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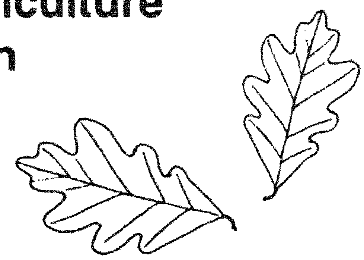

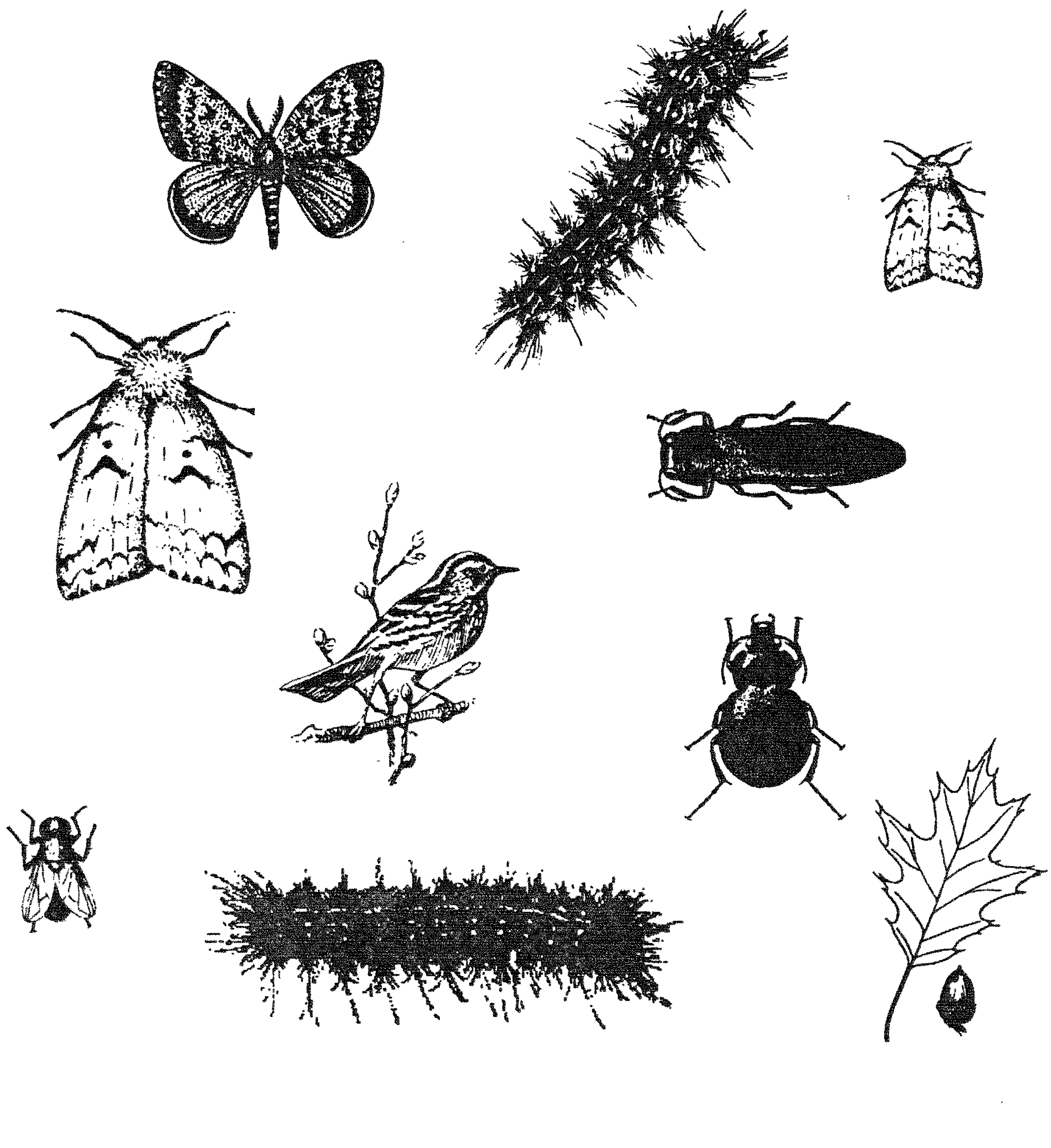

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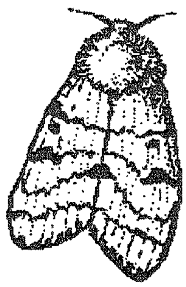

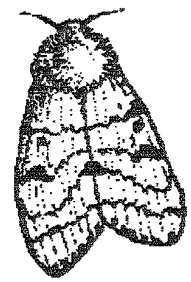

January 16-19, 1996
Loews Annapolis Hotel
Annapolis, Maryland

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

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FOREWORD

This meeting was the seventh 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)
R. Huettel, 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
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AGENDA

Tuesday Afternoon, January 16

REGISTRATION
POSTER DISPLAY SESSION I

Wednesday Morning, January 17

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

Welcome
Michael McManus, USDA-FS

Introductory Remarks
Max McFadden, USDA-FS

A National Commitment: Technology Development for Forest Health Protection
Ann Bartuska, USDA-FS

Oak Decline Around the World
Kurt Gottschalk, USDA-FS

Strategic Plan for Forest Health Research
Kurt Gottschalk and Max McFadden, USDA-FS

POSTER DISPLAY SESSION II

Wednesday Afternoon, January 17

GENERAL SESSION . . . . Moderator: E. DelFosse, USDA-APHIS

Worldwide Distribution and Significance of Gypsy Moth Parasitoids
Presenters: F. Hérard, USDA-ARS, Montpellier, France; G. Hoch, Institute of Forest Entomology, Austria; J. Novotný, Forest Research Institute, Slovak Republic; P. Schaefer, USDA-ARS; R. Fuester, USDA-ARS; W. Kauffman, USDA-APHIS; E. DelFosse, USDA-APHIS

POSTER DISPLAY SESSION III

Thursday Morning, January 18

GENERAL SESSION . . . . Moderator: E. Dougherty, USDA-ARS

Advances in Pheromone Research for Gypsy Moth and Related Lymantriids
Presenters: R. Dickens, USDA-ARS; J. Oliver, USDA-ARS; G. Gries, Simon Fraser University, British Columbia; R. Cardé, University of Massachusetts; B. Leonhardt, USDA-ARS; D. Leonard, USDA-FS

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

Status of Operational Techniques for Managing the Gypsy Moth
Presenters: J. Podgwaite, USDA-FS; R. Cibulsky and R. Fusco, Abbott Laboratories; R. Reardon, USDA-FS; A. Hajek, Cornell University

Thursday Afternoon, January 18

GENERAL SESSION . . . . Moderator: W. Wallner, USDA-FS

Asian Gypsy Moth: Research, Diagnostics, and Status
Presenters: D. Ferguson, USDA, National Museum of Natural History; R. Harrison, Cornell University; S. Bogdanowicz, Cornell University; V. Mastro, USDA-APHIS; T. McGovern, USDA-APHIS; M. Keena, USDA-FS

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

Potpourri of Research Reports
Presenters: H. Evans, Forestry Commission, United Kingdom; S. Thiem, Michigan State University; R. Malakar, University of Massachusetts; C. Davidson and J. Johnson, Virginia Polytechnic Institute and State University

Friday Morning, January 19

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

Exotic Pests: A Threat to the World's Forest Resources
Presenters: W. Wallner, USDA-FS; J. Cavey, USDA-APHIS; M. Montgomery, USDA-FS; K. Shields and T. ODell, USDA-FS; R.M. Muzika, USDA-FS; D. McNamara, European Plant Protection Organization, France; J. Handiside, Ministry of Forestry, New Zealand

CONTENTS

A NATIONAL COMMITMENT: TECHNOLOGY DEVELOPMENT

FOR FOREST HEALTH PROTECTION

Ann M. Bartuska

Director, Forest Health Protection, USDA Forest Service, Washington, DC

ABSTRACT

The need for new and improved technology has never been more apparent than now as the Forest Service reorganizes, restructures and downsizes both staffs and budgets. The key to our being able to effectively retain or even increase our levels of output lies in developing and implementing the technology that will allow us to "do more with less."

Forest Pest Management, now Forest Health Protection (FHP), has traditionally carried out technology development, improvement and application activities through our Technology Development Program (implemented at the Regional/Area level) and through three detached Washington Office units, the Pesticide Application Group in Davis, CA, the Methods Application Group in Ft. Collins, CO and the National Center of Forest Health Management in Morgantown, WV. In 1995, partly in response to the opportunities provided by Reinvention of the Forest Service: the Changes Begin, but mainly to centralize and strengthen our commitment within FHP to technology development, we proposed the establishment of the first Forest Service Enterprise Team. This was ultimately approved and in February, 1995, the Forest Health Technology Enterprise Team (FHTET) was created.

The Forest Health Technology Enterprise Team is made up of the three Washington Office detached units mentioned above. The mission of the Team is to: "Foster the development and use of technologies to protect and improve the health of America's forests." While the three units will remain in their current locations and retain their current areas of emphasis and responsibility, their skills and expertise have been consolidated. The result is a much more efficient and effective way to coordinate, develop and deliver new and improved technology. The units function together under the umbrella of the Enterprise Team to identify partners and cooperators to work with us to better address customer needs. Specific activities and work areas of the Enterprise Team include:

- Information Services
  - Database construction
  - Information retrieval
  - Informational letters (e.g. Timely Tips, FSCBG Technology Transfer Letter, FHTET Newsletter)

Technical Support Services
- Model runs
- Airborne video
- Airborne photo missions
- Data visualization
- Photointerpretation

Training and Education
- Certification and continuing education
- Forest Service national training
- Skill refresher courses
- Manual development

Technology Development
- FHP Technology Development Program
- NAPIAP Program
- Development and support of pest models, decision support systems, information display
- Pesticide application technology

Methods Improvement
- Biological control
- Biopesticides
- Non-target impact evaluations
- Environmental fate evaluations

Forest Health Protection is committed to developing and improving needed technology for better decisionmaking and for attaining and sustaining the health of our Nation's forests. Establishment of the Forest Health Technology Enterprise Team reinforces this commitment and will help keep the Forest Service on the cutting edge and allow us all to better redeem our responsibilities.

OAK DECLINE AROUND THE WORLD

Kurt W. Gottschalk[1] and Philip M. Wargo[2]

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WHAT IS OAK DECLINE?

Oak (*Quercus* spp.) decline is a malady related to the consequences of stress and successful attack of stressed trees by opportunistic (secondary) organisms (Wargo *et al.* 1983). It is a progressive process where trees decline in health for several years before they die. Houston (1981) developed a model of declines that is presented in Figure 1. So what is stress? It is pressure that brings about changes in a tree's physiology, form, or structure that predispose it to invasion by organisms that it ordinarily can resist. Stress can be biotic (defoliation by insects or fungi), abiotic (frost damage, defoliation by frost, drought, excess moisture, air pollution), or stand dynamics/life stage induced (low vigor due to competition or age). So what are secondary organisms? They are biotic agents, usually insects or fungi, that normally attack weakened trees and kill them but rarely can successfully attack healthy trees. These organisms play an important ecological role by killing trees that are weakened due to competition or other natural processes. Due to the numerous stress agents that can affect oak forests, oak decline can occur simultaneously in many different geographic areas, be triggered by entirely different or identical stress agents, and result in death of oaks from a wide variety of organisms.

One theory of decline is dependent on the simultaneous decline in vigor of entire stands or larger areas of trees that are all the same age (Mueller-Dombois 1986). This theory, called the cohort senescence theory, can be applied to mixed upland oak stands even though it was developed in Hawaii. As trees age and reach physiological maturity, their vigor declines. When vigor is reduced enough, the trees can be successfully invaded by secondary organisms and die en mass. Alternatively, a moderate stress could result in large-scale decline and mortality when stands are in this condition while a similar stress in younger stands would have little effect. The recent declines of scarlet (*Q. coccinea*) and black (*Q. velutina*) oak across the eastern United States may actually represent this type of decline as longer-lived oaks like white (*Q. alba*) and northern red (*Q. rubra*) were generally not affected even though they were growing in the same stands.

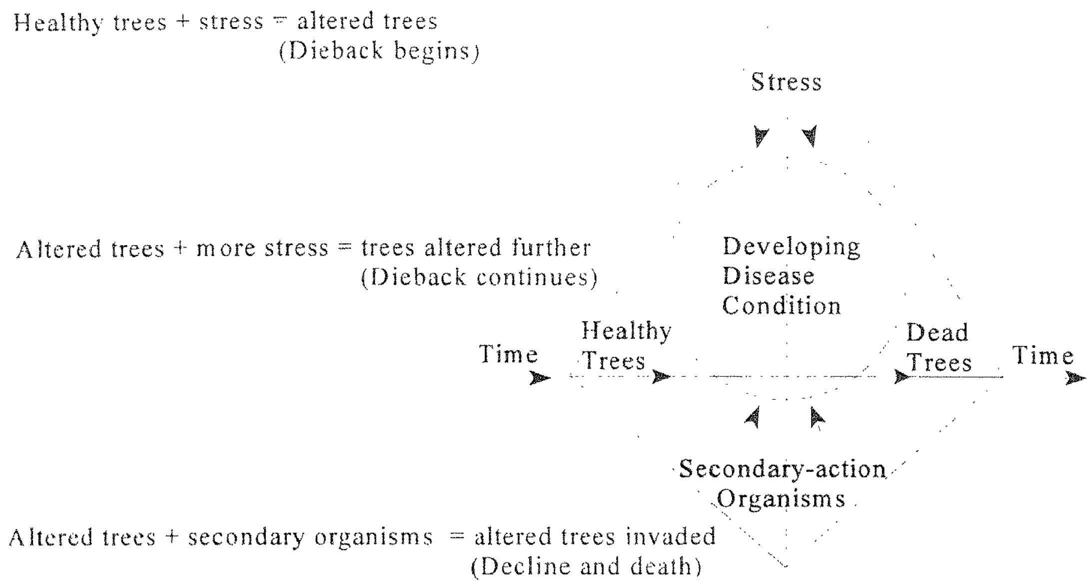

Figure 1. Decline disease schematic developed by Houston (1981).

OCCURRENCE OF OAK DECLINE

Oak decline is not a new phenomenon. Reports of declines of oak in Asia, Europe, and the United States have appeared in the scientific literature since the early 1900s. However, in the three most recent decades, reports of this malady in oak forests seem to have increased (Delatour 1983, Millers *et al.* 1989, Oleksyn and Przybl 1987, Ragassi *et al.* 1989, Tainter *et al.* 1984, Vannini 1987). In this section, we present the recent occurrences of oak decline throughout the world, the oak species involved, and their associations with stress agents and secondary organisms (Table 1).

Table 1. Recent occurrences of oak decline throughout the world including the oak species, stressors, and organisms involved in the decline process (see Literature Cited for sources).

Country/Continent	Species	Stressors	Organisms
North Africa			
Morocco & Tunisia	*Q. suber*	drought defoliation - insect	*Hypoxylon mediterraneum* *Cerambyx* beetles

Country/Continent	Species	Stressors	Organisms
Europe			
Portugal	*Q. suber* *Q. cerris* *Q. ilex*	drought cultural practices	*Armillaria* spp. *Hypoxylon mediterraneum* *Phytophthora cinnamomi*
Spain	*Q. canariensis* *Q. faginea* *Q. ilex* *Q. pyrenaica* *Q. suber*	drought	*Diplodia mutila* *Hypoxylon mediterraneum* *Phytophthora cinnamomi*
United Kingdom	*Q. petraea* *Q. robur*	drought frost damage	*Armillaria* spp. *Agrilus* spp.
Belgium/Netherlands	*Q. petraea* *Q. robur*	drought/excess moisture defoliation - fungal/insect frost damage	*Armillaria* spp.
France	*Q. ilex* *Q. petraea* *Q. pubescens* *Q. robur* *Q. suber*	drought defoliation - fungal/insect frost damage soil/site factors & off-site planting	*Agrilus* spp. *Armillaria* spp. *Ceratocystis* spp. *Ophiostoma* spp. *Collybia fusipes*
Italy	*Q. cerris* *Q. frainetto* *Q. ilex* *Q. pubescens* *Q. robur* *Q. suber*	drought defoliation - fungal/insect	*Armillaria* spp. *Collybia fusipes* *Ganoderma* *Diplodia mutila* *Hypoxylon mediterraneum* *Phomopsis quercina* *Stuartella formosa* Bacterial spp.
Germany	*Q. petraea* *Q. robur* *Q. rubra*	drought defoliation - fungal/insect frost damage excess nitrogen	*Armillaria* spp. *Ceratocystis* spp. *Agrilus* spp. Viruses
Poland	*Q. robur*	drought defoliation - fungal/insect frost damage	*Armillaria* spp. *Ceratocystis* spp. *Fusicoccum quercus* *Ophiostoma* spp.

Country/Continent	Species	Stressors	Organisms
Czech Republic & Slovakia	*Q. cerris* *Q. petraea* *Q. robur*	drought air pollution	*Agrilus* spp. *Ophiostoma* spp. *Diaporthe fasiculata*
Austria	*Q. petraea* *Q. robur*	mistletoe, *Loranthus europaeus* soil/site conditions	*Ceratocystis* spp.
Hungary	*Q. cerris* *Q. petraea* *Q. robur*	drought/excess moisture defoliation - fungal/insect frost damage soil/site conditions	*Armillaria* spp. *Ceratocystis* spp. *Ophiostoma* spp. *Collybia fusipes* *Agrilus* spp.
Romania	*Q. cerris* *Q. frainetto* *Q. pedunculiflora* *Q. petraea* *Q. pubescens* *Q. robur*	drought defoliation - fungal/insect soil/site conditions	*Armillaria* spp. Canker fungi
Bulgaria	*Q. cerris*	drought	*Armillaria* spp. *Ganoderma* *Diplodia mutila* *Hypoxylon mediterraneum*
Moldavia, Ukraine, Baltic States, & Western Russia	*Q. imeretina* *Q. longipes* *Q. petraea* *Q. robur*	drought defoliation - fungal/insect silvicultural manipulations	*Armillaria* spp. *Ophiostoma* spp. *Agrilus* spp.
Asia			
Far East Russia	*Q. dentata* *Q. mongolica*	drought defoliation - insect	*Armillaria* spp. ??
China	*Q. dentata* *Q. mongolica*	drought defoliation - insect	*Armillaria* spp. ??
Japan	*Q. serrata* *Q. mongolica*	drought	*Armillaria* spp. *Ophiostoma* spp. *Platypus quercivorus*

Country/Continent	Species	Stressors	Organisms
North America			
Western United States	*Q. douglasii* *Q. agrifolia* *Q. lobata* *Q. engelmannii* *Q. kelloggii*	drought/excess moisture leafy mistletoe - *Phoradendron villosum* air pollution defoliation - insect	*Armillaria* spp. *Phytophthora cinnamomi* *Diplodia quercina* *Cryptocline cinerescens* & *Discula quercina* *Agrilus* spp. ??
Canada	*Q. alba* *Q. coccinea* *Q. rubra*	drought defoliation - insect	*Armillaria* spp. *Agrilus* spp.
Eastern United States	North *Q. alba* *Q. coccinea* *Q. ellipsoidalis* *Q. palustris* *Q. prinus* *Q. rubra* *Q. velutina* South *Q. falcata* *Q. laurifolia* *Q. marilandica* *Q. nigra* *Q. phellos* *Q. stellata*	drought/excess moisture defoliation - frost/fungal/insect air pollution	*Armillaria* spp. *Hypoxylon atropunctatum* *Agrilus bilineatus* and other species

CONCLUSIONS

Oak decline occurs wherever oak occurs and because of the number and diversity of oak species, it occurs over a wide range of sites in most forested places of the northern hemisphere. While many oak species experience decline, some species are affected more than others, perhaps proportional to their abundance on the landscape, or their susceptibility to the major stress agents that affect oak.

The basic process of oak decline and therefore symptoms of oak decline are similar in all places where decline occurs. Trees are affected by stress agents, dieback in response to the stress, change in susceptibility to deleterious organisms, are attacked by these organisms, and decline and die. Drought and defoliation are the two most common stress agents and the *Armillaria* root disease fungus is the most common secondary organism associated with oak decline worldwide. Age or physiological maturity may be involved in the current worldwide episode of oak decline. Major climatic events also have been proposed but not yet confirmed as common triggering factors.

It should also be noted that declines are not limited to oak species and forests. Many other hardwood species undergo declines including ash (*Fraxinus* spp.), maple (*Acer* spp.), birch (*Betula* spp.), and beech (*Fagus* spp.) among others (Millers *et al.* 1989). A number of these species also are widespread around the world and have decline problems in many locations other than the United States. When viewed from this perspective, understanding and managing decline diseases is an important world forest health issue.

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NORTH AMERICAN GYPSY MOTH FEMALES: AN ADVANTAGE OF BEING FLIGHTLESS

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ABSTRACT

Gypsy moth larvae from North America (West Virginia, United States), Western Europe (Loiret, France), and Asia (Bellyk, South Siberia, Russia) were reared individually at petri dishes in a laboratory at Krasnoyarsk, Russia. They were fed ad libitum by the foliage of 4 host-trees species: *Betula pendula*, *Cotoneaster lucidus*, *Larix sibirica* and *Tilia cordata*. Each day foliage was collected from the same five trees of each species, carefully mixed and fed to the insects. Duration of instar and the weight of larvae and pupae were recorded for each larvae. Adult females were dissected with nearest 12 hours of eclosion and the number of fully formed eggs, with vivid chorion sculpture was calculated. The data are based on 324 females from 3 populations on 4 host-plants.

The differences in pupal weights of the 3 geographic populations was not consistent between the tree species. Asian female pupae were significantly heavier than European and North American on *Cotoneaster* and larch; on *Betula* the weight of the Asian and North American pupae was the same, but higher than that of European pupae; on *Tilia* there was no difference between European and Asian pupae, and all North American larvae died. Fecundity of Asian females was 40% lower on *Betula*, 25% lower on *Cotoneaster*, and 47% lower on *Tilia* than Euro-American strains. There were no differences in fecundity on larch. Weight specific fecundity (eggs per gram of pupal weight) of Asian strain was significantly lower than Euro-American strain on all hosts (ranging from 55 to 77%). This means that the Euro-American strain is approximately 30% more productive than the Asian one.

The weight of an individual egg mass was not measured in this experiment, but in other tests we have found that differences in the weight of an egg between populations is either small (< 10%) or not different. Thus, we do not believe that the Asian population is producing fewer but larger eggs. The lower relative fecundity of Asian females may be a cost for their ability to fly. The data suggest that the Asian gypsy moth females use a larger part of the resources accumulated by larvae to produce the mass of flight muscles and energy reserve to fuel flight. By being flightless, the Euro-American females can direct more of the resources in the pupae to the production of eggs.

THE EFFECT OF TWO APPLICATIONS OF *BACILLUS THURINGIENSIS* ON NON-TARGET LEPIDOPTERA

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ABSTRACT

Recent field studies have shown that a single application of *Bacillus thuringiensis* (*Bt*) has a detrimental impact on native, non-target Lepidoptera. The use of two applications of *Bt* to assure foliage protection may have even a greater impact on non-target Lepidoptera. The decision not to protect foliage and risk defoliation could also have an impact on non-target Lepidoptera by reducing food resources. The objectives of our study were to determine the effects of two applications of *Bt* and high gypsy moth densities on non-target Lepidoptera.

Fifteen plots were established in the Monongahela National Forest, West Virginia, in 1995. Five plots had low gypsy moth (GM) densities, five plots had high GM densities, and five plots had been treated with two applications of *Bt*. Sixty red oak trees and 20 maple trees were encircled with burlap bands. Two plots from each treatment had an additional 20 white oak trees banded. Plots were aerially treated with 24 BIUs of *Bt* from the 19th through 23rd of May and again on the 31st of May. Plots were sampled twice a week and only macrolepidopteran larvae were collected. No GM larvae were collected. The number of GM larvae resting under the bands was counted once a week as an indication of GM density.

Gypsy moth populations were suppressed by the *Bt*. The high GM density plots were beginning to show signs of defoliation when populations were suppressed by an outbreak of the fungus *Entomophaga maimaiga*. No defoliation occurred in any plot. A total of 1,476 macrolepidopteran larvae were collected: 895 from low GM density plots, 431 from high GM density plots, and 150 from treated plots. Weekly totals indicate that plots had similar larval collection numbers for the 2 weeks preceeding treatment. The total number of macrolepidopteran larvae collected from the low and high GM density plots did not differ significantly; however, they were significantly different from the treated plots. When larval

totals were examined by family, the results were mixed. Both the noctuids and geometrids had larval collection numbers that differed significantly between treatments. The lymantriids were not significantly different between low and high GM density plots, but they differed significantly from the treated plots. The highest number of arctiids was collected from the treated plots. The treated plots did not differ significantly from the high GM density plots but they were both significantly different from the low GM density plots. The majority of the arctiids that were collected consisted of one species, and do not appear to be susceptible to *Bt*. Forty species representing 31 genera and 8 families were collected from all the plots. Twenty-eight different species were collected in each of the low and high GM density plots, but only 16 different species were collected in the treated plots.

In conclusion, non-target Lepidoptera were significantly affected by two applications of *Bt*, and the impact seems to be greater than a single application. The effects of defoliation are still unknown since gypsy moth populations and subsequent defoliation were reduced by a fungus outbreak.

FORECASTING GYPSY MOTH DEFOLIATION FROM SURVEYS OF EGG MASSES ON TREE BOLES

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ABSTRACT

This study examined counts of egg masses on the tree bole as a cost-efficient way to forecast the need to suppress a population in order to prevent nuisance defoliation. Egg masses were measured on transects of 100 trees in 139 woodlots. New and old egg masses were counted on the lowest 2 meters of the tree bole and the length of two new egg masses was measured. A two-step sequential procedure provided the most reliable predictions of defoliation exceeding the nuisance threshold. In Step 1, egg mass densities below 0.2 per tree indicate no control is needed, while densities above 2 per tree indicate that control is needed to prevent defoliation. In Step 2, a regression model is used to predict defoliation at densities between 0.2 and 2 egg masses.

The model uses proportion of trees that are oak, the mean length of new egg masses, and the ratio of old to new egg masses to predict defoliation class. The protocol, based on 1992 data, was tested with 1991 and 1993 data by using a subsample of 25 trees to predict if the defoliation of all 100 trees in a transect would be above or below 37%. The accuracy of making correct control decisions was 86%. An experienced worker can sample 25 trees in about 25 minutes. Thus, the protocol offers good reliability at a labor cost comparable to the 5-minute walk method.

DEVELOPMENT OF AN OPTIMIZED BTK PRODUCT FOR FOREST INSECT CONTROL

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ABSTRACT

For the past 25 years, Btk products have been manufactured by fermentation for forest insect control and formulated into aqueous and oil-based suspensions. The potency for all Btk products has been expressed in international units (IU's) based on the cabbage looper (*Trichoplusia ni*) bioassay. It is well documented that individual insect species exhibit a differential response to various Bt toxins and that the international unit is only a guide to be used for a general comparison of product biological activity. Therefore, significant potential exists for product improvement by optimization of specific activity for key target forest pests.

Abbott Laboratories has recently focused on optimization of Btk activity for the control of key forest pests, including the gypsy moth (*Lymantria dispar*). The production (fermentation) process has been optimized by using a new analytical technique and an insect-specific bioassay to measure these improvements. As an example, for gypsy moth, all processes were identified and improvements made via diet and plant bioassays versus the target insect, *L. dispar*, rather than the cabbage looper. HPLC (High Performance Liquid Chromatography) is the first analytical tool which has demonstrated a high degree of correlation with target insect bioassay activity. There are still additional factors which impact Btk bioactivity and which cannot be measured solely by HPLC, including Bt crystal solubility, protoxin activity, synergy among toxins, number of spores, and Bt-related metabolites.

Abbott has completed, in cooperation with a number of outside forest research and development scientists, a series of diet bioassays, whole plant bioassays, and field trials to identify and improve Btk products for forest insect control. These assays supported the conclusion that differential activity could be identified between production processes and as measured by different target insect species. In addition, there is a significant difference in the dose response (slope) for gypsy moth larvae to these different processes.

Field evaluations were completed in the U.S. and Canada to confirm the lab bioassay results and demonstrated a differential activity under field conditions. Therefore, we are now proposing the potential use of a new activity designation, the FTU (Forest Toxin Unit) as the most valid measurement for Bt product activity for forest insect species. The product label potency will reflect equivalent FTU and BIU designations and the product should be utilized at equivalent spray volumes to the former BIU-based products.

APPLICATION OF A HIGH DENSITY REARING SYSTEM TO THE PRODUCTION OF GYPSY MOTH LARVAE FOR VIRUS

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ABSTRACT

AgriVirion has developed a high density insect rearing system, called HeRD, that enables the rearing of reproducible, synchronous populations of healthy insect larvae at densities not previously achievable. The system is efficient at a wide range of production scales and minimizes the use of diet, labor and capital resources. HeRD was developed to enable the efficient production of biological materials, including recombinant proteins encoded by genetically engineered viral vectors, and insect viruses for use as biopesticides. HeRD has been used to rear healthy larvae of *Trichoplusia ni* and *Spodoptera exigua* at densities of more than 9,000 and 12,000 per cubic foot, respectively. A study was undertaken to determine whether the HeRD system could be adapted successfully to the rearing of *Lymantria dispar* larvae for the production of Gypchek.

Early studies indicated that mature fifth instar *L. dispar* larvae preferred solid surfaces rather than the edges used in HeRD for other insects. HeRD modules were constructed to accommodate this preference, and densities of greater than 800 per sq. ft. and greater than 1,600 per cu. ft. for mature fifth instar larvae were achieved, compared to densities of approximately 150 per sq. ft. and 720 per cu. ft. reported for cup-based systems. The average pupal weight in the HeRD system was approximately 15% greater than that in cup-based systems. Preliminary work with fourth instar larvae has shown that they adapt readily to the flanged HeRD modules used with other insects, and will grow to densities of greater than 2,400 per sq. ft. and 6,750 per cu. ft.

These results demonstrate that the HeRD system can be used to rear *L. dispar* larvae, and that they grow more densely and larger than in other systems. Based on published recoveries of virus, fifth instar larvae grown in the HeRD system would yield approximately 2.2 times as many acre-equivalents per year per unit volume of rearing space as cup-based systems. Fourth instar larvae grown in the HeRD system would yield approximately eight times as many acre-equivalents as cup-based systems producing infected fifth instar larvae.

THE EFFECTS OF FOLIAGE DAMAGE ON TRANSMISSION OF THE GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS

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ABSTRACT

Foliage of red oak (*Quercus rubra* L.) and black oak (*Quercus velutina* Lam.) trees was contaminated with the cadavers of first instar gypsy moths (*Lymantria dispar* L.) killed by the gypsy moth nuclear polyhedrosis virus. We damaged the foliage using gypsy moth larvae enclosed on the foliage in mesh bags, or with scissors. Healthy third instar gypsy moths were confined on these branches in mesh bags and allowed to feed for one week, then removed and returned to the lab. Additionally, we performed laboratory bioassays by removing leaf disks from the foliage of trees used in the experiment described above, treating them with virus, and feeding them to healthy third instars held individually in petri dishes. All test larvae in these experiments were reared individually, and their mortality from virus was recorded. Assays for tannin content were performed on frozen samples of leaves from the leaf damage experiments described above. None of these experiments done over a 3-year period showed significant effects of foliage damage on virus mortality of test insects or tannin content of damaged leaves. Leaves at one of two naturally-defoliated sites were found to have higher tannin levels when damaged than when undamaged.

GYPSY MOTH DEFOLIATION AND ITS EFFECT ON TREE GROWTH AND MORTALITY IN COASTAL PLAIN MIXED PINE-HARDWOOD STANDS

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ABSTRACT

From its first introduction in 1868 until the early 1960's, gypsy moth activity has been concentrated in the northern hardwood and oak stands of New England and the Mid-Atlantic States. The expansion of populations into the southeastern United States has resulted in the defoliation of previously undisturbed forest types. Much of the southern Coastal Plain consists of either mixed pine-hardwood stands, or pure pine stands with a hardwood understory. Laboratory studies have revealed that some southern species, such as sweetgum (*Liquidambar styraciflua* L.), are preferred hosts. Southern conifers, such as loblolly pine (*Pinus taeda* L.), though considered to be resistant to defoliation may be consumed in certain situations. In 1992 a long-term study was initiated to determine the effect of gypsy moth defoliation on the growth and mortality of loblolly pine and associated hardwoods in mixed stands. Two stand types were selected for study, mixtures of loblolly pine and oaks (*Quercus* spp.) and mixtures of loblolly pine and sweetgum. Research plots were established in 47 stands in 1992 and 1993. Gypsy moth populations were active in 18 stands in 1993; however, by the end of the 1995 growing season only17 of these remained within the plot network.

Mean total stand defoliation for all stands increased from 15 percent in 1993 to 31 percent in 1994, then decreased to 27 percent in 1995. Pine-oak stands experienced their highest levels of mean defoliation in 1993 (23%) and 1994 (38%), while mean defoliation of pine-sweetgum stands was greatest in 1994 (24%) and 1995 (33%). In 1995, mean cumulative stand mortality was greater in pine-oak stands than in pine-sweetgum stands. On average, pine-oak stands lost 28% of their original basal area and 11% of the original number of stems, while pine-sweetgum stands lost 8% of their original basal area and 6% of their stems. Mortality rapidly increased during the second year following defoliation, a pattern that has been observed by previous researchers. In both stand types, susceptible species experienced higher average levels of defoliation and had a greater mortality rate than resistant species. Crown class and crown condition of individual trees appeared to play a role in whether mortality occurred subsequent to defoliation. In pine-oak stands, cumulative basal area mortality of dominant and codominant trees averaged 22%, while intermediate and suppressed trees lost 40% of their original basal area. In pine-sweetgum stands, dominant and codominant trees suffered only 4% cumulative basal area mortality, but intermediate and suppressed trees averaged 27% mortality. It is anticipated that mortality will continue to increase in both stand types, and further measurements are scheduled for 1996.

ELECTROPHYSIOLOGICAL STUDIES OF PHEROMONES AND ANALOGS IN *LYMANTRIA MATHURA* AND *L. DISPAR*

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ABSTRACT

Since introduction of gypsy moth, *Lymantria dispar*, into the United States and its attainment of pest status, research on the gypsy moth and other potential lymantriid pests has intensified. I report progress in our identification of the sex attractant pheromone produced by *L. mathura* females, and results of electrophysiological studies of *L. dispar* involving cyclopropane (CP) and aziridine (AZ) analogs of its sex attractant, disparlure [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane = (+)-disparlure = (+)-D].

Preliminary investigations of the sex attractant pheromone of *L. mathura* using coupled gas chromatography/electroantennograms (EAGs) with male moths revealed at least two active areas of gas chromatograms of ovipositor extracts. Identification of compounds responsible for the observed activity is in progress. Significant EAGs were not elicited by either enantiomer of disparlure. Dose-response curves constructed from EAGs of *L. dispar* males to (+)-D, the CP-, difluoroCP-, and dichloroCP-analogs were parallel but shifted closer to the abscissa for the analogs. Relative stimulus loads necessary to elicit equivalent responses for (+)-disparlure and its analogs were: (+)-D = 1, difluoroCP = 100, dichloroCP = 500, and CP = 1000. Dose-response curves for *cis*- and *trans*-AZ analogs were nearly identical, but shapes of these curves differed from those obtained for (+)-D and other analogs. EAGs elicited by (+)-*cis*-AZ [conformation corresponding to (+)-D] were greater than those elicited by (-)-*cis*-AZ. Our results demonstrate similar mechanisms for electrical responses elicited by (+)-D and the cyclopropane analogs. Greater activity for dihalogenCP analogs relative to the CP analog may be explained by inductive effects of the halogen atoms on neighboring carbons to mimic effects of oxygen of the epoxide pheromone. The fact that the difluoroCP analog was more active than the dichloroCP analog may be accounted for by the smaller size of the fluorine atom compared to chlorine; while greater effectiveness of the difluoroCP analog relative to the CP analog is explained principally by inductive effects since atomic radii of fluorine and hydrogen are similar. Greater EAG activity of (+)-AZ relative to (-)-AZ correlates with the relationship of these analogs with (+)-D and (-)-D. While dose-response curves obtained with the AZ analogs are more difficult to explain, nitrogen analogs are known to have interesting effects on olfactory systems of other insects. Biological activity of the disparlure analogs will be further evaluated in adaptation experiments, as well as in laboratory and field behavioral tests.

GENETIC POTENTIAL FOR DEVELOPMENT OF RESISTANCE TO *BACILLUS THURINGIENSIS* IN THE GYPSY MOTH

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ABSTRACT

The potential for development of resistance to *Bacillus thuringiensis* (*Bt*) in gypsy moth (*Lymantria dispar* (L.)) populations after its repeated use on infestations is being investigated. Nineteen populations in Pennsylvania, New Jersey, Virginia, and West Virginia were analyzed for susceptibility to *Bt*. Eleven of these had been sprayed with at least 16 to 24 BIU/acre of commercial *Bt* formulations for three to six generations between 1987-1993. The other eight populations, located in the same general areas, were never treated with *Bt*. Egg masses collected from all populations were separated into two aliquots; one was used immediately to bioassay the wild populations (1st generation), and the other was used to produce laboratory-reared progenies from each population for later more intensive comparative analysis, selection studies, and estimation of heritability.

Comparison between all wild populations indicated that there was no significant difference between slopes of the concentration-response lines. The average slope was 3.3 and ranged from 2.9 to 3.6, suggesting a high degree of similarity in concentration-response relationship between populations regardless of geographical location or *Bt* treatment history. Within-group susceptibility between populations varied significantly. The LC_{50} of treated populations ranged from 24 to 51 IU/ml of diet, whereas the LC_{50} of untreated populations ranged from 30 to 71 IU/ml of diet. Comparison between the progenies (2nd generation) of the wild populations also indicated a similar concentration-response relationship where slopes did not differ significantly. However, within-groups susceptibility did; the LC_{50} ranged from 10 to 70 IU/ml of diet for sprayed populations and from 20 to 64 IU/ml of diet for the untreated populations. Initial analyses of selected progenies of parents that survived an LC_{75} (110 IU/ml of diet) compared to the controls from the same families also failed to show any significant decrease in susceptibility; this was again due to within group variability for both unselected and selected progenies. A comparison of selected (150 IU/ml of diet) and control progeny after two generations of selection also failed to show any significant decrease in susceptibility to *Bt*, but the range of LC_{50} for the selected progeny (50 to 118 IU/ml of diet) was greater than that of the control progeny (65 to 80 IU/ml of diet). However, the variation in susceptibility to *Bt* of populations from North America (selected or unselected) is less than that for populations from Eurasia (LC_{50} ranges from 47 to 338 IU/ml of diet). Analysis of data to estimate the heritability of *Bt* susceptibility in North American populations has begun.

GYPSY MOTHS AND OTHER EXOTIC PESTS: THREATS TO UK FORESTS

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ABSTRACT

The islands of Great Britain and Ireland are protected by a natural aquatic barrier that reduces the probability of colonization by forest pests from mainland Europe and from the rest of the world. One of the greatest threats to the efficacy of this barrier is international trade in wood and wood products. Specifically, the UK imports approximately 90% of its timber requirements, representing very large volumes of wood.

Pest risk analyses and experience of interceptions of pests have provided information on those insects that pose the greatest threats to UK forest resources. Recent interceptions included gypsy moth (*Lymantria dispar*), which was found in gardens in London. This new finding and the measures taken to manage the infestation will be described. In addition, a campaign of increasing the awareness of both the public and local authorities to the potential of this pest has been mounted, with some success.

The eight toothed European spruce bark beetle (*Ips typographus*) is the European forest pest that poses the greatest threat to UK spruce forests. This has been intercepted or described in Britain since the mid 1800s but in recent years, with increasing trade and the greater dependence on spruce (particularly Sitka spruce and, to a lesser extent, Norway spruce) the risks have increased. Data from pheromone traps and timber inspections on importation have indicated a very large rise in the number of interceptions during the past two years (1994 and 1995). This will be discussed in the context of pest risk analysis and on the potential consequences to UK forests.

ANALYSIS OF GYPSY MOTH PARASITISM IN THE PENNSYLVANIA FOREST PEST MANAGEMENT STUDY PLOTS, 1984-1992

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ABSTRACT

Parasitism of gypsy moth, *Lymantria dispar* (L.), was monitored in 40 study plots in Pennsylvania from 1984 to 1992. Nine parasite species, all but one introduced from Eurasia, were commonly found. Parasitism by the egg parasite *Ooencyrtus kuvanae* (Howard) varied between regions, averaging ca. 30%. Parasitism by the bivoltine larval parasite *Cotesia melanoscela* (Ratzeburg) averaged 6.4% and 3% during the 1st and 2nd generations, respectively. Parasitism by *Phobocampe unicincta* (Gravenhorst) averaged 5.2%, and was highest in low stable gypsy moth populations. Parasitism by *Compsilura concinnata* (Meigen) averaged 7.9%, but fluctuated over time and was highest in low declining and low stable populations. *Parasetigena silvestris* (Robineau-Desvoidy) was the dominant larval parasite, with mean parasitism of 16%, but averaging 30% in low stable host populations. Parasitism by the remaining larval parasite, *Blepharipa pratensis* (Meigen), averaged 5%, but rose during the outbreak. *Brachymeria intermedia* (Nees) was the dominant pupal parasite, but mean parasitism was only 2%. Parasitism of pupae by *Coccygomimus disparis* (Viereck), the most recent addition to the parasite complex, was generally low (<1%), as was that by *Theronia atalantae fulvescens* (Cresson), the only indigenous species recovered frequently.

When statewide means for host density (egg mass counts) and apparent (peak) parasitism were arranged in a time series, the results were difficult to interpret because host population trends were not synchronized in all regions, and biases existed due to missing values for parasitism at low host densities in some plots in certain years. Density dependent responses were variable, and most plots of parasitism against host density showed a high degree of scatter, even in cases where very high levels of statistical significance were observed. Two species, *B. pratensis* and *B. intermedia*, showed direct density dependence, four species (*C. melanoscela, P. unicincta, C. concinnata, P. silvestris*) showed inverse density dependence, and three species (*O. kuvanae, C. melanoscela, B. pratensis*) showed delayed density dependence. Three additional species (*C. concinnata, P. silvestris, T. a. fulvescens*) exhibited delayed density dependence in high host populations. Multiple regression analysis of parasitism by *P. unicincta* and *P. silvestris* against egg mass counts in the same and previous year revealed that

strong negative direct density dependent responses were masking positive delayed density dependent responses. Therefore, nearly every species in the parasite complex had a delayed density dependent response, although some were more noticeable than others.

Density dependent responses of some species varied by year. First generation *C. melanoscela*, had strong responses in 1984 and 1991-92. Density dependent responses by *P. silvestris* were consistently negative, but weak in 1988 and 1992. Density dependent responses by *B. pratensis* and *B. intermedia* varied also, those for the former being strongest in 1988 and 1991; those for latter, in 1989 and 1991. Responses for most species were weakest during 1986-87, when statewide gypsy moth populations reached their lowest levels.

Several species appeared to be well adapted to certain physiographic regions. For example, *O. kuvanae* and *P. silvestris* to the Piedmont and Ridge & Valley Regions, *P. unicincta* to the Allegheny Mountains, and *B. pratensis* to the Unglaciated Allegheny Plateau. Parasitism by most imported species, however, was low in the Unglaciated Allegheny Plateau, the region most recently invaded by the gypsy moth. Regional differences affected density dependent responses in parasitism.

When separate regressions for density dependent responses by the dominant larval parasites (*C. melanoscela, C. concinnata, P. silvestris,* and *B. pratensis*) were run for building, stable, and declining host populations, an interesting pattern emerged. Usually, the strongest responses were observed in declining populations, the weakest responses in building populations, and intermediate responses in stable populations. The sole 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.

The relationship of parasitism to changes in gypsy moth populations was examined through stepwise multiple regression analysis. A six-variable global multiple regression model explained ≈37% of the variation in year-to-year per capita changes in gypsy moth populations. Disease and unknown mortality were the most important factors, but % nonviable eggs, parasitism by *O. kuvanae, P. silvestris* and *B. intermedia* were also of significance ($P \leq 0.05$). A two-variable regression model using % parasitism by *C. melanoscela* (both generations) as independent variables explained ≈40% of the variation in between year changes in egg mass density in low populations (< 100 egg masses/ha). Likewise, for medium density populations (99.9 < egg masses/ha < 1,000), a two-variable model with % parasitism by *P. silvestris* and *C. melanoscela* (1st brood) as independent variables explained ≈34% of the variation in year-to-year changes of gypsy moth abundance. A four-variable model for high (> 1,000 egg masses/ha) populations with % parasitism by *P. silvestris* and *B. intermedia* and % infection with NPV and unknown mortality explained 21% of the variation in year-to-year changes in gypsy moth abundance. These results suggest that parasitism is more likely to influence changes in host density at low densities than high densities. Building gypsy moth populations were characterized by low incidence of both parasitism and disease; declining and low stable populations, by high parasitism, particularly by *P. silvestris* and *C. melanoscela*.

OBSERVATIONS ON FEMALE-BIASED SEX RATIOS IN THE GYPSY MOTH

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ABSTRACT

In 1994, a population of gypsy moth, *Lymantria dispar* (L.) was observed at Angola Neck (Sussex Co.), Delaware, that exhibited a highly female-biased sex ratio. Proportions of females in samples of large larvae, prepupae, pupae, and postseason pupal remains taken from this population ranged from 94% to 100% and averaged 95%. Factors contributing to this unusual sex ratio could not be determined. Egg masses were collected at the same and nearby sites the following year, and the hatchlings were reared out to see if female-biased sex ratios would result. Larvae that died before reaching the pupal stage were preserved in alcohol and later dissected to examine developing gonads. Many such larvae could be sexed successfully. The *G*-test was used to test sex ratios of gypsy moths for homogeneity and departures from a theoretical 50:50.

Overall, sex ratios in these rearings were female-biased (avg = 61% females), and statistically significant differences from a hypothetical 50:50 sex ratio were noted in 10 of 36 egg masses with hatch and at 6 of the 10 sites monitored, including the site where the aberrant sex ratio was discovered in 1994. None of the sites had significant tests for heterogeneity, signifying that any deviations from expectations of sex ratios within sites were in the same direction or did not differ from one another. One egg mass yielded solely female progeny, but the low number of progeny obtained (n = 8), precluded speculation that all the progeny would have been female. Unfortunately, hatching failure and larval mortality resulted in significant proportions of individuals that could not be sexed (range: 2%-97%, average = 44.2%). These proportions were sufficiently high to account for the departures from a 50:50 sex ratio, but there was not any correlation between sex ratio and the different categories of mortality (egg parasitism, hatch failure, larval mortality, and total mortality). Unhatched, unparasitized eggs were embryonated, indicating that all egg masses had been deposited by mated females. The sex ratio in dead larvae that could be sexed averaged 63.7%, so it could not be concluded that there was a consistent bias in male mortality. Therefore, the results are consistent with the hypothesis that some females at some sites give rise to progeny with a female-biased sex ratio; however, the alternative hypothesis that females have a fitness advantage remains tenable.

MODELING POTENTIAL FOREST VEGETATION CHANGES DUE TO GYPSY MOTH WITHIN UNITED STATES ECOREGIONS

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ABSTRACT

A simulation of the effects of three gypsy moth outbreak scenarios on forest vegetation changes over 20 years was done for all ecoregions in the coterminous United States. Simulations were done using the Forest Vegetation Simulator of the USDA Forest Service and SE-TWIGS. Outbreak scenarios included no outbreak (baseline), moderate, and heavy. National Forest inventory data from the western U.S. and Forest Inventory and Analysis (FIA) data for state and private lands and eastern U.S. national forests were used. Under baseline, no outbreak conditions, susceptible trees increased slightly, decreased slightly, or maintained their relative position depending upon the ecoregion and successional status. Under moderate outbreak conditions, susceptible species decreased somewhat while resistant and immune increased slightly. When subjected to a heavy outbreak, susceptible species decreased significantly and resistant and immune species increased significantly. These results suggest that in the long term, U.S. forests will gradually become more resistant to gypsy moth as the proportion of susceptible species is reduced.

INFLUENCE OF GYPSY MOTH DEFOLIATION AND SILVICULTURAL TREATMENTS ON SEED FALL

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ABSTRACT

An evaluation of the effectiveness of silvicultural treatments to minimize gypsy moth effects on forests is ongoing at the West Virginia University Forest. Two treatments, sanitation thinning and presalvage thinning, are being tested. Estimates of defoliation, growth and mortality were measured on permanent plots. As part of the evaluation of defoliation patterns and silvicultural treatments, seed traps were used to measure the annual seed fall. Seed fall was analyzed for the influence of silvicultural treatment, defoliation, stand composition, and year effects. Year had a highly significant effect for total, oak, and yellow-poplar seed falls. Defoliation had a highly significant negative effect on total and oak seed falls, but not on yellow-poplar seed fall. Stand composition had a highly significant effect on yellow-poplar seed fall, a significant effect on oak seed fall, and no effect on total seed fall. Silvicultural treatment had no effect on total, oak, or yellow-poplar seed fall. In addition, some significant interactions, particularly defoliation times year contributed to understanding the seed fall produced in these stands.

PHYSIOLOGICAL AGE AFFECTS THE DEVELOPMENTAL RESPONSE TO TEMPERATURE IN DIAPAUSING GYPSY MOTH EGGS

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ABSTRACT

We are interested in developing a model of gypsy moth egg development that is capable of accurately predicting egg hatch over extremely large geographic areas. A model will only be geographically robust if it accurately describes the relationship between temperature and developmental rate. There is an underlying assumption in development vs. temperature experiments that there is an identical developmental response to a given temperature over time. We found that this underlying assumption was badly violated when we examined the developmental response of postdiapause gypsy moth eggs. As eggs advanced in postdiapause physiological age they showed an increasing developmental response to all temperatures (Fig. 1). Because this critically important assumption did not hold true in postdiapause development, we wished to estimate the effects of temperature and physiological age on developmental response during the diapause process.

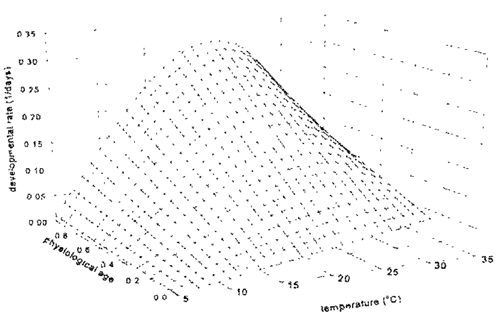

Figure 1.

We measured "instantaneous" developmental rates 0, 10, 30, 50, 60, or 75 days after diapause initiation at temperatures of -5, 0, 10, 15, 20, and 25°C. See Gray *et al.* 1995 for a description of the technique of measuring "instantaneous" developmental rates.

Physiological age had a profound effect on the developmental response to temperature in diapausing gypsy moth eggs. In contrast to the effect of physiological age in postdiapause, where developmental response increased at all temperatures with increasing age, response in diapause was not always positive. Developmental response to low temperatures (-5°C) increased initially, reaching a maximum around day 50, and then declined (Fig. 2, top). At high temperatures (25°C) there was no detectable developmental response at the onset of the diapause phase, but approached 0.1 day^{-1} 75 days later (Fig. 2, bottom). At intermediate temperatures there was a gradual increase in developmental response with increasing age.

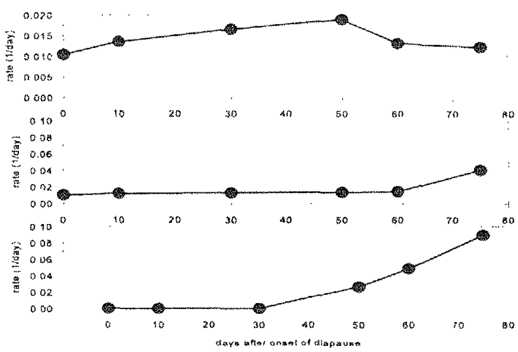

Figure 2.

The pattern of an initial increase in developmental response at low temperature, followed by simultaneous decline at low temperature and increase at high temperature suggests the diapause phase is composed of two processes. A low-temperature process predominates during early diapause and is gradually replaced by a high-temperature process. This results in an unexpected bi-modal developmental rate function mid-way through the diapause process where low AND high temperatures promote faster development than intermediate temperatures (Fig. 3). These data support the "cold-sensibilization" and "warm-reactivation" processes of diapause hypothesized by Zaslavski.

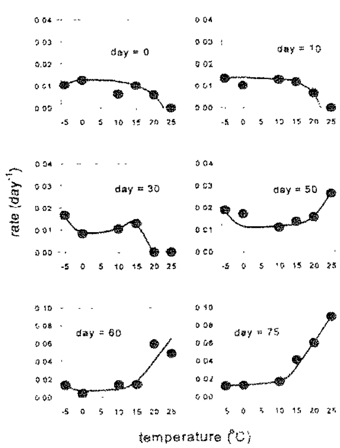

Figure 3.

We are currently in the process of developing a mathematical description of this complicated phase. Our three-phase model of gypsy moth egg development will be completed with this description, and we will begin to test our model for its geographic robustness.

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NOVEL SEX PHEROMONE COMPONENTS OF LYMANTRIID MOTHS

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ABSTRACT

Pheromone gland extracts of nun moth (NM), *Lymantria monacha* L., gypsy moth (GM), *L. dispar* L., and Douglas-fir tussock moth (DFTM), *Orgyia pseudotsugata* (McDunnough) were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD), high performance liquid chromatography, and by coupled GC-mass spectrometry in selected ion monitoring mode. In field tests of candidate pheromone components for NM and GM in the Czech Republic, a ternary blend of (+)-disparlure, (7*R*,8*S*)-*cis*-7,8-epoxy-octadecane [named here (+)-monachalure] and 2-methyl-*Z*7-octadecene at a 10:10:1 ratio synergistically attracted NM males, and was 10 times more attractive than previously known (+)-disparlure. Three epoxide [(-)-disparlure, (+)- and (-)-monachalure] and 2 hydrocarbon volatile components [2-methyl-*Z*7-octadecene and *Z*7-octadecene] synergistically prevented cross attraction of co-seasonal GM males, and imparted specificity to NM sexual communication. Identification of *cis*-7,8-epoxy-3-methyl-nonadecane as a second pheromone component in female GM, attracting GM but not NM males, provided the first evidence for species-specific pheromone components also in GM. In field tests of 3 candidate pheromone components, (*Z*)6,(*Z*)9-, (*Z*)6,(*E*)8- and (*Z*)6,(*E*)9-heneicosadien-11-one [*Z*6*Z*9, *Z*6*E*8, *Z*6*E*9] for DFTM in British Columbia, *Z*6*E*8 strongly enhanced attractiveness of previously known (*Z*)6-heneicosen-11-one. Because *Z*6 by itself attracts 7 species of tussock moths (2 sympatric with DFTM), a blend of *Z*6 plus *Z*6*E*8 may impart specificity to DFTM pheromone communication. Future use of multiple-component pheromone lures will allow highly sensitive and species-specific monitoring of NM, GM and DFTM populations.

SHOULD *ENTOMOPHAGA MAIMAIGA* BE CONSIDERED AS AN OPERATIONAL TECHNIQUE?

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ABSTRACT

Two life stages of the gypsy moth fungal pathogen *Entomophaga maimaiga* can be found in the environment: relatively short-lived conidia and overwintering resting spores. Resting spores, an environmentally resistant stage, are dormant after being produced in later instars and only begin germinating the spring following production. It is the resting spore stage that has been released to introduce *E. maimaiga* to locations where it is not yet established. Between 1990 and 1994, this fungus was released at over 140 sites in the northeast and mid-Atlantic areas with the majority of releases yielding successful establishment. *E. maimaiga* persisted and infection levels increased between 1991/1992 and 1994 at release sites in George Washington National Forest (GWNF), VA. After the GWNF releases in 1991/1992, defoliation in fungal release areas remained extremely low until 1995, when gypsy moth populations were abundant at many sites. However, these high populations experienced epizootics caused by *E. maimaiga* at the end of the 1995 season.

We have undertaken a project to investigate release of *E. maimaiga* with another objective: to augment fungal populations and thereby manipulate disease epizootics. This project was conducted on the eastern shore of MD by releasing resting spores at the bases of individual trees simulating plantings in urban/suburban homesites. Infection levels were higher in fungal release plots compared with controls early in the season when third instars were present. By the end of the season, *E. maimaiga* was found at equivalent levels in release and control plots because epizootics caused by this fungus occurred throughout the area. Defoliation was the most important variable for evaluating success of fungal releases since this study was conducted to evaluate gypsy moth control in residential areas. The lowest levels of defoliation were observed in fungal release plots while severe defoliation was only observed in control plots. Egg mass densities at the end of the season were equivalent in treatment and control plots.

In conclusion, *E. maimaiga* resting spores have been used successfully for both inoculative releases and augmentative releases in gypsy moth populations. For many fungal releases to

date, naturally produced resting spores occurring in soil at the bases of trees were collected and distributed. Using soil as a source of inoculum has short-comings including 1. Soil must be taken from areas without specific plant pathogens in the soil, 2. It is a lengthy and complex process requiring specific equipment to confirm the presence of resting spores in soil and quantify their density, and 3. To collect soil bearing resting spores, one must know which trees had previously been covered with cadavers of gypsy moths killed by *E. maimaiga*. As an alternative, for some releases that have been conducted, cadavers of gypsy moth larvae that had died from *E. maimaiga* infections were collected and released at new sites. While this method is preferable to collection and release of soil containing resting spores, this procedure requires planning because cadavers must be collected the June/July before the desired release. At present, mass production of *E. maimaiga* resting spores is not an option because this spore stage cannot be produced *in vitro* and we must understand dormancy requirements of *in vivo*-produced spores before *in vivo* mass production of a germinable product is possible.

MOLECULAR IDENTIFICATION TECHNIQUES: A POPULATION GENETICS PERSPECTIVE WITH OBSERVATIONS OF MICROSATELLITE LOCI

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ABSTRACT

Developing molecular diagnostics for identifying Asian gypsy moths (*Lymantria dispar* L.) in North America requires genetic markers that discriminate "pure" Asian and North American populations and robust baseline data from potential source and recipient populations. The ideal genetic marker would exhibit fixed allelic differences between source and recipient populations, but at many loci surveyed thus far, Asian and North American moths do not show fixation for alternate alleles. The absence of fixed allelic differences can be due to any one of several processes, including historical gene flow, ancestral polymorphism, and convergence. Even in the absence of fixed allelic differences, however, suspect moths can be reliably typed to source population by examining multiple loci. Hybridization of Asian and North American moths would complicate typing; an F1 hybrid resulting from the cross of two pure parents (one Asian and one North American) has a multilocus genotype that would be found at equal frequencies in both populations. Asian loci immigrating to North America are initially completely linked, and are thus associated with each other in a non-random manner, relative to their frequency in the population (linkage disequilibrium). Recombination among Asian and North American loci can rapidly (as quickly as 10 generations) decay this disequilibrium, effectively masking the initial Asian introduction.

Microsatellites are a class of DNA markers that have great potential for discriminating Asian and North American populations of gypsy moths. Microsatellites are regions of simple sequence repeats, often flanked by unique-sequence DNA and are co-dominant, highly polymorphic (for repeat length) and assayed by the Polymerase Chain Reaction (PCR). Data from three microsatellite loci indicate that Russian moths (both Far East and Siberia) are more variable than their North American counterparts, with most of the variability found in multiple alleles not present in North America. Allele frequencies are used to assign suspect moths to their most probable source population. Two moths captured in the Pacific Northwest in 1991 were assigned with high confidence to North America and Russia, respectively, consistent with typings based on mitochondrial DNA (mtDNA). A third moth collected in Germany contained European (=North American) mtDNA, but the assignment based on microsatellites was more ambiguous, indicating that the ancestry of this moth may be complex, and not easily traced to

a source population. The real challenge may not only be in finding the geographic origin of suspect moths, but rather in correlating a suite of genetic markers with threatening ecological and behavioral traits, a process that would involve generating a detailed linkage map of the gypsy moth genome.

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

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ABSTRACT

An evaluation of the impact of an exotic herbivore, the gypsy moth, on selected forest ecosystems in the South was begun in 1991. Six permanent plots along the southern leading edge of moth movement were established and rated for susceptibility to gypsy moth. Two plots were selected in each of three geographical locations: the coastal plain (Currituck Co., NC and Northampton Co., VA), piedmont (Lake Anna State Park, Spotsylvania Co., VA), and mountains (George Washington National Forest, Amherst Co., VA). All plots are in mixed hardwood forests with large oak components. Each plot consists of three lines of 25 one-tenth acre stations and stations are 25m apart; the over-all plot dimensions are 75m by 625m. For later comparison, the understory and overstory vegetation were measured. Three sites (two at GWNF and one at Northampton) have sustained some gypsy moth defoliation within the last four years; the others have not.

Because small mammals, in particular the white footed mouse, *Peromyscus leucopus* Rafinesque, are believed to be major gypsy moth predators, we surveyed their populations within all plots from 1992-1995. We used Sherman traps baited with a peanut butter-oatmeal mixture and unbaited pit-fall traps. Two surveys were conducted (early and late summer) for the first three years, but only one was done in 1995. Mast production within these plots was measured and the current year's nut catch was a good predictor of next year's mouse population. Between population surveys or within one week of single surveys, predation was measured by offering freeze-dried gypsy moth pupae at each trapping station. Pupae were

offered at four locations per station: within the litter and at 0.25, 1.0 and 2.0m on four randomly selected trees. These data (1992-1995) suggest that when vertebrates are at high densities they are more successful in preying upon freeze-dried pupae than are invertebrates. However, invertebrate predation could be partially masked by the manner in which mammals feed on pupae. The large contribution made by invertebrates is nonetheless rather remarkable when one considers the short exposure (3 days) to these pupae. During 1992, predation was predominately by vertebrates and there was an inverse relationship between vertebrate and invertebrate predation. Total predation was almost unchanged from 1992 to 1993, even though mouse populations were low to moderate. The major difference in 1993 was the prominent role contributed by invertebrates. For 1994, total predation was much higher in the piedmont and mountains, but again most predation was by invertebrates. During 1995, the major predators in the piedmont and mountains were vertebrates but invertebrates were the major predators in the coastal plains. The significant predation within all geographical locations, even when small mammal population was low to moderate, suggests that there is a richness of predators along the southern edge of moth movement. This is unlike information from the northeastern US (Bryant Mountain, VT, Cape Cod and Western MA) where predation decreased as small mammal populations decreased.

To establish baseline information, and document changes in gypsy moth parasites over time, approximately 7,000 F1-sterile gypsy moth egg masses were released in the three geographical locations in 1993 and 1994. Following three instar collections (2nd and 3rd, 4th and 5th, and 5th through pupae) all insects were individually reared on an artificial diet and held for parasite emergence. The parasitoid diversity between the three habitats and large differences in parasites reared from year to year within each habitat underscores the importance of maintaining long-term studies in this area.

Larvae and pupae were collected and analyzed for NPV and *Entomophaga maimaiga* in the 1994 survey. The release of F1-sterile eggs appears to be a promising technique for surveying for the fungus along the leading edge of moth movement.

NATURAL ENEMY IMPACT ON GYPSY MOTH

IN ALSACE, FRANCE, DURING 1995

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ABSTRACT

Natural enemies of gypsy moth were obtained from 1,638 larvae and pupae collected in Alsace, France, at two sites where egg mass densities and the proportions of each genotype (European, Asian, and hybrid) had been evaluated before hatching. Stratified collections were made on three tree species at two sites, and larvae were reared on artificial diet. Total mortality by causes other than parasitoids, mainly nucleopolyhedrosis virus, was equally high at both sites and was not affected by tree species or height in trees. The complete guild of parasitoids had similar impact on host populations at both sites, whatever the tree species or the stratum of collection. These two factors did not affect parasitism by any of the major parasitoids. In contrast, the site factor led to important differences in parasitism of gypsy moth by different species.

Three hundred thirty-two larvae were collected over time and dissected to study superparasitism, multiparasitism, and hyperparasitism. Comparison of levels of parasitism by rearing hosts and dissecting samples showed that mortality by virus masked 30-60% of real rate of parasitism. In high host density, the relatively low abundance of *B. schineri* was aggravated by competition with *P. silvestris*. Impact by *P. silvestris* was considerable, and successfully complemented the incidence of virus. However, most hosts attacked by *P. silvestris* were hyperparasitized by *Perilampus* sp.

All parasitoids ensured a very effective control of the defoliator at both sites. If 1995 is the last year of outbreak in Alsace, as it is expected because of the obvious population collapse, we assume that the two promising tachinids for introduction into the USA against gypsy moth, *Blepharipa schineri* and *Ceranthia samarensis*, very likely will become relatively more abundant during next years in low host populations.

NOVEL STRUCTURE OF THE GYPSY MOTH VITELLOGENIN GENE

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ABSTRACT

Vitellogenins (Vg) are precursors of vitellins (Vt), major components of egg yolk proteins in many oviparous vertebrates and invertebrates, including insects. Vg and their genes have been characterized from a number of taxonomically diverse group of animals. Most Vgs are large proteins (400-600 kDa) consisting of a large (140-190 kDa) and a small (35-60 kDa) subunit. The Vg genes are large and specify a single transcript that codes for a previtellogenin molecule of ~200 kDa. The previtellogenin undergoes proteolytic cleavage to generate the large and small subunits. A notable feature of Vg genes characterized to date involves the arrangement of the subunit specifying regions in the previtellogenin. The small subunit coding region is located at the N-terminal portion, and that of the large subunit at the C-terminal portion. This and other conserved features suggest that the vertebrate and invertebrate Vg genes have evolved from a common ancestral gene.

We have characterized the gypsy moth Vg gene by determining the complete nucleotide sequence of the gene and the VgmRNA. The analysis showed that the gypsy moth Vg gene differs from other Vg genes significantly. Using the sequence information of a partial length cDNA clone and those of N-termini of Vg165 and Vg36, overlapping cDNA fragments were prepared through rTh polymerase-mediated polymerase chain reactions and 5'- and 3'-RACE reactions. Sequence determination of these fragments and that of a genomic clone containing the Vg gene yielded the complete nucleotide sequences of the mRNA and the gene. The Vg gene consisted of 7 exons interrupted by 6 introns. The N-terminus of Vg165 was located in exon 2 while that of Vg36 in exon 6. The VgmRNA consisted of 5,579 nucleotides without the poly(A) tail. A single translation initiation codon ATG was present at position 60-62. This was followed by a single open reading frame of 1,747 aminoacids.

The most distinctive feature of the gypsy moth Vg gene was the arrangement of subunit coding regions: it is opposite of what has been observed in other systems. The gypsy moth Vg is the first reported example, where the subunit encoding regions are "switched". However, there is sufficient similarity between the gypsy moth and other Vg genes to suggest that they are related. This raises an intriguing question, how and when did this "switch" occur during evolution. The recently reported Vg gene from *Bombyx mori*, also a member of the order Lepidoptera, has the structure conforming to that of other Vg genes. Information about Vg genes from different lepidopteran families will be helpful in elucidating the evolutionary

divergence. Although it is difficult to speculate how such a "switch" could have happened, or possibility involves "exon swapping", where a particular exon is moved to a different locatio within the same gene. Generally, these events are presumed to happen during gene duplicatio events. But, the gypsy moth genome contains a single copy of the Vg gene. More work on th gypsy moth and other lepidopteran Vg genes is necessary to answer some of these questions.

CLONING AND CHARACTERIZATION OF THE GYPSY MOTH *BT*CRYIAC RECEPTOR

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ABSTRACT

Bacillus thuringiensis (*Bt*) endotoxins are the most widely used microbial biopesticides for managing gypsy moth in North America. Concern about the adverse effects on non-target species has resulted in a great interest for the development of improved, more specific and effective *Bt* endotoxins.

Bt toxins act by altering the permeability of the gut membrane. After solubilization, the toxins bind specifically to receptors on the surface of cells lining the midgut. This interaction is requisite for the first phase of action of the toxins to elicit the toxicity. Type and number of these receptors appears to determine the specificity and effectiveness of the *Bt* toxin.

Recent work has identified an aminopeptidase (APN-1) present in the gypsy moth midgut that specifically binds *Bt*CryIAc toxin, suggesting that it may function as a receptor. The protein was purified to homogeneity and used to raise polyclonal antibodies in rabbits. The antibodies were used to isolate a cDNA clone encoding the APN-1. A cDNA expression library was constructed in bacteriophage vector lambda Ziplox using poly(A) RNA isolated from the gypsy moth midgut. The library was screened using the antibody, and clones cross-reacting with the antibody were purified by plaque purification. One of the clones was further characterized by determining the complete nucleotide sequence of its insert. The cDNA insert was ~4.3 kbp long, which is consistent with the large size of the APN-1 protein. A single large open reading frame was present encoding a protein of ~120 kDa. A polyadenylation sequence, AATAAA, at position 4245 preceded the poly(A) tail. In *in vitro* transcription/translation analysis, the clone coded for proteins as large as 100 kDa. We plan to express this cDNA through LdNPV for *in vitro* production of active *Bt* receptor in cell culture systems. Availability of active receptor will facilitate development of an *in vitro* technique to screen naturally occurring *Bt* toxins and recombinant *Bt* toxins (generated through mutagenesis) for identifying toxins with higher specificity and increased insecticidal activity.

THE GYPSY MOTH AND ITS ANTAGONISTS AT DIFFERENT POPULATION DENSITIES IN THE OAK FORESTS OF EASTERN AUSTRIA

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ABSTRACT

Gypsy moth populations of different densities and their antagonist complexes were studied in three mixed oak stands near Eisenstadt, Burgenland (Austria) from 1993 to 1995. Gypsy moth eggs, larvae, and pupae were collected stage specific, reared at the laboratory and checked for parasitoid emergence or death due to pathogens or unknown causes. Predators were observed during larval collections. In 1993 samples of gypsy moth pupae were taken to estimate the impact of carabid beetles. At the latency site (in 1995 also at the former gradation site) *Lymantria dispar* egg masses and young larvae were exposed.

In 1993, the parasitoid complexes at the sites with elevated and low gypsy moth densities differed significantly. *Parasetigena silvestris* (Robineau-Desvoidy) (Dipt., Tachinidae) was the dominant parasitoid at the gradation site. The rates of total parasitism were low at the gradation site during the culmination of the population in 1993, but increased in the postculmination year. An expected population increase at one site did not occur. This was probably in part due to the impact of tachinid flies, especially *P. silvestris*. Hosts that were exposed at the latency site were highly parasitized. *Glyptapanteles liparidis* (Bouché) (Hym., Braconidae) was the dominant species there. Total parasitism was higher than at the other sites. In 1994, when population densities generally had decreased to innocuous levels, there were no marked differences between the frequencies of the antagonists at the various sites. The importance of braconid species increased. In the following year, besides *G. liparidis* and *P. silvestris*, *Phobocampe spp.* (Hym., Ichneumonidae) were the dominant parasitoids of gypsy moth larvae.

During the study period also the following other parasitoids were recovered frequently: *Glyptapanteles porthetriae* (Muesebeck) (Hym., Braconidae), *Phobocampe pulchella* Thoms., *Phobocampe unicincta* (Gravenhorst) (Hym., Ichneumonidae) and *Blepharipa pratensis* (Meigen) (Dipt., Tachinidae). *Cotesia ocneriae* (Ivanov) (Hym., Braconidae) was recorded at the latency site for the first time in Austria. Interestingly, we found no parasitoids of gypsy moth eggs at all. *Calosoma sycophanta* L. (Col., Carabidae) was an important mortality factor of late instar gypsy moth larvae and pupae at the outbreak site in 1993. Nuclear polyhedrosis virus was the most frequently observed pathogen at all sites but no epizootic of the virus occurred.

NON-TARGET EVALUATION AND RELEASE OF TWO NON-INDIGENOUS TACHINIDS

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ABSTRACT

Two exotic, gypsy moth parasitoids, *Caranthia samarensis* (Villeneuve) and *Blepharipa schineri* (Mesnil) (Diptera: Tachinidae) have been imported from Europe to North America, but not yet been released in the United States. These species represent "New Directions in Biological Control of Gypsy Moth," in that they: 1) were collected from foreign, endemic (non-outbreak) populations of gypsy moth in Europe, and 2) appear to be restricted in their host ranges, thus minimizing risks to non-target species. Heretofore, gypsy moth natural enemies imported to North America had been collected from high to moderate populations of gypsy moth, largely due to the relative ease of collecting hosts and their associated natural enemies. Consequently, introduced natural enemies that established are most effective during the increase and outbreak phases of gypsy moth populations when damage has already occurred, but are less effective at maintaining non-outbreak populations. It is becoming increasingly clear that distinctive natural enemy complexes are associated with outbreak and endemic populations, and that biological control of gypsy moth should include natural enemies capable of attacking gypsy moth throughout all phases of its population cycle. *C. samarensis* and *B. schineri* are key species that attack low densities of gypsy moth in Europe. Once established, they would fill a niche vacant in North American gypsy moth populations, with the expectation of better population management and slower foreward invasion. These two parasitoids are in quarantine at Newark, Delaware ARS where studies are underway to provide the biological data (e.g., host specificity, ovipositional cues, etc.) needed for future permit requests for field release in the United States.

In the mid-1980s, Canadian Forest Service and the International Institute of Biological Control (IIBC) initiated foreign exploration of *C. samarensis* in France with subsequent importation to Canada. Sentinel gypsy moth larvae were used to collect this low-density species, where it was the dominant parasitoid in low-density populations, with up to 45% parasitism. Its good attributes as a biological control agent of gypsy moth include: 1) responds quickly and effectively to local increases, and 2) only known hosts are gypsy moth and *Orgyia recens*. In Quebec, F. W. Quednau refined a protocol for laboratory rearing and storage conditions that supported limited field releases of *C. samarensis*. This rearing technique exposed live gypsy moth hosts on oak seedlings. Following a 10-12 day post-mating gestation period, female flies ovolarviposit directly onto small larvae (2nd and 3rd instars) of gypsy moth by placing eggs onto dorsal hairs. Eggs hatch immediately and the new maggots bore through the host's integument to develop internally. Mature maggots exit from late instar hosts and drop to the soil where they form puparia. *C. samarensis* has a high incidence of diapausing pharate adults inside the puparium (~83-90% facultative diapause). V.G.N. "mass released" ($\geq$300 gravid females) *C. samarensis* in southeastern Ontario into 10x10x15 ft. cages (1992-1994) and free releases (1995). Field evaluations demonstrated that successful parasitism occurred yearly. Permanent establishment has not yet been confirmed. Additional research is warranted in areas of: 1) survival of puparia in cold storage, 2) overwintering field survival, and 3) host specificity.

In 1993, scientists in USDA APHIS began collaborating with Canadian Forest Service on rearing and release strategies for implementing *C. samarensis* in N. America. The host exposure method developed by Canada was very time-intensive. Therefore, W.C.K., then at APHIS PPQs Otis Laboratory in Cape Cod, Massachusetts, tested a method for removing eggs from uteri of *C. samarensis* and artificially implanting them onto gypsy moth larvae. This technique was successful (25% of eggs parasitized hosts and produced puparia) for mature eggs that contained larvae with well developed mandibles, but failed with less developed eggs. Average time from implantation to pupation was 12.4 days ($\pm$0.51). This technique may be a useful tool for supplementing rearing or for biological studies (e.g., those that require exact time of oviposition).

R.W.F. is conducting host specificity studies on *B. schineri* (1995-1996) and *C. samarensis* (1996). *B. schineri* is widely distributed in continental Europe, Russia, China and Japan. Females deposit numerous eggs on leaves, and a suitable host larva must ingest an egg for parasitism to occur. Mature maggots emerge from pupae or large larvae, then drop to the forest litter to pupate. This species has an obligatory diapause as pharate adults inside puparia. *B. schineri* attacks both the European and Asian strains of gypsy moth and is known from only two other hosts, *Endromis versicolora* and *Dendrolimus sibiricus*. Two types of host specificity tests were conducted for *B. schineri* in 1995: 1) cues for oviposition, and 2) oviposition choice tests. In cues for oviposition, *B. schineri* laid more eggs on leaves with tethered gypsy moth larvae than on leaves with previous gypsy moth feeding, simulated feeding, or untreated control leaves. In oviposition choice tests of several hosts on different host leaves, *B. schineri* laid more eggs on red oaks with gypsy moths than on non-oaks with gypsy moths , or on either red oak or non-oak with non-target lepidoptera. Although oviposition sometimes occurred on bouquets with novel larvae and/or host plants of novel larvae, no progeny of *B. schineri* developed. It appeared that attraction to the host was stronger than attraction to a particular host tree leaf. These two experiments will be repeated in 1996. When completed, if these host specificity tests indicate that *B. schineri* or *C. samarensis* do not represent a significant risk to non-target lepidopteran species in the forest ecosystem, we will request permits to release in the United States.

BIOLOGY, BEHAVIOR, AND SUSCEPTIBILITY TO MICROBIALS OF GYPSY MOTHS FROM AROUND THE WORLD

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ABSTRACT

Gypsy moths (*Lymantria dispar* (L.)) from 5 North American, 34 European, and 8 Asian populations were reared in the USDA Forest Service's Quarantine Laboratory in Ansonia, Connecticut, to compare their biology, behavior, and susceptibility to *Bacillus thuringiensis* (*Bt*). Eggs from all of the North American populations required more than 60 days at 5°C for eclosion; eggs from European and Asian populations varied in the percentage able to eclose after only 60 days at 5°C. No geographical pattern of requirements for hatch was obvious. Larval growth rate and adult size were compared for individuals reared on artificial diet at 25°C and 60% RH. Populations from the most northern latitudes grew faster and weighed less, while those from the most southern latitudes grew slower and weighed more. Larval color of North American populations consisted solely of gray or yellow-gray forms. Larvae from European populations were predominantly gray and yellow-gray, though black and/or yellow larvae also were present in most populations. The gray and yellow-gray color forms were present in all Asian populations sampled, but ~70% of the larvae from the 2 most eastern populations were yellow. Two laboratory techniques were used to assess female flight capability: a free-flight test and a body flip test. No females from the 5 North American populations sampled were able to glide or sustain flight and $<$ 10% were able to flip themselves upright with difficulty. No females from the Portuguese, Swiss, Croatian, Bulgarian, Austrian, or Slovakian populations, and no females from 4 of the 5 French populations could sustain flight; $<$ 20% were able to glide even a short distance or flip themselves upright. Females capable of sustained flight were found in all of the populations from Germany, Poland, Lithuania, Russia, China, and Japan, and from 1 of the 5 populations from France. These populations also had some females that glided or never flew (except 1 Russian population); $\geq$ 90% of the females were able to flip themselves upright quickly. The relative susceptibility of larvae to *Bt* from the populations was determined by comparing the LC_{50} for each population with that of the standard strain (NJSS). Larvae from the North Carolina, German, Polish, and Chinese populations were as susceptible as the NJSS, while those from Lithuania were more susceptible than the NJSS. Larvae from Russia, Bulgaria, France, and Japan were less susceptible than the NJSS. There was little variation within and between populations from North America. Populations from Europe and Asia demonstrated substantial within- and between-population variation, though Eurasian populations not geographically separated by mountain ranges tended to be more similar. Movement of gypsy moths from east to west may have occurred. This could account for part of the variation observed in populations from Europe.

PURIFIED AMINOPEPTIDASE-N FROM GYPSY MOTH BBMV IS A FUNCTIONAL RECEPTOR FOR *BACILLUS THURINGIENSIS* CRYIAC TOXIN

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ABSTRACT

We have evaluated the binding of *Bacillus thuringiensis* CryIAc toxin to its purified receptor from gyspy moth (*Lymantria dispar*). Purified aminopeptidase-N (APN) from gypsy moth BBMV bound specifically to CryIAc protoxin and toxin, whereas neither the structurally related CryI toxins nor CryII toxin and coleopteran specific CryIIIA bound to APN. *In vitro* binding of CryIAc toxin to BBMV was inhibited by competition with APN. Inhibition of short circuit current (Isc) for CryIAc, measured by voltage clamping of whole gypsy moth midgut, was blocked by adding phosphatidylinositol-specific phospholipase while the toxic effect of CryIAa toxin was not changed. Addition of APN to the lumen side of voltage clamp chamber also blocked inhibition of Isc for CryIAc toxin only. These data suggest that APN is the functional receptor for CryIAc toxin in gypsy moth larvae.

CONTROL OF LOW-DENSITY GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) POPULATIONS BY MATING DISRUPTION WITH APPLICATIONS OF CONTROLLED RELEASE FORMULATIONS OF PHEROMONE

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ABSTRACT

A four-year study (1990-1993) in Giles County, Virginia demonstrated that low-density populations of the gypsy moth, *Lymantria dispar* (L.), were effectively suppressed by annual applications of 75g of racemic disparlure/ha formulated in plastic laminate flakes. In three replicate plots (14-64 ha each), all measures of population density (numbers of larvae and pupae, male moth captures in pheromone-baited traps, mating success of monitor females, and numbers of fertile egg masses) were substantially suppressed by the single application of pheromone in each of the four years while populations increased in untreated surrounding areas. These tests also showed that, when plots were treated with 150g of pheromone/ha in 1990 only and then left untreated for the following three years, populations continued to the suppressed in 1991-93 in the 1990-treated plots as compared with the populations in the control plots.

Since the laminate flakes emitted only 27-40% of the applied pheromone dose during male moth flight, a formulation with a higher release rate was desired to better utilize the applied pheromone. In 1993, a new pheromone formulation of polymer beads (AgriSense, now Biosys) was developed which delivers pheromone at a higher rate than does the flake formulation. The efficacy of a single application of the flakes at 50g pheromone/ha was compared with two applications (2 wk apart) of beads, each at 15g pheromone/ha, on 14-52 ha plots (4 replicates of each). The degree of population suppression compared with the control was high and essentially the same with both the bead and flake formulations. In the year following treatment (1994), the populations remained lower in the 1993-treated plots as compared with the control plots. On the basis of these tests, it is concluded that use of

pheromone as a mating disruptant provides effective control of low-level gypsy moth populations. The release characteristics of additional formulations of pheromone (micro sponges from Biosys and micro chips from Shin Etsu) were evaluated in 1995 but these formulations were judged to be less effective than the flakes and beads.

HOW TASTY ARE GYPSY MOTHS TO SMALL MAMMAL PREDATORS?

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ABSTRACT

Predation by small mammals on gypsy moth pupae has been documented as a major source of mortality in low density gypsy moth populations in North America. There is compelling evidence that changes in the density of low density gypsy moth populations are directly related to predation levels, which are in turn directly related to predator abundance. Campbell and Sloan hypothesized that predation by small mammals regulated gypsy moth populations at low levels. However, Elkinton *et al.* reported that predation on gypsy moths was characterized more by a type II functional response, rather than a type III response that would be necessary for population regulation. Smith found that gypsy moth larvae and pupae typically represent a minor component of the diet of most small mammal species and that gypsy moths are apparently less highly preferred than many other competing food items present in forests. In order to obtain a clearer picture of the functional response of predators to gypsy moth densities (and their subsequent ability to regulate gypsy moth populations) it would be helpful to understand how patterns of predation on gypsy moths compare with other food items that might be more highly preferred. Predation on gypsy moth pupae was simultaneously compared with predation on sunflower seeds and meal worms at a site on the Powdermill Run Nature Reserve, Carnegie Museum, Westmoreland Co., PA. Gypsy moth populations were extremely low in this area such that egg masses could not be found. Each prey item was individually glued to small burlap squares and placed on the forest floor over three nights. The fate of each prey item was recorded daily. Three trials were conducted in the spring, mid-summer, and late summer. Each prey item was replicated 80 times in each trial and deployed in a transect with 10 m between each item.

The functional response of predators to gypsy moth pupae was compared with the response to sunflower seeds at a site on the West Virginia University Forest, Monongalia Co., WV. Gypsy moth populations were also very low. At this site, prey items were deployed at both high density and low density. The low density consisted of a transect of food items with 10 m between each item; each item was replicated 80 times. The high density consisted of four 20 x 20 m grids containing 225 prey items (density = 5,625 per ha) glued to burlap bands and placed on the forest floor. Within each grid, 25 prey items were labeled and checked daily for three days in order to determine their fate (dead or alive).

At the Powdermill site, predation on gypsy moths was considerably less than on either sunflower seeds or on mealworm pupae. Predation by vertebrates on all prey items appeared to be lowest during the September trial than either the May or June trials. This difference could be due to either changes in small mammal abundance or due to changes in the availability of alternate prey items.

When gypsy moths and sunflower seeds were deployed at low densities at the WVU Forest, predation on sunflower seeds was less than on gypsy moths. However, when prey items were deployed at high densities, predation on gypsy moth pupae decreased but predation on sunflower seeds increased. We interpret this as evidence of the more desirable nature of sunflower seeds compared with gypsy moth. Presumably predators were actively seeking out sunflower seeds as their densities increased but the capacity of predators to feed upon gypsy moth pupae was apparently satiated. Thus the functional response of predators to gypsy moth densities appears to follow that of Type II response but the functional response of predation to sunflower seeds is more characteristic of a Type III response. Therefore, it appears likely that these predators have the capability of regulating populations of some prey items but gypsy moths are probably too undesirable of a food item for regulation to occur. We hope to continue these experiments to determine if these hypotheses are true.

ASIAN GYPSY MOTH

EXCLUSION, SURVEY AND ERADICATION ACTIVITIES

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BACKGROUND

The Asian gypsy moth (AGM) is a serious pest which can devastate forests, woodlands, and residential landscapes. It is viewed to be potentially more destructive than the North American variety of gypsy moth (NAGM), which was introduced from Europe in 1869. Significant behavioral differences, which include the female's capability to fly, the broader host range of the larval stage, and reduced chill requirements for egg hatch combine with other attributes to greatly enhance the AGM's ability to become established and spread rapidly.

AGM was first introduced into the United States (US) in Washington and Oregon in 1991. The source of the infestation was determined to be vessels that had been infested with egg masses while visiting Russian Far East ports. Based upon the Declaration of Emergency issued by the Secretary of Agriculture, a $19 million eradication project was implemented and successfully completed in 1994. In addition to the activities directly connected to the eradication project, a monitoring and exclusion program was established to help prevent additional introductions.

In 1993 a second major introduction occurred when infested military cargo from a US Army NATO base in Southern Germany was unloaded in Wilmington, North Carolina. Until this introduction occurred, no one was aware that AGM had been established in Europe. A second eradication project was initiated, which is expected to cost approximately $9 million over a three- to four-year period.

CURRENT ACTIVITIES

USDA AGM Policy - Due to the significant behavioral differences observed between NAGM and AGM, it was determined that the policy and program guidelines in place for NAGM were not sufficient to effectively deal with the Asian strain. At the direction of the APHIS, PPQ Deputy Administrator, work on a new policy for AGM was started during the winter of 1993-94. Utilizing a great deal of cooperator input, a final policy document was accepted by the US Forest Service and released as Departmental Policy in the spring of 1995.

The USDA AGM Policy Statement is used to provide guidance on how to deal with any AGM introduction, regardless of where it may occur. A key aspect of the new policy is that AGM is considered to be an exotic economic pest that is not currently established in the US. Accordingly, APHIS will make every effort to exclude it from entering the country and serve as the lead federal agency in any efforts initiated to eradicate known infestations. APHIS will also continue to conduct multifaceted exclusionary activities, supported by detection surveys at high risk introduction sites. Provided that the source of a new infestation is known, eradication activities against AGM will be initiated even within the area of the US that is considered to be generally infested with NAGM. Outside of the generally infested area, eradication activities will be aggressively implemented against all AGM detections. Due to the potential for spread posed by flight capable AGM females, pretreatment delimiting trapping will not be conducted. Eradication treatments will commence the following spring after detections are made based upon the best information available. Extensive post treatment delimiting surveys will then be conducted to determine if additional treatments are necessary.

Military Preclearance Program - This program is part of APHIS's overall strategy to prevent the introduction of economically important exotic pests into the US. In addition to quarantine inspections conducted at all ports of entry, APHIS maintains "preclearance" operations at certain foreign sites where significant amounts of material are being exported to the US. The Military Preclearance Program in Germany targets the movement of military goods that may be infested with AGM. APHIS maintains a full time presence in Germany to train and coordinate military inspection activities. The goods inspected prior to movement to the US include all types of military cargo, trucks, vehicles and other heavy equipment, as well as the privately owned vehicles and outdoor household articles of military personnel.

The 1993 introduction into North Carolina occurred in part because of the unfortunate coincidence of two major events. The first was the 1993 outbreak of moth populations (which included a significant Asian presence) in Southern Germany. The second event was the "stand down" of US Army forces in Europe. Between 1993 and 1995 over 200,000 military personnel, as well as all supporting equipment, were transferred back to the US as part of an overall plan to reduce our NATO forces. A recent report from the APHIS representative in Germany indicates that the stand down has been completed and the moth outbreak is apparently over; he also added that we may soon find out if AGM is in Bosnia.

Russian Far East AGM Monitoring Project - This project is a cooperative program involving the Forest and Quarantine Services of both the US and Russia. It was designed and implemented in 1993 to track population levels of AGM and other *Lymantriid* species in and around the Russian Far East ports that are involved in direct commercial trade with North America. The data and information generated from this work is used in the APHIS vessel exclusion program to prevent AGM introductions from Russia. The original 1993 cooperative agreement was renewed several times, and funding, provided mainly by the Forest Service, is in place to support the project through 1996. The project is expected to be continued through the next outbreak of the moth population cycle. Currently, populations are still low in the port areas but increases in trap catches in nearby forests indicate that populations are building. The Russians predict a full blown outbreak within the next three to four years.

APHIS Vessel Exclusion Program - The purpose of this program is to prevent the artificial spread of AGM from Russian Far East ports to North America. Based upon the information collected in the Russian Far East Project, APHIS inspects vessels that are considered to be at risk for AGM introductions. The risk occurs when AGM females lay egg masses on the ship superstructures while they are docked at Russian ports. Subsequently, larval hatch and blow may occur when the same vessels are docked at US ports. The 1995 AGM flight period lasted from July 15 through September 15 in the Russian Far East port areas. A list of vessels which called at these ports during this period has been developed using data from Lloyd's database MARDATA, the U. S. Coast Guard, the State Plant Quarantine Service of Russia and Agriculture Canada.

APHIS has asked shipping interests not to charter ships that called at Russian Far East ports during the egg laying period for voyages that would put the vessels in US or Canadian ports during the high risk egg hatching period (March to July). Any high risk vessel that arrives during this period will be boarded at sea and inspected prior to docking. If it is found to be infested it will be ordered to leave US waters immediately. Although APHIS has no regulation prohibiting the entry of AGM high risk vessels, the Plant Pest Act grants the authority to order infested vessels to leave US waters. Outside of the hatch period, inspections are done after the ships dock. A vessel will be prohibited from docking at all US ports if it is determined to be infested until after the expected hatch period ends. Agriculture Canada enforces a similar inspection and exclusion program.

APHIS AGM Port Survey - In addition to the risk posed by vessels coming directly from Russian ports, cargo and shipping containers from Europe may also be infested. Due to the large volume of traffic and the difficulty to effectively track the movement of all containers, APHIS is trapping likely sites of introduction to provide for early detection of new infestations. The focus of the survey includes ports, inland waterways and military bases that receive cargo or are visited by high risk vessels from AGM infested areas.

As part of the 1992 AGM eradication project in the Pacific Northwest, PPQ implemented a nationwide survey at all ports and high risk waterways in an effort to detect other AGM introductions. Trapping levels varied on the basis of whether the port was located inside or outside of the generally infested area and whether it had been exposed to ships that had visited high risk Russian ports during moth flight periods. Trapping densities ranged from 4-9 traps per square mile within a 5-mile radius of the ports. Black light traps were also utilized at ports within the generally infested area.

Based on this special survey, the APHIS AGM Port Survey is now conducted on an annual basis in order to provide a means of detecting exotic moth introductions in areas where we do not normally have coverage under the GM National Survey Plan. While this survey does not provide enough information to track introductions to individual containers or shipments, it will help to prevent the establishment of isolated infestations in areas where dispersal of goods to inland locations could occur in subsequent years. While local conditions are considered, the main trapping period will last from June 1 to October 1, with a minimum of 40 traps per port, deployed within a 3-mile radius of the port. Ports with a history of AGM interceptions warrant a higher level of detection monitoring and are trapped at a density of 9 traps per square mile within a 1-mile radius of dock area (28 traps) and 4 traps per square mile out to a radius of 5 miles (302 traps). All moths caught in the port survey traps are sent to Otis Methods Center for DNA analysis.

North Carolina AGM Eradication Project - 1995 project activities included the treatment of three sites for AGM in North Carolina, and one for NAGM in South Carolina. A total of 6,000 acres was sprayed twice using aerial applications of *Bacillus thuringiensis* (*Bt*). The 3-acre core area of the South Carolina infestation was also treated with two ground applications of Dimilin. Extensive post treatment delimiting surveys covering almost 2,000 square miles were conducted to measure the efficacy of the treatments and determine if any other AGM were in the area. These surveys were conducted at a density of 25 traps per square mile. Both North and South Carolina participated in the project.

The 1995 survey program resulted in the capture of over 140 moths. All of the catches were sent to Otis Plant Methods Center for DNA analysis; only three tested positive for AGM. Two of the Central Siberian AGM were caught at the dock area of the Sunny Point Marine Terminal and the third one was found near the mouth of the Cape Fear River. Analysis of the catch locations and recent survey history has led to the speculation that these catches probably represent new introductions that occurred after 1994 treatment and survey operations were completed. Each AGM catch site will serve as the center of a 640-acre treatment block that will receive two to three applications of *Bt* in the spring of 1996. Due to several years of negative survey for AGM in most of the project area, the 1996 delimitation survey will be reduced by over 50 percent to include the 900 square miles found in the eastern and southern portions of the original block.

OUTLOOK

All of the above mentioned activities are part of APHIS's multifaceted program to prevent new introductions of AGM and eradicate all known infestations as they occur. APHIS management recognizes the importance of this pest and will continue to support activities to prevent its establishment in the US. However, ever increasing demands on limited resources may require the development of new partnerships and innovative methods to continue an effective battle against AGM. Cooperation with all concerned parties will be needed for success.

EUROPEAN PERSPECTIVE ON EXOTIC FOREST PESTS

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ABSTRACT

The European and Mediterranean Plant Protection Organization (EPPO) was founded in 1951 by the countries of Europe to try to limit the spread of established pests and prevent the introduction of potentially dangerous exotic pests by means of international cooperation. Different Panels of experts have been created for different subject areas and the Panel on Phytosanitary Problems in Forestry is the one that deals with all aspects of plant quarantine in forestry. The Panel is composed of 10 forestry experts (entomologists and pathologists) from 10 different countries who meet usually once a year. In practice, its role is to: determine which pests could (if introduced or spread) be of economic importance; perform pest risk assessment on these pests to decide if they should be quarantine pests; and prepare management options to prevent the introduction of the pest from all likely pathways. Access to reliable information on the individual pests remains a problem, especially as they are generally pests not present in Europe and, therefore, not familiar to European scientists. Broader cooperation among different regions of the world could help to solve this problem.

Gypsy moth is an example of a quarantine subject recently discussed by the EPPO Forestry Panel. Because of the very severe outbreak in Germany in the early 1990s, with characteristics which suggested the possibility that there may have been an introduction of a different strain of the insect, the Panel needed to decide whether measures should be taken by surrounding countries to limit the spread of this strain.

The German outbreak followed several years with exceptionally high temperatures and low rainfall which would favor a more serious outbreak. The other "Asian" characteristics noted in Germany (wider host range, morphological variation and female flight) have often been recorded in the literature for European populations, and more frequently observed during major population explosions. The conclusion of the EPPO Forestry Panel was, therefore, that there did not appear to be any change in the gypsy moth situation in Europe and, thus, no need to take phytosanitary precautions to prevent spread. Results with RAPD PCR cluster analysis in Germany confirmed that the interrelationship between central European populations had not been disrupted by introduction of Asian genotypes.

It seems that palearctic populations possess a much wider range of genetic variability than American populations and, therefore, one should not refer to "Asian" and "European" gypsy

moth, but recognize that American populations differ from the Euro-Asian populations in lacking certain genetic features. EPPO would not, therefore, support the naming of the "Asian gypsy moth" as a quarantine pest, since it is not yet clear that such a thing exists but, more so, because quarantine pests should only be scientifically recognized entities.

HEMLOCK WOOLLY ADELGID: HISTORY, BIOLOGY, AND CONTROL

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ABSTRACT

The hemlock woolly adelgid, *Adelges tsugae* Annand, is a serious threat to hemlocks in eastern North America, *Tsuga canadensis* and *T. caroliniana*. This adelgid was first described from collections made in northern California in 1922. In 1937 it was found in Japan and Formosa. It is likely native to eastern Asia where it is distributed widely. In 1951 it was discovered in Richmond, Virginia, and now is distributed along the East Coast from North Carolina to Massachusetts and as far west as West Virginia.

The hemlock woolly adelgid is not considered a pest in Asia or on the U.S. West Coast, where a combination of tree resistance and natural enemies is believed to keep the adelgid below damaging thresholds. On the East Coast, populations build rapidly following infestation and cause slow deterioration of the crown. Tree death often occurs after 4 or more years of infestation. The adelgid population can be controlled on individual trees by thoroughly drenching foliage with horticultural oils, soaps, and other chemical pesticides, and by systemic pesticides applied to the soil.

Biological control is the most feasible option for forest situations. Adelgids have no known parasites, but several predators feed on them. In eastern North America, the previously introduced coccinellid, *Scymnus suturalis* Thunberg, and the native derodontid beetle, *Laricobius rubidis* LeConte, are the most common predators of *A. tsugae*. In Japan, an orbatid mite, *Diapterobates humeralis* (Hermann), and a coccinellid, *Pseudoscymnus* n. sp., are enemies of the adelgid; they were released in Connecticut recently. Several predators of the adelgid have been observed in China and Taiwan, and there are plans to assess their potential as biological controls of *A. tsugae* in eastern North America.

PERFORMANCE OF GYPSY MOTH FROM ASIA AND NORTH AMERICA AND THEIR HYBRID CROSSES ON HOST PLANTS

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ABSTRACT

My previous studies concentrated on the performance of newly hatched gypsy moth larvae because this stage is most sensitive to host suitability. These tests compared populations from central Siberia and Massachusetts on more than 50 tree species. The Asian population had higher survival and weighed more after 10 days than the North American population. Generally, if the survival of one population on a particular host was high (>85%), survival of the other population also was high. Conversely, if one population had poor survival (<10%), survival of the other population also was low on that host. On intermediate hosts such as *Pseudotsuga menziesii, Robinia pseudoacacia, Acer rubrum,* and *Prunus serotina*, survival of the Asian population was much higher than the North American population. Asian larvae were larger than North American larvae on all but one of the host species.

This year, larvae were reared from hatch to pupation on host plants. There were four populations: North American from the Appalachian Mountains near the North Carolina-Virginia border; Asian from the Russian Far East near Mineralni; and two reciprocal hybrid crosses between the populations. The parents of these four populations had been reared on artificial diet. The hosts were *Quercus rubra, Betula populifolia, A. rubra,* and *P. menziesii.* Measurements were made at 7-day intervals and at pupation. The data show that 7-day-old Asian and hybrid larvae were larger than their North American counterparts. Similar differences in size were observed at 14, 21, and 28 days. The weight of Asian and hybrid pupae generally was greater than the North American, though the differences were not as great or as consistent as observed for larvae. Larval development was more rapid for Asian and hybrid populations. For example, the mean time for the female larval stage was 43 *vs.* 40 days on oak, and 69 *vs.* 57 days on maple for the North American and Asian populations, respectively. The hybrids resembled the Asian parent more than the North American parent in growth rate, but were more variable than either parent population. In summary, Asian populations of the gypsy moth survive and grow better than the gypsy moth now established in North America. Differences are most apparent on host species that are marginal. Performance of hybrids between the Asian and North American gypsy moth is more similar to the Asian than the North American population.

DYNAMICS OF TWOLINED CHESTNUT BORER IN FORESTS DEFOLIATED BY GYPSY MOTH

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ABSTRACT

Abundance data for *Agrilus bilineatus* (twolined chestnut borer [TLCB]) were collected in 1989, 1991, 1992, 1993, and 1994. This period of time covers an outbreak of gypsy moth in West Virginia. Also, several stands were thinned prior to the defoliation. Populations of twolined chestnut borer escalated in 1991, following defoliation in 1990. The following year defoliation continued although at a reduced level, but TLCB increased substantially from the previous year. TLCB abundance related significantly with percent defoliation of oaks and this relationship improved with the second year of defoliation. Considerable within stand variation exists in this relationship however; on a plot level basis there is not a direct correspondence between defoliation and TLCB. Thinning did not appear to have a consistent effect on TLCB, and defoliation represented the controlling factor in determining TLCB density.

EXOTIC WEEDS IN THE EASTERN UNITED STATES

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ABSTRACT

Plant introductions into North America have been noted since the late 18th century, and the total number of exotic plants now rivals that of insects. Some states in the coterminous 48 have as much as 30% non-indigenous plant species in their inventories. Little has been done to alter the trend of increasing numbers of invasive plants. On the contrary, State natural resource agencies or game commissions have contributed substantially to the problem by their encouragement of non-native plantings. Exotic plants have been shown to outcompete native species, and can have adverse effects on species of limited distribution, or rare and endangered plants. Furthermore, the integrity of natural areas can be substantially modified by exotic plants. Traits that are characteristic of invaders include a high allocation to reproductive output, long range seed dispersal, and broad ecological amplitude. Habitats that are prone to invasion include grasslands, fragmented forests, or any highly disturbed ecosystem. Among the vulnerable habitats, i.e. areas where exotic plants may create problems are unique areas and areas with high endemism. Some functional properties that are modified by exotic plants range from primary productivity to alterations in food web structure to complete changes in disturbance regimes.

EFFECTS OF GYPSY MOTH DEFOLIATION AND FOREST THINNING ON GROUND-DWELLING ARTHROPODS

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ABSTRACT

From 1989 to 1992 pitfall traps were used to monitor populations of terricolous arthropod fauna: spiders, carabids, ants and phalangids (opiliones). During that time, gypsy moth (*Lymantria dispar* L.) populations increased, causing severe defoliation and considerable mortality. Also, several areas were silviculturally thinned in an attempt to reduce stand level susceptibility and vulnerability to the gypsy moth, allowing for contrast of the two disturbance types. In most cases, both defoliation and thinning appeared to evoke a similar response. The effect of canopy-opening disturbance was noticeable for ants and carabids - total abundance decreased but diversity increased. For spiders and phalangids, the effect of either defoliation or thinning was dampened by natural variation in the populations. Thinning, however, increased the abundance of phalangids, but the effect was evident only one year.

RESPONSE OF HERBACEOUS VEGETATION TO GYPSY MOTH AND SILVICULTURAL TREATMENTS

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ABSTRACT

While the effects of disturbance such as insect defoliation have been well documented in terms of overstory mortality and woody species regeneration, little is known about such disturbance effects on herbaceous flora, or non-commercial woody species. Since ground vegetation is often more sensitive to site and environmental conditions, such information may be valuable as indicators of forest resilience and extent of forest change as well as understanding successional development. To this end, we have studied vegetation in the lower structural layer for several years on oak / mixed hardwood stands that had been defoliated by gypsy moth (*Lymantria dispar* L.) in 1990 and 1991, and/or silviculturally thinned in 1990. There were also control stands with neither thinning nor defoliation. We sampled herbaceous vegetation in 1992, 1993 and 1995. Estimates of other ground cover species such as *Rubus*, ferns, and grasses were determined in 1989, 1990, 1991, 1992, 1994, and 1996. Increases in species richness were evident in thinned stands. Using detrended correspondence to examine herbaceous vegetation patterns, we found that defoliation has a greater influence than thinning in separation of stands. However, the separation was greatest on DCA axis 2, suggesting that factors other than disturbance more strongly control the herbaceous vegetation. Site factors and original overstory composition tend to determine the composition of the ground vegetation. Pronounced changes in coverage of some species occurred on stands that were defoliated, with significant increases in ferns and grasses, such that woody regeneration may be inhibited.

GYPCHEK: RESEARCH AND DEVELOPMENT TOWARD COMMERCIALIZATION AND OPERATIONAL USE

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ABSTRACT

Gypchek, the gypsy moth nucleopolyhedrosis virus (NPV) product, is not yet in commercial production because it is not cost-competitive with *Bacillus thuringiensis* products or diflubenzuron products for gypsy moth control. The prohibitive expense of *in vivo* production has been the biggest roadblock to the development of a commercial product. Over the past decade, modest improvements have been made through production line and process engineering, but the cost of producing NPV for a 1-acre treatment (4 X 10^{11} occlusion bodies) remains close to $10.00. Several genotypic isolates from Gypchek have shown properties of enhanced potency and speed of kill and may be candidates for *in vivo* production. Eventually, a commercial product that minimizes microbial contaminants and is easier to control than *in vivo* production will spring from *in vitro* production. Recent research has advanced the development of a serum-free medium, a stable gypsy moth cell line amenable to large-scale production, and a viral genotype capable of continuous culture minus a high frequency of low potency, few-polyhedra mutants. After a 2-year hiatus in the development of a commercial gypsy moth virus product, American Cyanamid Company is ready to scale-up an *in vitro* production of a gypsy moth virus and formulate a product for a small-scale ground test in 1996. We hope this will lead to an aerial test in 1997 and the marketing of a commercial product shortly thereafter.

FIELD-TESTING A SPRAY ADJUVANT FOR GYPCHEK

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ABSTRACT

A ready-to-use spray adjuvant (Carrier 038®) for Gypchek has been developed through collaborative research with Novo Nordisk. The adjuvant (now an Abbott Labs. product) is superior to the standard USDA Forest Service lignosulfonate-molasses formulation in ease of mixing and spraying, and also provides enhanced protection of the virus from sunlight. In an aerial application trial, we evaluated a double application of a Carrier 038 formulation of Gypchek, a double application at a reduced volume, a single application, and a double application of the standard USDA Forest Service formulation against dense larval populations of the gypsy moth in Virginia. Thirty 4-ha plots that supported dense gypsy moth populations were treated., Six replicates of each of five treatments were assigned in a randomized block design: Control (no spray), STD (Double application of the USDA Forest Service formulation at 5×10^{11} viral occlusion bodies [OB] in 19 liters/ha), 038 (double application of 5×10^{11} OB in 9.5 liters/ha), 038/2 (double application of 5×10^{11} OB in 4.8 liters/ha), and 038/1AP (single application of 1×10^{12} OB in 9.5 liters/ha). Treatment evaluations showed that there were no significant differences ($P > 0.05$) in NPV mortality in larvae collected from either the STD treatment plots (80%), the 038 treatment plots (86%) or the 038/2 treatment plots (83%). However, the single application treatment (68%) was significantly lower than the other 038 treatments. All Gypchek treatments were significantly different from the control (19%). Defoliation differences (18-23%) between Gypchek treatments were not significant ($P > 0.05$), but all were significantly lower ($P < 0.05$) than the control (37%). Similarly, numbers of surviving larvae were not significantly different ($P > 0.05$) between Gypchek treatments but were significantly different ($P < 0.05$) from the control. These results indicate that a Carrier 038 formulation of Gypchek applied twice at either 9.5 liter/ha (1 gallon/acre) or 4.8 liters/ha (0.5 gallon/acre) provides a level of efficacy comparable to the standard formulation applied twice at 19 liters/ha (2 gallons/acre). Thus, Carrier 038 is an attractive alternative to the latter for use in gypsy moth management programs.

CURRENT STATUS OF INSECT GROWTH REGULATORS FOR GYPSY MOTH

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ABSTRACT

Two ecdysis-targeted insect growth regulators, Diflubenzuron and Tebufenozide, are currently being used to manage low to high density populations of gypsy moth. Diflubenzuron is registered for forestry use by the US-EPA whereas Tebufenozide is applied to forests in the US using an experimental use permit.

Diflubenzuron (Dimilin 25 and Dimilin 4L) is marketed by Uniroyal and has been aerially applied to approximately 4.5 million acres as part of the Federal and State cooperative gypsy moth suppression program. The maximum dose is 1.0 oz AI/acre/year applied as 1 or 2 applications. Diflubenzuron is consistently efficacious insecticide for managing gypsy moth. An evaluation of potential impacts to selected non-target organisms on entire broadleaf watersheds was recently completed on the Fernow Experimental Forest, Parsons, West Virginia. Pretreatment (1989, 1990, 1991), treatment (1992) and posttreatment (1993, 1994) monitoring was conducted for 5 groups of non-target organisms: (1) fungi, bacteria and invertebrates in leaf litter and soil, (2) aquatic macroinvertebrates and fungi, (3) pollinating insects, (4) aquatic and terrestrial salamanders, and (5) canopy arthropods. In general, few and previously documented negative impacts were detected for two species of mites, several species of mayflies and stoneflies, yellowjacket workers, and macrolepidopteran larvae. Residue levels were not detected in soil and stream samples, persisted on foliage samples with approximately 30% remaining at leaf fall and persisted in litter samples over winter months and declined during the 2nd season.

Tebufenozide (Mimic 2F and Mimic 240 LV) is marketed by Rohm and Haas and has been evaluated for efficacy on small replicated plots established in Ohio (1994, 1995) and in Virginia (1995). The maximum dose is 0.09 lb AI/acre/year applied once. Tebufenozide evaluations of non-target organisms indicate negative impacts to several species of macrolepidopteran larvae. Preliminary studies suggest that residue profiles for Tebufenozide and Diflubenzuron are similar.

GENETIC STRUCTURE OF *COMPSILURA CONCINNATA* POPULATIONS IN THE NORTHEASTERN UNITED STATES

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ABSTRACT

Biological control strategies against exotic lymantriid defoliators of forests in the northeastern United States have been implemented without an evaluation of the genetic background of the natural enemies introduced. After population outbreaks of *Lymantria dispar* (L.) and *Euproctis chrysorrhoea* (L.) in the early 1900's, the tachinid *Compsilura concinnata* (Meigen), collected from *E. chrysorrhoea* larvae from eastern Europe, was released in the northeastern United States in 1906 and established within 4 years.

We examined the genetic structure of *C. concinnata* using an electrophoretic analysis of their allozymes in populations from different geographic regions in the Northeast. The populations in New Jersey, Pennsylvania, New York, Maine, Connecticut, and Michigan were sampled by collecting *L. dispar* host larvae placed as trap-hosts. Evaluating the genetic variability at 37 enzyme loci in this tachinid, we found seven polymorphic genes, namely at *b*-esterase, hexoseaminidase, and phosphoglucomutase enzymes loci.

The samples demonstrated no major differences in allozyme types and only slight variability in allele frequencies. Low genetic variability within populations and between sites reflected the homogeneity on genotypes present in the founding populations. Differences were found at protein loci between sites, but these differences could not be attributed to any distinguishing geographical characteristic of the sites. Monitoring populations of the natural enemies of pests is one component in the evaluation of biological control programs. Analyses of the genetic structure of non-native natural enemies also aids identification of genetically differentiable forms, reflecting the evolution of biological types.

MANAGING FORESTED ECOSYSTEMS IMPACTED BY HEMLOCK WOOLLY ADELGID

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand -- an exotic pest native to Japan and other parts of Asia -- was first noted in Connecticut in 1985 (Lapin and ODell, in preparation[1]). It is a small, aphid-like insect with long, piercing-sucking mouthparts. It inserts its mouthparts into the base of needles on eastern hemlock, *Tsuga canadensis* Carriere, and feeds on fluids derived from storage cells in the xylem ray tissue (Young *et al.* 1995). In addition to the specific damage that is caused by adelgid feeding, the weakened state of infested trees may render them more susceptible to attack by other insects, to attack by secondary organisms, and to abiotic stresses such as drought. Adelgid infestation, perhaps in combination with other stresses, is causing the decline and death of large areas of hemlock forest in the northeastern United States. It is destroying the hemlock trees that the gypsy moth, *Lymantria dispar* L., left behind.

Eastern hemlock is one of the most shade-tolerant trees in North America. It is found predominantly in riparian areas and in rocky ravines. It is a component of old-growth forests, it is dispersed throughout forests, and it is a popular and irreplaceable ornamental species. The scattered distribution of hemlock coupled with its presence in a great variety of habitats provides diversity on a landscape scale that will be lost if hemlocks are destroyed. Changes in forest ecosystems due to hemlock replacement are difficult to predict. With the rapid impact of the HWA, there is considerable concern that eastern hemlock will disappear from large areas of its former range. Because of all of these concerns, forest pest specialists and land managers are being deluged with requests for preferred management practices to minimize the impacts of HWA.

Our work has focused on the Lower Connecticut River Valley, an area of approximately 300,000 acres, and the site of the initial HWA infestation along the Connecticut River. The

[1] Most of the information presented in this report will be published in, "Ecosystem management in the Lower Connecticut River Valley: Impact of hemlock woolly adelgid on associated ecosystems," in preparation by B. Lapin and T. ODell as a General Technical Report, USDA Forest Service, Northeastern Forest Experiment Station.

entire basin encompasses 7.2 million acres and is located in four states. Eastern hemlock is an important component of this ecosystem. A cooperative project was established with the ultimate goal of protecting and restoring areas in the Lower Connecticut River Valley that are impacted by HWA. Specific objectives are to develop: experience-based best management practices for ecosystems impacted by HWA; knowledge necessary to understand the distribution and abundance of HWA at the landscape level of ecological integration; and science-based management technology for protecting and restoring forest ecosystems impacted by HWA. Many partners and cooperators are working to achieve these objectives.

The USDA Forest Service, Northeastern Forest Experiment Station, held a workshop in November 1994 to develop and consolidate information about suitable management practices for HWA. Natural resource experts, stakeholders, and landowners described desired future conditions that currently are provided by the presence of eastern hemlock at specific types of sites. These desired future conditions were critical for evaluating and selecting best management practices geared for aquatic and terrestrial systems, and to achieve specific management objectives. This information was summarized and consolidated into a management practices matrix for use by professional land managers. However, the information contained in the matrix easily can be transcribed into a variety of formats suited for specific audiences.

Along with our partners and cooperators, we are continuing to monitor and evaluate impacts of HWA on ecosystems and to further develop preferred management strategies for HWA.

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IDENTIFICATION OF A BACULOVIRUS POLYHEDRON FORMATION MUTANT WITH A NOVEL PHENOTYPE

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ABSTRACT

Once the processes of polyhedron formation and virion occlusion are understood, a means of manipulating these processes may be devised in order to generate viral strains with enhanced polyhedron formation and efficacy properties. We have identified and characterized at the organismal level several *Lymantria dispar* nuclear polyhedrosis virus (Ld*M*NPV) polyhedron formation mutants as a means of increasing our understanding of the processes of polyhedron formation and virion occlusion. One of those viral strains, Ld*M*NPV isolate PFM-S is described in this abstract. The attributes of budded virus synthesis, polyhedron production, polyhedron size, virion occlusion, and virion encapsulation exhibited by Ld*M*NPV isolate PFM-S were characterized. These attributes were also characterized for Ld*M*NPV isolates A21-MPV (a wild type polyhedron formation virus) and FP-A21-2 (a few polyhedra mutant), and compared to the attributes exhibited by isolate PFM-S.

Cross sections of polyhedra produced by isolate PFM-S were analyzed by electron microscopy and compared to polyhedra cross sections of wild type polyhedra and polyhedra generated by an FP mutant. Isolate PFM-S polyhedra were found to lack viral particles. However, in contrast to polyhedra generated by FP mutants and wild type virus a small percentage of isolate PFM-S polyhedra were encapsulated by a diffuse electron dense structure. Isolate PFM-S exhibited a budded virus $TCID_{50}$/ml of media of approximately 1.6×10^7. The amount of budded virus produced by isolate PFM-S was significantly greater than the amount produced by isolate A21-MPV, and was similar to the amount produced by a few polyhedra (FP) mutant.

Isolate PFM-S produced significantly fewer polyhedra per flask compared to wild type virus (approximately 3.5×10^7 and 1.2×10^8, respectively), but significantly more than an FP mutant (approximately 5×10^6). Isolate PFM-S produced an average of 70 polyhedra per cell. In contrast, the FP mutant A21-2 produced an average of only 6 polyhedra per cell. Polyhedra generated by isolate PFM-S were significantly smaller (average diameter of 1.1 μm) than polyhedra generated by wild type virus (average diameter of 2.1μm). Similar to FP mutants, isolate PFM-S was found to occlude very few viral particles into polyhedra. An average of

0.2 virions were present per μm^2 of polyhedra cross-section area in polyhedra produced by isolate PFM-S. The results of this study indicate that the phenotype of isolate PFM-S is unique in comparison to previously described baculovirus polyhedron formation mutants.

COMPREHENSIVE PROGRAM TO EVALUATE EXOTIC MICROSPORIDIA FOR CLASSICAL BIOLOGICAL CONTROL OF THE GYPSY MOTH

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ABSTRACT

Microsporidia (Phylum Microspora), obligately parasitic protozoa, are reported to be widely distributed in gypsy moth populations throughout Europe but have never been recorded from gypsy moth populations in North America. The cooperative projects described here are intended to assess the suitability of microsporidia as biological control agents of the gypsy moth in North America.

Foreign Exploration: In 1985, microsporidia were isolated from gypsy moths collected in Czechoslovakia and Portugal. During the years 1993-95, microsporidia were isolated from gypsy moths collected in Austria, Bulgaria, Romania, and Slovakia. No microsporidia were found in large numbers of gypsy moths collected in Siberia.

Taxonomy: Our foreign exploration activities for gypsy moth microsporidia have produced 11 distinct isolates, which we believe represent at least five species of microsporidia. We have

completed ultrastructural and life cycle studies on eight isolates and are in the process of completing rRNA sequences for all of the isolates. We are describing one of our isolates as a new species and, after some taxonomic revisions, we will characterize others as previously described species.

Modeling: The mathematical model of the epizootiology of the Portugal isolate of *Microsporidium* sp. is based on laboratory work carried out in Illinois and Michigan. We plan to test two models, one without canopy spatial dynamics and one that includes the movement of larvae and spores. Field data from Maryland and Michigan will be used to test the models.

Host Specificity: We are currently evaluating five isolates of microsporidia (probably different species), recovered from European populations of the gypsy moth, for release in North America. Our current studies indicate that many, if not most, infections that can be initiated in nontarget hosts in the laboratory are not necessarily typical of those in the natural host, and are not transmitted horizontally in the nontarget insects.

Field Release Studies in Slovakia: Because we currently do not have permission from regulatory agencies to release microsporidia permanently into North American gypsy moth populations, our information on the variables affecting the development of epizootics and the spread of microsporidia in large field populations of the gypsy moth must be obtained directly from studies in Europe. In small scale field evaluations of microsporidia in Eastern Slovakia, microsporidia applied to a 40 x 40 m plot spread to gypsy moths collected in the first 25 m area on two sides of the plot.

Field Release Studies in the United States: In 1992 and 1993, we released laboratory-reared gypsy moth egg masses into several small isolated field plots after contaminating some of the masses with spores of *Microsporidium* sp. Portugal isolate. Infection rates were low so we have studied the impact of UV light on contaminated egg masses, and a new technique for contaminating feral egg masses with spores sprayed with an air brush. We have also studied the host range in the field of the Portugal isolate of *Microsporidium* sp.

BACULOVIRUS HOST RANGE DETERMINANTS: IDENTIFICATION AND CHARACTERIZATION OF A HOST RANGE FACTOR FROM GYPSY MOTH NPV

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ABSTRACT

A major advantage of using baculoviruses for gypsy moth control is host specificity. Unlike *Bacillus thuringiensis* toxins, which can kill a variety of species, *Lymantria dispar* M nuclear polyhedrosis virus (LdMNPV) affects only gypsy moths. This makes it particularly attractive for use in environmentally sensitive areas. However little is known about the underlying mechanisms that determine its host specificity.

Work from Ed Dougherty's laboratory at the USDA-ARS in Beltsville, MD, demonstrated that LdMNPV could provide a helper function that allowed AcMNPV, to replicate in a gypsy moth cell line, IPLB-Ld652Y, that would not normally support its replication (McClintock, J. T. and E.M. Dougherty. 1987. Virus Res. 7:351-364). This suggested that we might be able to use this system as a means to identify a host range gene from LdMNPV.

Baculovirus infections can be initiated in cell culture by transfecting cells with naked virus DNA. To see if it might be possible to map the LdMNPV host range gene by transfection assay we tested the ability of transfected AcMNPV DNA to infect Ld652Y cells and LdMNPV DNA to provide the helper function. Transfected AcMNPV DNA could not initiate an infection in these cells. However when co-transfected LdMNPV DNA, AcMNPV was able to replicate, indicating that transfected LdMNPV was able to provide the helper function. This allowed us to use cloned pieces of LdMNPV genomic DNA in co-transfection assays with AcMNPV DNA to map the LdMNPV host range gene. The smallest LdMNPV clone that promoted AcMNPV replication was an 835 bp, *Pst*I-*Dra*I restriction fragment, that mapped between 43.3 and 43.8 map units of the LdMNPV genome. This fragment encodes a predicted protein of 218 amino acids (25.7 kDa). We named the gene *hrf*-1, for host range factor 1. No genes or proteins with similarities to *hrf*-1 were identified in searches of protein and nucleic acid sequence data bases. Nor were any common protein motifs identified in the protein that might suggest how this gene functions to permit AcMNPV replication in Ld652Y cells. Northern blot analysis of mRNAs isolated from LdMNPV-infected Ld652Y cells over time and in the presence DNA or protein synthesis inhibitors indicated that *hrf*-1 is transcribed as a delayed early gene, first observed at 6 hours post infection and absent in cells treated with the protein synthesis inhibitor cycloheximide.

determine if *hrf*-1 would allow AcMNPV to replicate in gypsy moth larvae we constructed ombinant AcMNPV bearing the *hrf*-1 gene. The polyhedrin gene was intact in this :ombinant so that we could test infectivity by feeding the virus. The recombinant viruses)licated in both Ld652Y cells and another gypsy moth cell line, IPLB-LdFB, that does not)port AcMNPV replication. In feeding assays, newly molted second instar larvae were fed ;h doses of PIBs. No mortality was observed until 24 days after infection. At 45 days after 'ection less than 40% of the larvae had died. Recombinant virus was recovered from half of :se, 20% of the infected-larvae. LD50s were not determined.

ABUNDANCE, DISTRIBUTION AND PARASITISM OF GYPSY MOTH EGG MASSES ON THE DELMARVA PENINSULA

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ABSTRACT

This study focused on factors influencing the distribution and survival of egg masses of the gypsy moth, *Lymantria dispar* (L.), a major pest of shade and forest trees in the Northeast. In March 1990, gypsy moth egg masses (N = 450) were sampled at 22 sites on the Delmarva Peninsula: 9 sites in New Castle Co., DE; 4 sites in Wicomico Co., MD; 3 sites in Caroline Co., MD; 3 sites in Kent Co., DE and 3 sites in Somerset Co., MD. Sites were grouped in the following habitats: (1) forests and woods, (2) forest openings, (3) park and woodlands, and (4) suburban areas. From one to ten egg masses were collected on 59 trees (17 tree species consisting of six food classes, 1=most favored, 2=favored, 3=intermediate, 4=less favored, 5=least favored, 6=inanimate objects). Parasitism, egg mass height above ground, egg mass abundance and egg mass size were compared for each site, habitat, tree species, and food class.

Egg masses tended to be placed higher on red maple (*Acer rubrum*) than any other tree species, and higher on trees in food class 1 (white oak group) than other food classes. The residential forest openings had the greatest egg mass height, while the suburban habitats had the lowest. This might be due to the presence of more man-made objects (poles, houses, lawn furniture, etc.) in the suburbs. Egg masses were more abundant on food class 2 (red oak group) trees than others. The leading species were southern red oak (*Quercus falcata*), willow oak (*Q. phellos*), and white oak (*Q. alba*). Habitat did not appear to affect egg mass abundance. Ash trees (*Fraxinus* spp.) had larger egg masses than other species, but there were no significant differences between food classes and habitats. Parasitism by *Ooencyrtus kuvanae* (Howard) ranged from 0% to 100% (avg = 33.06%) and was inversely related to egg mass size, but not significantly affected by tree species. Rates of parasitism were similar in all habitats. Parasitism was lowest on inanimate objects and highest on tree species in food class 5, particularly on dogwood (avg = 57%). Dogwood (*Cornus florida*) is an understory tree, and might be more accessible to parasites for this reason.

COMPARATIVE ANALYSIS OF *BACILLUS THURINGIENSIS* BINDING TO THE MIDGUT BRUSH BORDER OF RUSSIAN, LITHUANIAN, AND AMERICAN POPULATIONS OF GYPSY MOTH

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ABSTRACT

The target of *Bacillus thuringiensis* (*Bt*) insecticidal proteins (δ-endotoxins) in the gypsy moth (*Lymantria dispar*) is the brush border membrane of larval midgut cells. After ingestion by insect larvae, the δ-endotoxins are proteolytically activated in the midgut, and bind to specific receptors on the midgut epithelial membrane. Recently it has been recognized that these receptors play an important role in determining the specificity and insecticidal activity of various *Bt* δ-endotoxins.

We have previously reported that the gypsy moth midgut aminopeptidase-N (AP-N) acts as a receptor for the CryIA(c) δ-endotoxin. The receptor for another member of the CryIA δ-endotoxins, CryIA(a), was found to be a different molecule with an apparent size of 220 kDa. In this study, the binding of CryIA δ-endotoxins to the brush border membrane vesicles (BBMV) prepared from midguts of Russian, Lithuanian, and North American populations of gypsy moth was examined to determine whether there are any differences in the toxin-binding proteins that may be correlated with reported differences in their suseptibility to *Bt*.

SDS-PAGE analyses of hemolymph, midgut BBMV, and the midgut digestive fluid of Russian, Lithuanian, and two North American populations of gypsy moth were very similar. After SDS-PAGE, CHAPS-solubilized BBMVs were transferred to nitrocellulose and probed with biotin-labeled CryIA(a) and CryIA(c) δ-endotoxins. CryIA(a) δ-endotoxin bound to a 220 kDa binding molecule, whereas, CryIA(c) δ-endotoxin bound to a 120 kDa binding protein in BBMV of all four populations of gypsy moth. Immunoblot analysis using rabbit antibodies raised against purified gypsy moth APN confirmed that the 120 kDa receptor for the CryIA(c) δ-endotoxin present in all four populations is APN. Since *Bt* binding to the BBMV was indistinguishable, these results suggest that some other factor(s) such as the proteolytic processing of the δ-endotoxins by midgut proteases is responsible for the *in vivo* differences among gypsy moth populations in their susceptibility to *Bt*.

KINETIC BINDING CHARACTERISTICS OF *BACILLUS THURINGIENSIS* CRYIA TOXINS TO THE PURIFIED CRYIA(C) RECEPTOR (APN-1) FROM THE GYPSY MOTH

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ABSTRACT

Bacillus thuringiensis (*Bt*) has become increasingly important for suppression of gypsy moth. However, the use of *Bt* is restricted due to its selectivity and moderated efficacy. Current research is focused on means to improve these two qualities by understanding the molecular basis of selectivity and the insectidal properties of *Bt* δ-endotoxins.

The binding of a *Bt* δ-endotoxin to specific midgut epithelial receptors is prerequisite for conferring toxicity of a particular δ-endotoxin to an insect. The CryIA(c) δ-endotoxin receptor in the gypsy moth has been identified as the midgut brush border membrane aminopeptidase-N (APN). In this study, two APNs (APN-1 and APN-2) were purified from gypsy moth midgut brush border membrane vesicles (BBMV) and their ability to bind δ-endotoxins was examined by ligand binding, and an optical biosensor based on surface plasmon resonance (SPR).

Analysis of APN-1 and APN-2 revealed that only APN-1 can bind toxin. The kinetic binding characteristics of *Bt* toxin binding to APN-1 was determined in detail. The toxin-receptor complex association and dissociation were measured in real time permitting determination of kinetic rate constants, binding affinities, and binding site characterization.

Gypsy moth APN-1 specifically bound CryIA(c) demonstrating a moderately fast association rate ($7.18 \times 10^4 \text{ ms}^{-1}$), and a slow dissociation rate (2.32 s^{-1}), and an overall affinity of 3.23×10^{-8} M. Stoichiometric analysis of CryIA(c) binding revealed that the gypsy moth APN-1 possesses a single CryIA(c) toxin binding site which differs markedly from the aminopeptidase-N toxin-binding protein isolated from Manduca sexta larvae which can bind two molecules of toxin. No binding of either CryIA(a) and CryIA(b) was observed. APN-1 did not bind the coleopteran-specific toxin, CryIIIA. CryIA(c) binding to APN-1 was inhibited in the presence of N-acetylgalactosamine suggesting that it may form an integral part of the CryIA(c) binding site in APN-1.

EXOTIC FOREST DEFOLIATORS: SOME SPECIES OF CONCERN

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The introduction of the Asian biotype of gypsy moth into North America by unexpected pathways (grain ships and military equipment) demonstrated that new introductions are likely to be a continuing problem. We should not be naive and cannot expect that these unique pathways of introduction apply only to gypsy moth. Increasing international trade and developments in transportation technology ensure that the threat of additional exotic pests entering via these pathways will continue. The sheer volume of immigration (>1 million people/year), visitors (>4 million/year), and imports (>$700 billion/year) is steadily increasing as is the associated risk of pest introductions.

North America contains many plant genera common to Eurasia and thus can be expected to be susceptible to cross regional exchange of insects. Indeed, North America has been invaded by significantly more forest insect pests from Eurasia than vice versa. Reasons given for this disparity include more plant genera in North America than in Europe, more Eurasian plants naturalized in North America, simpler and more fragmented forests in Europe, and Eurasian insects are better competitors. Whatever the reason(s), these translocations have been very costly, economically and ecologically (Wallner, in press). Of the 70 major insect pests of U.S. forests, 19 were introduced (Pimentel 1986). It is not possible to predict with any certainty if an insect native to one country will assume pest status in another. However, if an insect is a pest in its native habitat, and a potential receiving country has similar host plant genera, it must be considered a threat. Although pest risk assessment procedures attempt to clarify pest transport, establishment, and reproduction, not all potential pest insects can be considered, nor their pestilence predicted.

The unpredictability of pest establishment and behavior is depicted by the browntail moth, *Euproctis chrysorrhoea,* which was introduced into Massachusetts in 1890. It infested an area of 150,000 km^2 by 1915 but later in the 1960s retreated to select maritime habitats and could be found on only 4.8 km^2. Since then, it has spread dramatically, from denizens of beach plum to widespread infestations on several hundred thousand hectares of mixed hardwoods, perhaps as a result of mild winter temperatures or the relaxation of control procedures. Nonetheless, browntail moth continues to be a pest in Europe in comparable habitats. Even though predicting the behavior of other exotic forest defoliators may be difficult, there is some justification in assuming that, once a pest, always a pest.

Three other Lepidopteran defoliators are likely to become problems if successfully transported into North America. Because commerce may play a crucial role (providing a variety of mechanisms for transporting pests and products), the behavior of these pests relative to risk of introduction are assessed.

The nun moth, *Lymantria monacha*, is closely related to gypsy moth in appearance and behavior. It is the classic insect pest of European coniferous forests where it not only kills spruce and pine but also defoliates deciduous trees as well (Bejer 1988). It is distributed over most of Europe across Siberia and the Russian Far East within a band between lat 43 to 57° N. It is a major pest in Central and Eastern Europe and southwestern Siberia, as well as Spain. For example, in Poland, during the period 1987-94, the nun moth caused enormous tree losses on more than 6.3 million ha. During 1993-94, more than 1 million ha were threatened and had to be protected with chemical treatments (Lipa and Kolk 1995). The frequency of outbreaks has increased to every 6 years vs > 40-year intervals. Climate, past use of hard pesticides, and extensive off-site planting of Norway spruce are believed to have contributed to the severity of the outbreaks. Both male and female adults are vagile and attracted to artificial lights; females lay an average of 120 to 180 eggs in clusters of approximately 40 eggs in bark crevices or under lichens on the bark. First and second instars are capable of dispersing over considerable distances.

Clearly, nun moth establishment in North America would be disastrous because of its polyphagous feeding habits, ability to colonize new habitats, and capacity to be spread rapidly by vagile females. It has the potential to be transported via commerce because eggs are laid in crevices of bark and other potential vehicles such as containers, pallets, ships, etc. Adults are readily attracted to artificial lights and have been observed on Russian ships in Far East ports. Regions of highest risk in North America, based on host plant availability and climate, include some 70,000 ha of western forests west of the Cascade Range, high-elevation spruce/fir/pines in the upper Midwest, and northeastern North America.

The pine (winter) and oak (summer) processionary moths of the genus *Thaumetopoea* are serious defoliators of Mediterranean forests (Frerot and Demolin 1993). Their feeding reduces tree growth and increases mortality; yet their major impact is on human health from urticating hairs. Potential host and climatic regions abound in southern California and Mexico. Although there are extensive oak and pine forests in the southeastern United States, the processionary moths are not likely to survive because of limiting winter temperatures colder than -12°C, which kill overwintering larvae. Thus, maritime locations are most susceptible. The potential for the processionary moths to be introduced probably is low; the greatest threat is from larvae in nests on host material. The browntail moth was introduced into the United States in this way on rose plants. There are a number of different *Thaumetopoea* species and ecotypes with variable life cycles. For example, *T. pityocampa* can exist for up to 7 years in the soil in pupal diapause. It is unlikely, however, that pupal introduction would be a successful pathway given the restriction of soil contaminants in international commerce.

There are approximately 1,000 species of Lasiocampidae (tent caterpillars) distributed fairly uniformly throughout the world. Among the most harmful are those in the genus *Dendrolimus*, which damage conifers. Although found throughout Eurasia, they are absent from North America. The major species are: *D. pini*, which feeds principally on Scotch pine in northern and central Europe; *D. superans* and *D. superans sibiricus*, which attack larch, pines, and firs in Siberia, the Russian Far East, and China; and *D. spectabilis* and *D. punctatus*, which damage various pines of Korea, China, Taiwan, and Japan. There is considerable taxonomic debate over the validity of individual species, but none in respect to the pest status of *Dendrolimus* spp. on conifers. Defoliation is usually extensive with damage occurring over thousands of hectares and is dramatic since just a few robust larvae can completely defoliate small conifers (Rozkov 1963). Additionally, larvae possess urticating hairs that pose a human health hazard.

Most *Dendrolimus* spp. are univoltine; some require 2 years per generation, while others such as *D. punctatus* from Taiwan, have three generations per year. Eggs are laid during the late summer on needles in Siberia and on branchlets and in bark crevices in Europe. Diapausing larvae overwinter in the duff or soil. The colonization potential of *Dendrolimus* spp. is high based upon their oligophagous feeding habits, and vagile adults ensure they could spread rapidly. The threat of *Dendrolimus* spp. being successfully transported and established is high based on the portability of eggs and diapausing larvae. The large number of different species and subspecies over such a wide geographic range elevates the risk of accidental introductions. Most regions of North America have forest, plantation, and ornamental plantings of susceptible coniferous hosts (various pines, spruces, and larch). Obviously, those regions of the Midwest, South, Southeast, and the far West are most vulnerable. Thus, given the numerous *Dendrolimus* spp., there is very high probability for successful establishment and spread should introduction occur.

Solutions to mitigating exotic pest introductions are complex. However, some general guidelines could mollify this problem.

<u>Mitigating Exotic Pests Domestically</u>

- Levy user fees to accelerate research and regulatory activities
- Enact legislation to litigate culpable party
- Focus technology for biotype identification/behavior
- Reduce import dependency upon raw products

<u>Mitigating Exotic Pests Internationally</u>

- Elevate concerns on a global basis
- Foster scientific collaboration on technology development
- Prioritize high-risk pests commodity/country
- Identify introduction pathways with importers/exporters

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SOIL NUTRIENTS AND SAPLING GROWTH BEFORE AND AFTER A GYPSY MOTH OUTBREAK IN SW PENNSYLVANIA

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ABSTRACT

Predicting forest responses to gypsy moth defoliation requires knowledge of the soil nutrient levels and sapling recruitment necessary for forest regrowth. I tested two hypotheses: (1) soil nutrient levels will increase during canopy defoliation; and (2) saplings will increase growth after canopy tree death. Annual mid-summer monitoring of soils and vegetation in 18 locations within three mature, deciduous forest sites occurred from 1986-92, before, during, and after a 1989-90 gypsy moth outbreak. Canopy defoliation ranged from 0-100% among locations and matched the patchy distribution of oaks.

Multiple analysis of variance tests for repeated measures of soil cations, pH, phosphorus and total nitrogen (N) showed highly significant year effects, but defoliation as a covariate was not linked to annual soil parameter shifts. This pattern indicates factors other than defoliation are more important in predicting annual changes in soil nutrients. Example: No soil N levels changed during defoliation; instead, during two drought years, N in the O horizon dropped to half the value in non-drought years.

The survivorship of saplings (0.5-3m tall) at the 3 sites was high (70-95%) two years before outbreak, declined during outbreak (50-70%) and dropped further after outbreak (16-60%). The lowest sapling survivorship occurred at sites with high overall sapling density before outbreak. The decline in leaf N levels in some sapling species after canopy defoliation indicates nutrient competition may explain low sapling survivorship after the gypsy moth outbreak. When small tree crowding increased due to the recruitment of new saplings following partial canopy death, saplings present prior to canopy defoliation had increased mortality risk.

In sum, gypsy moth defoliation had little measurable effect on soil nutrients, but subsequent partial canopy death substantially altered vegetation dynamics. Overall sapling abundance increased, as predicted, in sites where canopy tree death occurred; however, individual saplings present before canopy defoliation showed a mixed response to canopy tree death, probably due to the combined effects of decreased competition with canopy trees and increased competition among saplings.

REARING *CALOSOMA SYCOPHANTA* ON GYPSY MOTHS AND ARTIFICIAL DIETS: A COMPARISON

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ABSTRACT

In the last few years, forest releases of *Calosoma sycophanta* L. have been carried out in Michigan, West Virginia, Delaware and Maine. These beetles were reared in Connecticut using gypsy moth prey supplied by USDA-APHIS. The interest in *Calosoma* has prompted the development of an artificial diet for the beetle that has the potential for being less expensive and labor-intensive to use than are gypsy moths.

In most of the tests, hatching larvae of *Calosoma* were placed in individual Petri dishes and fed pieces of artificial diet that had been wrapped in plastic film and frozen. Pieces of diet were partially unwrapped before being placed with larvae. Other larvae were fed gypsy moth pupae reared on Cape Cod. The percent survival to adult and lengths of adult elytra were measured.

Of the diets tested (beef liver, calf liver, chicken, beef hamburger, pork, or combinations), the beef liver + chicken diet was as good as gypsy moth pupae for rearing larvae of *C. sycophanta*. An important priority for further progress is development of a package for the diet that beetles will accept. Also, an artificial diet needs to be developed for adults, because these consume a large number of gypsy moth larvae while reproducing.

HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE*,

STYLET INSERTION AND FEEDING SITES

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ABSTRACT

Stylet bundle insertion site, path traveled, and feeding site were examined for the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), on current and previous year's needles of eastern hemlock, *Tsuga canadensis* Carriere. The stylet bundle is comprised of four individual stylets -- two outer mandibular stylets and two inner maxillary stylets. *A. tsugae* inserted its stylet bundle on the adaxial side of eastern hemlock needles, proximal to the twig with respect to the leaf abscission layer. Insertions were primarily intracellular through epidermal cells. Once inserted, the stylet bundle then followed a mixed intracellular and intercellular pathway, with the latter predominant, when penetrating the plant tissue to the feeding site, the xylem ray parenchyma cells. We observed evidence of salivary secretions, tracks, and sheaths produced by *A. tsugae* in the plant similar to those produced by aphids and other adelgids in their host plants. Unlike other adelgids studied, which feed on cortical parenchyma cells and on solutes from phloem, *A. tsugae* seem to feed only on storage cells, the parenchyma cells that comprise the xylem rays. This suggests that *A. tsugae*'s intense impact on eastern hemlock may be due to factors other than its food consumption; that is, possible toxin effect and/or altered host-plant response to environmental conditions.

THE COMPLEX OF GYPSY MOTH (*LYMANTRIA DISPAR* L.) PARASITOIDS IN SLOVAKIA AND IN OTHER CENTRAL EUROPAEAN COUNTRIES

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ABSTRACT

Gypsy moth (*Lymantria dispar L.*) is a very serious pest of oak forests in the territory of Slovakia. The pest belongs to the original species in the Central-European region and the north border of the territory of its mass outbreak reaches the south part of Slovakia. The last outbreaks were in the years 1984 - 85 and 1993 - 94 and they reach high intensity - 10 000 ha of stands infested annually.

The parasitism of gypsy moth eggs masses was checked on 10 localities and parasitism of larvae and pupae on 4 localities in Slovakia from year 1991 till 1994. The dominant tree species on localities were *Quercus cerris* and *Quercus petraea* in undergrowth with *Prunus spinosa, Ligustrum vulgare, Acer campestre, Carpinus betulus* and *Robinia pseudoacaciae*.

We have collected the egg-masses in amount of 10 pieces per each locality individually. Egg-masses were stored in the laboratory conditions until the hatching out of the parasitoids. We have collected about 100 pieces of larvae of L1-2, L3-4 and L5-6 instar and 100 pieces of pupae from each locality (per year). The larvae were reared in the laboratory rearing boxes (glas cylinder), 30 per each box and the larvae were reared by oak foliage. The parasitoids were prepared and determined. The number of egg masses was checked and countered by the Turèek method in each locality in September.

We have recorded the occurrence of two species of the egg parasitoids *A. disparis Rusch.* and *O. kuvanae How.* The total parasitization was 0.34 % (0.01 - 2.02 %) of those *A. disparis* had a share 79.5 % and *O. kuvanae* 20.5 %. *A. disparis* was more frequent on the monitored localities (7 localities). *O. kuvanae* was rarer (4 localities) and its territory of occurrence was shifted to the west in comparison with the previous observations.

We have examined 3735 larvae and pupae of *L. dispar* from 4 localities in Slovakia and 602 larvae and pupae (cca. 16 %) of the collected were parasitized. The presence of a large spectrum of different parasitoid species was observed. *Parasetigena silvestris, Blepharipa pratensis, Cotesia melanoscelus* and *Glyptapanteles liparidis* were the most important species.

The Braconidae (*C. melanoscelus, G. porthetriae)* had a dominant position during the latency period. They caused 62.37 % population reduction of gypsy moth, together with Ichneumonidae (*Phobocampe* sp., *Hyposoter tricoloripes*). The Tachinidae have played a main role in all other gradation phases. The dominant part had an oligophagous species of Tachinidae (*P. silvestris, B. pratensis),* on the other hand the polyphagous species (*Zenillia libatrix, Compsilura concinnata, Drino incospicua*) were less frequent. The relationship between oligophagous and polyphagous increased from progression till regression phase. Specially *P. silvestris* is most dominant species of decline phase, but this phase is typical for increasing of the impact of Braconidae (*G. liparidis*) on gypsy moth population.

In latency, the gypsy moth mortality reached 92.32 % as a common effect of impact of parasitoids, pathogens and unknown reasons. The high mortality, which is in natural condition supported by weather conditions, inaparent infections, etc. is able to keep the population density of pest under ecology threshold for a relatively long time (4-8 years). In progression, the mortality of pest decreases very dramatically (72.48 %). We do not know if this was evoce by increasing of gypsy moth population activity or decreasing of breaking factor activity (pathogens, parasitoits and other factors). In culmination, the mortality reached 91.58 % a during regression it was 96.63 %. The parasitoits were less important than pathogenes and unknown reasons of mortality. The parasitoids had a dominant impact during the latency and regression periods.

In comparison with last gradation increased impact reason of some species of parasitoids, for example *B. pratensis* a *G. porthetriae* which were very important in 1992-94 were not observed in gypsy moth locality in 1985-87. On the other hand the decreasing of the other species as *Blondelia nigripes, Compsilura concinnata* were observed as well.

From literature which we have studied was elaborated a list of parasitoids and importancy of these parasitoids in several countries in Central Europe.

Table Heading Key:

SR - Slovak Republic, **PL** - Poland, **UR** - Ukraine, **HU** - Hungary, **YG** - Yugoslavia, **BL** - Bulgaria, **RU** - Europaean part of Russia

List of the Gypsy Moth (*Lymantria dispar*) Parasitoids Recorded in Slovakia and in Other Central Europaean Countries

	SR	PL	UR	HU	YG	BL	RU
EUPELMIDAE							
Anastatus disparis Rusch.	o					o	
ENCYRTIDAE							
Ooencyrtus kuvanae Howard	o					o	
PROCTOTRUPOIDEA							
Hadronotus lymantriae	o					o	
TACHINIDAE							
Exoristinae							
Exorista fasciata Fall.	o	o		o	o	o	
E. garndis Ztt. = *sorbilans* Wied.	o	o		o	o	o	
E. larvarum L.	o	o		x	xx	xx	xx
E. segregata Rond.						x	
Parasetigena silvestris R.-D.	xx	x	o	x	xx	xx	xx
Blondelia inclusa Htg.						o	
B. nigripes Fall.	o	xx		o	o	o	
Compsilura concinnata Meig.	x	o		o	x	x	o
Nemorila floralis Fll.	o					o	
N. maculosa Meig.	o					o	
Drino bimaculata Htg						o	
D. gilva Hert. (? Diprionidae)						o	
D. incospicua Meig.	o				o	o	o
C. gnava Meig. = *exavata* Ztt.	o					x	
C. lucorum Stein							o
Senometopia confundes R. = *Carcelia confundes* R.-D						x	
Senometopia excisa Fall. = *Carcelia excisa*						o	
Senometopia separata R. = *Carcelia separata*	o	o			o		
Zenillia libatrix Panz.	o	o	o	o	o	o	
Pales pavida Meig.	o				o	o	

	SR	PL	UR	HU	YG	BL	RU
Blepharipa pratensis Meig.	xx	xx		o	o	xx	xx
= *Sturmia scutelata* R.D.							
B. schineri Mesnil	o	o		o	o		
Baumhaueria goniaeformis M.		o		o		o	
Tachininae							
Tachina fera L.						o	
T. magnicornis Zett.	o	o		o	o	o	
Blepharomyia piliceps Zett.						o	
BRACONIDAE							
Doryctinae							
Doryctes leucogaster Nees.						o	
(? Coleoptera)							
Braconinae							
Oncophanes laevigatus Ratz.						o	
(? Tortricidae)							
Rhoganinae							
Petalodes unicolor Wesm.				o			
(?)							
Aphidinae							
Diaretis polygoni Marsh						o	
= *Aphidius matricariae* Hal.							
(Aphidoidea !!)							
Meteorinae							
Meteorus cintellus Spin.							o
(? Tortricidae)							
M. gyrator Thun.			o				
(? Noctuidae)							
M. pulchricornis Wesm.	o	o	o			o	
M. versicolor Wesm.	o		o			o	
Meteorus sp.						o	
Microgastrinae							
Cotesia melanoscelus Ratz.	xx	o	x	o	o	x	o
= *solitarius* Ratz.							
C. praepotens Halid.				o			
(? Geometridae)							
C. ocneriae Iw.			o				
C. glomeratus L.						o	
(? Pieridae)							

	SR	PL	UR	HU	YG	BL	RU
C. rubripes Halid. (? Geometridae)						o	
Glyptapanteles fulvipes Halid. (? Noctuidae)						o	o
G. inclusus Ratz.					o		
G. liparidis Bché.	x	o	o	x		o	xx
G. porthetriae Mues.	x	o	xx		o	xx	
G. vitripenis Curt. (? Noctuidae)		o				o	
Dolichogenidea lacteicolor Vier.						o	
Apanteles obscurus Nees (? Pyralidae)							o
Apanteles sp.						x	
Microgaster hospes Marsch. (? Tortricidae)						o	
Microgaster sp.						o	
ICHNEUMONIDAE							
Pimplinae							
Apechtis compunctor L.	o						
A. rufata Gmel.							o
Pimpla hypochondriaca R.	o	o	o			o	o
P. contemplator M ll.			o				
Iseropus inquisitor Scop.	o	o	o			o	
I. turionellae L.	o						
Itoplectis alternans Grav.							o
Itoplectis sp.							o
Theronia atalantae Poda	o		o			o	
Campopleginae							
Hyposoter tricoloripes Vier.	o	o					
Hyposoter sp.	o						
Phobocampe unicincta Grav. =*Hyposoter disparis* Vier.	x	o				o	
Phobocampe pulchella Thoms.							o
Phobocampe confusa Thoms.							x
Phobocampe sp. (*P. lymantriae*, *P. pulchella* ?)	o	o				o	
Campoplex sp.	o						
Casinaria tenuiventris Grav.						o	o

	SR	PL	UR	HU	YG	BL	RU
Casinaria sp.	o						
Ichneumoninae							
Lymantrichneumon disparis P.	o					o	
Ephialtes didymus Grav.			o				
Exolytus laevigatus Grav.							o
Perithous septemcinctorius Th.							o
SARCOPHAGIDAE							
Parasarcophaga portschinski R.						o	
P. uliginosa	o					o	
P. harpax			o			o	xx
Pseudosarcophaga affinis Fall.	o		o			x	xx
P. tuberosa Pand.						o	o
Kramerea schuetzei Kramer	o					o	
CHALCIDIDAE							
Brachymeria intermedia Nees	o		o			o	
Brachymeria picea Nik.							o
Brachymeria sp.						o	
Monodontomerus sp.	o						
Monodontomerus aeureus Walk.						o	
Chalcis forscolombei Duf.						o	
Chalcis minuta Lin.						o	

USDA Interagency Gypsy Moth Research Forum
January 16-19, 1996
Annapolis, Maryland

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