a phylogenetic tree for *Lymantria* spp. using ribosomal DNA sequencing
- determine the range of AGM in Germany
- test DNA markers for female flight in Europe with trading partners and military sites
- standardize the diagnostic procedure (i.e., which markers and interpretation of results)
- determine the specificity of genomic markers (i.e., the behavior with the same specimen)
- develop selectivity and sensitivity for candidate markers using "domestic" sample

Predicting Biological Events

Accomplishments

- model deployed for 1st instars dispersing from ships
- egg hatch model adapted to broad range of latitudes
- non-diapause AGM strain developed after first generation of selection
- Central Siberian strain develops faster than that from Far East
- bioassay for female flight developed

Current Planned Activities

- determine flight propensity for strains from the Russian Far East, Central Siberia, and Germany and hybrids with North American strain
- determine female flight propensity when food stressed, hatched from different portions of the egg mass, of different instar types (5 or 6 instars), early *vs* late hatch
- develop rapid technique for identifying female flight potential
- define diapause requirements for AGM and integrate into egg hatch model
- ascertain female flight propensity with flight mills

Research Needs/Development Activities

- compare female flight propensity using females reared on artificial diet with those reared on host foliage
- incorporate AGM developmental data into NAGM growth model
- determine population density and environmental effects on female flight
- determine the role of lipid metabolism in female flight assessment
- model female flight dispersal

The foregoing research/development listings were predicated on the concept that new strains could be prevented from establishing in North America. However, it is clear that with increasing international trade, military relocation, etc., that new or additional pathways of introduction will occur. The technology to manage new gypsy moth strains is untested, thus, the following activities are suggested in anticipation of future management needs.

Reducing the Impact of Established Asian/Siberian Infestations

Research Needs/Development Activities

- determine the risk of establishment and rate of spread in various North American ecosystems
- assess utility of NAGM technology
- monitor the potential AGM hot spots identified by molecular diagnostics
- evaluate management options in European infestations with scientific collaborators there

The Asian gypsy moth experience can serve as a model system for other Lymantriid moths that have similar habitats and pose comparable risks of introduction. An additional issue has been USDA-APHIS policy that frowns upon culturing potential pests within quarantine. Clearly, if this was not undertaken on Asian gypsy moth, the scientific advances made would not have been possible. Perhaps this will initiate a change in philosophy and regulations so that preemptive work can be conducted on other exotic pest Lymantriids such as pink gypsy moth and nun moth.

CHARACTERIZATION OF A SPECIES-SPECIFIC DNA PROBE FOR THE IDENTIFICATION OF *ENTOMOPHAGA MAIMAIGA*

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ABSTRACT

The zygomycete, *Entomophaga maimaiga* Humber, Shimazu & Soper, is the only member of the *Entomophaga aulicae* species complex to be taxonomically separated. A pathogen of the gypsy moth originally isolated in Japan, *E. maimaiga* has been identified as one agent responsible for the dramatic collapse of gypsy moth populations in the northeastern United States. This fungus is morphologically indistinguishable from other members of the *E. aulicae* species complex, but can be distinguished by isozymic composition, PCR-based techniques, and RFLP assays. We report here the identification of a DNA fragment cloned from a U.S. *E. maimaiga* isolate which hybridized to DNA of geographically diverse *E. maimaiga* isolates, but did not hybridize to DNA from other entomophthoralean fungi, including other isolates of the *E. aulicae* species complex. Sequence analyses revealed that the species-specific DNA fragment is 1962 base pairs in length, is 69.8% AT, and is composed of several simple sequence motifs including 5'-C(A_n)T-3' and 5'-T(A_n)C-3'. The DNA sequence contained several stop codons in all six reading frames, with the largest open reading frame potentially encoding a 63 amino acid protein. Although weak amino acid similarity was observed with a *Drosophila* LTR and a putative open reading frame terminating in the cloned sequence, no significant homologies at the nucleotide or amino acid levels were found in searches of existing databases. Quantitative dot blot hybridizations indicated that the sequence contained a repetitive element present at approximately 160±8 copies/*E. maimaiga* nucleus. The repetitive nature of this probe proved highly useful and allowed for the detection of an *E. maimaiga* infection within a single *L. dispar* larva. The development of this highly sensitive and rapid DNA-based assay for *E. maimaiga*-infected larvae has aided in the assessment of the role of alternative hosts in the production of epizootics in gypsy moth populations.

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DEVELOPMENT AND USE OF MOLECULAR MARKERS FOR THE IDENTIFICATION OF *ENTOMOPHAGA MAIMAIGA*

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ABSTRACT

The fungal genus *Entomophaga* includes several insect pathogens which are currently under consideration for use as biological control agents. *Entomophaga aulicae* (Reichardt in Bail) Humber and *Entomophaga maimaiga* Humber, Shimazu & Soper, are important mycopathogens of Lepidoptera. *E. aulicae* has been isolated from 10 families of Lepidoptera, including Lymantriidae, while *E. maimaiga* has been isolated from only the Lymantriidae, and primarily from the gypsy moth, *Lymantria dispar* (L.). Although morphologically indistinguishable, laboratory assays have shown that *E. aulicae* isolates are unable to infect gypsy moth larvae, while *E. maimaiga* isolates are unable to infect typical *E. aulicae* hosts. Studies regarding the presence and/or importance of alternative hosts in the life cycle of these fungi have been hampered by the lack of rapid and unequivocable methods for the detection and differentiation of these two fungal species. Furthermore, without intra-specific markers, it is extremely difficult to assess the effectiveness of specific strains in the production of natural or induced epizootics.

To address these problems, we have investigated the use of various DNA-based techniques to assess variability among species of entomophthoralean fungi. We have developed both species-specific and strain-specific DNA markers useful in the identification of *Entomophaga* isolates. Southern analyses using *E. aulicae* rDNA sequences as probe (ribotyping) identified three distinct ribotypes among 28 isolates of *E. aulicae*. All eight isolates of *E. maimaiga* showed a distinct fourth ribotype pattern. Isolates of entomophthoralean fungi representative of other species and genera had ribotype patterns distinct to each different species examined. In order to assess intra-specific variability among isolates of *E. aulicae* or *E. maimaiga*, we isolated cloned DNA fragments from *E. aulicae* genomic and cDNA libraries, and *E. maimaiga* genomic libraries and tested these as probes in Southern analyses. Variability was detected among certain *E. aulicae* isolates, including in some cases variability among isolates from the same epizootic. However, no variation was detected among the geographically diverse isolates of *E. maimaiga*. Further analyses using PCR-RAPD assays identified some differences between the *E. maimaiga* isolates, but variability was low compared to that observed among isolates of *E. aulicae*.

Finally, highly repetitive DNA elements specific for *E. aulicae* and *E. maimaiga*, as well as for other entomophthoralean fungi, were identified. The combinatory use of these repetitive element probes was shown to provide a rapid and unequivocable technique for the differentiation of *E. aulicae*- and *E. maimaiga*-infected larvae.

Supported by an NSERC Canada Strategic Grant (J.C.S.) and an NSERC Scholarship (S.R.A.W.).

HOW *E. MAIMAIGA* INFECTS HOSTS: A TALE OF TWO SPORES

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ABSTRACT

Entomophaga maimaiga, as is well known, produces two kinds of spores. Resting spores overwinter in the soil, germinating in the spring to infect gypsy moth larvae. Conidia are formed from fungal hyphae developing in hosts. Conidia released through the air can infect other larvae. Larger infected gypsy moth larvae also produce resting spores that are inactive until the next spring. To investigate the roles these spores play in infection of hosts, 2nd instar gypsy moths were exposed in cylindrical wire cages in a forest where gypsy moths and the fungus were prevalent. Cages were placed either on the soil to sample the germination of resting spores and subsequent infection of larvae, or about 2 m above the ground to detect infection due to conidia. Cages were exchanged daily throughout May and June, and exposed larvae reared out. Temperature and rainfall were also recorded daily at the site. Larvae in cages on the soil became infected with the fungus throughout the season, and usually one day after rainfall occurred. In contrast, larvae in aerial cages were infected mainly during June, and usually the same day that rainfall occurred. Infection rates of larvae collected weekly from the same site also mainly occurred in June. These results suggest that conidia produced by infected larvae were mainly responsible for the large mortality caused by the fungus at this site. However, the interaction between infection probability and rainfall, temperature, gypsy moth larval behavior, and host and pathogen phenology are complex, and this conclusion is probably simplistic. To obtain more information about these interactions, a computer model was developed (with the help of David Onstad) that was driven by temperature and rainfall values, and that predicted the dates when resting spores and conidia would have their greatest potential for infecting hosts. Runs using rainfall and temperature records for 1990 and 1991 gave infection times of larvae that were quite similar to field estimates. However, resting spores were largely responsible for infection of hosts. In general, results suggest that both resting spores and conidia are important for the successful suppression of gypsy moth populations caused by this pathogen.

CALOSOMA SYCOPHANTA DOES AFFECT GYPSY MOTH POPULATIONS

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ABSTRACT

Calosoma sycophanta is a specific predator beetle of the gypsy moth that has the potential for controlling high populations of the pest. Its phenology is closely related to that of the gypsy moth, and adults are only obvious where there are outbreaks. Procedures have been developed to rear the beetle with some success using gypsy moths from USDA-APHIS at Otis, Massachusetts (Gary Bernon). Previous releases of adult beetles have been made in Connecticut with encouraging results. However, pupal mortality caused by the released beetles was only transitory because the beetle is already well established in Connecticut. An ideal way to assess the effectiveness of *C. sycophanta* would be to release the beetle in areas where the gypsy moth but not the beetle is present. Such areas occur in Michigan (McCullough, Stehr), West Virginia (Butler), and Delaware (Fuester), and this last summer releases were carried out in these states. Results showed that gypsy moth pupal survival was inversely related to estimates of the numbers of immature *C. sycophanta* present in each of the plots. This provides strong evidence that the predator was able to have an impact on gypsy moths in these areas, and suggests that additional releases of the beetle along the leading edge of gypsy moth infestations would be useful.

INFLUENCE OF WEATHER ON THE SYNCHRONY OF GYPSY MOTH OUTBREAKS IN NEW ENGLAND

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ABSTRACT

Outbreaks of the gypsy moth were partially synchronous across the New England states from 1938 to 1992. To explain this synchrony, we investigated the Moran effect, a hypothesis that local population oscillations resulting from similar density dependent mechanisms operating at time lags may be synchronized over wide areas by exposure to common weather patterns. Time series analysis revealed defoliation series in two states as first-order autoregressive processes and the other two as periodic second-order autoregressive processes. Defoliation residuals series computed using the autoregressive models for each state were cross correlated with series of weather variables recorded in the respective states. Weather variables significantly correlated with defoliation residuals in all four states were minimum temperature and precipitation in mid-December in the same gypsy moth generation and minimum temperature in mid to late July of the previous generation. These weather variables also were correlated strongly among the four states. The analyses support the predictions of the Moran effect and suggest that common weather may synchronize local populations so as to produce gypsy moth outbreaks over wide areas.

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