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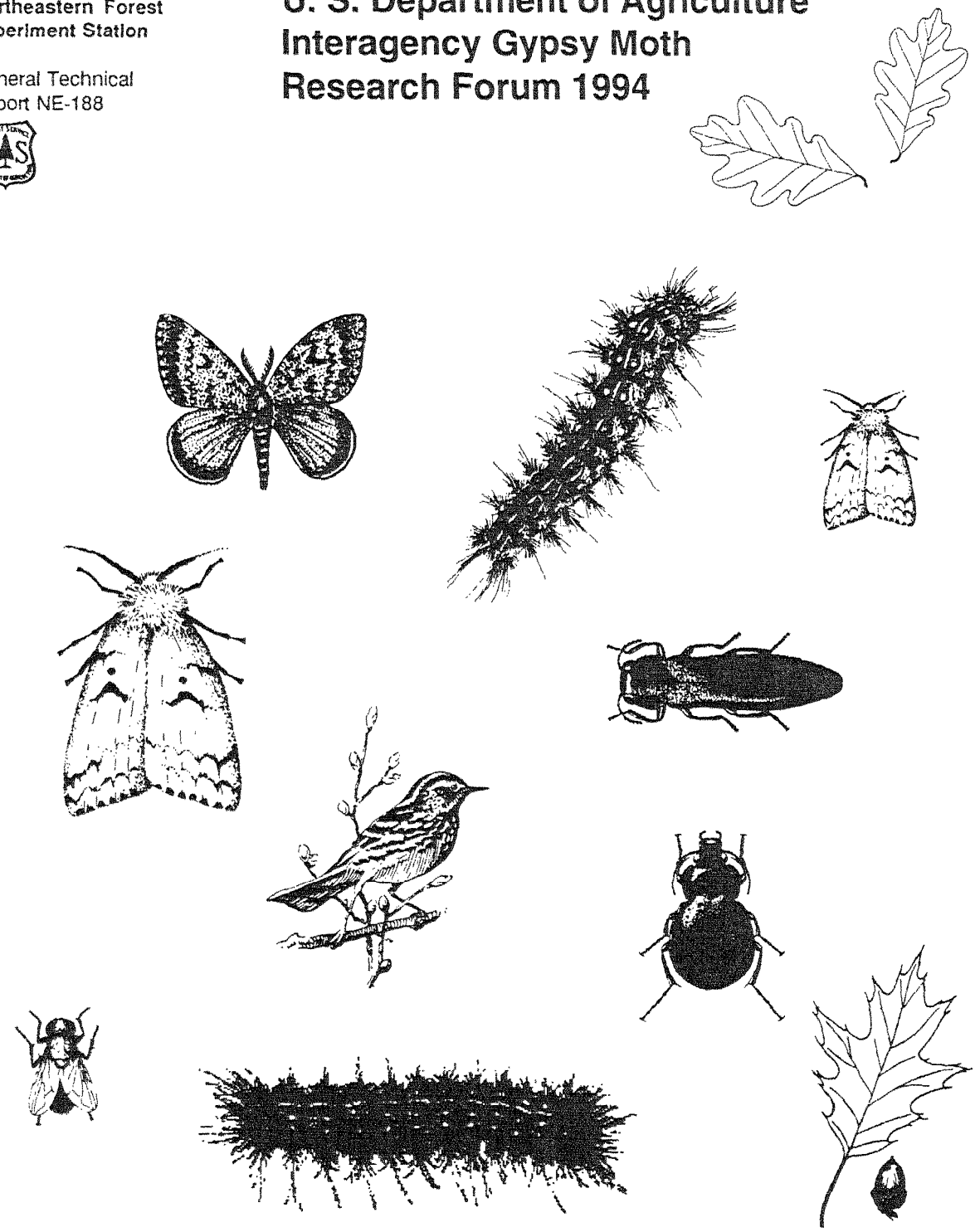
Report NE-188

# PROCEEDINGS

## U. S. Department of Agriculture Interagency Gypsy Moth Research Forum 1994



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# Proceedings
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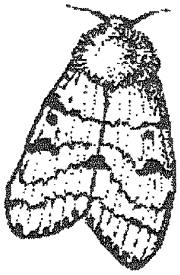
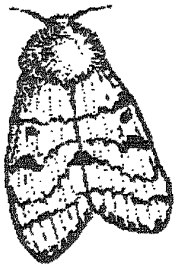
# 1994





January 18-21, 1994
Loews Annapolis Hotel
Annapolis, Maryland

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## FOREWORD

This meeting was the fifth 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)
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M. McFadden, Forest Service-Research (FS-R), Chairperson

USDA Interagency Gypsy Moth Research Forum
January 18-21, 1994
Loews Annapolis Hotel
Annapolis, Maryland

## AGENDA

Tuesday Afternoon, January 18

REGISTRATION
POSTER DISPLAY SESSION I
WELCOME MIXER

Wednesday Morning, January 19

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

Welcome
Michael McManus, FS-R

The Gypsy Moth as One of Many: the Nationwide Toll of Harmful Non-indigenous Species
Phyllis Windle, Office of Technology Assessment

Activities and Opportunities in International Forestry Research
Sheila Andrus, FS-WO

ARS International Biological Control Programs
James Krysan, ARS

Wednesday Afternoon, January 19

GENERAL SESSION ..... Moderator: E. Delfosse, APHIS

Regulatory Aspects of Biological Control
Presenters: G. Aplet, The Wilderness Society; M. Miller, Emory University School of Law;
J. Maddox, Ill. Natural History Survey

POSTER DISPLAY SESSION II

Thursday Morning, January 20

CONCURRENT SESSION I . . . . . Moderators: M. Shapiro, ARS and K. Shields, FS-R

Optical Brighteners: Potential, Problems, Prospects
Presenters: K. Shields, FS-R; E. Dougherty, ARS; M. Shapiro, ARS; R. Webb, ARS; J. Podgwaite, FS-R; J. Cunningham, Forest Pest Management Institute (Canada)

CONCURRENT SESSION II . . . . . Moderator: R. M. Muzika, FS-R

Economic Considerations for Managing the Gypsy Moth
Presenters: A. Liebhold, FS-R (presented by K. W. Gottschalk, FS-R); W. Leuschner, Clemson Univ.; R. Hedden, Clemson Univ.; W. Dickerson, North Carolina Dept. of Agriculture

POSTER DISPLAY SESSION III

Thursday Afternoon, January 20

CONCURRENT SESSION I . . . . . Cancelled

Impact of Diflubenzuron on Selected Non-targets Within Closed, Broadleaf Watersheds

CONCURRENT SESSION II . . . . . Moderator: A. Bullard, FS-NCFHM

Alternatives for Managing the Gypsy Moth: Status Report
Presenters: B. Leonhardt, ARS; V. Mastro, APHIS; B. Helson, Forest Pest Management Institute (Canada); C. Riegel/J. Slavicek, FS-R and E. J. Park/J. Burand, Univ. of Massachusetts

GENERAL SESSION

"Potpourri"
Presenters: V. Mastro, APHIS; J. Elkinton and V. D'Amico, Univ. of Massachusetts; D. Leonard, FS

Friday Morning, January 21

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

Asian Gypsy Moth - Recent Developments and Research Findings
Presenters: J. Harper, North Carolina State Univ.; H. Bogenschütz, Forest Research Institute (Germany); Y. Baranchikov, Sukachev Institute of Forest (Siberia) and M. Montgomery, FS-R; R. Cardé, Univ. of Massachusetts; M. Keena, FS-R; S. Bogdanowicz and R. Harrison, Cornell Univ.; K. Garner and J. Slavicek, FS-R; W. Wallner, FS-R

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# ECOSYSTEM INTEGRITY, ALIEN SPECIES, AND BIOLOGICAL CONTROL

Gregory H. Aplet

The Wilderness Society, 900 17th St. NW, Washington, DC 20006

Sustainability has become a fashionable buzzword in the last decade, as society has come to appreciate the limited ability of the earth's ecosystems to meet human demands. According to the Brundtland Commission in its landmark 1987 report, *Our Common Future*, achieving sustainability requires "meeting the needs of the present without compromising the ability of future generations to meet their own needs." While the intent is clear, it is difficult to put this standard into practice, since we cannot know the needs of future generations. Thus, we must interpret sustainability as meaning "maintaining the *potential* of earth's ecosystems."

In a 1992 workshop entitled "Defining Sustainable Forestry", convened by The Wilderness Society, American Forests, and the World Resources Institute, Jerry Franklin of the University of Washington identified two types of potential relevant to sustainability: physical or *productive* potential and biological or *genetic* potential. Productive potential includes such things as soil productivity, clean air, and clean water. Genetic potential includes the whole diversity of genes and the species they form. Because we cannot maintain all the world's species in zoos or artificial parks, conserving genetic potential requires maintaining the integrity of the world's ecological systems.

Biological diversity, the variety of life on earth, is distributed according to the actions of many factors, including climate, soils, and biotic interactions, combined with historical accidents of continental drift, island biogeography, dispersal, and evolution. The result of all these interactions is the distribution around the world of innumerable distinct combinations of species. These distinct combinations form unique communities and regional ecosystems. The integrity of a given ecosystem is defined as much by which species are *not* there as it is by which species *are* there. Species additions can destroy ecosystem integrity as much as species extinctions.

Throughout history, humans have been eroding ecosystem integrity through their effects on both extinction rates and the transport of alien species into novel ecosystems. The alien species issue has now reached crisis proportions and has finally captured the attention of policy-makers in Washington, as is evident from the Office of Technology Assessment's recent compilation of alien species effects in North America. But non-indigenous species are not just a domestic concern; alien invasions have had disastrous effects on the integrity of native ecosystems all over the planet.

What is the connection between exotic species problems and biological control? Clearly, biological control is one of the few available tools to combat the effects of alien species. Many alien problems result from artificially high population levels due to the escape of a species from

its natural enemies. Reuniting the pest with its natural enemies may be the only effective means of control in the long run. This traditional focus of biocontrol has met with spectacular success in some cases.

But biocontrol can also be part of the alien species *problem*. Every successful introduction adds another alien species to the ecosystem and further degrades ecological integrity. This may be worth the price if the long-term consequences of no action would result in significantly greater loss of integrity. But this is not always the case, and some biocontrol introductions can have disastrous and permanent consequences. Well known are the snafus of mongoose and mynah introductions in Hawaii. Many would argue that we now know enough not to make those kinds of mistakes again, but other cases, such as the extinction of endemic snails in the Pacific and the endangerment of native *Opuntia* from *Cactoblastis* in the Caribbean, are more recent and suggest that biocontrol remains a potentially very risky venture.

Biocontrols possess all of the characteristics of any other alien species. They have the same potential to develop unpredictable behavior as any organism undergoing ecological release in a novel environment. They are permanent and spread themselves. Once established, they cannot be recalled or controlled, except by the introduction of yet another species.

Perhaps more importantly, biocontrols possess characteristics that make them inherently dangerous organisms. Good biocontrols:

1) are capable of rapid breeding in response to an outbreak;
2) are voracious if predators and virulent or debilitating if diseases. Plant eaters must be able to kill whole plants or halt reproduction.
3) are either widespread and abundant or are efficient searchers.

These are the characteristics possessed by some of the most destructive alien pests. By their very nature, biocontrols should be of interest to those concerned about the effects of alien species.

Biocontrols can have other effects. If the biocontrol expands its host range to include non-target species, and if those host species are native, the biocontrol can have important effects on community and ecosystem processes.

Phytophagous insects, the target of many biocontrol organisms, form vital links in the food chain of forest communities, supplying the dominant food source for many forest birds. When a biocontrol is successful, it can dramatically reduce and stabilize the population of its host. Such reductions can decrease food abundance and change community relationships permanently.

Phytophagous insects also play a tremendous role in ecosystem-level phenomena. Many are an important cause of natural mortality in forest trees, and their foliage feeding, even at endemic levels, can dramatically affect nutrient cycling. In a report published in 1988 on genetically altered organisms, the Office of Technology Assessment warned that "the most serious

ecological impacts [of genetically engineered organisms] involve the disruption of ecosystem processes, such as energy flow and nutrient cycling." A biocontrol agent that significantly reduces populations of native species is likely to cause serious disruptions of ecosystem processes.

What is the lesson from these observations? Most importantly, it is to recognize that biological controls are alien species that carry the same concerns as some of the worst pests. Biocontrol is not necessarily the safe, environmentally benign alternative to chemicals that it is sometimes billed to be.

This is not to propose that biocontrols be banned. It is only to suggest that their risks be recognized, not just to particular economic or aesthetic species, but to the integrity of native ecosystems. Deference to native ecosystems must be paramount in the evaluation of potential biocontrol introductions.

Finally, the biocontrols that are most likely to have negative effects on native ecosystems are those agents aimed at native "pests". I strongly urge the biocontrol community to take a very critical view of the currently fashionable exploration of the use of alien species to control native pests.

# TREE SUITABILITY FOR ASIAN, EUROPEAN AND AMERICAN POPULATIONS OF GYPSY MOTH

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## ABSTRACT

The development of gypsy moth larvae from different geographic locations were compared on several species of trees. One set of tests were done in the U.S. Forest Service Quarantine Laboratory at Ansonia, Connecticut, on 46 tree and shrub species using gypsy moth obtained from Massachusetts and south-central Siberia. Other tests were done in Siberia on 20 host species using gypsy moth from Michigan, Germany, and south-central Siberia. Flying females have not been observed at the site where the Germany gypsy moths were collected.

In both locations, newly hatched larvae were reared on excised foliage for 10 days or until molt to the next instar. The relative growth rate (RGR), the gain in body weight/mean body weight/day, was a good overall indicator of host suitability and was highly correlated with body weight, development rate and percent survival. The following figures compare RGR of first instar larvae of the North American biotype with the biotype from other continents. Data symbols above the dotted line indicate that growth was greater for the Asian or European biotype than the North American biotype when reared on the same host.

For 19 of the 20 hosts tested in Siberia and 45 of the 46 hosts tested in Connecticut, the RGR was greater for the Asian biotype than the North American. The growth of gypsy moth larvae from Europe was very similar to the larvae from North America. Examples of host plants where big differences in growth occurred are: Scots pine, Asian biotype 5.2 times greater and survival 74% compared to 7% for the North American biotype; Douglas fir, Asian 2.4 times greater and survival 100% compared to 25% for the North American. In general, if the suitability of a tree species for one biotype is known, its suitability for other biotypes can be predicted.

# INTERACTIONS OF MICROSPORIDIUM AND GYPSY MOTH IN MICHIGAN FIELD PLOTS

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## ABSTRACT

In 1992 in the three oak woodlands with sparse gypsy moth infestations, 19%, 10%, and 8% of older larvae were infected at Dansville, Gregory and Rose Lake, respectively. In 1993, we performed the same experiment at the oak stand at Rose Lake in south central Michigan and at an aspen stand at Roscommon in north central Michigan. On 11 and 12 May, 400 ready-to-hatc egg masses were stapled at breast height to 9-10 tree trunks near the center of each plot. Twenty-five percent of the egg masses had been previously coated with a spore solution ($10^5$ spores/microliter) of *Microsporidium* sp. The initial prevalence of infection in hatching larvae was found to be 82% and 92% at Rose Lake and Roscommon, respectively, in randomly selecte sets of contaminated egg masses held in the lab. There were few if any feral eggs at Rose Lake, but Roscommon had a natural infestation of 150 viable egg masses per acre, 50% of which had hatched by 12 May. By May 20 all feral eggs had hatched, but none of the masses we had placed on the trees had hatched because of cold weather. On 18 May at Rose Lake and 20 May at Roscommon, several unhatched contaminated egg masses were returned to the lab and reared to determine infection levels. Initial prevalence dropped to 28% and 3% at Rose Lake and Roscommon, respectively. On 9 and 10 June, second and third instars were collected from the understory host plants within 30 m of the plot center. At Rose Lake and Roscommon, 113 and 452 larvae were collected, respectively, and the infection levels were 6% and 3%. On 22 and 7 July, larvae were collected under burlap bands placed on all large preferred hosts within a 30-m radius of plot centers. At Rose Lake and Roscommon 120 and 403 larvae were collected, respectively. The levels of infection in the older larvae were 0% and 1%, respectively, for Rose Lake and Roscommon.

# INTRODUCTION AND ESTABLISHMENT OF *ENTOMOPHAGA MAIMAIGA* IN MICHIGAN

Leah S. Bauer[1], David R. Smitley[2], Ann E. Hajek[3], Frank J. Sapio[4], and Richard A. Humber[5]

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## ABSTRACT

In 1991, late instar gypsy moth larvae were sampled and diagnosed for infections of *Entomophaga maimaiga* at 50 sites in Michigan. Approximately 1500 larvae were collected and reared from these sites, and no infections of *E. maimaiga* were detected. We also tested the efficacy of two inoculative release methods for *E. maimaiga* in replicated plots at two research sites in 1991 by 1) relocating soil containing *E. maimaiga* resting spores from Massachusetts to Michigan or 2) releasing inoculated larvae onto the bole of trees. Post-treatment sampling in 1991 showed that *E. maimaiga* was established in plots that received the inoculated larval treatment only. However, by 1992 establishment of *E. maimaiga* was confirmed for both inoculation methods, and there were no significant differences between the level of infection achieved by the two release methods, ranging from 9.3 to 11.7%. In addition, by 1992, infection was detected at low levels in the control plots (0.5 to 2.4%) which were located ca. 100 m away from the treatment plots. In 1992, we added a third research site to the study, using the method of relocating soil containing resting spores. Post-treatment sampling of that site in 1992 detected low levels of establishment at the epicenter only (5.5% infection). Continued monitoring of fungal establishment during the 1993 season revealed that epizootics of *E. maimaiga* occurred at the three research sites, with the incidence of infection ranging from 29 to 99% in the plots. Differences in infection levels between sites were correlated with the precipitation totals for two weeks or the hours of relative humidity ≥90% for the 10 days that preceded larval sampling. In 1993, the egg mass densities at the three research sites averaged 3- to 10-fold lower than in control plots. Our data also supports that *E. maimaiga* epizootics are less density dependent than gypsy moth NPV. In 1993, we also monitored the establishment and rate of spread of *E. maimaiga* at 20 release sites set up by the Michigan Department of Natural Resources in 1991 by the relocation of soil containing resting spores. *E. maimaiga* establishment was confirmed in six of the 20 release sites, and the rate of spread at was estimated at ca. 1.0 km per year.

# IDENTIFICATION OF FIVE MUTANT LOCI THAT GENERATE A FEW POLYHEDRA PHENOTYPE IN THE *LYMANTRIA DISPAR* MULTINUCLEOCAPSID NUCLEAR POLYHEDROSIS VIRUS

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## ABSTRACT

Two forms of virus are produced during replication of baculoviruses, a budded virus form and occluded virus. Budded virus is produced early in the replication cycle and is responsible for a systemic infection. Late in the replication cycle virus is occluded into protein crystalline structures termed polyhedra. Polyhedra are composed primarily of the protein polyhedrin and viral nucleocapsids. The processes of polyhedra formation and virion occlusion are poorly understood. These processes are likely to be complex yet only two proteins, polyhedrin and the AcMNPV 25K protein, have been identified to date that are essential for proper polyhedra formation.

Using a LdMNPV viral strain that exhibits enhanced polyhedra production stability during passage in cell culture, and other LdMNPV strains we generated several polyhedra formation (PF) mutants through passage of virus in *L. dispar* 652Y cells. These mutants were analyzed to test the hypothesis that there are several viral genes involved in the processes of polyhedra formation and virion occlusion. Virus was plaque purified and virus exhibiting typical FP mutant attributes and altered polyhedra morphology were selected for further study. Most of the isolates could be classified as FP mutants based on very low polyhedra formation. The other isolates produced fewer polyhedra than wild type virus, but more than FP mutants. Virion occlusion in polyhedra produced by all PF mutants was significantly reduced compared to wild type polyhedra. In addition, several isolates produced polyhedra with abnormal morphologies. 652Y cells were coinfected with two PF mutants and the resulting budded virus was analyzed for the presence of virus exhibiting a many polyhedra (MP) formation phenotype. Several combinations of PF mutants yielded MP e virus as a consequence of genetic recombination between the mutant viral strains. Marker rescue was performed with some of the PF mutants using a cosmid library of isolate A21-MPV. The approximate genomic location of the gene responsible for the PF mutant phenotype was identified for several PF mutant strains. Through genomic recombination between PF mutants and marker rescue, five genetic loci in LdMNPV were identified that impact polyhedra formation and virion occlusion. We are defining these loci as coding for polyhedra formation factors (pff) and have assigned the PF mutants identified in this study to pff groups 1 through 5. Studies are in progress to identify and characterize the mutations present in pff 1 through 5.

# THE GYPSY MOTH SITUATION IN GERMANY

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## ABSTRACT

At the Lymantria Conference in New Haven, Connecticut, in 1988, I reported an extraordinary gypsy moth outbreak in Southwest Germany. In 1985, an area of about 1,300 ha of oak forest was defoliated; never before has such a large area been damaged by gypsy moth in Central Europe. In 1993, the next gypsy moth outbreak in Germany was even more extensive; nearly 50,000 ha were infested and about 10,000 ha were completely defoliated.

In Europe, extended outbreaks of gypsy moth normally occur only in the hardwood forests of the Southeastern and Mediterranean countries, where the warm dry summers offer optimal conditions for gypsy moth development. Due to the high efficacy of the natural antagonists, the epidemic phase of the population dynamics does not last longer than 4 years. The last outbreak in Southwest Germany collapsed in the third year of the outbreak.

In the optimum region of Southern Europe, the population fluctuations of the gypsy moth are fairly regular with a mean periodicity of 7.7 years. Figure 1 shows the situation in Southwest Germany since the 1970s. Note that the absolute level of the defoliated areas in the three different forest districts is 7 or 8 years; the length of one cycle is the same as in the optimum region of Europe. This corresponding temporal regularity and the increased area of infested forests has led to the assumption that the south of Germany has become a favorable region for gypsy moth development.

This assumption is supported by the special weather situations in the last decades: an abnormal sequence of dry and warm summers, similar to the weather conditions in the optimum countries, had preceded both outbreaks in 1985 and 1993.

While everything seemed clear, an outbreak began in July 1993. Discussions on possible causes of the current gypsy moth outbreak again surfaced. What had happened?

On some warm nights, swarms of gypsy moth males and females, attracted by bright lamps, appeared in town and villages more than 10 km away from the infested forest. Streets and parking places were crowded with moths, so that car drivers had to switch on the windshield wipers. The next morning, the males had disappeared but the females laid their eggs on the walls of the buildings, posts of street lights, and on trunks of ornamental trees. Flying females were observed in more than 20 German communities indicating that this new situation is not

only of local importance. These records are concentrated in the area of Karlsruhe-Heidelberg-Heilbronn, an area where extended forests were defoliated last year, but not in the middle 1980s.

There have been some single records of females flying long distances from Hesse and Bavaria, but not from other European countries, where gypsy moth outbreaks previously have taken place.

When first confronted with this remarkable news, I began thinking about the flight ability of gypsy moth females in Europe. Is it possible that flying females just have been overlooked before? I find this hard to believe.

Although in Central Europe outbreaks were seldom before 1984, the gypsy moth always has been present in the communities of phytophagous insects in hardwoods. Therefore, it would be very astonishing not to find any record in the lists of entomologists using light traps for collecting moths. My search for such records was not entirely unsuccessful: In the State Museum of Natural History in Karlsruhe, I found seven notations of light-trapped gypsy moth females. They all were caught in the area of Karlsruhe-Heidelberg, the area of the current outbreak, between 1965 and 1984.

If it is true that only Asian gypsy moth females are able to fly, the origin of European gypsy moth populations should be reexamined in more detail using the modern techniques of DNA analysis. Specimens of older collections could supply these investigations with interesting material.

There are many questions to be answered concerning flight behavior and physiological characteristics of Asian gypsy moth in Europe. I hope that the planned Germany/U.S. mutual research program this season will result in a more thorough understanding of the problem. This is essential to resolving this very important international situation.

# GAS CHROMATOGRAPHY/MASS SPECTROMETRY CARBOHYDRATE ANALYSIS OF GYPSY MOTH TREHALASE

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## ABSTRACT

Trehalose is the largest carbohydrate reserve in gypsy moth. The glycoprotein trehalase (EC 3.2.1.28) catalyzes the hydrolysis of trehalose into glucose, thereby playing a major role in the metabolic homeostasis of the insect. Trehalase is known to exist in soluble and membrane-bound forms. In silkworm larvae, marked increases in trehalase activity coincide with the transformation of the membrane-bound form of the enzyme into a soluble form, occurring just prior to larval-pupal metamorphosis. The glycan component of trehalase may influence this intracellular compartmentalization of the enzyme, thus mediating biological activity.

An analysis of the carbohydrate moiety of the glycoprotein trehalase (EC 3.2.1.28) was undertaken to determine composition of its inherent glycans and their mode of linkage to the enzyme. Trehalase was purified to homogeneity from the soluble fraction (cytosol) of fifth instar larval gut using gel filtration and anion exchange chromatography. Mannitol was added to serve as an internal standard. Sugar monomers were released from 15 µg of the protein by methanolysis using 2M methanolic HCl. The sample was re-N-acetylated and derivatized using TMSI. Sugar monomers were then extracted into hexane. Two µl aliquots were analyzed using a 30 meter crosslinked 5% Ph Me silicone column. A temperature program progressed from 140° to 280°C at a rate of 8°/minute. Helium was the carrier gas at .9 ml/minute. Sugar standards were prepared in the same fashion. Identities of the sugar monomers present in trehalase were based on elution times as compared to TMS sugar standards, along with mass spectral matches of the carbohydrate peaks, generated from a mass spectral library data base.

The enzyme contains fucose, mannose, galactose, inositol, N-acetyl galactosamine, N-acetyl glucosamine, and sialic acid. Glucose was also identified, and is thought to be a contaminant from sephadex used during enzyme purification. The relative amounts of neutral sugars suggest that the glycan chains are complex in nature. The presence of the amino sugars N-acetyl galactosamine and N-acetyl glucosamine indicates that the enzyme has both N- and O-linked glycan chains. A small amount of inositol was also detected, suggesting that this enzyme, known to exist in both soluble and membrane-bound forms, may be anchored to a membrane lipid bilayer via an intervening glycan structure attached to the C-terminal amino acid of the protein. The enzyme also contains sialic acid, which is often the terminal sugar in both N- and O-linked glycans.

# DISPERSAL BEHAVIOR OF ASIAN GYPSY MOTH FEMALES

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## ABSTRACT

The mating and dispersal behaviors of the Asian gypsy moth (*Lymantria dispar*) were observed in Belyk, in central Siberia. The rhythm of female eclosion was bimodal, with most emergence occurring in mid-afternoon but with another peak in late afternoon. Mating usually occurred within one hour of eclosion. Females remained near their mating site until dusk, when they wing-fanned for several minutes before dispersal flight. Most females dispersed on the day of mating, but some females delayed dispersal for one day. Periodicity of male attraction to pheromone-baited traps is similar to its timing in the North American population, but the peak of attraction is centered around mid-afternoon and there is a second peak of attraction in the several hours following sunset.

Studies in the laboratory with flight mills (round-abouts) in quarantine confirmed that the initiation of flight occurs when sunset-like conditions are simulated. Some Asian gypsy moth females fly continually throughout the 8 hour scotophase, and F1 crosses also show similar patterns. There is considerable variation, however, among individuals and flight speed appears slower in both reciprocal F1 hybrids than in Asian strain females.

# A LABORATORY ASSESSMENT OF THE EFFECTS OF *BACILLUS THURINGIENSIS* ON NON-TARGET LEPIDOPTERA

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## ABSTRACT

The objective of our study was to determine the effect of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) on non-target Lepidoptera in laboratory bioassays. We evaluated the effects of Foray 48B on larvae of 41 non-target species, representing eight families. Fourteen species in six families were also evaluated for their susceptibility to infection by Dipel 8AF. Neat applications of Foray 48B or Dipel 8AF were applied to host seedlings or foliage bouquets. Larvae were confined in nylon mesh sleeves on treated or untreated foliage, and larval mortality was monitored daily for 5 or 7 days. A chi-square analysis was used to compare mortality on treated vs. untreated foliage. Significant mortality was recorded for 26 of the 41 species evaluated with Foray 48B. For 21 of these species, significant mortality occurred within 5 days of the start of the assay. Fifteen species were insensitive to Foray. Larvae of three of the four butterfly species tested succumbed within five days of treatment and the fourth butterfly species showed significant mortality before eclosion. Five of the seven geometrid species evaluated were significantly affected by the Foray treatment. Significant mortality was also recorded for one lasiocampid and three saturniid moths. Significant mortality was recorded for 13 of the 25 noctuid species evaluated. For 10 of the noctuids, significant mortality occurred within the first 5 days of the assay. Eight of the 14 species evaluated for susceptibility to Dipel 8AF showed significant mortality within the first 5 days of the assay. A single butterfly and two species of geometrid showed significant mortality. Four of the nine species of Noctuidae also showed significant mortality during the assay.

Our data indicate that it is probably unwise to generalize in terms of predicting the susceptibility of native Lepidoptera to *Btk*. For the Geometridae and Noctuidae we evaluated, susceptibility to *Btk* infection is not necessarily related to larval instar at the time of *Btk* treatment. In general, early (first or second) instar larvae were more susceptible than later instars. In terms of later instar larvae, most of the geometrids and noctuids we tested were immune to infection, although there were exceptions. At the generic level, we also noted significant intrageneric differences in *Btk* susceptibility among the eight species of *Catocala* we evaluated and in the three species of *Lithophane*. We conclude that *Btk* susceptibility must be dealt with on a species-to-species basis which will complicate management decisions involving the widespread use of *Btk*, particularly where species of "special concern" are involved.

# DEVELOPMENT OF AN EFFICIENT GYPSY MOTH SAMPLING PROTOCOL FOR FORESTS AND SUBURBAN AREAS

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## ABSTRACT

One hundred thirty nine transects have been established in 11 counties in northern Michigan. These transects are located in areas where the gypsy moth has been established for several years as well as areas where the infestation is relatively new. Each of these transects consist of 100 trees. Thirty one of these transects were established in the Spring of 1991 (i.e., prior to egg hatch 1991) and the remaining transects were established in late Spring and Summer 1991, Summer 1992, and Spring 1993. The following information has already been collected on each of the transects: 1) the width and length of each transect has been estimated; 2) the species and DBH of each tree within a transect; 3) the number of new and old egg masses on the first 2 meters of the bole; 4) the length of two egg masses per tree, when possible; 5) for transects established before egg hatch 1991, a five-minute walk was performed to provide a comparison of current procedures with any new protocol; 6) defoliation of each tree was estimated during late July and early August 1992; 7) 5-minute walks, 1/40 acre fixed-area plots, BAF10 and BAF20 variable-radius plot were surveyed adjacent to a number of transects in 1993.

Results from the 1992 field data indicate that an efficient method for predicting defoliation utilizing data collected from transects is feasible. Several sampling protocols were developed from data collected in 1992. One of the protocols developed uses a two step procedure to make control decisions. Step one involves making control decisions based on the mean number of egg masses per tree (i.e., XNEW $\leq$ 0.2- don't control; XNEW $\geq$ 2.0- control). The second step utilizes a model develop from the 1992 data to predict defoliation that is then used to make control decisions (i.e., if predicted defoliation $\geq$ 37.5%- control; if predicted defoliation < 37.5- don't control). This protocol was then used to make control decisions using data collected in 1991 and 1993. Validation of the 1992 sampling protocol indicates that the procedure was 88% effective (correct decision in 147 of the 167 transects) at making the right control decision. These results were produced using a sub-sample of 25 trees from the original 100 tree transects. The strength of the sub-samples in prediction defoliation means that the method may require sampling only 25 tree along a transect. This means that the time needed for sampling a transect will be only slightly more than current methods (i.e., approximately 15 minutes per transect).

As of January 1994, the development of a reliable, cost effect protocol is continuing. A great number of models have been examined. The best results have been those presented above. A simple decision rule based only on the mean number of egg masses (i.e., XNEW $\geq$ 2.0 - control; XNEW $\leq$ 2.0 - no control) was nearly as accurate as the two step procedure (i.e., 82.2% for 1992 data and 88.0% for the 1991 and 1993 data). However, this procedure produced more critical errors (not controlling when control was needed) than did the two step method.

The final goal of the project is to provide an efficient sampling protocol for making control decisions in both forests and suburban areas. Current results indicate a sequential sampling procedure is very likely the final product of this project.

Publications(s):

Chilcote, C. A., J. M. Scriber, M. E. Montgomery, G. Fowler. 1994. An efficient gypsy moth sampling protocol for forests and suburban areas. (in preparation for Northern Journal of Forestry)

Chilcote, C. A., J. M. Scriber, M. E. Montgomery. 1994. Comparison and effectiveness of gypsy moth sampling procedures for control decision making. (in preparation for Environmental Entomology)

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## AERIAL APPLICATIONS OF CYANAMID Ld NPV FORMULATIONS IN ONTARIO IN 1992 AND 1993

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### ABSTRACT

In 1992, 5 virus treatments and one *Bacillus thuringiensis (B.t.)* treatment were applied on woodlots in Simcoe and Aylmer Districts. All applications were double, 3 to 7 days apart, at 5.0 L/ha for the virus and 2.5 L/ha for the *B.t.* on first and second instar larvae. The treatments

were 1) a pilot test with Gypchek produced by the USDA Forest Service at $5x10^{11}$ PIB/ha in 25% molasses, 6% Orzan LS and 2% Rhoplex B60A or 2% Bond sticker (3 plots, 177 ha), 2) Gypchek at $5x10^{11}$ PIB/ha in Entotech 244 carrier (3 plots, 39 ha), 3) Disparvirus produced at FPMI at $5x10^{11}$ PIB/ha in 25% molasses, 6% Orzan LS and 2% Bond sticker (4 plots, 48ha), 4) Disparvirus at $5x10^{11}$ PIB/ha in Cyanamid Ld NPV wettable powder (4 plots, 43 ha), 5) Disparvirus at $5x10^{10}$ PIB/ha in Cyanamid Ld NPV wettable powder plus 1% Blankophor BBH (5 plots, 48 ha) and 6) Foray 76B undiluted at 50 BIU/ha (3 plots, 37 ha). Compared to 4 untreated check plots with 31% defoliation, there was no difference in the amount of defoliation in the treated plots which ranged from 24% to 30%. There was a 40% increase in egg mass numbers in the untreated check plots and population reductions due to treatment (Abbott's formula) were 78%, 76%, 66%, 74% and 84% in the five virus treatments, respectively, and 95% in the *B.t.* treatment. It should be noted that treatment 5 with the 10-fold reduction in virus dosage plus 1% Blankophor BBH performed as well as the other 4 virus treatments.

In 1993, three spray regimes using Cyanamid wettable powder formulated with Gypchek plus 1% Blankophor BBH were applied in 5.0 L/ha on 5 replicated 10 ha plots in Methuen Twp., Bancroft District when larvae were predominantly in their first instar. The applications were 1) $5x10^{10}$ PIB/ha, twice, 5 days apart, 2) $5x10^{10}$ PIB/ha once and 3) $10^{11}$ PIB/ha once and 4) a double application, 3 days apart, of Foray 48B at 30 BIU/ha in 2.4 L/ha applied on predominantly second instar larvae. Defoliation of oak was significantly less in all the treated plots, 20% to 23% defoliated in the virus treatments and 14% in the *B.t.* treatment compared to 50% defoliation in untreated check plots. There was a 77% decline in egg mass densities in untreated check plots due to overwinter mortality. All treatments had a significant impact on the gypsy moth population with reductions due to treatment (Abbott's formula) of 68%, 63% and 61% in the three virus treatments and 63% in the *B.t.* treatment. The low dosages of gypsy moth NPV with 1% Blankophor BBH gave effective gypsy moth control.

# A FIELD TEST OF GENETICALLY ENGINEERED GYPSY MOTH NPV

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## ABSTRACT

The gypsy moth (*Lymantria dispar* L.) nuclear polyhedrosis virus (LdNPV) was genetically engineered for non-persistence by removal of the gene coding for polyhedra production. A beta-galactosidase marker gene was inserted into this virus, so that larvae infected with the engineered virus could be easily tested for its presence using a chemical assay.

In May 1993, field tests were established in two 4 ha plots in Otis Air Force Base on Cape Cod. Gypsy moth larvae were released in both plots to serve as test populations. The virus was released in the center of the test plot by confining infected gypsy moth eggs on oak foliage in mesh bags. These bags were not removed until larvae hatching from infected eggs were 2nd instar. Most of these larvae became infected with the engineered virus. No virus was released in the control plot.

Weekly collections were made in both plots, beginning one week prior to virus release, and continuing for six weeks thereafter. Each week, several hundred larvae were collected at random from the control plot and 25 larvae were collected from each of the 33 sectors in the test plot. These larvae were reared individually and checked for mortality every second day. Parasitism by the wasp *Cotesia melanoscela* was high on both plots. Because we discovered that gypsy moth larvae infected with immature of *C. melanoscela* respond positively (turn blue) to the beta-galactosidase assay, the gypsy moth cadavers were tested for the presence of the engineered virus using both the chemical assay and restriction enzyme analysis. Throughout the seven collection weeks, the engineered virus was recovered in both early and late instars, but only from collection points within 50 m of the release point. No engineered virus was seen in the test plot in weeks 1 or 7. No virus was seen in the control plot at any time.

# EFFECTS OF GYPSY MOTH DEFOLIATION ON PINE-HARDWOOD STANDS

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## ABSTRACT

Since its introduction in 1869, the gypsy moth (*Lymantria dispar* L.) has expanded its range considerably. As it moves to the south and west, infestations will occur in stands whose species previously have been unaffected. Loblolly pine (*Pinus taeda* L.) and sweetgum (*Liquidambar styraciflua* L) are two of the most abundant species in southern forests. Therefore, knowledge of potential impacts of defoliation in stands containing these species is essential.

To quantify the impact of defoliation in mixed stands, we designed a study with the following objectives: 1) determine whether the pine component in mixed stands reduces or contributes to stand susceptibility; 2) determine whether hardwood defoliation promotes pine growth; 3) predict growth loss and/or mortality based upon the proportion of pine (or hardwoods) in mixed stands. One hundred and forty-one plots were established in loblolly pine/oak and loblolly pine/sweetgum stands throughout the Virginia/Maryland Coastal Plain. Two years of growth and defoliation information were collected for 75 plots and one year of data for 66 plots. In 1992, 52 percent of the plots contained active gypsy moth populations; population data for 1993 is unavailable. The number of egg masses ranged from 67 to 13,933 per hectare. Defoliation in loblolly pine/oak stands increased significantly from 1992 to 1993. However, only 20 percent of loblolly pine/sweetgum stands exhibited increased defoliation. Where defoliation occurred susceptible species (*Quercus* spp. and *L. styraciflua*) in both stand types were preferentially consumed. Resistant species (*P. taeda*) essentially experienced no defoliation in 1992. This continued into 1993 with the exception of a single pine/oak stand that received defoliation levels greater than 91 percent; here, defoliation among resistant species increased to 11 to 30 percent. A number of trees have been defoliated by other species of Lepidoptera, including forest tent caterpillar (*Malacosoma disstria* Hubner) and buck moth (*Hemileuca maia*). Both species were observed during the 1992 and 1993 field seasons, and if they continue to occur will likely influence the evaluation of gypsy moth impacts. During the next four field seasons, we will continue to monitor the progress of the gypsy moth in these stands and evaluate the effects of defoliation on tree growth and mortality.

# EFFICIENCY OF DEPOSITION OF PESTICIDE DROPLETS ON FLAT CARDS AND SPHERES

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## ABSTRACT

A spherical collector and a flat card have been used in collecting pesticide droplets aerially sprayed over forestry canopy (Miller et al., 1992). Knowledge of the efficiency of deposition of pesticide droplets on these types of collectors is required to quantify the efficacy of the spraying application. An attempt has been made in this study to evaluate the catch efficiency of Teflon spheres and Kromekote cards under field conditions.

The field experiment was undertaken at Las Cruces, NM, from 12 to 16 March 1992. An undiluted commercial preparation of *Bacillus thuringiensis* (*Bt*) was applied with the Cessna C-188 Ag Husky equipped with Micronair AU5000 rotary atomizers. Rhodamine tracer dye was added at a concentration of 0.4% (volume to volume), and the *Bt* applied at a rate of 59.26 BIU/hectare. Nominal spray heights of 6.1, 15.3, 30.5, and 91.5 meters were chosen . Kromekote cards (10.2x12.7 cm) with half of their area occupied by acetate sheets were used as targets mounted approximately 46 cm above the ground. A 274.5 meter sampling line was used. Twenty Teflon spheres in diameter of 1.92 cm were mounted side by side with Kromekote cards in the central portion of the sampling line. Droplet stains on the card portion of the cards were assessed by image analysis and the acetate portion of the cards and spheres were washed off with water, and assayed for Rhodamine dye content by fluorometry. Meteorological measurements were made at the site of application at 69 cm using a sonic anemometer and temperature and relative humidity sensors.

In a wind of 1.0 m/s, calculated collection efficiencies of deposition on a flat card can be as high as 90% for a droplet of 500 $\mu$m in diameter, while collection efficiencies are less than 20% for droplets smaller than 100 $\mu$m. Calculated collection efficiencies by the mechanism of inertial impaction on a sphere are less than 10% for a droplet of 10 $\mu$m in a wind as strong as 10.0 m/s and for droplets smaller than 90 $\mu$m in a wind of 0.1 m/s. The minimum values of collection efficiencies on a sphere by the mechanism of sedimentation and inertial impaction combined appear between a wind speed of 0.2 m/s and 0.5 m/s for droplets of 10 to 100 $\mu$m and between 0.5 m/s and 3.0 m/s for droplets larger than 150 $\mu$m. In a weak wind such as 0.1 m/s, contribution by the mechanism of sedimentation to deposition of material on a sphere is great, while contribution by inertial impaction is negligible. With increase in wind speed, the percentage of deposition contribution by sedimentation decreases, but the percentage of contribution by inertial impaction increases. For a droplet of a certain size, the collection

efficiency by the mechanism of sedimentation and inertial impaction combined has a minimum value at a particular wind speed.

The average collection efficiencies for cards decreases as the wind speed increases. The value of the average collection efficiency can be as low as about 30% at wind speed of 1 m/s. Results of regression show high linear correlation between the average collection efficiency and the wind speed ($R^2 = 0.91$) for cards. For spheres, the values of the average collection efficiencies are generally high compared with those for cards. While there is a trend that the average collection efficiencies for spheres decrease as the wind speed increases, the experimental data showed no linear correlation between the two phenomena ($R^2 = 0.07$).

# USING LIDAR TO TRACK SPRAY CLOUD DRIFT

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## ABSTRACT

A rapid scanning mini-LIDAR (Light Detection and Ranging) system was used to demonstrate the feasibility of laser technology in tracking spray clouds applied by aerial methods. The initial goal of the experiment was to see if the rapid scanning mini-LIDAR system would be adequate for detection of aerosol sized particles. Subsequently, it was desirable to know if the mini-LIDAR system would be capable of viewing spray cloud dynamics. The ultimate goal of the application of the LIDAR technology would be to view the pesticide spray shortly after its release from the aircraft spray nozzles and to be able to follow it from this release time until it began to penetrate into the canopy. It was hypothesized that by using the LIDAR system to make rapid series of scans, or "slices," in either the horizontal or vertical direction, the three-dimensional dynamics of the cloud could be visualized. This abstract describes the field experiment performed to demonstrate LIDAR effectiveness, the preliminary results, and the final goals of the field experiment.

The experiment was conducted in July 1993, in a forested region of north central Pennsylvania. The 18.0 m tall forest was predominantly oak and had undergone defoliation by gypsy moth for several years. At the site, a 37 m tall meteorological tower was instrumented at three levels within the canopy and at three levels above the canopy. A second tower was erected of scaffolding platforms for the LIDAR. The LIDAR platform was located several feet above maximum canopy height.

The spray plane applied *Bacillus thuringiensis* (*Bt*) about 15 m above the canopy. The LIDAR scans began within several seconds after the plane had passed the site. Using the sets of two-dimensional slices to provide a three-dimensional picture of the spray cloud worked very well. The swath of *Bt* from the plane was easily visualized. The dynamics of the spray, from wingtip vortex controlled movement at release to atmospheric controlled drift and deposition were well illustrated. Qualitative results highlighted the usefulness of the LIDAR. The data will be used to: (1) demonstrate spray cloud behavior from release point to canopy interception; (2) correlate atmospheric conditions with deposition a mount, canopy penetration and off-target drift; and (3) enhance and verify a Lagrangian stochastic model for predicting spray cloud transport in an unstable atmosphere.

# STAND STRUCTURE AND DEVELOPMENT AFTER GYPSY MOTH DEFOLIATION IN THE APPALACHIAN PLATEAU AND RIDGE AND VALLEY PROVINCES

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## ABSTRACT

Analyses of data collected from 27 stands in the Appalachian Plateau (AP) and Ridge and Valley (RV) provinces of PA, MD and WV, show that stand development patterns have been altered by the gypsy moth (*Lymantria dispar* L.) . The stands were located immediately behind the leading edge of a gypsy moth infestation. Stand structural data were collected in 1984-85 (pre-defoliation and again in 1989 (post-defoliation). Principal Component Analysis (PCA) was used to compare the pre-defoliation species distribution among the 350 plots in all stands. The PCA identified 10 tree species that accounted for about 90% of the variation in frequency of occurrence, and about 80% of the variation in basal area among plots; 11 species categories were created.

Physical site characteristics from the plot data, combined with soil survey information were used regroup the plots into species' assemblages based on site characteristics rather than stands. Basal area means among plots within each site characteristic were compared using ANOVA techniques. Initially, the factor loadings (eigenvalues) of the first six principal components of species basal ar were used; followed by the raw data (basal area sums) for the individual species that were significantly ($P<0.001$, $r> +/- 0.50$) correlated with each principal component. Both techniques produced similar ANOVA results. Species distributions and basal areas were significantly ($p<0.0$ affected by aspect in the AP province and by soil type in the RV province. Basal area of the oak (*Quercus*) species, particularly red (*Q. rubra* L.) and white (*Q. alba* L.) oaks, accounted for most of the differences among aspects, slope positions, and soil types. The plots in each aspect or soil-type group were then subdivided based on four levels of overstory density: <75 $ft^2$/A, 75-109 $ft^2$/A, 110-140 $ft^2$/A, and >140 $ft^2$/A. T-tests conducted between the total number of living trees ir pre- and post-defoliation structures showed significant decreases ($P<0.05$) in four site/density groups in the AP and one group in the RV. There were no significant decreases in the number of oaks in post-defoliation stands of the RV. However, there were significant decreases in red oak (five site/density groups) and white oak (one site/density group) in the AP. Pre- and post-defoliation height distributions were compared for species groups using the Pearson Chi-Square test of homogeneity. In the RV, white and red oaks showed significant changes in distributions for 7 soil/density groups, and red maple changed in 4 groups. In the AP, white and red oaks changed on three aspects for plots containing <75 $ft^2$ of basal area, while red maple changed in 7 aspect/density groups. These changes are attributed to both mortality and growth.

# APPARENT CAUSES OF DEATH IN GYPSY MOTH PUPAE STUNG BY THE INTRODUCED PARASITE *COCCYGOMIMUS DISPARIS* (VIERECK) [HYMENOPTERA: ICHNEUMONIDAE]

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## ABSTRACT

Observations on stinging behavior and incidence of successful parasitism by the recently introduced pupal parasite, *Coccygomimus disparis* (Viereck), were made in a field study at three sites in a forested community near the town of Hartly, Kent County, Delaware. Comparisons were drawn for ichneumonid parasitism and other mortality sources in hosts known and not known to have been stung by *C. disparis*. This species often stung and killed many pupae in addition to those successfully parasitized (i.e., in which progeny developed). The ratio between the total number of hosts stung by *C. disparis* and the number of hosts parasitized could not be calculated with certainty, because some stung hosts yielded the facultative hyperparasite *Theronia atalantae fulvescens* (Cresson), and we could not tell when attacks by *Theronia* had occurred.

Incidence of survival in hosts known to have been stung by *C. disparis* was significantly lower than in those not known to have been stung at two of the three study sites. The only site where no difference was noted had sustained heavy mortality (>80%), especially by *Calosoma sycophanta* (L.) and *Brachymeria intermedia* (Nees), so most of the experimentally stung hosts probably would have died anyway. Successful ichneumonid parasitism was consistently higher in hosts stung by *C. disparis*. Most hosts killed by stinging alone (i.e., excluding direct parasitism) appeared to have been desiccated or resembled diseased hosts. Sarcophagids were poor indicators of unparasitized hosts killed by *C. disparis*, and incidence of Diptera was higher in the general sample than in hosts known to have been attacked by this species. Stinging by *C. disparis* had no apparent effect on parasitism by *B. intermedia*. Attacks by *C. disparis* ranged up to nearly 18 minutes in duration, but averaged less than 4 minutes. Host feeding did not affect survival in stung hosts, but did prolong attack duration.

# A NUCLEAR DNA-BASED METHOD FOR IDENTIFYING ASIAN GYPSY MOTHS AND ASIAN/NORTH AMERICAN GYPSY MOTH HYBRIDS

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## ABSTRACT

Recent North American introductions of gypsy moths (*Lymantria dispar*) originating in Asia and Europe have necessitated the development of improved methods for specimen identification. A mitochondrial DNA-based method has been used; however, since mitochondrial DNA is maternally inherited, in hybrids the paternal genotype would not be detected. Therefore, a nuclear DNA genetic marker specific for the Asian or North American genotype would be preferable. We have used random amplification of polymorphic DNA by the polymerase chain reaction (RAPD PCR) to find strain-specific markers in gypsy moths. We have tested 280 arbitrary decanucleotide primers and have found several which appear to amplify diagnostic DNA fragments in one or both gypsy moth strains. We plan to characterize these amplified fragments and develop locus-specific PCR primers, which will improve the sensitivity and reproducibility of the method. To date, we have developed specific primers for one of these markers, which has two size variants: a larger fragment associated with the Asian strain and a smaller fragment found in North American specimens. We have begun analysis of large numbers of moth DNA samples to determine the distribution of these size variants throughout North America, Europe and Asia.

Of 39 Asian samples from far eastern Russia and Japan, 100% have the larger marker. Fifty-two known hybrid individuals have been tested and 85% of these typed correctly, having both the larger and the smaller marker. Some of the mistyped individuals had an Asian parent originating in central Siberia, suggesting the the "Asian" genotype may be less frequent in this region. In 20 samples collected in six sites in Europe, a mixture of the two markers has been found. It is not known at this time whether this reflects the genetic composition of the native European population, or whether Asian moths have spread recently throughout the European continent. Finally, in North America, 49 moths from Massachusetts, New Jersey, Pennsylvania, West Virginia, North Carolina and Michigan have all typed as North American. However, 4 of 16 individuals from Ashtabula County, Ohio have the larger "Asian" marker. It is unknown whether these individuals represent an Asian or European introduction or whether the larger marker is present in a small proportion of the North American population. More samples from sites throughout North America will be tested to answer this question and to establish levels of confidence for practical use of these markers. The RAPD PCR analyses carried out to date indicate that the gypsy moth strains are not well differentiated genetically. It may be impossible to find one marker which can distinguish all North American from Asian or European moths. However, use of several markers should allow statistically valid identification of suspect specimens.

# GYPSY MOTH DEVELOPMENTAL RATES IN POSTDIAPAUSE ARE AGE-DEPENDENT

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## ABSTRACT

Estimation of insect developmental rates has typically been done by measuring the time required to complete a recognizable ontogenetic process at a constant temperature, and then assuming that developmental response to the temperature (i.e. developmental rate) was uniform throughout the time. This technique has been unsatisfactory for investigations of gypsy moth development because: [1] developmental response to a given temperature clearly changes depending on whether an individual is in a diapause or non-diapause phase of development; and [2] it has been difficult to determine when diapause begins and ends.

Gray et al. (1991) developed a method of distinguishing the diapause and non-diapause condition, and are developing a three-phase model of egg development. In this model an egg must complete prediapause, diapause, and postdiapause phases before hatching and developmental response within each phase is governed by a unique rate function. Early experimental results suggested that developmental response to temperature was not uniform within postdiapause, but varied with advancing physiological age. Therefore, we measured "instantaneous developmental rates" at five constant temperatures at four times in the postdiapause phase.

An exponential model,

$$R_T(t) = R_T(0)e^{a_T t} \qquad [1]$$

was fit to these data, where $R_T(t)$ is developmental rate at constant temperature $T$ (°C) on day $t$, with $t = 0$ being the onset of postdiapause, and $a_T$ describes the amount of change in developmental rate per unit time at $T$. By expressing developmental rate as a time-independent function of physiological age, equation [1] reduces to the simpler model

$$R_T(A) = R_T(0) + a_T A \qquad [2]$$

where $R_T(A)$ is the developmental rate at temperature $T$ and at age $A$, $R_T(0)$ is the developmental rate at $T$ at the onset of postdiapause, and $a_T$ is as described for [1].

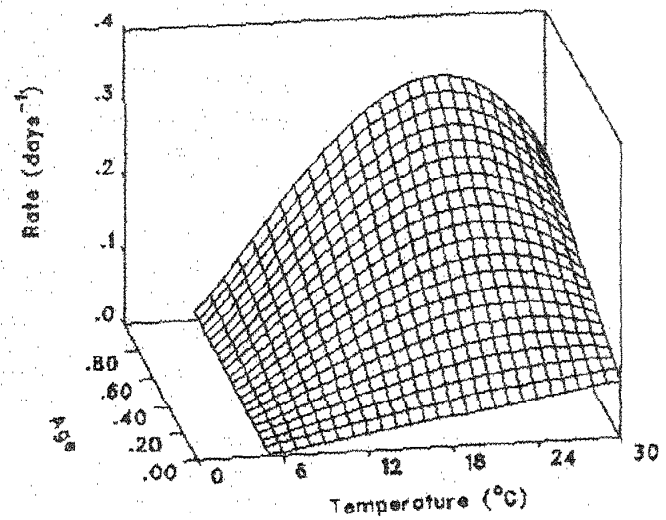


Fig. 1

This empirical model of gypsy moth postdiapause development describes an age- and temperature-dependent process where developmental rates are positively age-dependent at all temperatures, and positively temperature-dependent at all ages. However, response is initially slow and relatively insensitive to temperature (Fig. 1). The developmental response of eggs that are physiologically young in postdiapause is only slightly greater to warm than to cool temperatures. This serves to prevent warm days in late winter or early spring from promoting egg hatch. An equally warm day later in the spring has a greater effect since eggs are more physiologically advanced. For example, developmental rate at 22°C is 0.05 $days^{-1}$ at postdiapause initiation, and 0.25 $days^{-1}$ at physiological age 0.75.

## Literature Cited

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# PHYSIOLOGICAL HOST RANGE OF *ENTOMOPHAGA MAIMAIGA*

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## ABSTRACT

Presently, there is great interest in introducing the gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper to areas where it does not presently occur. However, researchers, land managers, and the public are concerned about the potential non-target effects of this recently discovered pathogen. Previous studies testing a limited number of host species demonstrated that Japanese strains of *E. maimaiga* are specific to Lepidoptera. We present results from 1992-93 laboratory bioassays evaluating the host range of northeastern U.S. strains of *E. maimaiga* by challenging a diversity of lepidopteran species. These bioassays were conducted under optimal conditions for infection and therefore results demonstrate an idealized range of hosts in which *E. maimaiga* can cause mortality and produce spores. A total of 78 species of Lepidoptera from 10 superfamilies were inoculated with *E. maimaiga* conidia; most of the native lepidopteran species tested were collected in Appalachian forests presently being invaded by gypsy moth, where *E. maimaiga* is not yet well-established. Mortality of larvae inoculated with conidia was greater than control mortality for 17 of the 72 species with controls. For seven of these 17, *E. maimaiga* did not produce spores although we assume that this fungus was at least partially responsible for these larval deaths. However, production of spores in or on cadavers was considered a definite sign that larval death had truly been caused by infection. Of the 78 species tested, only 27 (34.6%) produced spores after conidial inoculation. Infections occurred across a number of different superfamilies (Pyraloidea, Drepanoidea, Geometroidea, Mimallonoidea, Bombycoidea, Sphingoidea, and Noctuoidea) and usually included only some of the species tested in each superfamily. Spores were not produced in any of the butterflies tested. In all susceptible superfamilies except Sphingoidea and Noctuoidea, the levels of infection were low. Of the two sphingid species tested, one was not infected at all, while 96.3% of *Manduca sexta* (originating from a laboratory colony) became infected. Within the Noctuoidea, infection levels were low among some of the arctiids, noctuids, and notodontids tested; high levels of infection occurred across the four species tested from the family Lymantriidae. Alternate hosts were introduced to boxes containing abundant soil-borne resting spores to evaluate resting spore infection of species other than gypsy moth. Two of five species susceptible to conidial infection also became infected by resting spores, while one species infected by resting spores (10.0%) was not susceptible during conidial bioassays. During early spring, gypsy moth larvae placed over resting spores in the field first became infected two weeks before gypsy moth egg hatch. Infections due to *E. maimaiga* ceased in July, either one week before or approximately two weeks after the initiation of gypsy moth pupation.

# SPREAD OF *ENTOMOPHAGA MAIMAIGA*:

# AIRBORNE CONIDIAL MOVEMENT AND MODELING DISEASE SPREAD

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## ABSTRACT

Between 1989 and 1992, the distribution of the gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper increased dramatically. However, the mechanism for this spread was not known. Based on examples of rapid spread by fungal pathogens of plants and limited previous studies of other entomophthoralean pathogens of insects, one likely mechanism for spread was movement of airborne spores. During this study, we collected aerial samples of *E. maimaiga* spores (conidia) to determine both spatial and temporal variability. Weather data were collected adjacent to spore samplers, and, to determine whether airborne conidia were alive and infective, gypsy moth larvae were caged in the same vicinity.

Spore samplers were operated at four sites over two years; airborne conidia were only detected in the higher density gypsy moth populations sampled (ca. 2000 em/ha). At lower density sites (ca. 100 em/ha), cadavers of larvae dying from *E. maimaiga* may not have been abundant enough to produce conidial densities detectable with our equipment. During 1992, aerial sampling began wher third instars were abundant and peaks of airborne conidia occurred over 4 d periods, when fourth and fifth instars were present. Field sampling demonstrated that *E. maimaiga* infection in larval populations increased after airborne conidia became abundant. *E. maimaiga* conidia could be abundant at all vertical levels sampled (0.5-3.0 m) and conidial abundance was positively associated with relative humidity during and 0-24 h prior to sampling. A weak association occurred between density of airborne conidia and levels of infection among larvae caged at 0.5 m. This suggests that at least some of the airborne conidia sampled were alive and infective, but probably not all of them. In higher density gypsy moth populations, gypsy moth larvae caged at ground level became infected (by resting spores and conidia) on a regular basis until around pupation. In contrast, gypsy moth larvae in cages at 0.5 m became infected much more sporadically (due to sporadic occurrence of airborne conidia), yet infection levels at 0.5 m could become abundant. It is clear that both types of spores can be important throughout the field season although their relative importance varies from year to year. A model based on bioassay data for disease transmission due to conidia and resting spores with estimated conidial dispersal rates agreed well with data for short-distance disease spread during 1991. We conclude that short-distance spatial dynamics of this disease can be captured with knowledge of disease transmission and that conidial dispersal is a fairly simple process.

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

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## ABSTRACT

Small mammal live trap surveys were conducted in six sites, two each in the coastal plain, piedmont and mountains of Virginia / North Carolina during 1992 and 1993. Gypsy moth pupae predation was examined between the live-trap surveys by placing freeze-dried pupae at trap stations, at the litter layer, 0.25, 1.0, and 2.0 m. During 1992 total predation was high at all of the study sites, even where the mouse population was low. The lower predation by mice was, at least, partially compensated for by increased predation by invertebrates. There was an inverse relationship between predation by vertebrates and invertebrates. Our 1993 data corroborated these findings. During both years total predation remained about the same, even where low mouse density occurred. This constancy of total predation in these southern sites, despite radical reductions in populations of small mammals, would be consistent with an hypothesis of greater biodiversity in these sites. This is unlike information from Northeastern U.S. (Bryant Mountain, Vt.) where there was a direct decrease in predation as small mammals decreased. In 1993 gypsy moth F-1 sterile trap host plots were established at each site (mountains, piedmont and coastal plain) to determine the parasitic species in each habitat. It is notable that we found a number of parasites at Lake Anna, an area which currently does not have gypsy moth; also the abundance of *Eusisyropa virilis* in the coastal sites is of interest for two reasons: it is rarely found in gypsy moth in New England and it has a known host range of 30 species in 14 families of Lepidoptera. Gypsy moth parasitoid diversity in the three habitats suggest a need to investigate habitat effects on natural enemy populations. It seems important to establish the differences in biodiversity which exist as the gypsy moth moves from north to south. Such information could contribute to understanding relationships between this insect and its natural enemies and could have important implications in forest management. We also demonstrated the utility of releasing F-1 sterile egg masses to survey for parasites of subsequent gypsy moth life stages. It also seems that this technique could be used to survey for the natural spread of fungi and diseases of the gypsy moth.

# CLONING AND CHARACTERIZATION OF A cDNA ENCODING THE SMALL SUBUNIT OF THE VITELLOGENIN FROM THE GYPSY MOTH

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## ABSTRACT

The oligomeric structure of the vitellogenin (Vg) in the gypsy moth appears to be different from most lepidopteran Vgs, which consist predominantly of a single large type and a small type subunits. The native Vg from the gypsy moth (~490 kDa) is made up of three types of subunits of 190 kDa (Vg190), 165 kDa (Vg165), and 36 kDa (Vg36). The relationship among the three subunits and the structure of native Vg in the gypsy moth is not clear. Our earlier work indicated that the larger subunits (Vg190 and Vg165), which share extensive sequence homology and identical N-termini, had a precursor-product relationship. However, the smaller subunit (Vg36) was not related to the larger subunits, since the antiserum raised against Vg190 failed to cross-react with Vg36. Thus, Vg36 appears to be encoded by a separate gene in the gypsy moth.

To further analyze Vg36, we determined its N-terminal amino acid sequence. This sequence was confirmed using immunological methods. A synthetic peptide, corresponding to the N-terminal 15 amino acids, was synthesized using an Advanced Chemtech Model 350 peptide synthesizer. To facilitate its use for generating antibodies, the MAP resin technology was employed. Half a mg MAP-peptide, emulsified with Freund's complete adjuvant, was injected into rabbits. Serum collected after 8 weeks was used in Western analysis, which showed that the antibodies against the synthetic peptide cross-reacted with Vg36.

In order to clone and characterize the gene encoding the Vg36, a mixture of synthetic oligodeoxynucleotides, encoding the first six amino acids of Vg36, were prepared. These were used as primers along with the Universal Anchor Primer to amplify the cDNA coding Vg36 in a 3'-RACE reaction (Bethesda Research Lab., MD). A single ~1.2 kbp DNA was amplified in these reactions. This fragment was cloned into a T/A Cloning Vector (Invitrogen Corp., CA).

The cDNA fragment was labelled and used as a probe to investigate the nature of the Vg36 gene and its transcripts. In Northern analysis using total poly(A) RNA from L5D10 female larval fat body, the probe hybridized to a single RNA species of ~1.2 kb. The size of this RNA was consistent with the expected size for the Vg36mRNA. To characterize the Vg36 gene, a genomic Southern analysis was performed using high molecular weight DNA from the gypsy moth digested with various restriction enzymes. The results showed that, as in the case of the gene encoding the large subunit, a single copy of Vg36 gene was present in the gypsy moth genome.

# GENETIC CONTROL OF THE GYPSY MOTH

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## ABSTRACT

Several genetic approaches for insect pest control have been developed, but only a few of them have been successfully used. The most notable example is the employment of the sterile male technique. More sophisticated forms of genetic control such as hybrid sterility, sex-linked rearrangements coupled with conditional lethals, and others have been proposed and attempted. But, their application has been restricted to only a small number of pest species for which adequate genetic and biochemical knowledge is available to isolate and characterize these phenomena. A major limitation in applying genetic control techniques to lepidopteran insect pests has been the absence of sufficient basic genetic information of the pest species. Recent advances in molecular genetic analysis of insects, and techniques for transformation to produce transgenic insects have stimulated renewed interest in developing and applying novel genetic control techniques for lepidopteran pest control.

Our aim is to develop a genetic control strategy for the gypsy moth through the use of transgenic gypsy moth, whose genomes have been manipulated selectively by incorporating foreign insecticidal genes. These foreign genes, present in individuals in a target population, are expected to be expressed in a high population of individuals in ensuing generations resulting in their mortality. For such strategy to work, there are several important requirements. Proper gene promoters to drive the insecticidal genes have to be identified. These promoters should be such that they can be conditionally activated by controlling the experimental conditions such as temperature, hormones, metal ions, metabolites, etc. Otherwise rearing of such stocks carrying insecticidal genes will not be possible. A locus in the genome, which is not lethal to the insect's survival, has to be identified to insert the foreign gene. We have identified in the gypsy moth a gene whose promoter fits several of the above criteria. Vitellogenin (Vg) is a developmentally-regulated, abundantly-synthesized, sex-specific (expressed only in females) protein, whose genes are governed by the juvenile hormone in the gypsy moth. In order to isolate the Vg gene promoter, we constructed a gypsy moth subgenomic library using 12-15 kbp EcoRI fragments of the gypsy moth DNA. The phage library was screened using a cDNA probe corresponding to an internal portion of VgmRNA. One clone, E4VgL, appeared to contain the entire Vg gene. A restriction enzyme map of this clone was obtained and the region encoding the N-terminal portion of the Vg protein was identified by Southern analysis. Nucleotide sequencing of this region indicated that there was an intron of ~1.4 kbp immediately upstream from this region. In order to identify the promoter region, a cDNA representing the 5'-end of VgmRNA was synthesized and used as a probe. A SphI-BamHI fragment containing the promoter elements is being analyzed further by nucleotide sequencing. The promoter sequences will be used in the construction of a transformation vector for generating transgenic gypsy moth through microinjection techniques.

# COOPERATIVE REARING DEVELOPMENTS FOR *CERANTHIA SAMARENSIS*, A LOW DENSITY PARASITOID OF GYPSY MOTH

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## ABSTRACT

Forestry Canada and IIBC collected *Ceranthia samarensis* (Villeneuve) (Diptera: Tachinidae) from experimentally increased *Lymantria dispar* (L.) populations at Plancher Bas, France. *C. samarensis* is only known from low density, nonoutbreak populations of gypsy moth in Europe and has only two known hosts, *L. dispar* and *Orgyia gonostigma*. Forestry Canada's laboratory rearing of *C. samarensis* on gypsy moth larvae in cages is labor intensive and produces a limited number of individuals for release. Hence, we are collaborating with Forestry Canada to develop improved laboratory methods. The objective was to determine parasitism success of *C. samarensis* maggots artificially implanted onto *L. dispar* larvae and to compare its efficiency with that of the host exposure method.

We extracted maggots from uteri of nine gravid flies by removing maggots in cohorts of ten (i.e., maturity group 1 was most developed and maturity group 7 was least developed). We then implanted a single maggot into a 1.5ul droplet of Ringer's solution on the dorsal head/thorax juncture of a second instar gypsy moth. These 10 larvae with maggots from one maturity group were placed on artificial diet in a 2-tiered holding container. Last instar parasitoids exited host larvae and dropped into the lower chamber to pupate. For the host exposure method, we set up 15 cages each with two *C. samarensis* female flies (12 days post-copulation) and 30 gypsy moth second instars feeding on red oak seedlings. Each day for 14 days we replaced host larvae until both flies died. We fed larvae as before.

Implanted maggots from all nine flies parasitized hosts and developed into pupae, with a mean of 7.3 (range of 1-24) pupae per dissected fly. Twenty-five percent (65 of 259) of maggots from maturity groups 1-3 developed into pupae, whereas less developed maggots produced no pupae. Average time to pupation was 12.4 days (range of 9-18). We recovered pupae from only 3.8% (634 of 4440) of larvae exposed in cages, with a mean of 42.3 pupae per cage. Flies in all cages parasitized hosts, with a mean of 2.6 pupae/female/day. Time from host exposure to *C. samarensis* pupation averaged 15.4 days (range of 8-25). Mean time of death was 4.8 days for the first fly and 9.9 days for the second fly. In conclusion, mature maggots that were artificially implanted parasitized *L. dispar* larvae. Larvae exposed in cages were parasitized less, but perhaps this inefficiency can be remedied by larger cages, greater fly: host ratios, and exposing larvae void of oak foliage.

# GENETICS AND BIOLOGY OF ASIAN GYPSY MOTH AND ITS HYBRIDS

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## ABSTRACT

The following is a summary of progress on research to reduce the likelihood of the Asian race of the gypsy moth from establishing in North America. Most of this research is being conducted in the Forest Service Quarantine Laboratory located in Ansonia, CT. The source of the Asian gypsy moths used in these studies was grain ships from Russia that docked in the Northwest. The European gypsy moths from North America were from Massachusetts and North Carolina. Several researchers from the Forest Service's Northeastern Experiment Station are cooperators in this research program.

The Asian and European races of the gypsy moth are reproductively compatible, at least through one generation of hybridization. Gypsy moths from Russia, Asian race, weigh more than those from North America, European race. Russian gypsy moth larvae are equally or more susceptible to *Bacillus thuringiensis* and the gypsy moth nuclear polyhedrosis virus than are North American larvae. There is substantially more genetic variation in coloration and most other traits in the Russian populations than in the North American populations. Russian egg masses require a significantly shorter diapause chill period before hatch can be initiated than do North Americans. Adult Russian gypsy moth females are capable of extended strong directed flight and are attracted to lights. Russian females prefer to fly before they lay their eggs. The two races appear identical to the naked eye; laboratory analysis is required for positive identification.

First generation hybrids between the Asian (Russian) and European (North American) races of gypsy moths: 1) have similar diapause chill requirements to that of the Asian race which requires significantly less than does the European race, 2) resemble the Asian race in size (both as larvae and adults) and adult female wing length, 3) develop slightly faster than the parent races when crowded, 4) the majority (~99%) glide for a few meters vigorously flapping their wings but are not able to gain altitude or sustain flight, and 5) the larvae are intermediate in coloration to that of the two parent races except for the black form which appears only in families where one parent was the black form. In general, the hybrids are either intermediate to the two parents or resemble the Russian parent more than the North American parent. Number of genes involved and dominance issues can't be resolved at this point.

Gypsy moths collected from Germany, Switzerland, France and Austria are being evaluated in the quarantine for several traits and DNA analysis of samples has been initiated. Hatch characteristics of many egg masses collected from Germany and Austria indicate that they may have Asian race ancestry. DNA analysis of eggs from Europe is difficult to interpret at this time.

# DEVELOPMENTAL GENETICS OF *LYMANTRIA DISPAR*

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## ABSTRACT

We are studying the developmental genetics of *Lymantria dispar*. Over the last year we have focused on early embryonic development and on the appearance and behavior of chromosomes during gamete formation. Our observations are summarized as follows.

1. Whole mounts and serial sections of early embryos have shown us when cellularization, germband thickening, and embryonic segmentation occur. We are now working on techniques that allow us to identify these stages in living embryos. The germ bands of dechorionated embryos are visible using darkfield epi-illumination, which may facilitate injections and manipulations of the embryo using microsurgical techniques.

2. The germ cells of *Drosophila melanogaster* are sequestered as pole cells prior to the completion of embryonic cellularization. These cells then migrate to the midline of the germband, where they invaginate during the formation of mesoderm. Immunochemical makers for the product of the gene, *vasa*, identify pole plasm and germ cell determinants during all of this insect's development. We asked if germ cells and their precursors could be identified in gypsy moths by using this antibody. Western blots of proteins prepared from *D. melanogaster*, *Bombyx mori*, and *L. dispar* embryos of several stages were challenged with anti*vasa* antibody. Neither of the lepidopteran preparations provided any cross reaction with the antibody. This result has been obtained with antibody preparations to several other gene products that can serve as germ cell markers in other lepidoptera by other groups, suggesting that fundamental differences exist in the mechanisms which identify and sequester this cell population between the diptera and the lepidoptera.

3. We have previously described some aspects of the meiotic chromosomes of *L. dispar*. The males are chiasmate, while the females lack any physical indication of recombination. In general, the 31 elements of complement lack features which allow karyotic discrimination. Conventional staining with fluorescent dyes does not show heterochromatic blocks or the location of centromeres. However, we now have sufficient observations to assert that the females are the heterogametic sex, and that the WZ pair bears a nucleolus organizer. This

suggests that polymorphisms in the structure of the ribosomal RNA cistrons may behave as if they are sex-linked.

4. The seven nurse cells and the oocyte all proceed through the first meiotic division of the female. At some point in Zygotene/Diplotene the chromosomes decondense to form long diffuse structures on which chromomeres persist on some of the chromosomes. This stage seems most suited for continued karyotypic examination using banding and *in situ* techniques.

5. CsCl gradient equilibrium banding of total DNA isolated from adult females produces two well separated bands of equal concentration. The least dense of the bands (1.686 gms/cc) is approximately equal in density to mitochondrial DNA isolated from other insects. Isolation of DNA from nuclear preparations virtually eliminates this band, suggesting that it is largely cytoplasmic in origin. The remaining main band is symmetrically dispersed about a mean density of 1.699 gms/cc. Since nuclear satellite DNAs of low density usually define heterochromatin, this observation is consistent with the dearth of heterochromatic blocks seen in our chromosome preparations. The analysis suggests that cloning and molecular analysis should be restricted to DNA taken from nuclear preparations, since DNAs of high AT content (low density) can contribute to artifacts.

6. We examined meiosis in inter-racial hybrids of Asian and North American isolates. The prophase chromosomes of $F_1$ females were fully synapsed. The complements contained the normal chromosome numbers and there were no figures that implied inversion or translocation polymorphisms between the complements. Previous reports of size differences between the chromosome sets of different races could not be repeated in our materials. For instance, there were no heteromorphic bivalents or pairs which formed incompletely.

7. Prophase figures were rare in testis preparations from the $F_1$ male progeny of inter-racial matings, suggesting that the time spent in these stages is abbreviated. Where available, the chromosomes were achiasmate, and showed variable numbers of elements. It is not clear whether the apparent deviations from diploidy resulted from mitotic errors in premeiotic divisions, or from the breakdown of pairing in the achiasmate bivalents. later division stages were often highly irregular, with many spermatid clusters bearing anomalous chromosome numbers. Nonetheless, sperm maturation was evident and morphologically normal. The high frequency of aneuploidy observed here would explain some early reports of sex reversals in gypsy moths, and would predict that many DNA markers will be required to follow the process of genetic introgression of Asian moths into the North American populations.

# PRELIMINARY RESULTS ON THE EFFECTS OF IRON BIOAVAILABILITY ON TOXICITY, ELECTROPHYSIOLOGICAL AND BINDING CHARACTERISTICS OF *BACILLUS THURINGIENSIS* ON *LYMANTRIA DISPAR* L.

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## ABSTRACT

We have examined the biological activity of *Bacillus thuringiensis* insecticidal crystal proteins, CryIAa, CryIAb and CryIAc against gypsy moth (GM) using several assay systems. These include bioassay, receptor binding and voltage clamping with GM midguts. In these studies we observed the activity ratio of CryIA toxins as: CryIAa > CryIAb >> CryIAc. All of these studies were performed on a culture of GM showing abnormal performance syndrome (APS; 1) caused by rearing on an iron deficient diet. More recently, we compared APS-free cultures of GM with the previous APS culture. We have detected a reversal in activity of CryIAa and CryIAc toxins related to the iron bioavailability. The physiological basis of this reversal of specificity and the relation of susceptibility of GM reared on oak leaves, will be investigated in future studies.

# GYPSY MOTH CONTAINMENT PROGRAM: AN ECONOMIC ASSESSMENT

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## ABSTRACT

A rapid and approximate assessment was performed considering potential gypsy moth economic impacts on management activities, timber, recreation, and residences. Infestations for the next 25 years were simulated for six rates of spread, from 2.5 to 15.0 miles per year in 2.5 mile increments. Impacts were estimated and summed for each rate of spread. One rate of spread is chosen as the "without GMCP" scenario and a second as the "with GMCP" scenario. The difference between any pair of impacts is the estimated GMCP potential benefits.

Arbitrarily, the difference between 15.0 and 2.5 miles a year spread is chosen as the Best Case scenario, 12.5 and 5.0 as the Mid-range scenario, and 10.0 and 7.5 as the Worst Case scenario. The Equivalent Annual Expenditure was calculated. This is the amount which, if spent each year for the 25 years to implement GMCP, would cause benefits to equal costs or, equivalently, a 1.0 B/C ratio. The approximate values are:

| Scenario | Potential Benefit (present value) | Equivalent Annual Expenditure |
|---|---|---|
| Best | $500 million | $32.0 million |
| Mid-range | $300 million | $19.2 million |
| Worst | $100 million | $6.4 million |

About 60 percent of the potential benefits come from residential impacts, 30 percent from reduced management activities, and the remaining 10 percent from reduced timber and recreation impacts. Estimates are biased low because, except for management activities, only impacts occurring during the first year an area enters the Generally Infested Zone are considered.

[2]The author gratefully acknowledges the significant contributions of Dr. F. W. Ravlin and his team, most particularly John Young and Sally Walden; the FIA projects at the USDA Forest Service Experiment Stations; Mark Young at the Outdoor Recreation & Wilderness Assessment Project; the Technical Team; and many others.

# LOCATION OF THE *LYMANTRIA DISPAR* L. REGION OF *BACILLUS THURINGIENSIS* CRYIIA DELTA-ENDOTOXIN

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## ABSTRACT

*Bacillus thuringiensis* insecticidal crystal protein CryIIA has both high mosquito activity and gypsy moth activity, while CryIIB, which is 87% homologous, displays no mosquito activity and has a 3-fold lower gypsy moth activity. By the simple introduction of two new restriction enzyme sites and reciprocal exchange of subdomains of the two genes we have located the "gypsy moth specificity" determining region of CryIIA. There are only 14 amino acids that differ between the two genes over this region.

# USING FOREST INVENTORY AND ANALYSIS DATA TO PREDICT SUSCEPTIBILITY TO GYPSY MOTH DEFOLIATION

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## ABSTRACT

The gypsy moth is a polyphagous insect; North American populations feed on over 300 different shrub and tree species. Despite this wide breadth of host preference, there is considerable variation within northeastern North American forests in their susceptibility to defoliation. Considerable work has focused on characterization of the various components of stand susceptibility. These studies have implicated tree density, host species tree density, the abundance of tree structural features (e.g. bark flaps), and various site characteristics (e.g. soils) as important factors for predicting defoliation in a given stand. Unfortunately, most of the stand-level variables used by previously developed susceptibility rating systems are typically not available in most areas where gypsy moth management is contemplated.

As part of the forest inventory and analysis (FIA) program, the USDA Forest Service conducts inventories of forest resources in every state with the use of permanent plots where tree diameters and other measurements are collected periodically. In the East, these inventories are usually conducted every 5 to 15 years at about 1,000 irregularly spaced plots in each state. In this study, we explore the use of these data, as well as other ancillary data, for interpolation of stand composition.

COUNTY-LEVEL SUSCEPTIBILITY RATING: Herrick and Gansner determined that forest stands are susceptible to considerable gypsy moth defoliation when the proportion of basal area of preferred gypsy moth host species exceeds 50% of the total stand basal area. Therefore, we used FIA data from the eastern US to estimate the total number of acres of forested land where preferred species compose 50% or more of the total basal area. We then divided this number by the total acreage of the county to obtain an estimate of the proportion of the county where heavy defoliation by the gypsy moth is likely.

These analyses indicated that forests in the central Appalachian mountains are generally of high susceptibility which agrees with current experiences of heavy defoliation in that area. Our

analyses indicated that these predictions of susceptibility are statistically validated by historical levels of defoliation in that area. The analysis also indicates considerable areas of high susceptibility in the northern portions of the lake states of Minnesota, Wisconsin and Michigan. These areas are dominated by aspen and only small portions of the region are currently infested. The analysis also indicates an area of high susceptibility in the Ozark Mountain area of Arkansas and Missouri.

INTERPOLATION OF FIA PLOT DATA: In order to use forest composition data for decision making in a gypsy moth management program, forest composition estimates need to be made at the stand level. This requires a much higher spatial resolution than is provided by the above county-level estimates. Several statistical methods are available for the interpolation of spatial data. We used a geostatistical technique, kriging, to estimate host basal area at unsampled 1 ha grid cells as weighted averages of measurements at nearby locations. Unfortunately, kriging tends to generate very "smooth" surfaces of estimates; real surfaces of tree species composition are much more discontinuous due to small-scale variation in topography. Therefore we explored the use of topographical data to provide more precise estimates of forest susceptibility. Estimates of slope, aspect, and topographical position were derived from digital elevation models distributed by the US Geological Survey. We fit a variety of linear and non-linear models that predict proportion host basal area from these topographical measurements at 1 ha grid cells. The validity of these estimation methods are currently being evaluated. Preliminary results indicate that a combination of regression with kriging of residuals may be the most desirable method for estimating susceptibility.

# HOW TO USE YOUR GIS TO PREDICT GYPSY MOTH DEFOLIATION

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## ABSTRACT

In most current gypsy moth management programs, the decision to aerially suppress populations is based upon estimates of gypsy moth egg mass densities that exceed some specific threshold that is expected to cause intolerable levels of defoliation. These densities are averages of samples taken over forest stands that may be very large and treatment decisions largely ignore conditions in nearby stands and/or population levels in the previous year. Geographical information systems (GIS) are computer programs that are increasingly being applied in the management of natural resources over large areas; potentially they may be of great use in integrating gypsy moth census data, as well as forest composition data, in a way that promotes more sound management decisions. In this study, we compared three different spatially explicit models for their ability to assess regional population conditions and predicting defoliation. These models are designed to use spatially explicit data available in a GIS.

The three types of models evaluated were: 3-dimensional kriging, cellular transition models, and logistic regression models. The 3-dimensional kriging model extrapolated future defoliation maps from previous defoliation maps based upon geostatistical models of the spatial and temporal autocorrelation of defoliation. Cellular transition models simulated probabilities of transitions of individual cells from non-defoliated to defoliated based upon the defoliation status of the same and neighboring cells in the previous year, as well as upon egg mass densities, interpolated from an irregular network of egg mass density measurements. Logistic regression was used to fit a linear model for the probability of defoliation in a given cell based upon the presence of defoliation in the previous year, as well as upon interpolated egg mass densities.

These models were empirically derived from historical gypsy moth defoliation and egg mass data collected in Massachusetts from 1961-1991. All three methods behaved similarly, though the logistic regression and transition models performed slightly better, perhaps because they incorporated information about densities in the next generation (egg mass data). A similar logistic regression model was derived using data collected during the Appalachian Integrated Pest Management (AIPM) program. It was shown that this model can be used to more correctly make management decisions than can be done with current egg mass density thresholds.

# EFFECTS OF ASPEN CHEMISTRY ON GYPSY MOTH SUSCEPTIBILITY TO *BT*

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## ABSTRACT

The chemical composition of aspen (*Populus tremuloides*) foliage strongly influences gypsy moth larval performance. Larval growth and pupal weights are inversely related to foliar phenolic glycoside concentrations. Condensed tannins, however, do not appear to directly affect gypsy moth performance. This research program investigates the effects of aspen defensive chemistry on efficacy of the microbial insecticide *Bt* (*Bacillus thuringiensis*).

Laboratory studies determined $LD_{50}$s for larvae reared on foliage collected from one of five different aspen trees. We found 100-fold variation in $LD_{50}$ values. Foliar phenolic glycoside concentrations showed a positive relationship (nonsignificant trend) with *Bt* toxicity. Condensed tannins showed a strong, negative relationship with *Bt* toxicity. A parallel study with phenolic glycosides and *Bt* incorporated into artificial diets showed that phenolic glycosides strongly augment *Bt* toxicity.

Field studies on the effects of aspen chemistry on gypsy moth susceptibility to *Bt* were conducted in northern lower Michigan in 1993. Newly hatched larvae were reared in fine mesh bags on 17 aspen trees. Toxicity trials were conducted using three doses of *Bt* (four replicates of 20 larvae per dose). Larval mortality and foliar chemical composition varied significantly among trees. Regression analyses, however, did not reveal significant relationships between *Bt* toxicity and aspen chemistry. Lack of detectable relationships under field conditions may be due to: 1) difficulty in applying a specific dose of *Bt* to leaves, 2) large variation in larval mortality within individual trees, and 3) relatively low variation in chemistry among trees.

# CANOPY DEPOSITION CHARACTERISTICS OF THREE FORMULATIONS OF NPV AERIALLY APPLIED TO A MIXED HARDWOOD FOREST IN MICHIGAN IN 1993

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## ABSTRACT

The Manistee National Forest in Michigan was the site of an experiment where two commercial formulations of Nuclear Polyhedrosis Virus (NPV), one prepared by American Cyanamid Inc. and one prepared by Entotech Inc., were compared to the Forest Service standard formulation of GYCHEK. The formulation of GYCHEK has long been used by the Forest Service to control the gypsy moth (*Lymantria dispar* L.). The three formulations were sprayed by aircraft to plots of mixed hardwood forest. The goal of the sampling was to compare the deposition parameters of the three formulations on foliage and match these potential differences to biological differences that might occur in plots sprayed with the same formulations. Samples of foliage were harvested by shooting down small branches with shotguns. Twenty trees were sampled in each plot and six leaves per tree were collected. There were two replicated 30-acre plots for each formulation. In order to have a permanent record of deposition, each leaf was photographed after sampling. The photographs were then analyzed by image analysis to determine the size and number of droplets on each leaf.

Deposition parameters calculated were vmd (volume mean diameter), nmd (number mean diameter), droplet density, and volume density. All three formulations had average droplet densities of 4.8 drops/cm$^2$ or greater. The American Cyanamid formulation had the highest average vmd and the lowest average nmd of the three formulations. The highest droplet and volume densities were found in the plots sprayed with the Entotech NPV formulation. The Forest Service's GYCHEK had the highest variability of deposition parameters because of weather conditions at the time of the spray.

# AN EVALUATION OF OPPOSING VIEWPOINTS OF CLASSICAL BIOLOGICAL CONTROL

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## Introduction

I was invited to give one of three presentations in this symposium dealing with safety and regulation of nonindigenous biological control agents. Although the symposium was initially intended to have a debate atmosphere, I am happy that a true debate never developed and that all three presentations in the symposium had more in common than I expected. Rather than a debate I consider my paper a discussion of some of the arguments relative to the safety and regulation of nonindigenous biological control agents.

Although vertebrates are often cited as examples of catastrophic nonindigenous biological control agents, the introduction of exotic vertebrates has never been suggested or condoned by biological control specialists. In this paper the term "biological control agents" means insect parasitoids, predators, herbivores, and pathogens of insects.

## The Issues: Opposing Viewpoints

Everyone knows that opposing viewpoints exist in every facet of human endeavor. Politics, religion and even science contain many examples of diametrically opposed views that seem irreconcilable. Science should actually thrive on alternative views that can be stated in the form of hypotheses since this is the very basis of scientific inquiry. Although I am a strong advocate of biological control, I will attempt to objectively define what I believe is the basis for the two opposing views: 1) classical biological control is a safe successful method for controlling pests and 2) classical biological control is an inherently dangerous process that has caused irreparable environmental damage.

**Biological control is a safe, successful method for controlling pests.**

Many successes. No one can disagree that classical biological control has been very successful in controlling or moderating the undesirable effects of many species of insects. There are hundreds of documented examples that demonstrate the success of classical biological control.

Excellent safety record. Biological control is the safest method of pest control know. Classical biological control has been used as a strategy for pest control for over 100 years and there are there are very few documented examples of undesirable effects. Biological control agents are

harmless to humans and other vertebrates. They do not build up in the food chain as do many chemical pesticides. They do not produce secondary pests by destroying a wide spectrum of primary biological control agents. Finally, there is no documented evidence that insects or insect pathogens used as classical biological control agents have caused the extinction of any other organism.

Inexpensive. The introduction of classical biological control agents has saved billions of dollars in agricultural and forest systems. The cost of foreign exploration, laboratory evaluation, and introduction for all exotic biological control agents considered for introduction is only a fraction of the savings achieved by all of the successful introductions of nonindigenous biological control agents. When we compare the tax dollars spent on classical biological control with the savings achieved by controlling insect pests, classical biological control is a very inexpensive endeavor.

Permanent. One of the reasons classical biological control is so inexpensive is that established biological control agents are usually permanent. Once established, the biological control agent becomes an integral part of the insect pest population, acting as a permanent cycling biological control agent. This is almost always the objective of classical biological control introductions.

**Classical biological control is an inherently dangerous process that has caused irreparable environmental damage**

Permanent. While permanence is considered a virtue by proponents of biological control, it is considered a major liability by many who question the safety of classical biological control. If an introduced nonindigenous organism causes undesirable effects, those effects are permanent and the outcome is both undesirable and permanent. We are constantly reminded of this when we see the list of introduced, and usually unwanted, nonindigenous weeds and birds.

Limited monitoring. Some people who question the wisdom of classical biological control may admit that while there is little, if any, empirical evidence demonstrating that classical biological control has undesirable side effects, this is simply because there have been few long term studies monitoring the releases of introduced nonindigenous biological control agents. After their initial introduction, most insect biological control agents have not been extensively monitored for extension of either geographical range or host range. An additional criticism has been that most monitoring has been restricted to hosts that are pest species.

Few studies involving complex ecosystems. This criticism, directly related to the above, is that the limited monitoring studies have rarely included studies on ecosystem effects. I believe this is an appropriate criticism.

Expensive. Some critics of biological control have argued that if the introduction of a classical biological control agent is unsuccessful, the specific unsuccessful project is expensive, because it produced no useful results.

Extinctions? Numerous claims have been made that introductions of nonindigenous biological control agents have produced numerous extinctions of native organisms, either directly or indirectly. There is no documented evidence that the planned introduction of an insect parasite, predator, herbivore, or pathogen has caused the extinction of a nontarget host, or for that matter even a target host. Of course one could argue that if we have not monitored carefully or studied complex ecosystems, how can we know that extinctions have not occurred.

## The Issues: How Can We Resolve Opposing Viewpoints

Obviously the introduction of nonindigenous biological control agents has not caused massive environmental damage. Some have suggested that nonindigenous biological control agents are more dangerous than chemical pesticides because they are permanent and chemical pesticides are gradually degraded. I believe this is an irresponsible criticism given the extensive experimental evidence of the harmful effects of chemical pesticides. Cancer, severe effects on animals at the end of a food chain, production of secondary pests, and direct cause of human death are only some of the undesirable side effects of misusing chemical pesticides. Nevertheless, I do not believe that responsible biological control scientists can disregard responsible criticism of classical biological control. Limited monitoring of introduced exotic biological control agents and few studies involving the effect of these agents on complex ecosystems are legitimate criticisms.

I do not believe we can fully resolve these opposing viewpoints until we have more long term data sets on the releases of introduced nonindigenous biological control agents and their effects on ecosystems. It is actually to the advantage of biological control scientists to address these questions because a more thorough understanding of the distribution, host ranges, and effects on ecosystems will allow us to make better decisions about targets, biological control agents and methods of introduction.

I believe we should be more concerned about the future than about which viewpoint will ultimately prove to be correct. It is much more important that we work together to develop procedures that will reduce the possibility of mistakes in the conduct of classical biological control. Our ultimate objectives should be to develop guidelines and procedures that reduce the possibility of introducing a biological control agent that will do harm while at the same time not eliminating agents or groups of agents that are safe and potentially useful.

## Regulatory Concerns

It is probably overly simplistic to suggest that regulatory concerns can be reduced to only two questions, but I believe that most regulatory concerns are directly or indirectly related to 1) selecting the biological control target and 2) host specificity of the biological control agent.

**Selecting the target**

Is it a pest? No organism should be the target of biological control if it is not a true pest. Identifying an organism as a pest is not always as obvious as it may seem. In some cases there is no doubt about the pest status of an organism. Gypsy moths, corn borers, and alfalfa weevils are unquestionably pests. They frequently cause significant damage to their host plants, their host plants are valuable, and they are primary pests, not induced by the unwise use of pesticides. Other pest situations are not so obvious. For example, most secondary pests, generated when broad spectrum pesticides kill natural enemies, should not be the targets of classical biological control until an attempt is made to eliminate the actions that caused them to become secondary pests in the first place. Likewise an organism that causes minor and sporadic damage may not be an appropriate candidate for biological control We must be careful not to exclude organisms as candidates for biological control just because they attack minor crops (small acreage crops). A major pest of a minor crop may be an appropriate candidate for biological control if it fits all of the criteria used to select biological control candidates.

Can we live without it? This may seem to be a redundant question. Once we have identified the organism as a pest and as a target for biological control, why bother to ask this question, especially since there is no evidence that the planned introduction of a nonindigenous biological control agent has ever caused the extinction of a target host.

I believe it is important to pose this question because the concept of eliminating or reducing a target organism is the basis of some of the criticism directed at classical biological control. If our target for classical biological control is the gypsy moth, our goal is to reduce or eliminate gypsy moth populations. If we could eliminate the gypsy moth tomorrow (everyone knows this is not possible) there would be a cascading series of events caused by this action. Some of us may lose our jobs, or at least have to work on another entomological problem, but more seriously, and from an ecological perspective, many organisms that have grown to depend on the gypsy moth as a part of their livelihood would be affected. If we can not agree that significant reduction of gypsy moth populations is a desirable outcome, that the beneficial effects outweigh the negative effects, then we must revisit our choice of the gypsy moth as a target of biological control. I believe the gypsy moth example is an easy decision. We would all benefit if we could eliminate the gypsy moth; it is the ultimate objective of our workshop. Similar questions about other pests and pest situations are not so easy. I believe this issue is responsible for the some of the contentious disagreements that have developed about the use of classical biological control agents. It is important that we view all classical biological control actions within the framework of a cost/benefit scenario. This will not eliminate disagreements, but it should allow us to approach these disagreements in a logical, scientifically based manner.

Will classical biological control be effective? We can answer this question in the affirmative for groups of pests that have been particularly amenable to classical biological control. We are much less able to predict that a biological control agent will be ineffective. Scientists who work in specific cropping systems or deal with specific host/biological control agent interactions probably can make reasonable predictions now, but as we accumulate more long term data bases

on the relative success of classical biological control introductions for a variety of pests and in a variety of situations we should be able to make general predictions about the probability of success with classical biological control agents.

**Host specificity**

Host specificity is the major issue relative to the safety of classical biological control agents. How we deal with questions involving host specificity will ultimately determine how and to what extent we use classical biological control. Our safety questions are about ecological host specificity (host specificity in the field) but estimates of ecological host specificity are usually based on laboratory experiments. Ecological host specificity is known with absolute certainty only after the biological control agent has been released into the environment. These issues are complex and there are seldom yes or no answers. I believe that it will be impossible to develop a single general host specificity requirement for classical biological control agents before they are released. Each group of agents must be evaluated by scientists who are familiar with the unique characteristics of specific groups of biological control agents. The following is a very brief summary of what I consider to be the most important host specificity considerations relative to the regulation of classical biological control agents.

Host specificity effects. In my opinion most questions about the host specificity of an introduced biological control agent fall into one of three categories: 1) Does it colonize the nontarget host? 2) Does it kill the nontarget host? and 3) If either 1 or 2 occur, what effect does the biological control agent have on the community into which it is introduced?

The first question, colonization, involves cycling in the nontarget host. If the introduced agent is able to parasitize, infect, etc. the nontarget host and is subsequently able to persist by reproducing and cycling in that host, it has successfully colonized the nontarget host.

The second question, "does it kill the nontarget host?" is relatively simple. Most biological control agents that colonize nontarget hosts kill at least some of the hosts, but it is also possible for a biological control agent to kill individuals within a nontarget host population without colonizing the nontarget species. Depending on the circumstances, his may or may not be an important safety issue.

The third question involving community effects is much more difficult to address experimentally. If the biological control agent does not colonize or kill nontarget hosts, this is not an issue.

Estimating host specificity. One of the criticisms of classical biological control is that laboratory host specificity experiments do not accurately reflect ecological host specificity and that after an exotic agent is established, it is usually permanent. Although long term monitoring has not been conducted for many introduced exotic biological control agents, the data that are available suggest that estimates of host specificity have been reasonably accurate. This is especially true for insect herbivores used as classical biological control agents of weeds. Extensive laboratory

studies on the host specificity of this group of biological control agents has been very predictive of their ecological host specificity. The ecological host specificity of nonindigenous biological control agents is usually estimated by information obtained from phylogenetic relationships, data bases from the native country, and laboratory experiments.

Phylogenetic relationships can be one of the most accurate predictors of host specificity for some groups of biological control agents. Some groups of biological control agents are very host specific to certain host taxa. This is not only important in directly estimating host specificity, it is also important in designing appropriate laboratory experiments on host specificity.

Data bases from the native country of the biological control agent, when available, may be invaluable in estimating host specificity of a nonindigenous biological control agent. Unfortunately, such data bases are often not available and nontarget host taxa may be absent or not well represented in the country of origin.

Laboratory host specificity experiments are often one of the most important sources of information used to estimate ecological host specificity. The information generated in laboratory host specificity experiments is extremely useful for estimating ecological host specificity, but interpretation of the results of laboratory host specificity experiments is usually not straightforward. It is not possible to cover all of the nuances involved in relating laboratory host specificity to ecological host specificity. This will be discussed extensively in two publications resulting from a Workshop on Host Specificity sponsored by the Audubon Society. These publications are mentioned in the acknowledgements. Some of the important considerations relative to the design and interpretation of laboratory host specificity experiments are as follows:

- Experimental design must fit the biological control agent. It is impossible to develop specific methods for use with all biological control agents. In order to properly evaluate the host specificity of a biological control agent, the nature of the interaction between the agent and the host must be understood and conditions necessary for successful utilization of the host provided.

- The laboratory study must be statistically sound and have some predictive value. Yes or no answers are often of little value unless they are supported statistically. The experiments should also be clearly described and the outcome should have predictive value. For example, it is not useful to conduct a laboratory experiment evaluating the effect of a biological control agent on a nontarget host which is in a taxonomic group known to be refractive or to expose the incorrect stage of a nontarget host to the biological control agent.

- Laboratory host specificity experiments that are correctly designed, produce infection in a much larger number of nontarget hosts (both individuals and species) than is produced in natural settings. Generally, as the arena in which the host specificity experiments are conducted becomes more complex, the more specific the biological control agent

becomes. Single choice experiments (one host species) are less host specific than multiple choice experiments (more than one host species). We should continue to test this hypothesis with every biological control agent released into the environment.

## Funding Concerns

It would be unrealistic to avoid the problems associated with funding the studies I have suggested. It would be tantamount to shutting down classical biological control if we demand that many additional studies be conducted without providing adequate funding to conduct these studies. One of the reasons long monitoring studies and community ecoloty studies are rarely conducted is that biological control has traditionally been funded at marginal levels. Funds have simply not been available for these studies. Both critics and supporters of biological control should insist that funding be made available to conduct studies on long term monitoring and community effects.

## The Existing Legal Basis for Regulating the Introduction of Nonindigenous Biological Control Agents

Because I have no legal qualifications, I do not pretend to fully understand nor will I attempt to explain all of the laws that are currently the basis for regulating nonindigenous biological control agents. It is, however, well known that existing biological control regulations are based on interpretations of five different pieces of legislation. These legislative acts are as follows:

- The Plant Quarantine Act of 1912
- The Federal Plant Pest Act of 1957
- The Federal Insecticide, Fungicide, and Rodenticide Act of 1947 (FIFRA)
- The National Environmental Policy Act of 1969 (NEPA)
- The Endangered Species Act of 1973

These laws were not written with biological control agents in mind. The Federal Plant Pest Act of 1957 is the law most often used for regulating classical biological control agents. Its purpose was to prevent the introduction of organisms that damage plants, not to provide a regulatory function for biological control agents. It does not provide an adequate scientifically based framework for properly evaluating biological control agents.

## Five Old Laws Versus A New Biological Control Law

The possibility of replacing the existing regulatory structure for regulation of biological control agents with new legislation, designed to deal with the specific characteristics of biological control agents has often been the subject of informal discussions among biological control specialists. I believe that until several years ago the general consensus was that while the old laws were cumbersome and often illogical, familiar problems with the existing regulations may be better than the uncertainty of how an entirely new law might affect biological control researc and implementation. In my opinion, the view that a new biological control law may be worse

than regulations governing the use of classical biological control agents has begun to change and I believe the stimulus for this change was the issuance of several challenges questioning the safe use of classical biological control agents. Our current laws, not written for the purpose of regulating biological control agents, have not dealt well with these challenges. There is no effective mechanism for resolving the issues. Challenges have not been posed as testable scientific hypotheses and decisions have been postponed. A law designed specifically to deal with classical biological control might be an acceptable alternative.

Characteristics of our current system for regulating classical biological control agents. Following is an admittedly incomplete list of some of the characteristics of our current regulatory system.

- It is an adaptation of several federal acts, none of which was designed to regulate biological control.
- Within this regulatory system there are inconsistent and biologically illogical regulatory situations.
- Several regulatory agencies are sometimes involved.
- The regulatory agency (APHIS) is also involved in introduction of exotic biological control agents.
- With all of its faults the current system has functioned reasonably well for a long time.

Possible characteristics of a new biological control law. A new biological control law could take one of many forms. The final product will probably be influenced by the input from a wide range of lobbyists and special interest groups, including both critics and proponents of classical biological control. Under these circumstances good science does not always prevail. Following is another incomplete list of some of the characteristics a new law might possess.

- It could specifically address biological control issues.
- Depending on one's point of view, it could be much better or it could be much worse that our current system of regulations.
- It will probably create a new regulatory structure which may require a long learning curve for both regulators and regulated.

## A New Biological Control Law

I realize that it is presumptuous for an insect pathologist with absolutely no political influence to suggest a new law, but I believe both biological control proponents and critics could benefit from a logical scientifically based biological control law. The development of such a law will not be easy. Scientists engaged in biological control research and implementation must realize that we are subject to federal regulations. We should insist that the regulations be based on reasonable hypotheses supported by empirical data. Critics of classical biological control must be willing to pose reasonable and testable alternative hypotheses and both critics and proponents must be willing to abide by decisions based on the best science available at that time.

As a proponent of biological control I believe a new law should contain at least the following features.

- It should promote classical biological control.
- Decisions should be based on a risk versus benefit analysis supported by scientific evidence.
- Important decisions must seek input from user groups and environmental groups as well as from biological control scientists.
- It should support long term monitoring studies after the release of every biological control agent.
- It should encourage the philosophy that "every release be an hypothesis testing experiment" (This is a concept proposed by Dr. Peter McEvoy, University of Oregon, at the Audubon Society Host Specificity Workshop, Washington DC, 1993).

## Conclusions

It is important to understand that the ongoing "debate" about classical biological control is not between two homogenous groups. It is not an argument between biological control advocates and environmentalists. I am both a biological control advocate and an environmentalist, as are most other scientists engaged in biological control research. Most scientists active in biological control research believe we should insist on long term monitoring of biological control releases. Many also believe we should include studies on how classical biological control activities affect insect communities. Most environmental groups also support the concept of classical biological control. I hope that everyone involved in discussions about classical control agrees that we share the ultimate objective of controlling pests with methods that cause minimal environmental disruption. If we work together, I believe we can achieve this goal.

**Acknowledgments**

Although I am solely responsible for all of the comments in this paper, I am indebted to many of my colleagues in biological control with whom I have had numerous discussions about how biological control agents should be regulated. I am especially indebted to the biological control scientists who participated in a Workshop on Host Specificity sponsored by The Audubon Society. Many of my comments in this paper were influenced by discussions held at this workshop. Proceedings of the Host Specificity Workshop will be published during 1994.

# INTERACTION BETWEEN TWO PATHOGENS OF GYPSY MOTH : *ENTOMOPHAGA MAIMAIGA* AND NUCLEAR POLYHEDROSIS VIRUS

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## ABSTRACT

Despite yearly epizootics of *Entomophaga maimaiga* Humber, Shimazu and Soper beginning in 1989, defoliation by gypsy moth in Massachusetts increased each year through 1991 and many populations remained at high density for four or more years. Normally such populations collapse from epizootics of the gypsy moth nuclear polyhedrosis virus (LdNPV) (Campbell 1963, Doane 1970). We suspected that this fungal pathogen may suppress LdNPV epizootics in the same way as applications of *Bacillus thuringiensis* (Woods et al. 1988).

We conducted a laboratory study to observe whether infection of *E. maimaiga* influences the proportion of gypsy moth larvae dying from LdNPV. When gypsy moth larvae were fed LdNPV and then injected with *E. maimaiga* 10 days later, they died from LdNPV (based on microscopic examination) several days earlier than if they were infected with LdNPV alone. Furthermore, the proportion of larvae that died and contained LdNPV increased when larvae were infected with both, than if they were infected with LdNPV alone.

In field experiments , we initiated epizootics of *E. maimaiga* and LdNPV among healthy gypsy moth larvae by enclosing them in mesh bags with cadavers of larvae recently killed by either of these two pathogens alone or with both types of cadavers. In one experiment we varied the density of cadavers; in another we applied simulated rain to half of the bags. There was no significant density effect, but simulated rain significantly increased mortality from both LdNPV and *E. maimaiga*. We found no consistent difference between the proportion dying from LdNPV when that pathogen was present alone, than when both pathogens were present. The marginal attack rate of LdNPV (Elkinton et al. 1992) was not affected by the presence of *E. maimaiga*. Thus we conclude that *E. maimaiga* had no discernible effect on the impact of LdNPV in these bugs-in-bags experiments. We further conclude that any detrimental effect of *E. maimaiga* on LdNPv epizootics, must arise indirectly from the effect of the fungal pathogen on the density of gypsy moth larvae.

# CHARACTERIZATION OF A *LYMANTRIA DISPAR* MULTINUCLEOCAPSID NUCLEAR POLYHEDROSIS VIRUS VARIANT THAT EXHIBITS ENHANCED POLYHEDRA PRODUCTION STABILITY DURING SERIAL PASSAGE IN CELL CULTURE

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## ABSTRACT

During propagation of baculoviruses in cell culture a class of viruses with an altered plaque phenotype, termed few polyhedra (FP) mutants, arise at high frequency. FP mutants exhibit the characteristics of formation of few polyhedra, the occlusion of few or no virions in polyhedra, and the synthesis of greater amounts of budded virus compared to wild type (many polyhedra, MP) virus. The enhanced production of budded virus is thought to be the basis for FP mutants becoming the predominant virus type during serial passage in cell culture. The rapid formation of FP mutants during propagation in cell culture is an impediment to the production of baculoviruses in this system on a commercial scale.

The *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (LdMNPV) rapidly mutates to FP mutants during serial propagation in *L. dispar* 652Y cells. After only three serial passages of LdMNPV in 652Y cells greater than 90% of the virus present exhibited the FP phenotype (Slavicek et al., submitted). Development of LdMNPV viral strains refractory to mutation to the FP phenotype would facilitate production of virus in cell culture systems. This abstract describes the isolation and characterization of a LdMNPV strain, A21-MPV, that exhibits enhanced stability of polyhedra production in comparison to wild type virus during serial passage in the *L. dispar* 652Y cell line. The attributes of budded virus titer, polyhedra production, the percentage of infected cells that produce polyhedra, and the proportion of virus exhibiting a FP phenotype during serial passage in 652Y cells were determined for isolates A21-MPV and A21 (a wild type isolate). During 5 serial passages isolate A21 exhibited an increase in budded virus titer, a decrease in polyhedra production, a decrease in the percentage of cells that produce polyhedra, and a decrease in virion occlusion. These traits are typical of those displayed by FP mutants. In contrast, isolate A21-MPV exhibited no change in budded virus titer, polyhedra production, the percentage of cells that produced polyhedra, or virion occlusion during serial passage. After only the second passage greater than 80% of isolate A21 exhibited a FP phenotype. In contrast, less than 1% of isolate A21-MPV exhibited a FP phenotype after passage two. These results demonstrate that A21-MPV exhibits enhanced polyhedra production stability in comparison to wild type LdMNPV. Studies are in progress to identify the genotypic change responsible for the enhanced stability of polyhedra production exhibited by A21-MPV.

I. The Hostility of Biological Control Researchers Towards Increased Regulation

Biological control researchers at the Gypsy Moth Research Forum offered views about possible regulation ranging from open hostility, to dissatisfaction with current regulations, to interest in how good regulations, anchored in careful science, might operate.

Thoughtful critiques about current regulations and constructive discussions about possible regulations are rare in the biological control literature. Perhaps the 1994 Gypsy Moth Research Forum will spur an open, vital discussion, at conferences and in print, about the wise regulation of biocontrols. A fine start in this new dialogue appears in the presentation and paper by Joe Maddox at the Forum (1994. See also Harris, 1991).

Much of the published literature still seems to reflect a general hostility towards regulation, first documented in the Miller and Aplet article (1993). In 1992 Jack Coulson, a frequent commentator on biological control policies, wrote that "[b]iological control scientists are rightly concerned that regulations may be developed that will adversely affect classical biological control, which has proved to be a successful, environmentally sound, and cost- effective form of pest control." (Coulson 1992). R.W. Fuester, research leader at the Agricultural Research Service (ARS) Beneficial Insects Research Laboratory in Newark, Delaware, wrote in 1991 that a "disturbing trend appears to be a potential for greatly increased complexity in the approval process for release of candidate natural enemies from quarantine with what many fear will involve excessive and, in some cases, unnecessary host range testing." (1991 at 5).

The defensive perspective was captured by Coulson and Richard Soper, writing in 1989, when they suggested that the Technical Advisory Group (TAG) guidelines for weed controls and similar proposals should be encouraged by researchers "before more strict legislation or regulations are developed by non-specialists." (Coulson & Soper, 1989; see also Coulson, 1992).

Even Coulson and Soper have acknowledged that some difficult issues are unlikely to be resolved through guidelines developed by scientists working in the field.

> Some controversial biological control subjects ... include introduction of host-specific vs. polyphagous or broad spectrum natural enemies; multiple vs. single species introductions; competitive displacement; preselection and postevaluation studies; control of native pests with introduced natural enemies; and the role of secondary parasites. *Because of the controversial nature of most of these subjects, only some of these can be addressed in protocols and guidelines.* (Coulson & Soper, 1989 (emphasis added)).

The limitations on what informal guidelines are likely to achieve confirms the importance of a more formal legal framework for review of biocontrol research and application. The challenge is to develop a process and standards that are open, efficient, and have as their ultimate goal the development and application of wise and modest science.

II. Why Biological Control Specialists Should Demand Regulation

A. A Lawyer's View: When is Regulation Appropriate?

For lawyers, the need for regulation varies depending on the nature of the activity. Regardless of what people involved in regulated activities may think, lawyers typically are not fond of regulation for regulation's sake. Lawyers consider regulation unnecessary where markets produce rational choices that take account of most private and public costs and benefits of the activity, where the government role in the activity is minimal, where the threat from the activity is minimal, where the threat to the activity should harm occur is small, and where an activity is occurring without evident problems. Conversely, lawyers consider regulation appropriate where markets do not seem to weigh substantial costs and benefits from a given activity (for example where there are substantial externalities imposed), where the government is already centrally involved in the activity, where potential harm from the activity is great (and, again, unlikely to be accounted for by market forces), where the activity is important and worth protecting, and where problems are apparent in practice.

The application of these factors to development and implementation of biological controls seems clear. Government has been centrally involved with biological control efforts since the earliest experiments. Peter Harris identified some of the reasons why private markets have had only a modest role in developing biocontrols:

> The agents do not respect property boundaries and the prospects of getting a return on developmental costs through market sales are small as the agent finds the weed itself. Thus, classical biocontrol is largely done by government or with government funding in the public interest. Government is also responsible for protecting public interests to ensure that desirable plants and the ecology are not harmed. (Harris, 1991).

The substantial and increasing economic importance of biological pest control is also clear. For example, federal government funding of biological control research has increased over the past decade. D.E. Meyerdirk reports that funding within APHIS to support biological control has increased from $1.6 million in 1980 to $7.2 million in 1991 (Meyerdirk 1991).

Some biological control researchers argue that there have been few problems in practice, and therefore the activity should be left unregulated. Yet the assertion of safety and progress rests on a weak foundation, given the widely acknowledged absence of follow-up research on biocontrol failures. (Coulson 1992). The absence of follow-up appears equally true of biological control successes, where the agent succeeds in reducing or eliminating the target pest, yet where no evaluation is done on the impact of the agent on other parts of the ecosystem.

Nor can biological control be considered a scientific backwater out of the public eye. The recognition of demands for increased regulation appear in recent conference proceedings on biological control quarantine, which noted that "[t]he demand for biological control of pests

using their natural enemies is increasing as agriculture in the United States moves toward ecologically benign methods of pest control." (ARS Conference Proceedings 1991).

In September 1993 the Office of Technology Assessment, a non-partisan research arm of Congress, published a long report titled Harmful Non-Indigenous Species in the United States (OTA 1993). OTA confirmed that, on the more general question of species introductions, "the Nation has no real national policy on harmful introductions; the current system is piecemeal, lacking adequate rigor and comprehensiveness." (OTA, Summary p. iii). OTA identified the central regulatory issue as the need for government to decide which non-indigenous species "to keep out, which to release, and how to control those that have unexpected harmful effects." (OTA Summary p. 7). The Report noted proposals (including Miller & Aplet, 1993) for increasing regulation of biological controls. In January 1994 OTA began a new study, planned for 18 months, focusing specifically on biological controls. (OTA 1993b).

Increasing public and government interest in environmental issues makes biological control a likely area for public debate in the near future. Biological control is likely to emerge as a prime candidate for more formal legal control. One reason, therefore, for researchers to consider what good laws and regulations might look like is that the only alternative may be laws and regulations developed without their expertise. There are other reasons, however, why biocontrol researchers should aggressively support the development of new laws and regulations.

B. Reasons for Biological Control Researchers to Support Regulation

1. Protection of Sound Biological Control Research

One of the greatest threats to careful research and development of biological pest controls is the possibility that shoddy work by someone else -- a private firm or a government researcher -- will go terribly wrong, and result in a readily identifiable disaster of the kind documented repeatedly with older biocontrol efforts. The threat to future work may come not from actual harm to ecosystems but from perception of harm if, for example, biocontrol work should come to be perceived in public debate to be similar to use of genetically altered organisms.

The possibility that some scientists or companies might make a mistake cannot be dismissed. One state official at the Fifth Annual Gypsy Moth Research Forum (January 1994) described a stream of requests by private companies to introduce new organisms to storage, laboratory and testing facilities -- requests which, he said, were routinely denied for lack of adequate assurance about the quality of the biological knowledge on which the request was made (and lack of assurance about quality control standards that would prevent the accidental introduction of harmful organisms or diseases not subject to review). Suggesting a similar lack of care and potential for error, Coulson describes "current 'high-tech' pest control programs in which historical summaries or recognition of past biological control efforts seem of little importance or relevance to some scientists and administrators" (Coulson 1992 at 197).

Sound laws and regulations may prevent unwise introductions from taking place. Equally important, if a disaster occurs, regulations may provide a shield to protect against unjustified criticism of sound efforts to develop biological controls.

2. Limitation of Tort Liability

In the absence of more complete laws and regulations, the legal system could nonetheless become the sword that slows or halts work on new biological controls. Under traditional principles of tort law, the possibility always exists that a person may claim harms from a control effort, or threat of harm when seeking an injunction to block the use of a biocontrol agent. It was precisely such a dispute between the Australian cattle ranchers, who supported use of beneficial insects to control *Echium plantagineum*, a weed they called Paterson's Curse, and beekeepers who wanted to protect the same weed which they called Salvation Jane, that led to the development of a biological control law in Australia. (See Miller & Aplet, 1993).

Ernest Delfosse, the current head of the United States National Biological Control Institute whose career has largely been spent in Australia, added an important note at the Fifth Annual Gypsy Moth Research Forum. All biological control work, he observed, had been placed on hold for eight years during the pendency of the litigation over *Echium*. Delfosse noted at the conference that he was aware of at least two potential legal battles involving current control efforts in the United States.

Neither the legal system nor the media is likely to draw fine distinctions between improper biocontrol applications and thoughtful research. All who work with biocontrols bear the risk of errors by anyone who works with biocontrols -- a risk that can best be reduced through sound standards and procedures for control of research and application of biocontrols in the field.

3. Focus for Additional Funding

There may be substantial advantages to greater visibility that would follow from a law addressed specifically to biological controls. Coulson (1992) and others have noted the difficulty of funding important biocontrol work, especially less glamorous aspects such as documentation and follow-up research. A biological control law would justify separate funding lines in the federal budget, and would help to focus arguments in favor of additional support.

4. The Opportunity for Biological Control Researchers to Contribute to Emerging Models of Environmental Management

Policy makers and environmentalists increasingly raise notions of biodiversity and other newer conceptions of environmental management. The knowledge and expertise of biological control researchers may provide the kind of focus necessary to make clear, accessible, and practical, and therefore more influential, important concepts such as biodiversity. (See also Harris, 1991 at 62) (suggesting seven reasons to make the review of proposed controls more public.)

## III. The Need to Focus on Process

Greg Aplet and I have described the absence of current legal review of biocontrols and the difficulty of adapting current environmental laws to create a process that would both encourage and structure future biocontrol research and application. (Miller & Aplet, 1993). We proposed the creation of a new program to collect information and review biocontrol proposals. We suggested a handful of central principles that might govern such a process.

Some participants in the Fifth Annual Gypsy Moth Research Forum assumed that any new law of biocontrols would necessarily be massive, cumbersome, costly, and rigid. But a new law need not occupy one hundred pages in the United States Code, nor must new regulations promulgated under such a law fill several volumes of the federal register. Instead, much simpler laws and regulations can offer a structure and a process which identifies relevant factors and guarantees that they will be considered in the context of specific proposals.

Coulson (1992) argues that "[c]lassical biological control programs could be improved by more careful record-keeping, often lacking in the past, and by making the information readily available." He explains that biocontrol failures are poorly documented, as are experiments which cease for other reasons. He observes that better record-keeping is needed both for pre-release review and for consideration of post-release effects.

Yet Coulson describes a system now being developed which seems primarily archival and not intended to serve as the basis for better informed pre-release decisions. Coulson explained that, at present, data are not available about importation or release of biocontrols for up to nine years. He writes that "it is hoped that eventually this delay will be only 2-4 years." (Coulson 1992 at 203). Apart from the delay in availability, Coulson admits that current data collection does not include information on "establishments, and [] on the impact of establishments." (1992 at 203). What purposes are served if information is only available after controls have been applied? What learning can be achieved if no data is collected on establishments and ecosystem impacts?

The first priority for a new biological control law is to guarantee that adequate information is developed, recorded and made accessible on proposed shipment, testing, and introduction of biological controls, including a requirement of follow-up on all field tests and other introductions.

The title of our 1993 article, *Biological Control: A Little Knowledge is A Dangerous Thing*, was intended to emphasize the central role that information and understanding should play in encouraging wise use of biocontrols. Alexander Pope, from whom we stole the thought, explained the result of more careful study.

*A little learning is a dangerous thing,*
*Drink deep, or taste not the Pierian spring:*
*There shallow draughts intoxicate the brain,*
*And drinking largely sobers us again.*

The concept of biological control is enticing; the successes are intoxicating. Adequate learning will provide a foundation on which sober decisions can be made.

The second priority for a new biocontrol law is to make sure that decisions are made on the basis of the collective wisdom in the biological control field, that each decision take into account not only potential benefits but also potential costs, and that each decision be articulated, recorded, and subject to public debate and review.

Who might make decisions on new research and introductions? Participants at the Fifth Annual Gypsy Moth Research Forum argued that research on biological controls is enormously complex and highly varied depending on the nature of the target, the nature of the control, and a host of other factors. Regulations need not decide all or most issues ahead of time: they can identify issues that must be decided and who should decide them, leaving the application to case-by-case consideration and imposing simply a requirement of reasonable articulation of the basis for each decision by the approving authority.

One familiar process that might be used to review biocontrol projects is the professional peer review model used for publication throughout the sciences. Biologists comfortable with submitting the written product of research to peer review should be comfortable with submitting critical stages of that research to review as well. A review board, located in a variety of possible government agencies, could use peer review, structured around a predetermined set of questions, as a primary basis for approving or rejecting proposed projects.

## Conclusion

Biological controls are too important, too complex, and potentially too dangerous to be left in the regulatory purgatory of the current regime. This article suggests that more formal legal regulation of biocontrols may be closer to heaven than to the hell that some researchers hint at in their writings when addressing the possibility. Perhaps not. But if nothing else, increasing public debate and congressional interest in the introduction of non-indigenous species, and in biocontrols in particular, suggest an urgency for those who care about and understand the field to become centrally involved in the crafting of new laws.

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# SPATIAL AND TEMPORAL DIVERSITY OF INVERTEBRATE PREDATORS OF THE GYPSY MOTH

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## ABSTRACT

Little is known about the functional roles of ground dwelling invertebrates in forest ecosystems. Even less is understood about the potential significance of these in relation to disturbance, including defoliation or thinning. In addition to maintaining species diversity, sustaining populations of forest arthropods may be significant for the predatory roles these organisms serve in forests where gypsy moth defoliation is likely. The purpose of this research is to determine the potential impact of gypsy moth defoliation, mortality, on select invertebrate populations, and to examine the effect of silvicultural thinning on the populations. Four groups: Araneae (spiders), Opiliones (harvestmen), Formicidae (ants), and Carabidae (carabid beetles), were sampled using pitfall traps over an 11-week period, within each of 16 stands on the WV University Forest. The study spanned a period of four years, but only the first two years will be reported here.

Preliminary results indicate that spiders are the most numerous and diverse of the four groups. Temporal diversity trends were pronounced for spiders, with the greatest diversity early (May), and a decrease during June, increasing again in July. The other groups showed slight trends toward greater diversity later during the collection period, but were relatively similar throughout. Inter-stand diversity trends were notable also, particularly for spiders. Stands with basal area of oak > 50% had greater diversity among most groups of invertebrates in 1990 (the year following thinning). This was likely due to both thinning and defoliation. In general, thinning altered the temporal variation slightly, when compared with the previous (unthinned) year, and thinned stands typically had greater diversity, particularly with spiders.

# DEFOLIATION AND MORTALITY PATTERNS IN FORESTS MANAGED FOR GYPSY MOTH

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## ABSTRACT

Appalachian Plateau forests provide a variable resource for the gypsy moth. The mixed hardwood forests support one of the most diverse communities of woody plants in North America, but the composition and relative dominance of the forest changes substantially with slight changes in physiography, soil type, or climate. The intent of this research is to examine defoliation and mortality patterns of thinned and unthinned forest stands dominated nearly exclusively with oak and stands with oak and an admixture of associated hardwood species. Defoliation and mortality patterns over a five-year period appear to be very stand-dependent. While the general trend indicates that heavy defoliation of susceptible species occurred in 1990 and 1991, only six of the 16 stands sustained heavy defoliation. Defoliation patterns were nearly identical for both thinned and unthinned low oak stands, but with slightly greater mortality in the unthinned stand. The thinned high oak stands had greater defoliation and subsequent mortality than the unthinned counterparts. In most cases, both defoliation and mortality were slight in the resistant and immune preference classes. As gypsy moth populations build up, and mortality of preferred species continues, defoliation and mortality of the resistant and immune classes will likely increase.

# *AGRILUS* AND *ARMILLARIA*: AGENTS OF MORTALITY IN OAK DEFOLIATED BY THE GYPSY MOTH

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## ABSTRACT

*Armillaria* spp. colonization and *Agrilus bilineatus* Web (twolined chestnut borer [TLCB]) infestation are dominant causes of mortality following defoliation in hardwood forests. The primary objective of this research is to develop an understanding of the roles and relationships of these two organisms with each other and as affected by gypsy moth defoliation and thinning. Four mixed hardwood (low-oak) stands, and four oak stands were thinned in 1989. Subsequent defoliation was severe in some stands in 1990 and 1991, but tapered off in 1992. Results of sticky band traps used to assess TLCB populations indicated that populations were predictably low prior to thinning and prior to defoliation. These increased significantly each year to 1992 and declined to near 1989 levels in 1993. Thinning seemed to have little effect on levels of TLCB infestation thus far as TLCB were more abundant on the thinned stands than the unthinned counterparts. This may be a function of the stress of thinning as well as other stresses, e.g. drought or TLCB populations may be related to an increase in breeding habitat. Relationships between defoliation and TLCB were significant only when both were high. Autopsy results indicate that 98% of the dead trees were either infested with *TLCB* or colonized by *Armillaria*, and 75% showed evidence of both organisms.

# THE EFFECT OF IRON BIOAVAILABILITY IN A HOST, GYPSY MOTH (*LYMANTRIA DISPAR* L.), ON REPRODUCTIVE SUCCESS OF A PARASITOID, *GLYPTAPANTELES LIPARADIS* BOUCHÉ

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## ABSTRACT

The braconid wasp, *Glyptapanteles liparadis* Bouché, a gregarious parasitoid of gypsy moth, has been in continuous culture at the Forest Service's Insect Rearing Facility, Hamden, Conn., since importation from the People's Republic of China in 1987. A standard rearing technique, utilizing the gypsy moth laboratory strain, NJSS, as host, was developed (Abdul Hamid, unpublished), and has been used consistently since 1989. Between 1989 and 1992, recovery of *G. liparadis* cocoons and adults fluctuated widely. During this same period, production of NJSS was adversely affected by Abnormal Performance Syndrome (APS), characterized by unpredictable periods of poor hatch, reduced survival, and slow, asynchronous growth (straggling). Since parasitic stages are closely integrated with the physiology and biochemistry of their hosts, we suspected that the variation in *G. liparadis* reproduction was linked to APS in gypsy moth. Reduced bioavailability of iron (Fe) was found to be the cause of APS (ODell et al. 1993). We tested the hypothesis that reduced bioavailability of Fe in a host (gypsy moth) reduces the reproductive success of a natural enemy, the gregarious endoparasite, *G. liparadis*.

Fe bioavailability in the host (gypsy moth) can significantly affect reproduction and viability of the parasite, *G. liparadis*. When hosts were reared on diet containing crystalline ferric phosphate ($FePO_4$), i.e., reduced Fe bioavailability, significantly fewer parasites emerged and the proportion of these that pupated was significantly less than when hosts were reared on diet containing amorphous $FePO_4$. The bioavailability of iron in the diet of parents of hosts had similar effects regardless of the diet the hosts were reared on. The results of this study provide evidence of interaction between the diet of a herbivore (gypsy moth), the herbivore, and its natural enemy, *G. liparadis*.

ODell, T. M., M. A. Keena, J. A. Tanner, and R. B. Willis. 1993. Gypsy moth rearing problems linked to iron (Fe) in diet. Gypsy Moth News, 32: 3-5.

# POTENTIAL REDUCTIONS IN POPULATIONS OF NONTARGET LEPIDOPTERA CAUSED BY *BT* APPLICATIONS OR GYPSY MOTH DEFOLIATION IN ILLINOIS

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## ABSTRACT

The Database of Illinois Lepidoptera created by Cashatt and Godfrey was searched for all species in the families Hesperiidae, Lycaenidae, Lymantriidae, Notodontidae, Saturniidae, and Sphingidae collected in northeastern Illinois and in Pope County, IL, near Shawnee National Forest. The records for the 173 species were used to estimate larval phenologies. Information on host plants and phenological information for the non-target Lepidoptera and gypsy moth allowed us to predict the potential vulnerability of each species to a single application of *Bacillus thuringiensis* (*Bt*) directed against young gypsy moth larvae or to starvation due to defoliation by an outbreak population of gypsy moth. Data of John Peacock of the Forest Service allowed us to improve the prediction of *Bt* vulnerability for three species based on observed *Bt* toxicity. We chose to perform a conservative analysis with inclusion on the vulnerable list more likely than exclusion. All of the indigenous Lycaenids are potentially vulnerable to one or both of the two disturbances. Only four of the Notodontids are not expected to be vulnerable to either defoliation or a *Bt* application. We concluded, however, that 15 native skippers (Hesperiidae) are not expected to be vulnerable at either of the two sites in Illinois; another seven skippers are not vulnerable to both disturbances at one of the two locations.

# MODULATION OF ECDYSONE BIOSYNTHESIS DURING GYPSY MOTH VIRAL REPLICATION

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## ABSTRACT

Ecdysteroid UDP-glucosyl transferase (EGT) is a viral-coded enzyme which catalyzes the transfer of sugar from UDP-sugar to ecdysteroid, an insect molting hormone. EGT expression in gypsy moth (*Lymantria dispar*) virus-infected larvae is observed in the early stages of infection. The prothoracic glands from virus-infected larvae produce ecdysone at high levels and continue to secrete hormone resulting in high levels of ecdysteroid in the hemolymph. The hemolymph ecdysteroid titer in virus-infected larvae is significantly higher than in control insects. These hemolymph ecdysteroids are composed mostly of an ecdysone-glucose conjugate, the product of EGT. Our morphological, ultrastructural, and biochemical observations reveal that the prothoracic glands remain in an active state during viral replication.

# EVALUATION OF AERIALLY-APPLIED GYPCHEK FORMULATIONS CONTAINING A STILBENE OPTICAL BRIGHTENER

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## ABSTRACT

The USDA Forest Service (FS) and the USDA Agricultural Research Service (ARS) are collaborating with American Cyanamid Company (AC) in the evaluation of Gypchek formulations that contain the stilbene Blankophor BBH. If proven efficaceous, the use of this adjuvant would allow dramatic reductions in field dose rates. In 1993, evaluations of aerially-applied Gypchek-BBH formulations were conducted in Maryland, West Virginia and Michigan.

In Maryland, replicate 1-ha plots were treated once with either the FS "standard" molasses-based Gypchek formulation, or the same formulation containing 0.5% BBH. There were no significant differences ($P>0.05$) in either larval mortality or egg mass population changes between the two formulation treatments. In West Virginia, replicate 1-ha plots were treated once with FS Gypchek that was formulated by AC. Treatments were formulations with and without BBH. Defoliation was significantly lower ($P<0.05$) in plots treated with either formulation than in unsprayed control plots. However, larval mortality estimates were not significantly different ($P>0.05$) among treatments. In Michigan, four AC formulation treatments were compared to unsprayed controls on 12-ha replicated plots. The treatments were (1) two applications of $5X10^{11}$occlusion bodies (OB)/ha, (2) two applications of $5 \times 10^{11}$ OB + BBH/ha, (3) two applications of $5 \times 10^{10}$ OB + BBH/ha and (4) one application of $1 \times ^{1011}$ OB + BBH/ha. There were no significant ($P>0.05$) defoliation differences among treatments and egg mass populations dramatically increased in all treatments (between 119 and 357%). Control populations increased 23%.

Results indicated that the addition of BBH to the formulations tested provided only a marginal boost to efficacy. It is likely that the amount of BBH deposited per unit area of foliage is too low t be consistently effective.

# DELETION OF THE *LYMANTRIA DISPAR* MULTINUCLEOCAPSID NUCLEAR POLYHEDROSIS VIRUS EGT GENE ENHANCES VIRAL KILLING SPEED

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## ABSTRACT

The LdMNPV is a baculovirus currently used in the biocontrol of the gypsy moth. This virus is highly specific for the gypsy moth, and can be formulated for aerial spraying. However, it has a lower efficacy than other pesticides currently being used to control gypsy moths. One possible means to enhance viral efficacy has been previously studied in the related baculovirus *Autographa californica* multinucleocapsid nuclear polyhedrosis virus. O'Reilly and Miller (1991, Biotechnology **9**: 1086-1089) deleted a viral gene which modifies the hormone balance of infected larvae. This gene produces a protein, called ecdysone UDP-glucosyl transferase, which inactivated ecdysone by glycosylation; thereby preventing molting and pupation in infected larvae. Deletion of this gene led to a 20% improvement in viral efficacy, as well as lower weight gain in infected larvae. We have previously identified a homolog to this gene in LdMNPV, and showed that infected gypsy moth larvae contain glycosylated ecdysone at high levels. Therefore, we deleted this gene from LdMNPV.

The recombinant egt minus virus was generated by replacing the middle of the egt gene with B-galactosidase, a marker gene. This gene was inserted in such a manner as to create a translational fusion with the beginning of the egt gene under the control of the egt gene. A recombinant EGT minus virus containing the B-galactosidase gene was identified by DNA hybridization analysis. B-galactosidase enzymatic activity was not generated by the recombinant virus, and Northern analysis failed to detect transcriptional activity of the B-galactosidase gene. These results were not the anticipated result from this construct. There are three possible reasons currently being explored as to why no B-galactosidase activity was detected. These are a mutation during the construction, that the B-galactosidase fusion RNA is extremely unstable, or that some sort of downstream transcriptional control region has been disrupted. The activity of the recombinant virus was tested both *in vivo* and *in vitro*. Unmodified ecdysone was detected in larvae, or tissue culture cells, infected with the recombinant virus, in contrast to the glycosylated ecdysone found in larvae infected with wild type virus. In bioassays, neonatant larvae infected with the recombinant virus exhibit a 40% improvement in viral efficacy compared to those infected with the wild type virus (Lt50's of 4.3 vs 7.2 days). Late instar larvae show a 15% improvement in killing speed. Further work is needed in studying the effects of recombinant

virus infection on larval weight gain, and ultimately in field tests of the recombinant virus. If the field results reproduce those obtained in the lab this virus would yield a significantly improved product for the biological control of gypsy moths.

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## CHARACTERIZATION OF TEMPORAL GENE EXPRESSION AND DNA REPLICATION OF THE *LYMANTRIA DISPAR* MULTINUCLEOCAPSID NUCLEAR POLYHEDROSIS VIRUS

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### ABSTRACT

The gypsy moth (*Lymantria dispar*) is a major introduced forest pest in the northeastern United States. The baculovirus *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (LdMNPV) is a pathogenic to the gypsy moth and has a narrow host range. Baculoviruses go through a two stage life cycle, consisting of a budded viral form which acts inside a single larva, and an occluded viral form (polyhedra) which is environmentally stable and responsible for insect to insect transmission. Within a given cell there are four phases of gene expression. These are the immediate early phase, for which no viral proteins are required, the delayed early phase, which requires expression of other viral proteins, the late phase, which requires DNA replication, and the hyper-expressed late phase which produces large amounts of two proteins during the late phase.

We examined the viral life cycle of four different isolates of LdMNPV and obtained the same results with all four. We visually detected polyhedra 48 hours post-infection (h p.i.). In contrast, they are detected 24 h p.i. in AcMNPV (a related, much more studied baculovirus). An immediate early gene was detected at the end of a one hour virus infection period, a result identical to that obtained with AcMNPV. A delayed early gene was detectable by 16 h p.i., in contrast to the appearance of a delayed early gene in AcMNPV as early as 3 h p.i.. Both late and hyper-expressed late genes were first detected in LdMNPV infected cells 24 h p.i., while the same genes are detectable 6 h p.i. in AcMNPV. We also examined the time course of DNA replication in LdMNPV. DNA replication started 20 h p.i. in LdMNPV infected cells. In AcMNPV infected cells it started 6 h p.i.. In summary, although the initial phase of virus expression is unchanged from that found in AcMNPV infections, the rest of the viral life cycle, both before and after the onset of DNA replication, is significantly delayed.

# FACTORS AFFECTING GYPSY MOTH SPREAD RATE

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## ABSTRACT

An uni-dimensional spatio-temporal simulation model of gypsy moth spread has been developed. The following processes are considered: first-instar larval dispersal, constant survival to adult stage, male dispersal, random encounters of males with females, oviposition with constant fecundity. Egg density may not exceed maximum value of 10 millions eggs per hectare. Parameter values are the following: survival = 0.036 (Campbell 1981), proportion of females = 0.0664 (Campbell 1981), fecundity = 540 eggs/female (Montgomery 1991, Hough and Pimentel 1978, re-estimated), male search rate = 0.019 ha/male (our experiments), proportion of dispersing first-instar larvae = 0.05 (Taylor and Reling 1986, re-estimated), average distance travelled by dispersing larva = 3 km (estimated from Mason and McManus 1981, Taylor and Reling 1986), proportion of dispersing male moths = 0.5 (arbitrary), average distance travelled by dispersing males = 3 km (arbitrary).

The model predicts population expansion rate of 6.5 km/year. If distance of larval dispersal is equal to 4 km and survival is increased to 0.20, then the model predicts expansion rate of 14 km/year. These values are close to actual expansion rate (2.82 - 20.78) measured by Liebhold et al. (1992). Previously Liebhold et al. (1992) used Skellam's model and received the spread rate of 2.5 km/year. They underestimated the distance of larval dispersal because they used experimental data of Mason and McManus (1981) without extrapolation beyond 180 m. The gaussian puff model which was validated using these data (Mason and McManus 1981) shows much further larval dispersal.

Our model supports the hypothesis that gypsy moth in North America spreads by larval dispersal. However, other mechanisms, like egg mass transportation by humans, may occasionally increase population spread rate. The gradient of egg mass density across the leading edge is steeper than the gradient of male density because of male dispersal. Gypsy moth larvae arriving at some distance from the leading edge, originate from a large area between their destination and populations with outbreak density. It makes difficult to reduce immigration rate by suppressing source populations. Spread rate of gypsy moth in the model increased proportionally to the average distance of larval dispersal, as in the Skellam's model. However, Skellam's model underestimates spread rate 4 times as compared with our model because it considers continuous organism movement with random direction change. Larval dispersal distance has the greatest effect on population spread rate. The power coefficient in equation of larval dispersal has a strong negative effect. Mating success has almost no effect on population

spread because of male immigration in low-density populations. Mating success is the major factor in persisting of isolated low-density populations. Gypsy moth spread rate decreases with distance between favorable patches in heterogeneous environment. At distance of 1-1.5 km between patches, population spread rate is halved. However, the increase of distance between patches from 1.5 to 17 km has almost no effect on population spread rate.

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## OPTICAL BRIGHTENERS: EFFECT ON PERITROPHIC MEMBRANE

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### ABSTRACT

The peritrophic membrane lines the midgut of insects and encloses the food bolus. There have been no studies of gypsy moth peritrophic membrane, but in most insects it is made up of protein and chitin which may be complexed with polysaccharides and digestive enzymes. Its purported function is to protect midgut cell surfaces from abrasion; in addition, it may prevent pathogen access to midgut cells and may serve to anchor digestive enzymes (Richards and Richards, 1977).

Selected optical brighteners have been shown to greatly enhance the virulence of gypsy moth nuclear polyhedrosis virus (LdNPV) (Shapiro and Robertson, 1992), but the mode of action is unknown. This study determined whether an optical brightener fed separately, or in combination with LdNPV to gypsy moth larvae, resulted in changes in the surface structure of the peritrophic membrane.

Newly molted instar II gypsy moth larvae were reared on 30 ml of high wheat germ diet which had been overlaid with 1 ml of sterile distilled water (control), 1 ml of 0.5% Blankophor BBH (BBH), 1 ml of 1x10.6 PIB/ml LdNPV (LdNPV), or 1 ml of BBH and 1 ml of LdNPV (combination). In addition, some larvae were fed droplets of the above suspensions so that samples could be taken shortly after imbibition. Larvae were dissected and intact peritrophic membranes removed at intervals from 30 minutes until 8 days post-ingestion. Membranes were fixed, dehydrated, and critical point dried using standard histological procedures, and were examined using scanning electron microscopy.

At low magnifications the gypsy moth peritrophic membrane exhibits occasional surface wrinkles, but at higher magnifications appears very smooth and lacks any obvious pores.

Exposure to LdNPV resulted in no significant changes in surface structure until 8 days post-ingestion, when areas of some membranes appeared to be slightly lamellate. Exposure to BBH resulted in a gradual thickening of the peritrophic membrane, and the appearance of separate lamellae. These changes were first noticable 72 hours post-ingestion, and became increasingly obvious with time. Exposure to the combination treatment of LdNPV and BBH resulted in degradative changes which were detectable 2 hours post-ingestion. Initially, the peritrophic membranes had a roughened appearance, were heavily wrinkled, and some tears and slits were evident. By 12 hours post-ingestion, the membranes were heavily blistered and many of the blisters were ruptured. Large fissures, revealing multiple lamellae were apparent within 36 hours, and massive deterioration had occurred by 72 hours post-ingestion. Larvae in the combination treatment group did not survive longer than 6 days, at which time the peritrophic membrane was completely disintegrated, with only isolated strands remaining.

Continuous dosing with a combination of BBH and LdNPV resulted in progressive deterioration of the peritrophic membrane, but it is not known whether this treatment simply degrades the membrane or whether it also affects peritrophic membrane synthesis.

Richards, A. G. and P. A. Richards. 1977. The peritrophic membranes of insects. Ann. Rev. Entomol. 22:219-40.

Shapiro, M. and J. L. Robertson. 1992. Enhancement of gypsy moth (Lepidoptera:Lymantriidae) baculovirus activity by optical brighteners. J. Econ. Entomol. 85:1120-24.

# HOST SPECIFICITY OF THREE SPECIES OF EXOTIC GYPSY MOTH MICROSPORIDIA TO NATIVE NONTARGET LEPIDOPTERA

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## ABSTRACT

Host specificity information is necessary to evaluate the safety of pathogens used as biological control agents. Laboratory experiments are used to estimate host specificity in the field, but it is difficult to assess ecological factors. The evaluation of microsporidian pathogens poses additional difficulties because development in host tissues varies with the microsporidian species, the host species, the developmental stage of the host, and the initial concentration of the infective spores. The development of infection may be altered or terminated, resulting in an ecological "dead end" in a nontarget host. We studied the physiological effects of infection in the laboratory (physiological host specificity) of native North American forest lepidopterans by three species of European gypsy moth (*Lymantria dispar*) microsporidian pathogens. Our objectives were to use the results to estimate the ecological host specificity of these microsporidia. Twenty-two species of lepidopteran larvae were fed spores of *Microsporidium portugal*. Only in the gypsy moth were sufficient spores produced to expect transmission to conspecifics. Ten nontarget species developed atypical infections, infections in which abnormal spores were produced and/or the disease progression was terminated in early stages of the disease, often resulting in death of the host. Eleven nontarget species were completely refractive to the pathogen. Results were similar in 16 species of lepidopterans exposed to *Vairimorpha lymantria* although two nontarget species developed infections similar to those seen in the gypsy moth. *Endoreticulatis schubergi* produced an "all or nothing" response. Nine nontarget hosts were completely refractive to the microsporidium and six species developed heavy infections. This generalist microsporidium has been recovered in field collections of other lepidopteran hosts in North America. We believe that horizontal transmission of microsporidia can only occur when many environmentally resistant infective spores are produced by an infected host. Even when *M. portugal* and *V. lymantria* infected nontarget hosts, the progression of disease was altered in most species tested and small numbers of spores were produced. Our studies support the hypothesis that physiological host specificity is broader than ecological host specificity. Susceptibility and infection do not necessarily mean that horizontal transmission can or will occur, and ecological host range can be inferred only if horizontal transmission can occur. When the requirements for intraspecific horizontal transmission and known ecological parameters are considered, the ecological host range of *M. portugal* and *V. lymantria* may be quite narrow. From the standpoint of using microsporidia as classical biological control agents, the results are very encouraging.

# MAPPING A *LYMANTRIA DISPAR M* NUCLEAR POLYHEDROSIS VIRUS GENE REQUIRED FOR REPLICATION OF *AUTOGRAPHA CALIFORNICA M* NUCLEAR POLYHEDROSIS VIRUS IN A GYPSY MOTH CELL LINE

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## ABSTRACT

The slow kill rate of baculoviruses when compared to chemical insecticides and *Bt* is one limitation of baculovirus insecticides. Engineering baculoviruses to stop insect feeding more quickly may overcome this limitation. An advantage of baculovirus insecticides, particularly in sensitive ecosystems is their specificity. Most baculoviruses have limited host-ranges usually infecting a few closely related species. *Lymantria dispar M* nuclear polyhedrosis virus (*LdM*NPV) for example appears to be specific for the gypsy moth. Release of genetically modified baculoviruses and further efforts to improve baculovirus insecticides requires a better understanding of how host-specificity is determined.

The mechanisms that govern baculovirus host-specificity are poorly understood. One model system for identifying viral genes that are required for host-specificity is the study of virus infection in non-permissive cells. A number of studies using cultured cells indicate that baculoviruses can enter non-permissive cells, suggesting that mechanisms other than attachment and entry of the virus have roles in determining cell specificity. The gypsy moth cell line Ld652Y does not support replication of *Autographa californica M* nuclear polyhedrosis virus (*AcM*NPV). A cell line frequently used to propagate *AcM*NPV, SF21 cells, will not support replication of *LdM*NPV. However *LdM*NPV can provide a "helper function" that will allow *AcM*NPV to replicate in Ld652Y cells. We used transfection studies to determine the location of the *LdM*NPV "helper function" gene. *AcM*NPV is produced when *LdM*NPV and *AcM*NPV are cotransfected into Ld652Y cells. Chimeric viruses, comprised of a fragment of *LdM*NPV inserted into the *AcM*NPV genome, are produced when *LdM*NPV and *AcM*NPV are cotransfected into SF21 cells. Chimeric viruses will replicate in both cell lines. The identity of the *LdM*NPV fragment found in the chimeric viruses was determined by Southern blot analysis using cosmid clones of *LdM*NPV as probes. The inserted fragment is located between 20 and 60 map units (mu) on the *LdM*NPV genomic map. To precisely locate the "helper

function" gene, we employed a marker rescue technique. *AcM*NPV genomic and *LdM*NPV cosmi DNA were transfected into Ld652Y cells. "Helper function" was provided by each of two cosmid clones that overlap between 34 and 46 mu. Transfection with plasmid subclones of this region hav further narrowed the location of the "helper function" gene to an *Eco*RI/*Hind*III fragment between 42.2 and 45.7mu of the *LdM*NPV genome. Cloning and sequencing this gene will be useful in determining its function in the determination of LdMNPV host- or tissue-specificity.

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## RESEARCH ON THE MECHANISM OF THELYTOKY IN A UNIPARENTAL STRAIN OF THE GYPSY MOTH PARASITE *METEORUS PULCHRICORNIS* [HYMENOPTERA: BRACONIDAE]

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### ABSTRACT

The purpose of this research was to find the mechanism responsible for sex determination in a uniparental strain of *Meteorus pulchricornis* (Wesmael) from the Far East. Since previous study of certain other Hymenoptera (e.g., *Trichogramma* spp.) has suggested a microbial cause for thelytoky, experiments similar to those used in previous studies were used to see if microorganisms were causing thelytoky in the uniparental strain of *M. pulchricornis*. In previous studies, treatment with certain antibiotics or heat caused a reversion from thelytoky, in which females arose from unfertilized eggs, to arrhenotoky, in which unfertilized eggs produced males. In this present study, five generations of the uniparental *M. pulchricornis* were treated with streptomycin, rifampicin, tetracycline HCl, and erythromycin. Two generations of this strain were treated with temperatures above 30°C. No males were produced from either treatment, indicating no change in reproduction. Some interesting effects of the antibiotics wer noted; tetracycline greatly shortened the longevity of the females, and rifampicin lengthened their longevity. Temperatures above 32°C had adverse effects on the development of offspring within the host.

# REINFESTATION OF TRUNK BARRIER-PROTECTED TREES FROM ADJACENT CANOPIES

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## ABSTRACT

Sticky trunk barriers are presently viewed as a potentially attractive alternative to the use of ground-based insecticide sprays for the protection of individual trees from gypsy moth, *Lymantria dispar* (L). Our work with trunk barriers indicates that, while they consistently reduce gypsy moth larval density in oak canopies, reductions in defoliation are not always achieved. The number of larvae on treated trees is typically reduced by less than 30%. This may not be enough to provide consistent foliage protection. Properly applied and maintained trunk barriers made of duct tape and Tanglefoot™ are highly effective at preventing larvae from ascending tree trunks. One possible reason that the effect of the trunk barriers is not greater is that reinfestation may occur through the canopies of adjacent trees. The results of three separate experiments involving trunk barriers are presented here to provide insight into the question of whether reinfestation of trunk barrier-treated trees occurs through the canopies of adjacent untreated trees.

Experiment 1. In 1990, a test of the effects of banding on gypsy moth populations was conducted on four pairs of square wooded plots ranging in size from 0.14 (1 pair) to 0.25 (3 pairs) ha in Greenbelt, MD. In 1991, the test was repeated on five pairs of 0.25 ha square wooded plots in Harford County, MD. Each of the two plots in each pair was either banded or left unbanded. In the banded plots, all oak trees (1990), or all trees of any species (1991), with a diameter at breast height (dbh) of >5 cm were banded during April before the onset of egg hatch. Oaks, primarily white oak (*Quercus alba* L.), were the dominant trees in all plots. (Thorpe et al. 1993, J. Econ. Entomol. 86:1497-1501).

Experiment 2. This study was conducted at two locations in 1991 and at one location in 1992. Each location was divided into isolated canopy and contiguous canopy areas. Within each area, 10 oak trees were treated with sticky trunk barriers and 10 oak trees were untreated.

Experiment 3. This study was conducted at a single location in 1993. An extremely high density gypsy moth population occurred at this site, and many trees were 100% defoliated. Again, some oak trees were treated with sticky trunk barriers and some were left untreated. The number of points of contact with adjacent understory and overstory trees was noted for each treated tree.

In all studies, defoliation was measured subjectively on individual oak trees in 10% increments. In experiment 1, defoliation was estimated on 10 randomly-selected oak trees in each plot. In experiments 2 and 3, defoliation was measured on each tree included in the experiment. Tree trunks were banded by wrapping them with duct tape (52 mm wide) at a height of ca. 1.5 m, and then applying by gloved hand a 10-20 mm wide, 1-5 mm thick band of Tanglefoot™ (The Tanglefoot Company, Grand Rapids, MI) to the center of the tape. Staples were used to hold the tape in place on rough-barked trees. Larval population density was estimated using the frass drop/frass yield method. Frass drop over a 12-16 hour sampling period was estimated at random locations beneath oak canopy. Frass yield (number of frass pellets per larva) was determined by placing larvae individually along with 1-2 oak leaves in plastic cups with cardboard lids at the site.

In the first year of Experiment 1, only oak trees were treated with sticky trunk barriers. Gypsy moth larval density was reduced from an average of 176 larvae / $m^2$ to 114 larvae / $m^2$. In the second year, all trees in the experimental plots were treated with sticky trunk barriers. Larval density was reduced from an average of 107 larvae / $m^2$ to 89 larvae / $m^2$. The year x treatment interaction effect was not significant, indicating that the trunk barriers were no more effective when all of the trees were treated than when only oaks were treated. The results of this experiment do not show any evidence of reinfestation of treated trees from adjacent canopies.

In Experiment 2, on trees with isolated canopies, where no reinfestation was possible, gypsy moth larval density was reduced from an average of 69 larvae / $m^2$ to 57 larvae / $m^2$. On trees with contiguous canopies, where reinfestation was possible through the canopies of adjacent trees, larval density was reduced from an average of 81 larvae / $m^2$ to 53 larvae / $m^2$. A significant canopy x treatment interaction indicated that the effects of the trunk barriers were different in isolated- versus contiguous-canopy trees. However, this difference was because larval populations were reduced by the trunk barriers more in the contiguous-canopy trees than in the isolated-canopy trees. If reinfestation through the canopy occurred, then the trunk barriers should have been more effective in the isolated-canopy trees. Therefore, the results of this experiment do not show any evidence of reinfestation of treated trees from adjacent canopies.

Gypsy moth population density was high in Experiment 3, with more than 200 larvae per $m^2$ of ground surface. The entire area was moderately to severely defoliated, and many of the trees in the study, including those treated with sticky trunk barriers, were completely defoliated. The sticky trunk barriers reduced larval density from an average of 255 larvae / $m^2$ to 209 larvae / $m^2$. Defoliation was reduced from 83 to 65% on treated trees. There was no apparent relationship between the number of points of contact with adjacent understory and overstory trees and population density. A significant positive correlation would have suggested that reinfestation of treated trees from adjacent untreated trees occurred.

# GYPSES: A DECISION SUPPORT SYSTEM FOR GYPSY MOTH MANAGEMENT

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## ABSTRACT

GypsES is a decision-support system for gypsy moth management being developed by the Northeastern Forest Experiment Station. GypsES provides decision support to gypsy moth managers by identifying areas of concern, recommending areas to monitor, recommending areas for suppression, and producing maps and tabular summaries . The GypsES system uses GRASS to handle all geographic data, and an original database system that reads any .dbf file. We have also developed a generalized report generator that uses information from both the GIS and the database. We have also developed an original on-screen map editing facility called MapEdit, which edits raster, site, and vector files.

The three major components of the GypsES system are Hazard Rating, Survey, and Treatment. The Hazard Rating component classifies susceptibility to defoliation, estimates vulnerability to damage, determine hazard from gypsy moth based on management priorities, and determines current risk based on insect populations. The Survey component works in two different modes, Eradication and Suppression, according to the situation. In Eradication mode the system provides advice on setting and collecting data from pheromone traps. In Suppression mode the system provides similar advice on egg mass surveys and data management. The Treatment Component allows the user to draw spray blocks based on risk ratings, supports decisions by incorporating budgetary constraints in recommendations, and incorporates timing estimates from the phenology model to help plan suppression specifications. Also incorporated are a spray deposition model to assist in designing spray blocks and a simulation model for estimating damage to stands from defoliation.

Three modes of operation are anticipated among the different user groups. GypsES can be used to identify areas where silviculture and other non-insect-focused activities may be beneficial to National Forests and other forest management organizations even before arrival of the gypsy moth. GypsES can also be used to plan and implement eradication projects where the insect has been found but is not yet established. The program can also be used in conjunction with suppression programs to allocate resources to survey activities, identify treatment blocks, and monitor success of treatments.

Development of version 1 will be complete by the end of FY 1994. Current emphasis is on completion of an Eradication system, the Database entry and reporting system, improvement of program stability, and incorporation of the simulation models FSCBG and GMLSM Stand Damage. The program is currently usable, and one test site (Prince William County, VA) does use GypsES in its suppression program.

We plan to deliver GypsES to National Forests and other sites where needed. Primary among the other sites are county programs in Virginia and elsewhere, where active suppression programs already exist, and the local managers are requesting GypsES for their use. Each National Forest will decide how a local gypsy moth program could benefit most from GypsES. The software may be installed for use of the Forest Health Specialist, a Forest Silviculturist, or by District personnel, as required by local needs.

# CHARACTERIZATION OF THE MIDGUT PROTEASES OF GYPSY MOTH LARVAE

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## ABSTRACT

Since proteases are vital for the digestive process, there has been considerable interest in the characterization of the insect enzymes to facilitate the screening and identification of inhibitors that could adversely affect the growth and development of lepidopteran larvae.

The principal midgut proteinases of the gypsy moth, *Lymantria dispar*, were identified and the subcellular distribution of the enzyme activities was determined. The endoproteinase activity of fifth-instar larvae was largely attributed to two serine proteases, trypsin and an elastase-like enzyme. Exproteinase activity was attributed largely to a leucine aminopeptidase. The effects of a variety of inhibitors on the purified gypsy moth enzymes was distinct. Most of the trypsin was found in the luminal fluid, which is consistent with findings in other lepidopteran larvae. However, a significant amount of trypsin was localized in the brush border membrane which is consistent with the model where trypsin is initially bound to the secretory vesicle membrane before secretion into the lumen. Trypsin was purified from the luminal fluid by using a benzamidine-Sepharose affinity procedure. The gypsy moth enzyme resembled other trypsins with respect to size, substrate specificity and inhibition by specific trypsin inhibitors. N-terminal amino acid sequence of the gypsy moth trypsin to the 38th residue revealed high similarity to trypsins of the spruce budworms, *Choristoneura occidentalis* and *C. fumiferana*.

The distribution of elastase was similar to trypsin in that most of the enzyme was found in the lumen. However, there was less elastase associated with the brush border membrane suggesting that elastase may be present as a zymogen in the secretory vesicles. Gypsy moth elastase was purified by FPLC and studies of its substrate specificity and N-terminal sequence analysis were carried out. Purified elastase exhibited a size (Mr=24 kDa) slightly smaller than trypsin (Mr=25 kDa). Succinyl-dialanyl-prolyl-leucine-p-nitroanilide was one of the best substrates for the gypsy moth elastase which is characteristic of an elastase-2. The purified gypsy moth elastase was essentially free of trypsin-like activity, displayed little chymotryptic activity and was able to hydrolyze elastin-congo red. Exoproteinase activity was attributed to a leucine aminopeptidase (LAP), a membrane-bound enzyme in the microvilli of the larval midgut cells. LAP was purified after solubilization of the brush border membrane protein using the zwitterionic detergent CHAPS. SDS-PAGE and Superose-6 gel filtration studies of purified gypsy moth LAP indicated that the active molecule was a dimer with a subunit size of approximately 110 kDa.

# EFFECTS OF PROTEINASE INHIBITORS ON MIDGUT PROTEINASES AND THE GROWTH AND DEVELOPMENT OF GYPSY MOTH LARVAE

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## ABSTRACT

The trypsin-like serine proteases found in lepidopteran larvae are inhibited *in vitro* by a several types of plant inhibitors. It has been suggested that the plant inhibitors potentiate resistance to insect pests and that protease inhibitors can be used to inactivate insect midgut proteases and serve as an effective means for insect pest control. Certain serine proteinase inhibitors have been shown to affect the growth and development of insect pests in feeding trials. In addition, expression of trypsin inhibitor in transgenic plants have shown to protect these plants from insect attack. In this study, we examined the effect of incorporating proteinase inhibitors in artificial gypsy moth diet on the growth and development of the larvae from 3rd-instar through the 5th-instar, and the effect of these inhibitors on the enzymes in the insect's digestive system.

Newly-molted 3rd-instar gypsy moth were reared on artificial wheat germ diet with various inhibitors incorporated at 0.1% (w/w) of each inhibitor. Individual weights and developmental time were monitored daily at the same time. After 9 days, the larvae were bled, dissected, and the midguts were removed. The luminal fluid enveloped by the peritrophic membrane was removed. Midgut brush border membrane (BBMV) and cytosolic fractions were obtained by MgEGTA precipitation and differential centrifugation. The effect of the inhibitors on the midgut luminal enzymes; trypsin, chymotrypsin, carboxypeptidase, and elastase, was determined by using specific synthetic substrates. The effects of these inhibitors on the midgut brush border membrane bound (BBMV) aminopeptidases, esterase and alkaline phosphatase were examined. In addition, the total and the specific activity of these enzymes in the cytosolic fractions were measured. Plant proteinase inhibitors, such as soybean trypsin/chymotrypsin inhibitors, appeared to have little effect on larval development larval growth. Of all inhibitors tested, the microbial enzyme inhibitor leupeptin, was the most effective in suppressing gypsy moth larval weight gain and development. Analysis of the luminal proteinase activities indicated that leupeptin inhibited all the luminal proteinases including trypsin, chymotrypsin, and elastase. The results were unexpected since leupeptin is a poor inhibitor of elastase *in vitro*. In contrast, incorporation of chymostatin, a microbial inhibitor of gypsy moth elastase, into artificial diet did not have any significant effect on the growth and development of the gypsy moth larvae. These results are comapatible with the hypothesis that midgut trypsin is essential for normal growth and development.

# A FIELD ASSESSMENT OF THE EFFECTS OF *BACILLUS THURINGIENSIS* ON NON-TARGET LEPIDOPTERA

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## ABSTRACT

Effects of a single *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*) application on non-target Lepidoptera were studied under field conditions on five paired 20 ha blocks (labeled A-E) in Rockbridge County, Virginia. One of the plots in each block was sprayed with *Btk* on 14 May 1992 and the other served as an untreated control. Lepidopteran larvae were collected from foliage samples and from beneath burlap bands. Foliage samples were taken from three strata of the forest: the canopy (scarlet oaks), subcanopy (scarlet oaks), and understory (blueberry). Burlap bands were fastened to boles of scarlet and chestnut oak. The sampling was repeated in 1993 to determine if any post-treatment effects occurred.

In 1992, a total of 10,942 lepidopterous larvae were collected: 5,791 from untreated foliage, 4,256 from treated foliage, 672 from under burlap bands in untreated plots, and 205 from bands in treated plots. In 1993, a total of 5,919 lepidopterous larvae were collected: 2,193 from untreated foliage, 1,867 from treated foliage, 1,140 from bands in untreated plots, and 719 from bands in treated plots. We believe between-year differences in total numbers are primarily the result of natural year-to year population fluctuations and not treatment effects since larval numbers in both control and treatment plots were less in 1993 than 1992.

Our 1992 data show evidence of a reduction in larval numbers due to *Btk* on canopy Macrolepidoptera for blocks C, D, and E and in the subcanopy for blocks B, D, and E. For Microlepidotera there was a reduction in larval numbers in blocks A, C, D, and E in the canopy and in blocks B, D, and E in the subcanopy. We believe that much of this variation between blocks can be attributed to uneven aerial spray application. For the two treated plots in blocks D and E for which good spray coverage was attained there was a consistent effect of *Btk* on all Lepidoptera and strata analyzed. Although analyses have not yet been completed for the 1993 data, it appears that the lepidopteran fauna largely recovered by the summer of 1993.

Macrolepidoptera recovered from burlap bands were appreciably less common in the treatment plots in 1992. The differences between control and treated plots were most notable in the Noctuidae, but differences were also seen for the Lycaenidae, Geometridae, and Lasiocampidae. Low Noctuidae numbers were again noted in 1993.

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## ASIAN GYPSY MOTH RESEARCH PLANNING AND ACCOMPLISHMENTS

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### ABSTRACT

In November 1991, an interagency, international research group met in East Windsor, CT, to identify research critical to the successful response by regulatory agencies to the introduction of Asian gypsy moth (AGM). A series of research topics were identified in response to the introduction of Asian gypsy moth on Russian grain ships and research action was undertaken. The research areas identified and accomplishments achieved are listed. While these research themes were addressed for the Asian race of gypsy moth, the introduction into North America of a race of unknown origin from Germany raises the question of the need for additional review of research efforts. While considerable research continues on the European biotype of gypsy moth (EGM) in North America, AGM is significantly different necessitating unique emphasis.

### Detection and Delineation

#### Accomplishments

- verification of the attractancy of males to disparlure
- differences between AGM and EGM male periodicity
- adoption by the U.S. and its trading partners of uniform inspection protocols for vessels and cargo

#### Research Needs

- relationship of male AGM captures to female location
- factors influencing female flight distance and direction

## Prevent AGM from Entering North America

### Accomplishments

- lighting devices less attractive to females identified
- monitoring system established for Russian ports
- shipping schedules determined by female flight period

### Research Needs

- highly attractive lighting devices for sampling females
- egg mass sampling procedure for containers
- suppression of AGM populations around infested ports
- testing of less attractive lighting in ports or other sensitive areas, i.e., container terminals

## Eradication of AGM from North America

### Accomplishments

- effectiveness of *Bt* and NPV documented
- utilization of the EGM pheromone monitoring system for AGM

### Research Needs

- impacts of eradication activities on non-target arthropods
- more precise delimitation of AGM infestations

## Determine Susceptibility of Host Plants and Forests

### Accomplishments

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

### Research Needs

- assess AGM and EGM crosses on host utilization
- test additional U.S. tree species, especially southern pines and western species
- determine the genetic basis for host plant preference

## Consequences of Hybridization

### Accomplishments

- AGM and EGM are sexually compatible
- <1% of AGM/EGM F1 hybrids capable of directed flight
- egg chill of hybrids is intermediate of their parents
- hybrid larval color intermediate
- black-backed variant is family related
- F1 hybrids develop slightly faster than their Asian parents

### Research Needs

- predict retention of Asian characteristics with inbreeding
- compare behavioral and physiological traits between AGM from the Russian Far East and Germany
- ascertain the behavioral and genetic characteristics of hybrids for AGM from Germany and EGM

## Diagnostic Methods to Identify AGM

### Accomplishments

- mtDNA procedure for batch samples
- nuclear gene markers identified
- head capsule color discrimination developed
- wing venation characterization

### Research Needs

- expand baseline reference material for evaluating various diagnostic methods
- develop additional nuclear gene markers
- identify nuclear gene marker for female flight
- develop methodology for processing field samples
- clarify taxonomic relationships
- compare and contrast various diagnostics with one another
- evaluate cuticular hydrocarbon separation of AGM/EGM

## Predicting Biological Events

### Accomplishments

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

### Research Needs

- define diapause requirements for AGM and integrate into egg hatch model
- determine population density and environmental effects on female flight
- incorporate AGM developmental data into EGM growth model
- ascertain female flight propensity with flight mills

Research support funding for AGM has been on an agency by agency basis. Redirection of resources has permitted the accomplishments noted above. However, the introduction of an AGM biotype on military munitions and equipment, the lack of information in its distribution in Europe, and potential future introductions via differing pathways places considerable responsibility on regulatory and research organizations. Continuing introductions and associated costs for eradication have their limits and beg for equitable solution. It is the hope that, through the continuing leadership and support provided by the National Gypsy Moth Management Board, a cohesive approach to solving this international problem will occur.

# FEMALE FLIGHT: EVALUATION OF ASIAN GYPSY MOTH AND ITS HYBRIDS

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## ABSTRACT

Bioassays of 12 females per 45-minute session were conducted in a primary quarantine facility. Mated and unmated females were placed individually on 2-foot hickory sapling stem sections, mounted in a vertical position in an 8 X 7 meter room. Incandescent floodlighting in the room was reduced to 30 percent (approximately 1 ft candle) which stimulated preflight and flight activity.

Asian females initiate wing fanning at varying intervals after light reduction, fan for approximately four minutes, (elevating their body temperature 10-15°C), walk to the top of the perch and launch into flight. In one series of bioassays, the mean time to flight at 30 percent light was 15.8 minutes. Tests were also run at higher light intensities (30-70 percent) which produced a longer delay before fanning and flight (18-21 minutes). Unmated females may initiate similar flight activity or commence calling for a period of time before flight.

Whether mated or unmated, > 90% of pure Asian females exhibit strong directed flight at 30 percent light. Higher light intensities produced a lower percentage of strong directed flight (e.g. 62.5% flight at 70 percent light). $F_1$ females and a large percentage of $F_2$ females (Asian/North American crosses) generally have gliding flight or no flight. Initial tests show that < 1% of $F_1$ females produce strong directed flight as opposed to 10-15% for $F_2$ females.

$F_1$ and $F_2$ females frequently exhibit walking and wing fanning behavior that is rarely seen with pure Asian females. Many of these females will continue to wing fan for 30 minutes or more; most will not attempt flight.

**Conclusions**

A dependable bioassay protocol has been developed after flight tests of more than 3000 females. It will serve as the basis for determining the genetics of flight of Asian and North American crosses and back crosses as well as evaluation of source material from Germany.

# AERIAL EVALUATIONS OF GYPCHEK FORMULATIONS WITH AND WITHOUT SMALL QUANTITIES OF BLANKOPHOR BBH, AN ENHANCING ADJUVANT, SUPPORTED BY A GROUND DOSE RESPONSE STUDY

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## ABSTRACT

Three cooperative field studies to evaluate formulations of Gypchek, the gypsy moth nuclear polyhedrosis virus (NPV) product registered by the USDA Forest Service (USFS), were conducted in the spring/summer of 1993. The experiments were: (1) a ground application experiment in Dorchester Co., MD, in which different rates of an American Cyanamid formulation of Gypchek (virus provided by USFS for all three studies) and of a stilbene-derived experimental adjuvant (Blankophor-BBH) were applied, (2) an aerial application experiment in the George Washington National Forest, WV, in which an American Cyanamid formulation of Gypchek was applied at 2/5th recommended rate with and without Blankophor BBH, and (3) an aerial application experiment in Dorchester Co., MD, in which the USFS standard formulation of Gypchek was applied at 1/10th recommended rate with or without Blankophor BBH. (The recommended rate for Gypchek in 1993 was $5 \times 10^{11}$ occlusion bodies (OBs) per acre applied twice, for a total application rate of $1 \times 10^{12}$ OBs per acre.)

Results from the ground application experiment confirmed the positive results obtained with Blankophor BBH in a similar individual tree treatment study done in 1992 when Blankophor BBH is used as a 0.5% solution additive. A reduced rate (0.1%) of Blankophor BBH also significantly increased mortality of gypsy moth larvae over that seen in controls; however, the lowest dose of Blankophor BBH (0.02% concentration in the approximately 20 gal. solution applied per tree) did not have a measurable effect. Amounts of Blankophor BBH adhering to foliage after application was measured at 1322, 227, and 37 ug per gram dry weight of leaf for the three doses used.

Significant foliage protection was obtained by the virus product in the West Virginia aerial application study, while a small but significant increase in larval mortality due to NPV in treated plots compared with controls was measured in the Maryland aerial study. However, no effect of the presence or absence of Blankophor BBH was noted in either aerial study. Extrapolations made from the ground study indicate that the amount of Blankophor BBH applied in the aerial studies (17 g/A in West Virginia, 38 g/A in Maryland) may have been well below the threshold of activity for this material.

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## CAN ANTS CONTROL GYPSY MOTHS? A TWO-YEAR STUDY OF THE POSSIBILITY

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### ABSTRACT

In 1992 and 1993, experiments were carried out to manipulate ant populations in either a young forest (1992-trees about 3 m high) or a more mature forest (1993-trees about 30 m high). Ants, particularly various species of *Formica*, were encouraged by spraying sugar solutions in some plots, discouraged by establishing fluon-coated fences around some plots and reducing ant numbers by use of poison baits. In 1992 an attempt was made to force ants to forage in trees by removing the litter layer. In both years, ants were more numerous in the sprayed plots than in the fenced ones, showing that ant numbers can be manipulated by these treatments. Also in both years, gypsy moth larval numbers tended to be greater in the fenced plots than in the sprayed plots. Finally, correlations between ant numbers and gypsy moth larval numbers were significantly negative in both years. These results show that ants can affect gypsy moth numbers in two rather different forests. The two years' data demonstrate the potential that forest ants have for keeping low gypsy moth populations low, and thus their importance as biological control agents of this pest.

# POTENTIAL CHANGES IN SPATIAL DISTRIBUTION OF GYPSY MOTH OUTBREAKS IN PENNSYLVANIA UNDER CLIMATIC CHANGE

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## ABSTRACT

Changes in the geographical ranges and spatial extent of outbreaks of pest species are likely consequences of climatic change. We investigated potential changes in spatial distribution of outbreaks of the gypsy moth, *Lymantria dispar* (L.), in Pennsylvania using maps of historical defoliation, climate, and forest type in a geographic information system. Maps of defoliation frequency at a resolution of 2×2 km were assembled using historical aerial reconnaissance data. Weather maps for mean monthly temperature maxima and minima and precipitation over 30 years were developed by interpolation. A relationship between defoliation status and the environmental variables was estimated using linear discriminant analysis. Five climatic change scenarios were investigated: an increase of 2° C, a 2° increase with a small increase and a small decrease in precipitation, and projections of two general circulation models (GCM) after 100 years at doubled carbon dioxide levels. With an increase in temperature alone, the projected defoliated area decreased relative to ambient conditions. With an increase in temperature and precipitation, the defoliated area increased. Conversely, the defoliated area diminished when temperature increased and precipitation decreased. Results for the GCM scenarios contrasted sharply. For the GCM developed by the Geophysical Fluids Dynamics Laboratory, defoliation was projected to increase and cover the state area completely. For the GCM developed by the Goddard Institute for Space Studies, defoliation remained about equal to that under ambient conditions. The results are discussed in terms of potential changes in forest composition.

# METHODS TO MEASURE IRON BIOAVAILABILITY AND ITS EFFECT ON GYPSY MOTH GROWTH

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## ABSTRACT

For many insects, iron (Fe) is a dietary essential because it functions as a co-factor in enzymatic reactions. In the laboratory, reduced bioavailability of Fe causes slow, asynchronous growth (straggling) of gypsy moth (ODell et al. 1993) indicating its importance as a micronutrient. Straggling occurs when both parent and progeny are reared on diet containing crystalline ferric phosphate ($FePO_4$), i.e., reduced iron bioavailability. Straggling is significantly reduced when parental diet is supplemented with amorphous $FePO_4$. This suggests that adequate reserves of Fe have been passed during the reproductive process (e.g., transovarially), and implicates parental growth conditions and food quality as factors predisposing larvae of subsequent generations to differential performance. In preliminary tests, larvae reared from field collected eggs, on diet containing crystalline $FePO_4$, exhibited significant straggling but siblings reared on diet containing amorphous $FePO_4$ did not. This suggests that reduction of Fe bioavailability occurred in the parent in the field. We summarize here methods being developed to measure, quantitatively and qualitatively, dietary iron, and the insect's response to Fe bioavailability (Willis and Montgomery, in press).

A method for measuring amorphous ferric phosphate ($FePO_4$) in complex salt mixtures and insect diets has been developed (Willis and Montgomery, in press). The procedure uses citrate solutions for extraction of salt mixtures and tartrate solution for extraction of prepared diet. Iron in the solution is then determined colorimetrically. Crystalline ferric phosphate, which has no iron bioavailability, is not extracted by either solution. Thus, the procedure can determine if the amorphous form, which has a high iron bioavailability, is present. This procedure is being used with measurements of insect growth, i.e., incidence of straggling (see ODell et al. 1993) to quantitatively determine iron bioavailability in hosts.

ODell, T. M., M. A. Keena, J. A. Tanner, and R. B. Willis. 1993. Gypsy moth rearing problem linked to iron (Fe) in diet. Gypsy Moth News 32: 3-5.

Willis, R. B. and M. E. Montgomery. In press. Measurement of the amorphous form of ferric phosphate as an assessment of iron bioavailability. Analytical Chemistry.

# VERTICAL CANOPY-AREA PROFILES OF HARDWOOD FORESTS IN THE EASTERN UNITED STATES

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## ABSTRACT

Efforts were made in this study to systematically characterize the canopy area profiles for the major hardwood forest types in the Eastern United States for general simulation studies on forest-atmospheric exchanges, such as the Forest Service Cramer-Barry-Grim (FSCBG) aerial spray model.

The canopy analyzing system developed at the University of Connecticut was modified and used in this study to collect the vertical distribution data of hardwood forest canopies. The facility mainly included a mobile, up-down lifting tower with an auto-leveling platform to host a group of sensors. LAI-2000 canopy analyzers were used to measure the cumulative canopy area index above a given elevation in canopy. Fifteen major hardwood forest types were selected, from Minnesota to Florida, to include the boreal hardwood, northern hardwood, upland oak, and bottomland hardwood types recognized by the Society of American Foresters. Eight of the 15 types were measured in 1993. For each forest type, measurements were taken at 10 to 24 locations, at least 20 m apart from each other, and randomly chosen. The Weibull cumulative distribution function (CWDF) was used to fit and describe the vertical profiles of the cumulative canopy area index with the depth (downward distance from the canopy top) as the independent variable with the statistical software STATISTICA.

The vertical profiles were found significantly different among forest types. Mean leaf area index was just 2.7 for an aspen stand, and above 4.2 for white oak forests. While canopy area was found to have a large portion located in the lower part of the canopy for paper birch, most of the leaves were found near the top of the canopy in the aspen stand, although the total leaf area for both types were approximately the same. Every forest type showed some unique characteristics in canopy area distribution. Therefore, a generalized vertical profile is not suitable for all hardwood forest types. The CWDF was found to well represent the vertical profiles of hardwood canopy area, by explaining more than 97.5% variations in all cases. The profile data and CWDF fitting parameters will be consolidated into a digital canopy library of forest types along with a data base of the associated forest stand information for general simulation purposes.

## USDA Interagency Gypsy Moth Research Forum
## January 18-21, 1994
## Annapolis, Maryland

### List of Attendees

Anderson, George, Virginia Dept. of Agriculture, 307 Church Street, Blacksburg, VA 24061
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