United States
Department of
Agriculture

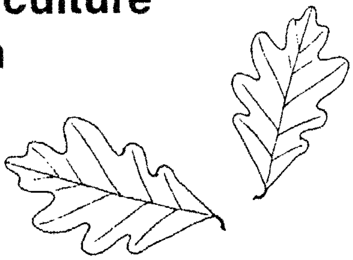
Forest Service

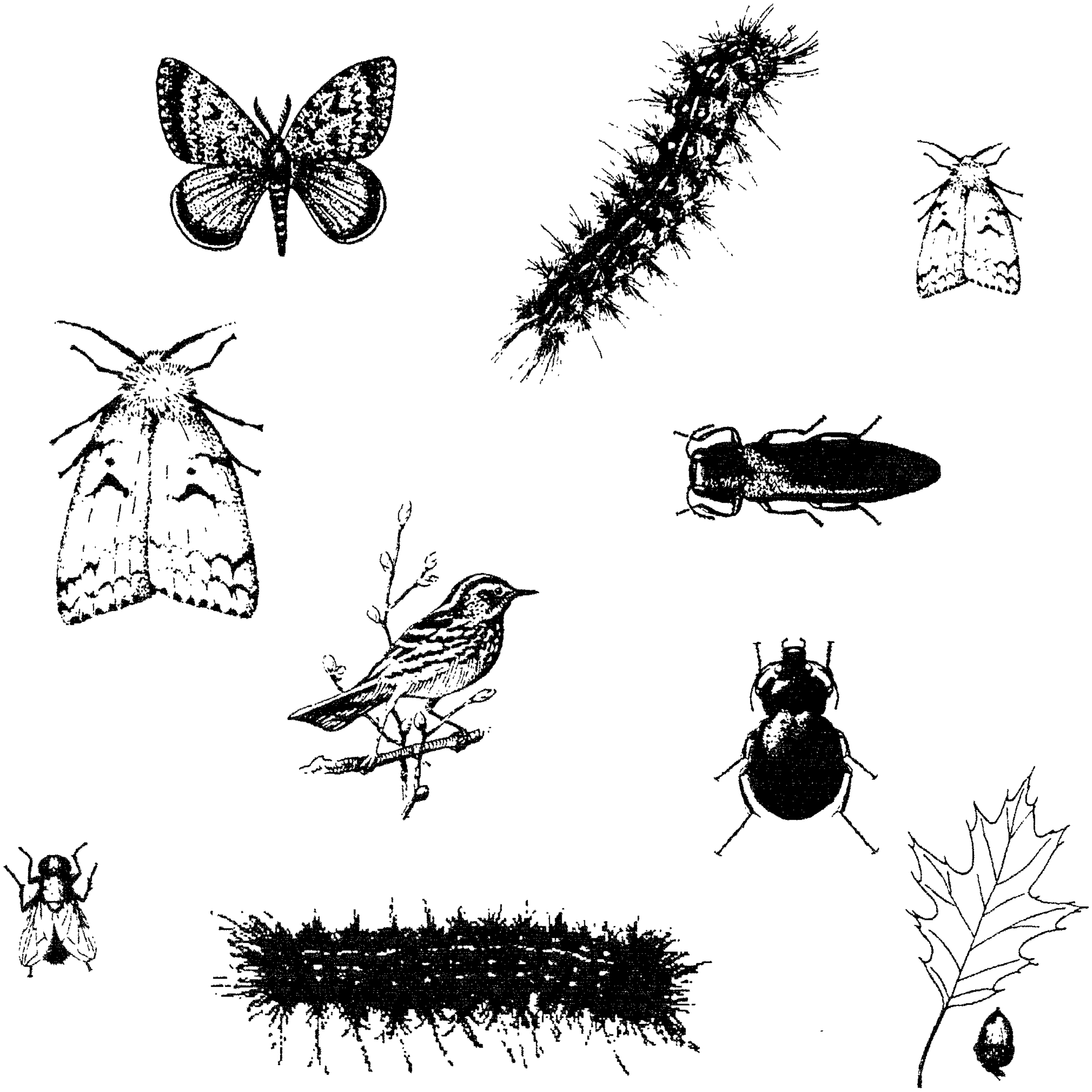
**Northeastern Forest
Experiment Station**

General Technical
Report NE-179

PROCEEDINGS

U. S. Department of Agriculture Interagency Gypsy Moth Research Forum 1993





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ACKNOWLEDGEMENTS

We would like to thank Dr. Mark J. Twery for providing the cover and title page design.

USDA Interagency Gypsy Moth Research Forum
January 19-22, 1993
Loews Annapolis Hotel
Annapolis, Maryland

AGENDA

Tuesday, January 19

REGISTRATION
POSTER DISPLAY SESSION I
WELCOME MIXER

Wednesday, January 20

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

Welcome
Michael McManus, FS-R

Research in Forestry Canada
Les Carlson, Forestry Canada

Status of Ecosystem Management: A National Perspective
Ann Bartuska, FS-WO

The National Biological Control Institute and Biocontrol in the U.S.
E. Delfosse, APHIS

The Abnormal Performance Syndrome Case
Melody Keena, FS-R

Forest Health Management Center Concept
A. Bullard, FS-AIPM/FHP

GENERAL SESSION . . . . Moderator: Les Carlson, Forestry Canada

Asian Gypsy Moth
Presenters: T. McGovern, APHIS; M. Holmes, APHIS; W. Wallner, FS-R; R. Cardé, Univ. of Massachusetts

POSTER DISPLAY SESSION II

Thursday, January 21

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Gut Physiology and Its Influence on Gypsy Moth Control Strategies
Presenters: H. Appel, Penn. State Univ.; M. Wolfersberger, Temple Univ.; D. Dean, Ohio State Univ.; W. McCarthy, Penn. State Univ.; R. Lindroth, Univ. of Wisconsin; K. Shields, FS-R; J. Burand, Univ. of Massachusetts; M. Shapiro, ARS

CONCURRENT SESSION II .... Moderator: T. ODell, FS-R

Gypsy Moth Biocontrol: Synthesis and Evaluation for Developing Research Priorities
Presenters: R. Reardon, FS-AIPM/FHP; R. Fuester, ARS; H. Smith, FS-R; J. Maddox, Illinois Natural History Survey

OPEN DISCUSSION WITH EXPERT PANEL

Panel: E. Delfosse, APHIS; J. Elkinton, Univ. of Massachusetts; W. Metterhouse, New Jersey Dept. of Agric.; J. Krysan, ARS

CONCURRENT SESSION I .... Moderator: V. Mastro, APHIS

Genetics and Systematics
Presenters: P. Schaefer, ARS; F. Proshold, ARS; D. Prasher, APHIS; J. Slavicek, FS-R; D. Ferguson, ARS

CONCURRENT SESSION II .... Moderator: J. Podgwaite, FS-R

Advances in Microbial Control
Presenters: D. Miller, Univ. of Connecticut; N. Dubois, FS-R; R. Webb, ARS; J. Podgwaite, FS-R

CONCURRENT SESSION I .... Moderator: K. Gottschalk, FS-R

Indirect Effects of Defoliation on Forest Resources
Presenters: P. Wargo, FS-R; D. Thurber, Nat'l Museum of Natural History; M. Vaughan, US F&WS

CONCURRENT SESSION II .... Moderator: J. Pierce, Environmental Action

Non-Target Concerns
Presenters: A. Hajek, BTI; L. Butler, West Virgnia Univ.; J. Peacock, FS-R; D. Wagner, Univ. of Connecticut

Friday, January 22

GENERAL SESSION . . . . Moderator: D. Gray, VPI

Gypsy Moth Life System Model: Status and Future Direction
Presenters: J. Colbert, FS-R; A. Sharov, VPI; J. Wilder, West Virgnia Univ.

Population Dynamics: What Causes Outbreaks and Collapses
Presenters: M. Montgomery, FS-R; A. Liebhold, FS-R; J. Elkinton, Univ. of Massachusetts

GypsES: Update and Future
Presenter: M. Twery, FS-R

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ABSTRACTS OF PRESENTATIONS AND POSTERS AND WORKSHOP SUMMARIES

RESEARCH IN FORESTRY CANADA

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INTRODUCTION

To get a feeling for the extent of Forestry Canada's research program, you should first of all have a little understanding of the vastness of the country and with that the diversity of problems that are faced by the forest managers. So let us take a little refresher on Canadian geographic and economic situations.

The total area of Canada is 997 million ha, of which 453 million ha are forested. And of these, some 244 million ha are productive forests that support a $40 billion a year forest industry. It is interesting to note that about 65% of all Canada's forest products exports are to the United States. Thus, to us, our continued cooperation with the Forestry Service is quite important. The fact that we share the longest border in the world means that we have to seek ways to continue to work together to solve both our cultural and resource problems. The total harvest each year is about 1,000,000 hectares. This varies from year to year depending on the state of the forest economy. Needless to say that because of the remoteness of our forests, much of the harvest is done around the over 350 single industry communities. In addition to losses due to harvesting we lose about 1,000,000 hectares of forest a year to forest fires and another 2,000,000 a year to insects and diseases. Admittedly, not all the losses are in the highly productive commercial forest areas. Reclaiming the areas harvested and burned depends primarily on natural regeneration of the forest. However, almost half of the harvested areas are planted back to the similar species.

As a forested nation, Canada is committed to the overall concepts of sustainable development. This is seen in the statement of Canada's forestry community goal:

"Sustainable Forests:A Canadian Commitment"
"Our goal is to maintain and enhance the long-term health of our forest ecosystems, for the benefit of all living things both nationally and globally, while providing environmental, economic, social, and cultural opportunities for the benefit of present and future generations".

This sounds familiar, as right it should. The concept is that as espoused in the Bruntland Commission's report, "Our Common Future".

The message is clear for our research mission and the direction it should take now and in the future. Note that we talk about the long-term health of the forest ecosystems. So it is no wonder that we have shifted much of our program to areas that promote environmentally sound management practices in our forests. While to many of you health means free from insects and diseases, it also means using management practices that do not harm the forest ecosystems to the point that they can not be repaired. What then are the major shifts in Forestry Canada's research program that puts it into this picture of ensuring the sustainability of the country's forests?

To many an eye not much has changed, but an examination of the kinds of things we are doing now may very well show that we are moving in a different direction. Our research program has five primary thrusts:

1. Environment - Included in this are such forest health issues as acid rain, air pollution, environmental impacts of forestry practices and our major monitoring programs in forest health.
2. Protection - Much of our traditional insect and disease research, but now using the state-of-the-art technologies for pest control development.
3. Fire - Continuing research into ways to effectively assess forest fire potential and to better predict fire behaviour.
4. Forest management practices - The use of new methodologies to more closely manage the forests.
5. Forest Products research - Support for the development of product standards and creating an atmosphere for product innovation.

The overall Forestry Canada research program depends on many excellent hard working scientists. The science related cadre of our department has a rough count of 825 staff members. Of these, 170 are research scientists with another 250 professionals in forestry officer or biological officer types of jobs. Along with the remaining 420 technical and support people, they run our eight research centres across the country, located in St. John's, Newfoundland; Fredericton, New Brunswick; Quebec, Quebec; Chalk River, Ontario; 2 at Sault Ste. Marie, Ontario; Edmonton, Alberta; and Victoria, British Columbia.

The specific strengths of each of the centres varies with some overlap due to the seriousness of particular problems within the regions. These are: Newfoundland - hemlock looper, spruce budworm, pheromones; Maritimes - spruce budworm, forest health, genetics; Quebec - spruce budworm, tree physiology, forest health; Ontario - forest management, forest health, fire; Northwest - fire, regeneration, climate change; Pacific - root rots, mountain pine beetle, pinewood nematode; Forest Pest Management Institute - pest management, *Bt*, viruses; and Petawawa National Forestry Institute - tree improvement, fire, inventory.

SPECIFIC PROGRAM AREAS

ENVIRONMENT

As noted earlier, our environmental programs involve studies on air pollution (acid rain), climate change, the effect of forestry practices on the environment, and forest health monitoring. We also consider our work on biological controls for pests as part of an overall approach to the way in which we structure our research program, but I will discuss this under a different heading.

A long-standing environmental research activity has been our LRTAP program (Long Range Transport of Air Pollutants). We spent considerable time looking for the effects of air pollution, primarily sulphur dioxide and sulphate deposition, on our forests. In the beginning, we had three catchment studies to monitor the day-to-day and year-to-year changes in the forests. They were located in Ontario, Quebec, and Nova Scotia. However, the direct effects were only seen in such areas as Sudbury, Ontario, and a few other point sources of sulphur emissions. While we still maintain the catchment near the Sault, our work has changed to looking at a whole suite of air pollutants that include NOX, ozone and other volatile organic compounds (VOC).

One project is being carried out on the effect of sulphates and ozone on the cuticular structure of some of our conifers. The premise is that thinner cuticles affect the uptake of pollutants, and for that matter any other organic compounds that may be presented to the surface of the needles. This work certainly has relevance to the use of insecticides or herbicides in areas that may be impacted by specific air pollutants. Another related project is looking at the effect of acid fog on the growth of birch along the Bay of Fundy. These fogs are known to have an acidic value as low as pH 2.9. The birch in the region are not of commercial value, but play an important part of the coastal forest ecosystem.

In another important project, we are determining the target loading of sulphates and nitrates for our more sensitive forests in eastern Canada. This work is being done in collaboration with the forest industry and the University of New Brunswick. It is hoped that this information will let us know just how much pollution our forests can accept.

Rolling out of the concern about air pollution and its potential effects on the forest ecosystems are two biomonitoring programs. Through the ARNEWS (Acid Rain National Early Warning System) program we monitor over 100 permanent sample plots for any changes in the health of the trees in them. We monitor for air pollution effects as well as for insects and diseases. The past year saw the first report which commented on the state of change in Canada's forest as reflected by the ARNEWS plots. We can proudly say that our forests are still healthy.

Another program is a joint one with our eastern provinces, the eastern states and both federal governments in which the health of the maples are monitored annually. NAMP, the North

American Maple Project, is similar to the ARNEWS monitoring program, but restricted to species of maple, in particular sugar maple. The monitoring has chronicled the maple decline situation over the past five years.

Our climate change program is relatively young and we are trying to determine the capacity of our forests to sequester and store carbon dioxide in the forest soils and to some extent in forest plants. As you might expect, the former is a little more difficult to determine given the tremendous variability of our forest soils. The basic program is to supply enough data to be able to develop a model of the fate of carbon dioxide in the Canadian forest ecosystems.

FOREST MANAGEMENT

Research on the effect of forestry practices on the environment is another aspect of the Forestry Canada program. Research projects include studies on the effect of steep slope logging, effects of clear cutting practices in Canada's forests, and the overall effects of selective harvesting in some our very sensitive west coast sites.

The project dealing with steep slope logging in British Columbia zeroed in on the building of roads that do not cause excessive deterioration to the forest ecosystems. Just logging will cause some detrimental effects on the forests, but the object is to develop mitigative measures that will allow the forests to return to their original state as soon as possible. The road building program, done with the cooperation of the Forest Engineering Research Institute of Canada (FERIC), resulted in road bases that could withstand the eroding effects of the wet west coast weather. Similar research on the environmental effects of road building has been carried out in Newfoundland in the past decade.

Work carried out in the boreal forest, where most of the clear cutting operations take place, have shown that the effects of clear cutting can be mitigated through the use of strip cutting programs that provide for better regeneration and enhanced cover for wildlife such as upland game birds and moose. This work has been done out of our research lab in SSM. Along with this research, we have invested a considerable amount of time and energy in the development of efficient and effective reforestation systems for those areas that have been clear cut. One final word on the clear cut syndrome is that in most cases the pines and the spruces of the boreal forest are "clear cut" by either wildfire or by indigenous insects.

Another project that we are carrying out, in a way similar to the catchment studies under LRTAP, is the monitoring of the a specific management unit on Vancouver Island. Here our scientists have looked at the ecosystem before, during, and after harvesting. The studies have tried to delineate the effects of types of harvesting, regeneration, and tending (i.e. fertilization, thinning, etc.) on the soils, soils microorganisms, tree growth, tree establishment, and insect populations. This project has been delivering excellent results for the past 20 years. In particular, it has given us an insight on the complexity of the ecosystems, yet it has shown that these ecosystems can and do recover their productive capacity.

Since research on many of the forest management practices has taken an environmental slant these days, it is hard to differentiate the line between environmental research and forest productivity research. Certainly one of the most important economically effective research programs deals with the development of forest management models that are used by the industry to plan their daily activities. The use of decision support systems (DSS) that interface with geographical information systems (GIS) have placed a powerful set of tools in the forest manager's tool kit for the management of the forests. These systems can now take into account the environmental, soil, and climate characteristics of a site or a region in developing a comprehensive management plan. The modelling aspects are also applicable to the understanding of how fires spread and how insect and disease problems develop.

One of the more successful tools developed for forest management has been the Forest Fire Danger Rating System and its application to the readiness of the forest fire prevention and protection programs. In addition, computer based expert systems are helping our fire fighters to effectively deploy their fire fighting equipment.

Models are also of interest in the field of insect problem management. There is a joint project between Forestry Canada and some scientists in the United States on the development of a model on the control of the jack pine budworm. We are actively working on a model for spruce budworm. In addition, we have made substantial progress in the development of a DSS for management of the hemlock looper in Newfoundland. A basic model has been developed using long-term FIDS data which can fairly accurately predict how various stands in Newfoundland will react to various levels of looper infestations.

FOREST PESTS

Our pest research and related control programs make up about 40% of the Forestry Canada Research effort. It will not be possible to cover all the related projects in this discussion. The priority projects that I see in Forestry Canada's protection research are the Gypsy Moth complex, spruce budworm, mountain pine beetle, root rots, and stem rusts. Another underlying priority is that of biological control of insects, diseases, and weeds. I will discuss this further in the next section of my talk.

It is no surprise that I put the gypsy moth complex issue at the top of the priorities, particularly given the events of the past year with the AGM. Many of you are familiar with most of the gypsy moth research being done in Canada, but I will just mention three areas of concentration.

First is the intensive study we have been doing to try and develop, along with our American counterparts, a practical and definitive test for the identification of the AGM. The work of Harrison, from Cornell University, on the use of mitochondrial DNA was a first major step and we have developed a lab in our Victoria, British Columbia centre using this technology to carry out the identifications of gypsy moth on our west coast. In addition, we are in the process of looking for funding to support work on the development of nuclear DNA markers to be used in the identification of potential hybrids of the EGM and the AGM.

Second, at our FPMI in SSM, Dr. Gary Grant is working with other pheromone specialists to develop a technique to more accurately assess the gypsy moth populations. The methodology will use far less pheromone plus a pheromone inhibitor to simulate the natural attraction and discouraging activities of gypsy moth females. This should allow us to better compare the annual egg mass counts with the actual population of the moth. We would like to test this approach in the eastern part of Russia on the AGM over the next few years, but at the present time we are restricted by lack of available funding.

Third, as many of you are aware, Forestry Canada has a program for the development of the DisparVirus, which is essentially the same as Gypchek. This work is also being done at our institute in SSM.

The spruce budworm research program is still our biggest entomology research program. The budworm populations have collapsed in many parts of eastern Canada and our research activity is more focused on the population dynamics of the insect. We know that it will become a problem again in the near future as it has been in the past after several collapses over the past 30 years. The situation has given us some time to look at the native parasites and pathogens that may have caused the collapse. Those that we are concentrating our effort on include a species of *Actia* (an insect) and one of *Nosema* (a pathogen).

The mountain pine beetle is one of the major pests of our western interior lodgepole pines. Our work there is a continuation of trying to understand the biology of this insect. It is not easy to approach this problem other than as a management issue. Our people in the Victoria lab have done a lot to help the managers to understand the development of the insect, but we don't have any sure-fire controls other than clear cutting or salvage logging. Our efforts on biological control have also been weak. So the beetle continues to be a challenge to our scientists.

Our work in forest pathology has several important thrusts, however I will only mention two of them. Root rot research has been carried out over a long period of time in Forestry Canada, particularly on *Armillaria* and *Phellinus* root rots. The work on *Armillaria* over the past few years has concentrated on the systematics and management of the fungus. In the case of *Phellinus*, work has been done on damage assessment and modelling its development in order to predict future losses. The stem rust research has been directed at the development of hazard ratings for white pine blister rust and early screening for resistance to gall rusts.

BIOLOGICAL CONTROL OF FOREST PESTS

Over the years we have had some very good success with the use of classical biological controls. The control of the European spruce sawfly, larch sawfly, mountain ash sawfly, and the winter moth are marks of our progress in this area. Our present program calls for searches for the parasites on gypsy moth, spruce budworm, and *Pissodes* spp.. Our Ontario and Quebec Regional centres have a program for the introduction of a fly, *Ceranthia*, as a parasite for the gypsy moth. It works quite well at moderate populations of the moth, but is not a cure-all for epidemic conditions. Another parasite being developed commercially in Ontario in collaboration with Ciba-Geigy is *Trichogramma*, an egg parasite for spruce budworm. These avenues suggest that control of insect pests with effective parasites, predators, or pathogens is possible.

Forestry Canada places a lot of hope in the area of classical biological control for our future pest management programs.

The continued research on biorationals is a strong point of our institute in SSM. They have major programs in the development of more effective biologicals such as *Bt* and baculoviruses. The efforts in Forestry Canada are focused on the use of molecular genetic technologies to improve the effectiveness and efficiency of these biorationals.

There is one final aspect of biological control that Forestry Canada researchers are involved in, and that is the development of various fungi for the control of weeds. Our people in the Victoria centre are in the final process of developing a fungus for the control of woody weeds in conifer regeneration stands. This approach will give us another environmentally sound pest control practice that we can use in the forests.

Much more could be said about our various research programs, but I can not report them here because of time constraints. The best way would be to contact my team in Ottawa and we will be able to put you in contact with scientists in specific research areas.

THE PROCESS - HOW WE DEVELOP AND CARRY OUT OUR RESEARCH

The funding: Forestry Canada, like any other government department in the world, operates on a special allocation from the government, which we call our A-base funding, and on special program funds, which we call soft money. The soft money is sometimes call "pass through" funds as they are administered by the Forestry Canada with little of the funds staying within the government department for internal programs.

A-base: These funds are derived from an annual operational plan which spells out the goals of the department for the up-coming year and verifies the progress made in the previous year. This plan is finalized within the department and sent to the Treasury Board for approval. The results are usually in the hands of the departments by early February in time to start our fiscal year on the first of April.

The plan is developed in recognition of the needs of our research and administrative programs. It doesn't mean that we get all the money we ask for or thought we had planned for. Barring any particular government austerity program, we are assured of approximately the same A-base funds from year to year. Thus our basic program of research doesn't vary, except for responses to new priorities. This stability is a two-edged sword in that there is security for some funds, but not the inflationary support needed for the conduct of science with modern technologies.

The funds are generally distributed on the basis of the size of the staff at each of the eight research centres across Canada. Program development for national issues becomes a little bit of a problem as the decentralization requires the regional centres to pay particular heed to the local clients of our research, i.e. provincial and industrial agencies. However, we do administer several national programs from our A-base from Headquarters that do enlist regional scientists.

Soft funding: These funds for research are equal to about 30-40% of the operational funding outside of the salaries, which are from the A-base. The type of programs that exist are two-fold in that some of the soft funds do come from reserves in our A-base program (albeit small, about 2 million out of 90 million), while the other comes to the department from special federal government programs such as the Green Plan. For Forestry Canada, the latter has been very helpful in the further development of our environmentally related research projects and to address emerging priorities. Approximately 8 million dollars a year are being pumped into our program over the next five years. Of course, the Green Plan money does have to have a direct connection to the sustainable development of Canada's forests.

The soft internal funds come from programs such as the development of energy from the forests (ENFOR), a science and technology opportunities fund, and specific allocations for problems like the AGM and the PWN.

The ENFOR program is specific and funds are allocated by a panel of research scientists to cover projects that enhance the productivity of forests for energy production. The S&T Opportunities Fund is a program that solicits proposals for research from the regional centres in specific priority areas. These funds are used to lever additional regional funds to develop programs that have a national overview.

The Green Plan funds have been given to the department to develop research programs that support sustainability of our national resource. Within this we have programs on LRTAP, IFPM, DSS, Forest Management Practices, Climate Change, Ecology Research, Seed Bank, and Fire.

The LRTAP and IFPM (Integrated Forest Pest Management) programs probably exemplify how we manage the development of our research agenda for Forestry Canada. In both cases we have a working group that represents each of the research centres with the appropriate research expertise. These people collectively develop the program of research through a system of calling for and evaluating proposals for project development. However, the first criteria is that the working group decides on and recognizes the priority needs of the science community and those of the national research management. The work after that is usually less difficult, but no less agonizing when very good proposals are put before them and there is not enough money to cover all submissions.

The IFPM working group has several sub-committees that develop "mini-programs" in the same manner that the main group develops the overall program. These sub-committees include such areas as classical biological control, biological control of weeds, biological control of pathogens, pheromones, and spruce budworm ecology.

Forestry Canada support of university programs: There are several ways in which the department supports universities. The first is through a joint Forestry Canada/Natural Sciences & Engineering Research Council fund that encourages the universities to enlist industrial partners in the research program. In this fund, the joint government council will supply two thirds of the funding for the project, while the industry supplies the other third. The second is the straight forward contracting with them for a particular piece of research. The third is a small, but effective, subvention program to universities with forestry faculties. This fund is less than 100 thousand dollars a year, but can be directed to specific projects rather quickly.

FUTURE FOR FORESTRY RESEARCH IN CANADA

Historically, Forestry Canada (often referred to as the Canadian Forestry Service) has been an organization that has been in constant movement from one government department to another over the past 30 years (a total of nine departments). However good or bad that may have been, the Service has always had a strong research component, with emphasis on protection and forest management. It is highly unlikely that there will be significant changes with any new moves to another department or agency.

From the funding standpoint, the immediate future will see more and more dependence on soft funds. They will be from government programs, such as the Green Plan, that cut across a number of science-based departments, to industry and other private sector support for particular types of research. This can be considered as a good move as many of the immediate forest management problems will be addressed. On the other hand, short-term funding adds a bigger planning workload on individual scientists. As any university professor who continually works with soft funds knows, the amount of non-research energy that has to be done to compete for those funds will increase significantly. At some point the government will have to ask what research game they should be in. Increasingly, we hear that funds are being reserved for programs that provide for the security, health, and safety of the country. It will be argued that a strong economy will provide these needs. And it will be also argued that you can't have a strong economy unless it has a good science base. So we will eventually come full circle to stronger government support for research.

Part of that concern on the economy is reflected in our major science thrust in the next few years, ensuring sustainable development of the country's forest resources. Science will have to help in the development of both environmental and political solutions to environmental problems. In all our zeal to follow the concepts of sustainable development, we have to make sure that our forest industries can operate effectively within these concepts and stay healthy. A special challenge.

Finally, the globalization of our research commitments as seen through the concepts of sustainable development will require applying our knowledge to more than local or national issues. Last year's UNCED conference in Brazil indicated that if we are going to make an impact on the global environmental problems, we will have be ready to work with the developing countries. Tropical forests are a source of income for many, while at the same time their exploitation creates some of the biggest environmental problems.

Globalization will also demand that our science-based departments will not only have to forge partnerships with science agencies in countries of our trading zone, but also with those advanced industrialized countries of Europe and Asia.

As researchers we have many science issues to address. I look at this meeting on the subject of the gypsy moth as an important one for my understanding of the present research being carried out in the United States. So I will be with you for the rest of the week and I wish you the best in your presentations and discussions. Thank you.

STATUS OF ECOSYSTEM MANAGEMENT: A NATIONAL PERSPECTIVE

Ann M. Bartuska

USDA Forest Service, P.O. Box 96090, Washington, DC 20090-6090

ECOSYSTEM MANAGEMENT

Ecosystem management means using an ecological approach to achieve the multiple-use management of national forests and grasslands by blending the needs of people and environmental values in such a way that national forests and grasslands represent diverse, healthy, productive, and sustainable ecosystems.

Direction for ecosystem management will be in Forest Service regulations, Forest Service manuals and handbooks, and Forest plans. Direction for Forest Service Research on ecosystem management is in the Strategy for the 90's. Strategic guidance for ecosystem management and ecosystem management research is in the 1990 Resources Planning Act Program.

AIMS OF ECOSYSTEM MANAGEMENT

Aims for ecosystem management include:

1. Take care of the land by continuing to restore and sustain the integrity of its soils, air, waters, biological diversity, and ecological processes.
2. Within the sustainable capacity of the land, meet the needs of people who depend on natural resources for food, fuel, shelter, livelihood, and inspirational experiences.
3. Within the sustainable capacity of the land, improve the well-being of communities, regions, and the nation through diverse, cost-effective, and environmentally sensitive production, use, and conservation of natural resources.
4. Seek balance and harmony between people and the land with equity between interests, across regions, and through generations, meeting this generation's resource needs while maintaining options for future generations to also meet their needs.
5. Improve the effectiveness of public participation in land and resource decision-making.
6. Expand conservation partnerships between Forest Service managers, other agencies, and the publics they serve in carrying out ecosystem management.

7. Strengthen teamwork between managers and scientists, including the integration of social, biological, and physical science disciplines.

EXPECTED RESULTS OF COORDINATING ECOSYSTEM MANAGEMENT

Ecosystem management should ensure that production of resource products, values, services, and uses desired by people from the National Forest System is done in ways that sustain healthy and productive ecosystems for future generations. Coordination of ecosystem management should also produce the following organizational results:

National Forest System Management shows:

- Better awareness, understanding, and use of ecological principles for sustaining diverse, healthy, and productive ecosystems.
- Increased environmental sensitivity and commitment to land health in producing natural resources and sustaining their uses.
- Improved compatibility and balance in resource uses and values at site, landscape, and regional scales over long time periods.
- Better use of scientific knowledge and management experience in implementing and refining land and resource management plans.

Forest Service Research shows:

- Better teamwork between researchers and managers in developing and using new scientific knowledge in forest plans and projects and assuring that management needs are reflected in research priorities.
- Better integration of biological, social, and physical sciences in research to develop scientific knowledge and technologies needed for ecosystem management.

The operating style of Forest Service personnel shows:

- Open and constructive dialogue with people so that their ideas are used in shaping Forest Service thinking and management of the public's National Forest System.
- Stronger partnerships with people, conservation groups, and resource user groups to assure that their needs and expectations are met.
- A Forest Service culture that better reflects the diversity of values and needs of the American people.

THE ABNORMAL PERFORMANCE SYNDROME CASE

Melody Keena

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I hope you enjoy a good mystery, because I am going to tell you the story of how the Abnormal Performance Syndrome Case was solved. Just sit back and relax, but not too much.

It was a cold winter day in December of 1988 when I was first told about the case. I was in Hamden, CT, to present a seminar and interview for a position, when one of the detectives (better known to you as researchers) told me that large numbers of domesticated gypsy moths were not growing and many more were dying before they reached adulthood. This unpredictable growth and survival problem they called Abnormal Performance Syndrome, APS for short.

The case intrigued me, so when offered a position in the Forest Service Laboratory at Hamden, I gladly accepted. When I arrived I knew very little about gypsy moths. So I began by reading everything about gypsy moths I could get my hands on, then spent hours observing and working with them.

After learning as much as possible about the gypsy moth, I decided to trace the history of APS. I asked around the agency and was told that there were files on the gypsy moth back to when they were first domesticated. After digging through dusty file after dusty file, with the help of other personnel at the Forest Service Laboratory at Hamden, CT, and the APHIS Methods Development Laboratory at Otis, MA, I found that APS was as unpredictable as they had told me. In addition, I found that at one time or another APS had viciously hit all the major sites where gypsy moths have been domesticated: APHIS site at Otis, MA; ARS site at Beltsville, MD; and the Forest Service site at Hamden, CT. I will just refer to the city when talking about each site from now on. It also appeared that no strain or sub colony of domestic gypsy moth had been spared a bout with APS.

In March of 1989, Tom ODell, a Forest Service detective who had been working on the case, drafted a problem analysis on APS. In the problem analysis he identified the extent of the problem, described the symptoms, and identified four types of possible suspects. This document was recently published, but as you might guess it is not on the best seller list.

APS was having a significant impact. Costs of producing Nuclear Polyhedrosis Virus about doubled, further development of the F1-sterile program was stopped, and the quality and quantity of domestic gypsy moths (NJSS strain) available for research was drastically reduced. Something had to be done to stop APS.

The symptoms of APS included the following. First, reduced hatch of embryonated eggs and dead neonates within the egg mass. Due in many cases to the inability of the neonate to chew out of the egg or the neonate running out of energy just after chewing out of the egg. Second, slow asynchronous development. In families (which are equivalent to an egg mass) with APS, many individuals are still first instars after 10 days, while unaffected individuals are large third instars. And at the time that all individuals should be pupae, some small larvae still remain in families stricken with APS. Third, there is a significant increase in mortality from hatch to adult eclosion. Many first instar larvae have problems settling down to feed and die of starvation as they wander aimlessly around their containers. The larvae that grow slowly often die because of molting or pupation problems. Finally, many individuals from families with APS have severe pupal deformities and several never make it to adulthood. Some of the adults that do make it are weak or deformed.

In August of 1989, a team of detectives was chosen and given three years to determine the cause of APS and develop ways to prevent it from happening. Detectives from three agencies within the USDA (the Forest Service, Animal & Plant Health Inspection Service, and the Agricultural Research Service) were brought together. I was asked to be part of this team.

In October of 1989, the team invited expert witnesses from around the US to a strategic planning session in Hamden, CT. For two days we discussed and developed a plan of action. A multitude of possible suspects to follow up on emerged. APS would be a tough case to solve. The team met a couple of months after the meeting and selected eight primary suspects to investigate. Now let me introduce you to the suspects and tell you the questions we hoped to find answers to as we investigated each suspect.

1. **Mr. Diet** Has the quality of the dietary ingredients been changed so that it causes APS? Does the formulated diet go bad as it ages and cause APS? Does what the parents eat have an affect on their offspring's' health?

2. **Mr. Rearing Environment** Do the rearing conditions (temperature, humidity, and air flow) change the incidence of APS in the parents or their offspring?

3. **Egg Treatment Jr.** Do the environmental conditions during egg embryonation, acclimation, or storage cause APS? Do dehairing or infestation methods affect the incidence of APS? Does surface sterilization of the egg mass with 10% formalin cause APS?

4. **Mr. Larval Density** Does overcrowding cause APS in either the parent or their offspring?

5. **Ms. Container** Does the size or construction of rearing containers used cause APS?

6. "**Granny**" **Inbreeding** Does inbreeding in gypsy moth colonies cause APS?

7. **Mr. Phenotype** We thought that he was involved in some way along with one of the other suspects. But, we needed to prove that there was a genetic basis to APS.

8. **Dr. Pathology** Is APS caused by a pathogen?

THE SUSPECTS

1. Mr. Diet
2. Mr. R. Environment
3. Egg Treatment Jr.
4. Mr. Larval Density
5. Ms. Container
6. "Granny" Inbreeding
7. Mr. Phenotype
8. Dr. Pathology

Now as Holmes would say, "the Game's afoot!"

We made many initial stabs in the dark and collected reams of data. Some of the first evidence collected was on Dr. Pathology. We found the following evidence. First, on examination of larvae with APS, their food and frass, we found no evidence of bacteria, fungi, or polyhedral viruses. Second, when diet was amended with oxytetracycline and/or heat (33°C) and treatments to eliminate Rickettsia-like organisms (which have been implicated in developmental problems in other species) were used, APS was not eliminated. Finally, surface contaminates of egg masses, such as the *Penicillium* spp., were tested and found not to cause APS. On the basis of this evidence we decided to eliminate Dr. Pathology as a suspect. Now we were down to seven suspects, any one or combination of which could have been responsible for this unpredictable problem.

What about Egg Treatment Jr.; did he cause APS? Here is the evidence on Egg Treatment Jr. First, we found that APS was not caused directly by the environment parameters used during egg embryonation, acclimation, or storage. Second, we found that dehairing and egg infestation methods did not affect the incidence of APS. And third, surface sterilization of egg masses with 10% formalin did not cause APS, even though it is potentially harmful to humans! On the basis of this evidence, Egg Treatment Jr. was eliminated as a prime suspect, but may be later investigated further to see if he was an accomplice.

What about Mr. Larval Density; do the crowded conditions that domesticated gypsy moths are forced to live in cause APS? The evidence suggests that he didn't do it. We found that neither the female density per container nor the number of individuals per container affected the incidence of APS in the parent or their offspring. On the basis of this evidence we eliminated Mr. Larval Density as a suspect.

Well, if it isn't the crowed conditions, could it be the type of container used to house the gypsy moths? Could there be a criminal mind behind the make-up of Ms. Container? Ms. Container does have a darker side, but she didn't cause APS. We found evidence that the container used to rear the parental generation can affect progeny developmental rates and survival, but that it was an overall reduction in fitness rather than a change in the incidence of APS. Other evidence suggested that larval rearing container could affect male mating ability, but not incidence of APS. Therefore, Ms. Container was eliminated as a suspect.

Only four suspects left, but still no break in the case. Many areas of investigation were still under way but we seemed to be getting nowhere fast since it took eight months to get results to some tests due to the long generation time of the gypsy moth and the need to run tests for two generations.

The evidence on inbreeding was very slow in coming since we had to investigate three generations, but once all the evidence was in the results were surprising. We found that inbreeding, resulting from brother and sister matings, initially increased the incidence of APS, but that after two generations fewer inbred families had APS than did randomly mated ones. Other genetic forces, like selection for individuals less susceptible to APS, must have also been acting.

As expected, we found that outcrossing between sub colonies of domesticated gypsy moth had no effects on the incidence of APS. But, if wild strains were crossed with domestic strains, the incidence of APS increased. Wild strains must have more individuals that are susceptible to whatever was causing APS.

Because of these findings, we could eliminate "Granny" Inbreeding as a prime suspect. We suspected that she might influence APS in some way, but inbreeding did not cause it directly. We began to wonder if the case would be solved within the three years we had been given.

Then there was a break in the case. The gypsy moth colony at Hamden, CT (NJSS-FS), had been plagued with APS for over a year and APS had been virtually eliminated from the colony in Otis, MA (NJSS-A(APHIS)), for the same period of time. Now we could compare colonies, their environments, and their diets in these two cities and hopefully determine the cause of APS.

The first thing we did was to recruit gypsy moths from both cities to take part in a series of experiments designed to expose the culprit. John Tanner, an APHIS entomologist at Otis, and Tom ODell and myself at Hamden conducted the experiments.

We chose cohorts of 20 individuals from each of 25 families (minimally) for each treatment. The gypsy moths were all reared 10 to a 6 oz. cup with 40 or 80 ml. of diet in each. The cups were held in environmental chambers where the temperature and relative humidity were maintained at 25° C and 50-60%, respectively. The lights were kept on for 16 hours each day.

In the first comparison, we took cohorts from the same family from each colony (NJSS-FS and NJSS-A) and reared them at Hamden and at Otis. This would tell us if the colonies were different, and if so, were these differences maintained when moved to the other environment (air flow, micro climate, etc.) and reared on the diet made at that site. At Otis, both sub-strains developed fairly normally. A few individuals in some of the NJSS-FS families had symptoms of APS. At Hamden, the NJSS-A sub-strain developed normally but the NJSS-FS sub-strain (the one normally reared there) had a high incidence of APS. This meant that something at the Hamden site was causing APS but that it took more than one generation to do so. We could now start eliminating site differences one by one to find the culprit.

In the second comparison, cohorts from the same families of both sub-strains were again reared at both sites, but now we had two diet treatments at each site as well. By exchanging diets we could find out if it was the diet or the rearing environment that was causing APS.

When the results came in they were very interesting. At either site, the only individuals that had APS were from NJSS-FS families reared on Hamden diet. We now knew that diet caused APS to be expressed but there still had to be something that was causing only the NJSS-FS to be susceptible to the Hamden diet. Thinking that we might find the answer in the parental generation we continued both comparisons to another generation.

Let's review what we already knew about Mr. R. Environment and Mr. Diet. The results of the two comparisons, as well as other tests, had told us that rearing conditions could affect developmental rate and survival of both the current and next generations. This affect, however, was an overall affect rather than a change in the incidence of APS. For example, all gypsy moths reared at Otis grew a little faster because their temperature relative to Hamden's was slightly higher, even though both chambers were set at the same temperature. On the basis of this evidence, we eliminated Mr. R. Environment as a suspect. This left only Mr. Diet and Mr. Phenotype.

Let's look at what we already knew about Mr. Diet. From studies conducted before or during the two comparisons, we knew the following:

1) The fatty acids found in the wheat germ did not change significantly with diet processing or age.

2) What were thought to be oxidation products, as evidenced by darkening of the diet, did not affect the incidence of APS.

3) Vitamin C in the diet declines rapidly with diet age, but does not affect the incidence of APS.

4) APS is caused by a problem with some dietary component, but which one?

With fatty acids and vitamin C ruled out, we turned to a systematic elimination of each diet ingredient and checked to see if it was a problem with how the diet was prepared or the ingredients were stored.

The ingredients in the gypsy moth diet used at Otis and Hamden are: Agar, Methyl Paraben, Wesson Salt Mix, Water, Wheat Germ, Sorbic Acid, Casein, and Vitamin Mix. Some of the ingredients used at the two sites come from different vendors and each site stores the ingredients in a slightly different way.

The first thing we did was to have the water from each site analyzed. There were qualitative and quantitative differences in the water samples. We then compared diet made from Hamden ingredients but processed at Hamden and Otis using their respective water. We found no significant differences in the two diets. Therefore, we eliminated water as a possible cause of APS.

Which ingredient or ingredients should we test next? To develop a priority scheme to test ingredients most likely to be causing APS, we looked at the criminal record of Mr. Diet. We found that Sorbic acid and Methyl Paraben, the anti-bacterial, anti-fungal compounds, can be toxic if too much is used and had even caused some APS-like symptoms in other insects. So we exchanged the two ingredients between the two sites and substituted them either singly or in combination into both diets (Hamden and Otis) at both sites, then reared cohorts of individuals from the same NJSS-FS families on each diet treatment at each site. Sorbic Acid and Methyl Paraben substitutions made no difference in the incidence of APS, so we eliminated them as possible causes.

During the same time we were running the Sorbic Acid/Methyl Paraben experiment, we produced diet in several different ways to see if diet processing made a difference. We found that some processing methods accentuate APS expression but did not appear to be the cause of APS.

Next we exchanged and substituted, in all possible combinations, wheat germ, casein and vitamins from one site into the diet of the other site at that site. We ran the normal Otis and Hamden diets as standards, at each site.

We found some small differences in the incidence of APS when Wheat germ, Casein, and Vitamin Mixtures were substituted, but no single ingredient or combination of these ingredients was the primary cause of APS. In addition, we compared wheat germ stored in different ways to eliminate the possibility that the small differences we saw were caused by differential water weight gain while in storage, but found no differences. We were now left with two ingredients to test: agar and Wesson salt mix.

A test substitution test, similar to the previous one, was set up for agar and Wesson salt mix. We found that the agar did not cause APS, but that individuals reared on diet that contained Wesson salt mix from Hamden had APS while individuals from the same families reared on diet containing Wesson salt mix from Otis did not have APS. We now knew that Mr. Diet had somehow been substituting an inferior salt mix into the diet that caused APS.

We now had enough information to obtain a warrant for Mr. Diet's arrest. In addition, we could use the Wesson salt mix, from the vendor that Otis uses, to begin to eliminate APS from the Hamden colony. But before we could completely eliminate APS and prevent it from happening again, we had to find out what was wrong with Wesson salt mix that Hamden had been using and why NJSS-A was not immediately susceptible.

Wesson salt mixture calls for specific percentages of each of the following ingredients: Calcium Carbonate, Copper Sulfate, Ferric Phosphate, Manganous Sulfate, Magnesium Sulfate, Potassium Aluminum Sulfate, and Potassium Chloride. This salt mixture was originally developed for vertebrates, but is used world wide in many insect diets. Some of the metal ions are not normally found in the leaves that wild gypsy moths would feed on, so are suspect. Not being chemists, we went to Ray Willis, a Forest Service chemist, for help.

Ray analyzed samples of both salt mixtures. He first tried to dissolve the salt mixtures in 3% HCl (Hydrochloric Acid) so that he could determine the concentrations of each of the metal ions present. The salt mixture from the vendor that Otis was using produced a cloudy solution that could be cleared by further treatments, while the salt mixture from the vendor that Hamden was using produced a clear solution with crystals on the bottom.

Ray filtered both solutions and analyzed the concentrations of metal ions in each. There were only two metal ions that had concentrations that were different enough to be suspect; iron and aluminum. He then tried to dissolve the crystals, from the Hamden salt mixture, that had not gone into solution and analyzed them for metal ions. The crystals' major component was iron. A literature search revealed that there were two possible forms that Ferric Phosphate could exist in and how to tell the two apart.

After Ray told us that the Ferric Phosphate was the problem, we called the two vendors. The vendors did not really know which form of Ferric Phosphate they had, Ferric Phosphate was just Ferric Phosphate to them. So we ordered salt mix from each vendor without ferric phosphate and had them send us samples of the ferric phosphate they used.

After we received the ferric phosphate, Ray identified what form each was. The vendor that Otis got its salt mix from used amorphous ferric phosphate (which has a powdery, flour-like texture) and the Hamden vendor used crystalline ferric phosphate (which has a texture similar to table salt). The crystalline ferric phosphate was the one that did not go into solution very well.

To determine if the incidence of APS is determined by the form of ferric phosphate used in the salt mix, each salt mix was obtained from the vendor with and without the ferric phosphate as part of the mixture. Each form of ferric phosphate was substituted into each salt mix base and compared with both premixed, salt mix standards. When amorphous ferric phosphate was added to either vendor's ferric phosphate free mixture, larvae reared on diet containing it did not have APS. On the other hand, if crystalline ferric phosphate was used, the gypsy moths being reared on the diet containing it had APS.

To be sure that it was an iron deficiency and not a phosphate deficiency, we tested the two ferric phosphates against ferric citrate by adding them to one of the ferric phosphate free salt mixtures. We found that larvae reared on diet containing ferric citrate grew as well as, or better than, those reared on diet containing amorphous ferric phosphate. APS was caused by an iron deficiency in the diet when crystalline ferric phosphate was used.

The only thing that we still had to investigate was why the NJSS-A sub-strain did not have APS when it was reared for the first time on iron deficient diet. To determine if there was a parental effect and understand the underlying genetic components that were involved, we designed the following experiment.

Cohorts of 10 neonates from each of 25 families, whose parents had been reared on diet containing amorphous ferric phosphate, were reared on either diet containing crystalline or amorphous ferric phosphate. Then the adults were mated using a complicated scheme which minimized possible genetic differences across treatments and produced full and half-sib families.

Females from family #1 reared on each diet type were both mated to either a male from family #2 reared on diet containing crystalline ferric phosphate or amorphous ferric phosphate. The same pairings were then carried out for females from family #2. Eight egg masses were produced per family pair. All of the progeny were then reared on diet containing crystalline ferric phosphate so that families with APS could be easily identified.

When the female parent was reared on diet containing crystalline ferric phosphate (shown here in red), the progeny had APS, but when the female parent was reared on diet containing amorphous ferric phosphate (shown here in green), the progeny did not have APS, regardless of the male parent's diet. This indicated that there was a significant maternal effect involved in APS. In addition, in families where APS was expressed in the progeny, there was a significant paternal effect in addition to the strong maternal effect. We also found that genetic differences in susceptibility to iron availability existed both between and within families. This indicated that the phenotype of each individual (whether it had APS or did not have APS) was partly determined by an underlying genetically determined sensitivity to dietary iron availability.

We now knew how Mr. Phenotype had conspired with Mr. Diet to cause APS. From all the evidence we had gleaned, we developed the following protocols that could be used to prevent APS from occurring. **First**, when Wesson salt mix is ordered the formula of all ingredients should be specified. Most important is that the ferric phosphate must be in the amorphous form, and vendors should certify that the ferric phosphate forms a cloudy dispersion in water and does not settle out as crystals. Ferric citrate may be a good alternative iron source, to replace of ferric phosphate, but we are waiting on the results of a test now in progress before any recommendations can be made. **Second**, specifications for all diet ingredients should be determined and used consistently in purchasing to avoid vender substitutions. **Third**, storage of diet ingredients should ensure that specifications are maintained.

We also have some cautions for everyone involved in rearing gypsy moths, as well as those rearing any other insect. First, when a change in diet or rearing procedures is contemplated, each change should be tested independently for a minimum of two generations. If we had not looked at more than one generation when investigating APS, we would not have been able to solve the case. Second, when tests are conducted, a minimum of 20 families and 20 individuals per family should be used when working with either wild or domestic gypsy moths. If you do not use enough families, you can miss important variation and may get erroneous results. Finally, variability in colonies should be conserved and/or increased so that inadvertent selection for sensitive genotypes and inbreeding depression are avoided. During the course of our investigation, we utilized a very inbred, nondiapause strain to get preliminary data on second generation effects and this colony was much more sensitive to APS then the standard colonies.

I would like to leave you with this question to ponder. Is APS out there in the forest lurking behind every tree just waiting to strike some unsuspecting wild gypsy moth?

INTERACTIONS OF MICROSPORIDIUM AND GYPSY MOTH IN MICHIGAN FIELD PLOTS

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ABSTRACT

In 1992, three oak woodlands less than 10 acres in size and with sparse gypsy moth infestations were selected as sites for this study. On 12 and 13 May, 400 ready-to-hatch egg masses were stapled at breast height to eight to nine oak tree trunks near the center of each plot. Using the methods of Jeffords *et al.* (1988), 25% of the egg masses had been previously coated with a spore solution (10^5 spores/microliter) of *Microsporidum* spp., the same species studied in Maryland in 1986 (Jeffords *et al.* 1988). The initial prevalence of infection in hatching larvae was 32.4% in a randomly selected set of egg masses held in the lab. Thus, 32/400 = 8% of the total population added to each plot was initially infected. On 1 and 2 June, 2nd and 3rd instars were collected from the understory host plants within 30 m of the plot center. At Dansville, Gregory, and Rose Lake, 286, 294, and 328 larvae were collected, respectively, and the infection levels were 2.1%, 5.1%, and 6.1%. On 22 and 23 June, 4th and 5th instar larvae were collected under burlap bands placed on all large preferred hosts within a 30-m radius of plot centers. At Dansville, Gregory, and Rose Lake, 369, 312, and 319 larvae were collected, respectively. Only three of the young larvae were parasitized by 2 June, but 40-70% of the older larvae were parasitized by 23 June. The levels of infection in the older larvae were 1.1%, 1.9%, and 3.5%, respectively, for Dansville, Gregory, and Rose Lake. On 29-30 June, prepupae were collected in each plot. Preliminary data (n=69) indicate that 19%, 10%, and 8% of these larvae were infected at Dansville, Gregory, and Rose Lake, respectively. In the field study performed in Maryland in 1986, 10.5% of the young larvae and 7% of the older larvae were infected.

Jeffords, M. R., J. V. Maddox, M. L. McManus, R. E. Webb, and A. Wieber. 1988. Egg contamination as a method for inoculative release of exotic microsporidia of the gypsy moth. J. Invertebrate Pathology 51: 190-196.

EFFECTS OF SOUTHERN TREE HOSTS AND CLIMATE ON GYPSY MOTH SURVIVAL AND DEVELOPMENT

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ABSTRACT

Some common southern tree species were evaluated in feeding trials as potential hosts for the gypsy moth, *Lymantria dispar* L.. Larval survival and pupal weights showed that water oak, *Quercus nigra* L.; post oak, *Q. stellata* Wangenh.; southern red oak, *Q. falcata* Michx.; sweetgum, *Liquidambar styraciflua* L.; and river birch, *Betula nigra* L., were as good as or better than white oak, *Q. nigra* L., which is generally regarded as a good host in the northeastern United States.

Tests of loblolly pine, *Pinus taeda* L., showed that first instar larvae did not survive when placed directly on pine. However, 2nd-5th instars transferred from southern red oak showed lower survival (25%) only as 2nd instars relative to those reared entirely on southern red oak. Gypsy moth hatch was measured from egg masses collected in Pennsylvania and West Virginia in summer and late winter, sent to Georgia and caged, and compared to hatch from egg masses from moths reared in Georgia. Tree host phenology was also measured on four species and compared to egg hatch. All egg masses hatched at the same time and exhibited similar hatch profiles regardless of geographic origin or exposure to different climatic conditions. Furthermore, hatch began in synchrony with budbreak and leaf expansion of tree hosts. Evaluations of understory hardwoods (water oak and sweetgum) growing under a pine overstory were made to determine if they might support low gypsy moth populations. Survival and pupal weights indicated that the understory trees as gypsy moth hosts were as good as codominant trees of the same species on the same sites. Indications are that at least as far south as northern Georgia, gypsy moth will not be limited by climate, tree hosts or moth/host phenology.

SEQUENCE CHARACTERIZATION AND TEMPORAL EXPRESSION OF THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS IMMEDIATE-EARLY GENE IE-I

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ABSTRACT

Baculovirus expression studies indicate that viral genes are expressed in a temporal cascade, where the immediate-early genes encode regulatory proteins that control the transcription of all other viral genes. Identification of these regulatory proteins is an important step in understanding the molecular aspects that govern viral potency. Many of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) immediate-early genes have been identified and sequenced. These genes are transcribed by the host RNA polymerase and therefore have regulatory elements and promoters that resemble those in eucaryotes. Promoters of viral delayed-early and late genes have regulatory elements that resemble each other but do not resemble those of the immediate-early genes.

Temporal analysis of transcripts in the *Lymantria dispar* nuclear polyhedrosis virus (LdMNPV) had identified three putative immediate-early genes which were localized between 6.0 and 13.7 map units on the viral genome. One of the genes, designated IE-I because it is located within the EcoRV I fragment, was chosen for further study. IE-I has been cloned, sequenced, and found to encode a 32 kDa protein with no strong homology to any known protein.

Analysis of the DNA sequence indicates potential early and late promoter elements upstream of the IE-I coding region. The early promoter elements include a putative TATA box (ATTAT) approximately 150 nucleotides (nt) upstream of the IE-I initiation codon, and a potential transcription start site (CATT) 18 nt downstream of the TATA box. Furthermore, 35 nt upstream of the TATA box is a region with the sequence AACGTGAC. This sequence is similar to regions, with the consensus sequence A(A/T)CGTGTR (where R is a purine residue), that have been identified upstream of many immediate-early genes in AcMNPV. Another region approximately 50 nt upstream of the IE-I coding region has the late promoter sequence ATAAG which may drive expression of IE-I during the late phase of viral replication. Temporal expression studies indicate that the IE-I gene codes for a 950 kb transcript that is expressed at 4 hr postinfection (p.i.). This transcript continues to be expressed throughout infection. Additional larger transcripts that contain IE-I can be identified during the later stages of viral replication (>48hr).

PURIFICATION OF THE GLYCOPROTEIN TREHALASE FROM *LYMANTRIA DISPAR* GUT

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ABSTRACT

The non-reducing disaccharide trehalose provides the largest carbohydrate reserve for energy metabolism in many insects, including *Lymantria dispar*. Trehalase (EC 3.2.1.28) is the only known enzyme catalyzing the hydrolysis of trehalose to glucose, thereby maintaining metabolic homeostasis by supplying glucose for energy-requiring processes in insect muscle and midgut. The enzyme also maintains hemolymph trehalose stores by converting any trehalose which diffuses into the gut lumen back to glucose, which can then be reabsorbed.

Trehalase exists in soluble and membrane-bound forms. In silkworm larvae, marked increases in activity coincide with the transformation of the membrane-bound form of the enzyme into a soluble form, which occurs prior to larval-pupal metamorphosis. Carbohydrate or glycan side chains attached to polypeptides can modulate the proteolytic processing of precursor glycoproteins to active forms and also influence the biological activity of proteins. Many membrane-bound glycoproteins are anchored in the membrane lipid bilayer via a covalent linkage between a phosphatidylinositol molecule, having its 1,2-diacylglycerol moiety embedded in the lipid bilayer, and an intervening glycan structure attached to the C-terminal amino acid of a protein. Studies exploring the nature and function of the glycan moiety of trehalase can provide a foundation for exploitation of this enzyme for the purpose of biocontrol of *L. dispar*.

Trehalase from larval gut of fifth instar *L. dispar* caterpillars was purified to homogeneity using gel filtration followed by anion exchange chromatography. Greater than 90% of enzymatic activity was present in the soluble (cytosolic) form, presenting as a single band of activity in nondenaturing polyacrylamide gels. SDS-PAGE indicates an approximate molecular weight of 60 kDa, consistent with the size of gut trehalase reported for *Bombyx mori* and *Blaberus discoidalis*. Enzyme immunoassay detection of the enzyme by incubation with digoxigenin-succinyl-*-amidocaproic acid hydrazide followed by binding with polyclonal sheep anti-digoxigenin Fab fragments verifies the presence of glycan moieties. Purified trehalase is also detected following SDS-PAGE and Western blotting with concanavalin A, a lectin which has a high affinity to high mannose or hybrid-type glycans.

NUCLEOTIDE SEQUENCE AND ANALYSIS OF P18, A NOVEL GYPSY MOTH VIRUS GENE ENCODING A ZINC BINDING DOMAIN

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ABSTRACT

Nucleotide sequencing of the *Bgl*II-P fragment (3,804 nt) of *Lymantria dispar* nuclear polyhedrosis virus clone g (LdNPV-g) revealed several ORFs, including a small ORF that could encode a polypeptide of 155 amino acid residues, and was designated p18. The nucleotide and deduced amino acid sequences of p18 do not share significant homologies with entries in GenBank (release 71.0), including baculovirus entries. One putative TATAA, one CCAAT boxes, and two GATA motifs were identified immediately upstream of the p18 ORF. Also, three sequences homologous to early baculovirus consensus transcription initiation sites were identified, suggesting that p18 may be an early gene. Five polyadenylation consensus sequences (AATAAA) were identified immediately downstream of the ORF. Further analysis of the deduced amino acid sequence of p18 revealed a putative Zn-binding domain of the C2H2 type near the carboxyl terminus, between residues 108 and 129. The tetrahedral coordination of a zinc atom could take place between cysteines 108 and 111, and histidines 128 and 129. These results indicate that p18 is a novel baculovirus gene with a DNA binding domain, that may play a role in the regulation of transcription of baculovirus genes.

INTERACTIONS BETWEEN THE GYPSY MOTH VIRUS AND INSECT MIDGUT CELLS

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ABSTRACT

The early interaction between polyhedra-derived baculovirus (PDV) and the host midgut epithelial cells has not been well studied. In our work, we have provided evidence for specific binding of *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) PDV to tissue culture cells as well as on *L. dispar* brush border membrane vesicles (BBMV). In addition,

we have obtained evidence for PDV entry into cells and vesicles by direct membrane fusion. The specificity of binding between PDV and cell membranes was investigated using fluorescence-activated cell sorting (FACS) analysis. We found that FITC-labeled PDV bound in a saturable manner to the gypsy moth cell line, IPLB-LdElta, and to *L. dispar* BBMV. Unlabeled PDV competed effectively with labeled PDV for limited receptor sites, further supporting the specificity binding. Using results obtained from these experiments, we calculated that there are approximately 106 PDV-specific receptor sites per IPLB-LdElta cell. This PDV specific binding is thought to involve a protein receptor since major reductions in virus binding (as high as 70%) were found after protease treatment of cells and vesicles. PDV fusion to cells and BBMV was examined using a fluorescence dequenching assay employing octadecyl rhodamine B (R18)-labeled PDV. PDV fusion to BBMV occurred throughout the pH range of 4-11 with dramatic increase in the level of fusion (3x) under alkaline conditions.

USE OF PCR TO DETECT VIRAL DNA ON EGGS AS A MEASURE OF VIRUS LEVELS IN GYPSY MOTH POPULATIONS

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ABSTRACT

We have used polymerase chain reaction (PCR) to detect gypsy moth virus DNA on the surface of individual eggs. In this study, we have performed preliminary work to determine if there is a correlation between the number of individual eggs that are found to be positive for viral DNA by PCR and the number of insects hatched from eggs that die from virus. Gypsy moth egg masses from four different field populations were collected and individual eggs from three to six egg masses were recovered and analyzed by PCR. Twenty eggs from each egg mass were used in PCR experiments while an additional 20 eggs were allowed to hatch and scored for mortality 7 and 14 days later. The primers and conditions used for PCR were chosen for the specific amplification of the gypsy moth virus polyhedrin gene. The percent mortality for an individual population ranged from 9.4 to 48.6% and the percent of eggs positive for viral DNA had a similar range (5.6 to 41%). For individual egg masses, there was a very good correlation ($R^2 = 0.76$, $p < 0.001$) between the percent mortality and the percent found to be positive for viral DNA by PCR. These results indicate that PCR can be used to accurately determine the level of infectious virus on the surface of gypsy moth eggs. Further work is needed to determine if this approach can be used to determining the precise level of virus in gypsy moth populations.

DIMILIN IMPACT ON LEPIDOPTERA AND OTHER CANOPY ARTHROPODS: PRELIMINARY RESULTS

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ABSTRACT

A number of studies have been conducted to assess impact of forest application of Dimilin to non-target arthropods. Martinat *et al.* (1988) found that Dimilin reduced abundance and species richness in mandibulate herbivores, especially Macrolepidoptera larvae. In North Carolina, Blanton (1988) showed that the mean number and richness of arthropods in an untreated canopy were significantly greater than in a Dimilin treated canopy. Sample (1991) found that 11 of 30 non-lepidoptera taxa collected by blacklight trap were reduced in Dimilin treated plots. Total species richness and species richness in four of eight Lepidoptera families were reduced. In a current study at Cooper's Rock State Forest in West Virginia, we are comparing impact of Dimilin, *Bt* and defoliation on non-target arthropods in single 300-acre blocks. Sixty percent fewer Macrolepidoptera larvae were taken from the canopy of the Dimilin treated block than a nearby heavily gypsy moth defoliated block despite very low Dimilin coverage on the foliage.

In 1989, the USFS began funding a Dimilin impact study on four small (30-100 acres) closed watersheds at the Fernow Experimental Forest near Parsons, WV. Among the studies was ours to determine Dimilin impact on canopy arthropods. Baseline data were collected from 1989 through 1991 by means of foliage pruning and banding of mixed oaks, red maple, black cherry and black birch, and in 1990 and 1991 by blacklight trapping. Two of the four watersheds were treated with Dimilin 4L at 2 oz. formulation per acre on 17 May 1992. Canopy arthropods were sampled until mid-August 1992 by foliage pruning, tree bands and blacklight traps.

Preliminary analyses indicate that total taxa richness and abundance of canopy arthropods were significantly reduced on Dimilin treated watersheds in the treatment year. Affected were Lepidoptera larvae in the families Noctuidae, Geometridae, Notodontidae and Lyonetiidae; all Symphyta, some herbivorous Coleoptera, Psocoptera, predatory Thysanoptera, some Homoptera, Diptera, Orthoptera, and Arachnida. Apparently, some of the taxa are being affected directly through disruption of molting or egg inviability while other taxa (predators) are being reduced through loss of available food.

ADULT BEHAVIOR AND ITS RELATIONSHIP TO NORTH AMERICAN PROGRAMS

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ABSTRACT

The pheromone communication systems of *Lymantria dispar* in the Russian Far East and the established North American population appear quite similar. Field tests of attraction in Mineralni, Primary Krai to synthetic pheromone and mixtures of pheromone with known antagonists of attraction showed that increasing doses of (+)-disparlure elevated trap catch and that (-)-disparlure and the olefin analog added to the attractant diminished trap catch. However, the two populations differ somewhat in the timing of the diel rhythms of male attraction. In the Russian Far East the gypsy moth initiates attraction late in the mornings, has a peak of attraction in mid afternoon and is attracted to pheromone through the first half of the night. Thus the rhythm of diel attraction in this population occurs later in the day and extends farther into the night than in North America.

Female dispersal behaviour was monitored by placing females that had emerged from collected pupae on tree trunks approximately 1.5 meters above the ground. At dusk females initiated wing fanning and walking while wing fanning. Over several minutes females ascended the tree walking while wing fanning, reaching heights of 10 meters or more before dispersal flight.

EGG MASS SAMPLING PLANS FOR GYPSY MOTH MANAGEMENT PROGRAMS

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ABSTRACT

The goal of this research was to develop gypsy moth egg mass sampling plans that reflect the influence of habitat, changes in egg mass distribution, and provide population densities or density categories for making control decisions. Sequential egg mass sampling plans for gypsy moth management programs in urban and suburban habitats were developed from 0.01-ha, fixed-radius plot samples collected in Loudoun, Fairfax, and Arlington Counties, Virginia. The sampling plans were developed from Wald's sequential probability ratio test and are based on a negative binomial distribution. Operating characteristic and average sample number curves were used to determine the acceptability of the sampling plans. Three sampling plans were developed for the action thresholds of 612, 1,236, and 2,471 egg masses/ha.

The use of binomial sampling for low density (<618 egg masses/ha) gypsy moth populations in continuously forested habitats was examined. Fixed- and variable-radius plot egg mass samples were collected in 28 study areas in Virginia, Maryland, and Massachusetts. The relationship between egg mass density and the proportion of trees with zero egg masses was developed. Binomial sampling resulted in a higher relative variability and lower relative efficiency than fixed- and variable-radius plot sampling. Binomial sampling was determined not to be an effective sampling method for gypsy moth populations below 618 egg masses/ha. Fixed- and variable-radius plot egg mass samples were taken when leaves were present (summer) and absent (winter) in 136 sample sites in Virginia. A significant difference between summer and winter counts was determined. The relationship between summer and winter counts was quantified using nonparametric statistics. Winter egg mass counts were found to be 14 to 34 percent higher than summer egg mass counts. The probability of a summer egg mass count exceeding an action threshold was constructed by fitting a logistic curve to empirical data for the action thresholds of 618 and 1,236 egg masses/ha. Egg mass counts need to compensate for differences between summer and winter counts. Alternatively, the probability of a summer egg mass count exceeding an action threshold should be utilized.

AN EFFICIENT TREE-BASED GYPSY MOTH SAMPLING PROTOCOL FOR PREDICTING DEFOLIATION

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ABSTRACT

During 1991 and 1992, 148 transects were established in 10 counties in the northern part of the lower peninsula of Michigan. Within these transects, measurements of egg mass numbers on the first 2 meters of the boles of 100 trees (>2 inches in DBH) are being made. These measurements are being used to develop regression models to predict subsequent defoliation in the transects. Preliminary analysis of the 1992 data has produced a multiple regression model that is a "good fit" ($r^2 = 0.764$; $F = 65.268$).

The ability of the current model to predict defoliation from data collected in 1991 is limited (i.e., $r^2 = 0.1197$; $F = 3.943$). However, the current model would have correctly placed 20 of the 31 transects into the correct decision category for spraying. Only four of the transects would not have been sprayed when actual defoliation indicated spraying was warranted. Seven other transects would have been sprayed when defoliation indicated that spraying was not necessary.

The current models show promise in their ability to predict defoliation from tree-based data. The high degree of relationship found in the regression models is encouraging. Model reduction and refinement continues and more accurate predictions of defoliation are anticipated.

The project is continuing to collect data on the transect-based sampling protocol. In addition, data is being collected to relate transect-based results with results from 1/40-acre, fixed-radius plots and 5-minute walks.

THE GYPSY MOTH LIFE SYSTEM MODEL:

STATUS AND FUTURE DIRECTION

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ABSTRACT

Within the past year we have added two additional models to the gypsy moth life system collection. You can now select any of the following: full life system model, medium or abbreviated life system model, stand-damage model, and the differential equations model.

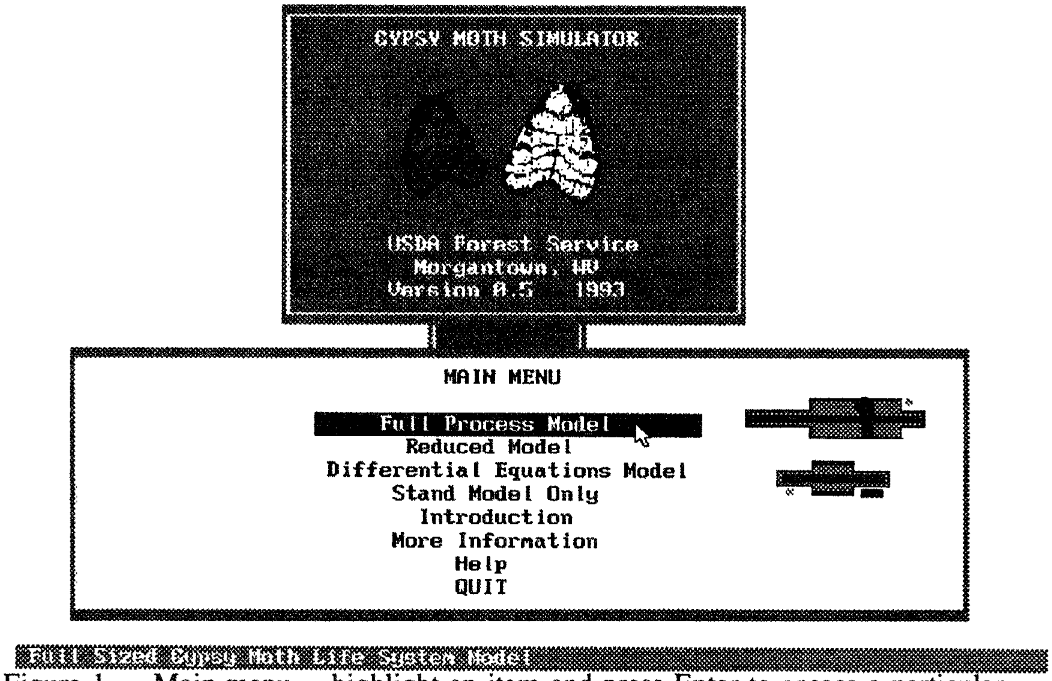

Figure 1. Main menu -- highlight an item and press Enter to access a particular model or further information about the gypsy moth life system models.

Each model is available from a central menu (Fig. 1). The access and maintenance of input and output for each formulation is handled in the same consistent fashion so that as you become familiar with a specific model, accessing and using other components will be easier.

With each model formulation there are advantages and disadvantages; more detail means more variables and parameters to consider altering, less detail means less ability to make direct or detailed ecological or biological interpretations.

The full model contains the most detail of gypsy moth, forest, and natural enemy dynamics: gypsy moth growth, feeding, and population dynamics as driven by daily heat accumulation from a weather submodel or weather data. Foliage growth is also driven by weather. Each species or guild of natural enemies is modeled in sufficient detail for users to manipulate the details of these interactions. Individual natural enemy submodels can also be removed completely, if desired. This model can be linked to the stand model to obtain foliage biomass (gypsy moth food source), simulate the effects of defoliation on tree growth and mortality, and the feedback of defoliation effects on future foliage dynamics.

The medium model, developed by Alexei Sharov, carries less detail than the full model but still has considerable detail. It models populations as five discrete stages through a year (eggs, small larvae, large larvae, pupae, and adults) and does not carry the details of feeding or natural enemy dynamics. Defoliation is a function of number of large larvae. Because it carries less detail, it can be used to produce simulations faster. Natural enemies and related mortality factors are modeled in less detail but the majority of natural enemy dynamics are maintained. This model may also be linked to the stand model, and as such, it will be provided foliage biomass and will provide defoliation effects in return.

The stand model can be run separately. In this case, the user supplies the defoliation scenario and is provided the effects on growth and tree mortality. The stand model (in linked versions above, as well) input system provides the user with the means to schedule management entries and describe cutting practices to be applied. Weather affects tree growth but only total heat accumulation for each year is required. Again, users can choose to use prescribed weather summary data or simulate weather from random variations about mean values.

The differential equation model is much simpler than either of the other models of gypsy moth dynamics. Gypsy moth, foliage, and natural enemy biomass densities are the three variables associated with the three differential equations. There are but a dozen parameters for the whole system but we have discovered a robust assortment of behaviors over what appear to be a quite reasonable range for the parameters.

With each model, as a simulation is taking place, the user is provided with a screen to follow output graphically. It is possible to stop a simulation and rescale, add, remove, or change the lines within a graph, split the screen and add another graph, or change to viewing other relationships before returning to the active simulation. This tool permits users to see a simulation as it unfolds and make decisions immediately about the value of a particular set of conditions. If one finds a particular scenario is not providing the desired results, one can quit the active simulation, return to the parameter management screens, make desired adjustments, and restart a simulation.

SIMULATION RESULTS

I would like to describe some simulation results from the full gypsy moth life system model and in so doing, describe some of the features available and graphic outputs that can be used to interpret results of simulations. First, as you choose the full model (or any of the others), there is a complete set of defaults available. This means that a first time user could merely select run and see what the defaults produce before making any changes. The stand is described by selecting species and then entering stem counts for diameter classes. By selecting species that are preferred hosts, one can produce a stand that the model gypsy moth population will defoliate significantly. Running that stand and viewing the egg mass density each year will show the populations across an outbreak. Adding the "Ln[K value]" graph will show you how mortality factors changed, natural enemies responded to changes in gypsy moth density, and virus eventually became the controlling factor in terminating the outbreak. By returning to the control panel and selecting the addition of simulated application of pesticide, one sees that the default parameters values are those used by Podgwaite *et al.* (1992a,b) in tests of Gypchek (see Fig. 2 and 3) or by Dubois *et al.* (1988) in tests of new *Bt* strains. Users may choose to alter any of these default parameters though. In reviewing the

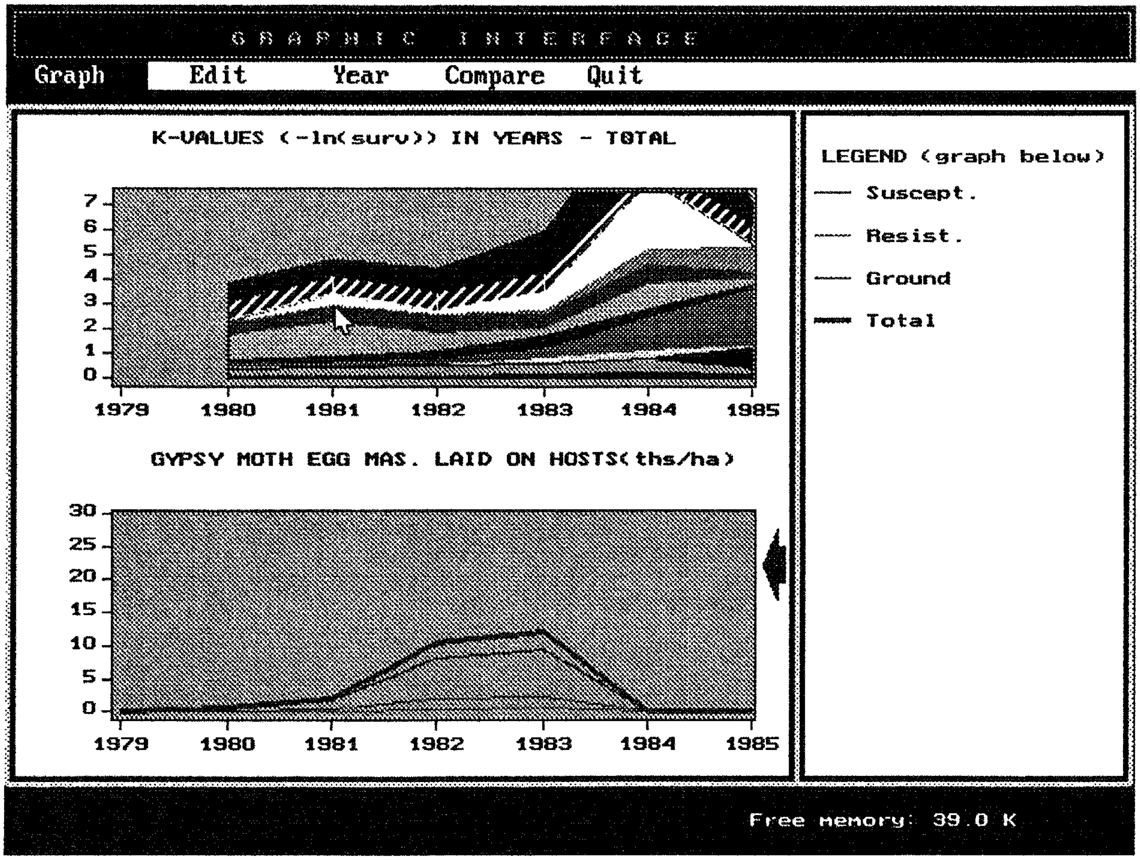

Figure 2. Simulated spray of viral insecticide Gypchek in 1981. The white area in the upper graph represents the relative mortality due to virus each year.

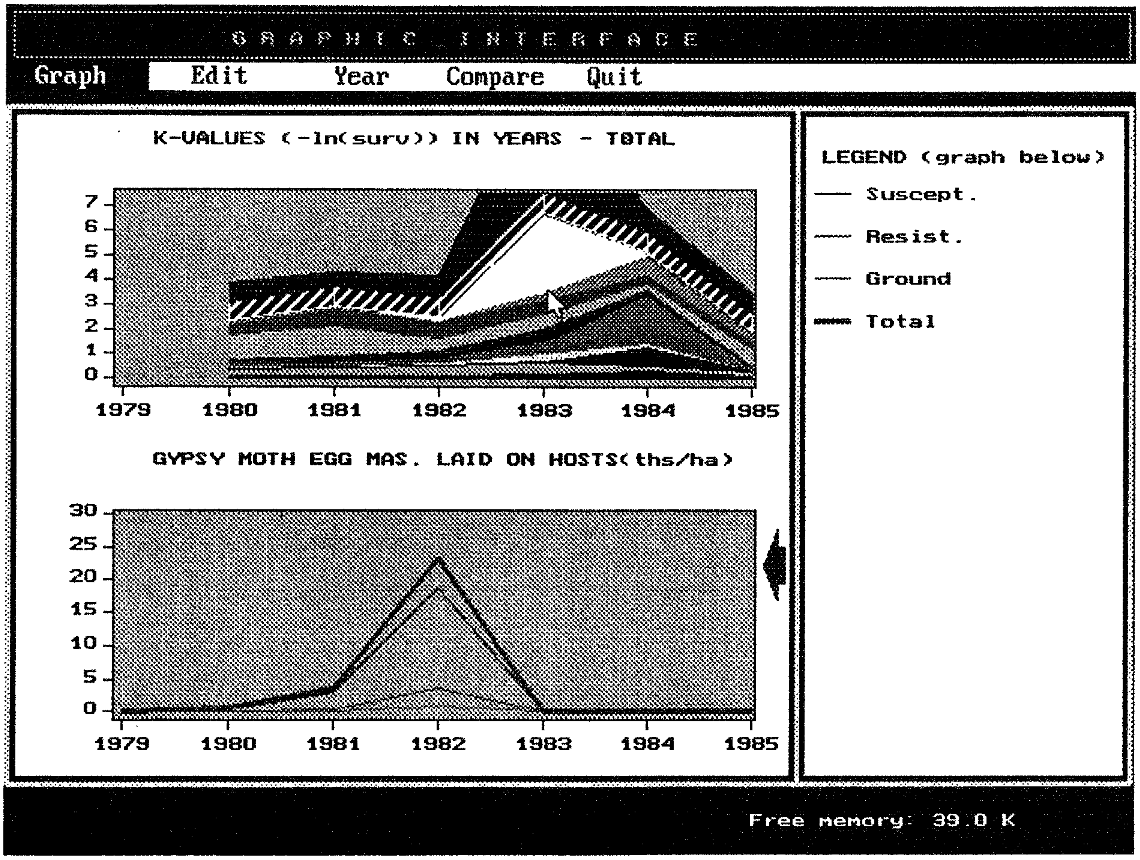

Figure 3. Simulated spray of Gypchek in 1983. The white area in the upper graph represents the relative mortality due to virus each year.

results of natural enemy submodels, one sees that the dynamics of parasites in the model compare well with the available literature (Williams *et al.* 1990, 1992). By changing the year that one chooses to have a simulated spray application or resetting the timing parameters within a year, one can see the effects of various strategies. To further understand the effects of the feedback of individual natural enemy species or guilds, one can remove specific submodels and if desired, replace the dynamic interaction of natural enemies with fixed mortality rates. When other natural enemies are removed or ineffective, one sees virus and starvation becoming the controlling mortality factors and defoliation becoming extreme (approaching 100%). By using the default natural enemy submodels, but altering the stand compositions to those described by Williams *et al.* (1990), one sees that the models also exhibit a similar range of defoliation levels as a gypsy moth outbreak comes and goes.

FUTURE DIRECTIONS

We are in the process of testing the implementation of the user access portions of these models. Documentation, including model formulations, biological bases, user's and programmer's guides, is being drafted and reviewed. We will begin testing these models and specific component submodels in the coming months and years. We are currently developing data to test the effect of defoliation on tree mortality and will follow that with a test of defoliation effects on tree growth. We are interested in working with any parties that have an interest in testing or just using the models and are actively pursuing cooperative efforts to develop field tests for validations of components.

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GYPSY MOTH LIFE SYSTEM MODEL:

A TOOL FOR ANALYSIS OF POPULATION DYNAMICS

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ABSTRACT

The Gypsy Moth Life System Model (GMLSM) was designed to simulate the principal ecological processes driving local gypsy moth population dynamics. It covers three trophic levels: host trees, gypsy moth as a primary consumer, and natural enemies of gypsy moth. It can be used for: 1) testing hypothesis about mechanisms of gypsy moth population dynamics, 2) forecasting population trends and dynamic patterns, 3) testing and optimization of management tactics and strategies, and 4) evaluation of aggregated parameters of simple models. The object of simulation is a life-system - a system which includes the population and its effective environment, i.e all major factors and processes that affect directly or indirectly the dynamics of this population. Life-system is considered in a homogeneous stand on an area of 1 hectare. Ecological processes are simulated separately in different tree species (or tree classes) and in different strata (overstory, understory, ground, and tree boles). The model predicts seasonal and long-term dynamics of the gypsy moth life system.

In 1992, the model was demonstrated in several universities and Forest Service laboratories. Later, it was improved on the basis of received recommendations. The major changes include the following: 1) the input/output interface has been developed for changing model parameters and managing input and output files, 2) the graphic output interface has been improved using the C-scape software, 3) growth of small trees was incorporated into the Stand submodel, 4) classification of tree species on the basis of their relation to gypsy moth was introduced, 5) dynamics of gypsy moth adults were incorporated into the model, 6) ecological processes related to sterile insect release and pheromone application were made time-dependent, 7) preliminary versions of the Fungus and Calosoma submodels have been developed, and 8) the algorithm for simulation of multiparasitism has been improved. As an example of using the GMLSM, we tested the hypothesis of Lance *et al.* (1987) that fast development of gypsy moth larvae in outbreak areas is caused by temperature change in the microhabitat. The model showed that temperature changed too late in the season to cause observed acceleration of larval development. Thus, the model does not support this hypothesis.

FACTORS INFLUENCING GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS TRANSMISSION

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ABSTRACT

Previous research has shown that horizontal transmission of gypsy moth NPV (LdNPV) within a host generation is a fairly straightforward process resulting in two waves of mortality in the gypsy moth larval stage (Woods and Elkinton 1987). This mechanism of transmission is embodied in the Gypsy Moth Life System Model, which holds great potential for predicting mortality from LdNPV. However, knowledge of the effects of a variety of factors on the transmission process is required before such predictions are possible. We have been exploring these effects by way of "bugs in bags" experiments using infected and uninfected larvae reared in mesh bags.

One such experiment looked at the effects of foliage density and NPV-killed cadaver density on NPV mortality. NPV mortality among healthy test larvae was found to be significantly affected by both foliage density and cadaver density. Transmission coefficients calculated for a modified Anderson and May model using these data were seen to decrease with increasing cadaver density, although these coefficients were expected to remain constant over a range of densities.

Another experiment using artificial rainfall found that rain was capable of significantly reducing NPV mortality by washing virus off leaves, and had a marginally significant spreading effect. This spreading effect was not confirmed in later experiments.

To quantify NPV decay rates, infected cadavers were exposed to natural conditions on foliage for 6, 17, and 27 days. Healthy larvae were then used to bioassay the contaminated foliage. Mortality was seen to decrease with time, suggesting a half-life of approximately one week for virus in an outdoor setting.

Experiments looking for an effect of instar on NPV mortality found that 4th instar larvae experience greater mortality than neonates when placed on foliage contaminated with equal amounts of viral inoculum. This result is surprising, since the LD_{50} for 4th instars is higher than the neonate LD_{50}.

IMPACTS OF DEFOLIATION IN PINE-HARDWOOD MIXTURES

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ABSTRACT

As gypsy moth populations move further into the southeastern United States, they will continue to have an increasing impact on stands containing a mixture of both pines and hardwoods. One hundred and fifty 0.04-hectare plots are to be established in mixed loblolly pine-oak and loblolly pine-sweetgum stands in the Atlantic Coastal Plain of Virginia and Maryland.

The study objectives are to determine 1) whether the pine component in mixed stands reduces or contributes to the susceptibility of the hardwood component; 2) whether defoliation of the preferred hardwoods results in increased pine growth, or subsequent pine defoliation; and 3) whether the proportion of pine (or hardwood) is a useful variable in predicting growth loss and/or mortality of the hardwood species. Seventy five plots have been established in mixed stands in Virginia and Maryland.

MODE OF ACTION OF *BACILLUS THURINGIENSIS* TOXINS ON GYPSY MOTH: RECEPTOR BINDING AND CHANNEL FUNCTION

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ABSTRACT

The tertiary structure of one of the *Bacillus thuringiensis* δ-endotoxins (the CryIIIA protein toxin) has recently been revealed (Li *et al.* 1991). It is assumed that most *Bt* toxins follow the general structural features of the CryIIIA toxin; that is, three domains and predominant loops in domain II. The predicted functions associated with the structural domains of the *Bt* insecticidal crystal proteins, according to Li *et al.* (1991), are: domain I, the pore formation (assumedly the potassium channel functions); domain II, receptor binding; and domain III, structural integrity.

We have tested aspects of this model by site-directed mutagenesis in domains II and III. We have observed that mutations in one of the loops of domain II affects receptor binding. The binding constant of the mutant toxins was measured as $K_d = 36$, compared to $K_d = 3.9$ for the wild type toxin. These mutations also dramatically affect toxicity but not the structure of the toxins. The mutant toxin, as the wild type, was resistant to trypsin digestion, indicating that no reduction in structural stability was caused by the mutations. Mutations in beta sheet 17 of domain III were observed to affect potassium channel function. This beta sheet was chosen for mutagenesis because it is highly conserved among Cry toxins and because its amphipathic nature, with an arginine-rich face, is reminiscent of the conserved S4 region of classical ion channels. Mutations that convert the outer-most arginines of this beta sheet to lysines were observed to dramatically affect the toxicity and the voltage-clamp response (inhibition of short-circuit current) of these toxins, but not the receptor binding ability or the structural stability. Mutations in the central two arginines were found to be critical to structural stability and inhibited proper folding. In conclusion to this section, we have demonstrated that the loop region of domain II plays a role in receptor binding and that domain III is involved in potassium channel function.

In other research on binding studies and voltage clamp studies with gypsy moth midguts, we have compared the activity of the same three CryIA toxins (the components of the bipyramidal toxin of HD-1). As illustrated in Table 1, CryIAa and CryIAb are the most active toxins (LC_{50} values), while CryIAc is very weak. Despite the relatively weak biological activity of the pure Cry toxins to gypsy moth, binding studies indicate that the CryIAa toxin displays very good binding to gypsy moth BBMV, comparable to the best binding reported on the most sensitive insects (e.g. *B. mori*). We also observe that CryIAb displays the poorest binding, despite the fact that it has biological activity comparable to CryIAa. This confirms the finding of Wolfersberger (1990) that CryIAb toxin is unusual in having strong toxicity, despite weak binding. Preliminary voltage-clamp studies on gypsy moth midguts indicate that the order of activity of the CryIA toxins (i.e. the relative ability to inhibit short-circuit current) is CryIAa = CryIAb > CryIAc. This indicates that the strong insecticidal activity of CryIAb is related to its potassium channel function, again confirming the prediction of Wolfersberger (1990) that CryIAb made up for its weak binding by possessing a stronger alternate function.

These findings give rise to the question: if CryIAb is a good toxin despite its weak binding, might it not be better if its binding properties were improved? This is a question we intend to explore by exchanging the amino acids in the aforementioned loops of domain II of CryIAb for those of the toxin with greatest binding potential, CryIAa.

Table 1. Comparison of CryIA toxin binding and toxicity to *Lymantria dispar* and *Bombyx mori* (LC_{50} values on *L. dispar* are tentative)

	[---------*L. dispar*---------]			[----------*B. mori*----------]		
Toxin	Kd	Bmax	LD50	Kd	Bmax	LD50
CryIAa	0.442	9.26	20	3.5	7.95	0.37
CryIAb	3.65	3.33	20	nd	nd	nd
CryIAc	1.25	5.72	120	33.7	1.95	>150
CryIAb*	19.8*	2.7*	1.08*			
CryIAc*	2.03*	3.69*	425*			

* data from Wolfersberger (1990)

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GYPSY MOTH PUPAL COLLECTIONS ALONG THE FOREST-TO-CITY CONTINUUM WITH REFERENCE TO *COCCYGOMIMUS DISPARIS*

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ABSTRACT

As the gypsy moth (*Lymantria dispar* L.) range is extended, new host species, climate conditions, and habitats are encountered. Of concern is the movement of gypsy moth into urban forests. The focus of research at Delaware State College has been to examine the potential for biological control along the following habitat continuum: (1) native forest and woodlot, (2) residential forest opening and strip development, (3) park/recreation and residential woodlot, (4) residential suburban and city suburban, and (5) urban forest. The research focuses on biological control and the movement of predators and diseases along the continuum. The most recently introduced pupal parasite, *Coccygomimus disparis* (Viereck), has received special attention since it was collected for the first time on the DelMarVa Peninsula in 1989. *Coccygomimus disparis* occurred overwhelmingly in pupal collections in the residential forest opening and strip development habitat sites: 100% in 1989 (only one specimen), 82% in 1990 (80 of 97 specimens), 95% in 1991 (41 of 43 specimens), and 90% in 1992 (9 of 10 specimens). All *C. disparis* recovered in 1992 were from pupae collected under burlap. Annual *C. disparis* parasitism was 0.13% in 1989, 4.02% in 1990, 0.84% in 1991, and 0.97% in 1992. The parasite was recovered at 1 of 26 pupal study sites in 1989, 12 of 17 in 1990, 7 of 16 in 1991, and 3 of 11 in 1992. During the course of the study, *C. disparis* was present in pupae collected from 13 tree species. These host trees include eight oak species: white oak (*Quercus alba* L.), chestnut oak (*Q. prinus* L.), black oak (*Q. velutina* Lam.), northern red oak (*Q. rubra* L.), water oak (*Q. nigra* L.), willow oak (*Q. phellos* L.), southern red oak (*Q. falcata* Michx.), and Japanese sawtooth oak (*Q. acutissima* Carruth.). Hickory (*Carya* Nutt. spp.), weeping willow (*Salix babylonica* L.), and three species of pine (Eastern white pine (*Pinus strobus* L.), Scotch pine (*P. sylvestris* L.), and loblolly pine (*P. taeda* L.)) have also served as host trees. Water oak, willow oak, southern red oak, and loblolly pine are southern coastal plain species new to gypsy moth infestation. Four species not native to the DelMarVa Peninsula include Japanese sawtooth oak, weeping willow, white pine, and Scotch pine. We are investigating these southern native and horticultural species in relation to the health and maintenance of urban forests which, with gypsy moths, become doubly stressed ecosystems.

NEW LABORATORY AND FIELD DEVELOPMENTS IN *BACILLUS THURINGIENSIS* AND HOST SUSCEPTIBILITY

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ABSTRACT

The cryIA group of Insecticidal Crystal Proteins (ICP) produced in *Bacillus thuringiensis* subspecies *kurstaki* (*Btk*) are particularly toxic to Lepidoptera larvae. Most *Btk* formulations used against the gypsy moth, *Lymantria dispar* L., contain at least the cryIA(a) and cryIA(c) ICPs (and probably the cryIA(b) as well). Synergism between *Btk* spores and cryIA(a) and cryIA(c) ICPs and their active chimeric proteins was reported previously. Current studies with non-entomopathogenic bacterial representatives of the forest microflora suggest that the bacterial-ICP interaction is not specific to *Bt* spores. Many but not all bacteria tested were synergistic with the cryIA ICPs, resulting in lethal septicemia. The results indicate that the ICPs effected a non-specific facilitated invasion by the bacteria into the hemolymph. They also suggest that gypsy moths that ingest a non-lethal dose of sprayed *Bt* may be compromised and be susceptible to infection by the forest microflora. Laboratory bioassays show that *Bt* is effective against older, more mature 3rd and 4th instar gypsy moths. Albeit, with some formulations, the dose necessary to effect significant mortality at either the LC50 or LC95 level of effectiveness is significantly higher than with 2nd instar. Results of field studies conducted in the last two years with Thuricide 48LV and 64LV and Foray 48B against 3rd and 4th instar larvae suggest that *Btk* could effectively protect the infested forest stand from further assault. Foray 48B and Thuricide 64LV effectively prevented further defoliation; their estimated Foliage Protection Efficiency (FPE) was 100% and 87%, respectively. The larval and pupal densities were also significantly reduced by 93% and 71% when treated with Foray 48B. Both Thuricide formulations were less effective and larval densities under burlap bands were not significantly different from the controls. Population densities, as measured by egg mass densities in blocks treated with Thuricide 48LV and Foray 48B were reduced by 72% and 94% from their prespray density levels. However, because of concurrent reductions of 90% and 53% in untreated blocks, these reductions could not be attributed to treatment but rather to a general population collapse. Untreated controls associated with the Thuricide 64LV study increased by 54% to approximately 4,000 egg masses per acre and treated blocks were reduced to 800 egg masses per acre. Although this was a significant treatment effect, the 46% reduction was insufficient and retreatment will be required the following year. Taken individually, these estimates may seem unconvincing; nonetheless, together they do suggest that Foray 48B, at least, can effectively protect infested forest stands from further deterioration by gypsy moths when treatment is unavoidably delayed until the larval population matures to the 3rd or 4th instar.

SUSCEPTIBILITY OF GYPSY MOTH, *LYMANTRIA DISPAR* L., TO CRYIA(A) AND CRYIA(C) INSECTICIDAL CRYSTAL PROTEINS, COMBINED WITH *BACILLUS THURINGIENSIS* SPORES AND OTHER BACTERIAL SPECIES ISOLATED FROM THE FOREST ENVIRONMENT

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ABSTRACT

Synergism in the gypsy moth between non-entomopathogenic bacteria and the insecticidal crystal proteins (ICPs) encoded by the *Bacillus thuringiensis* subspecies *kurstaki* cryIA genes was evaluated. The bacteria, representing five families, were isolated from a mixed red maple stand and were fed to second instar gypsy moths at 25 and 250,000 CFU per cm^2 of diet surface. Alone, they had no measurable effect on the larvae. When they were combined with 40 or 10 ng of cryIA(a) ICP or 100, 40, or 10 ng of cryIA(c) ICP per cm^2 of diet surface, which alone effected limited mortality, significant lethal septicemia was observed in the first five days after exposure. Generally, synergism was limited by either bacteria or ICP concentration and there were differences between the bacteria-ICP combinations. Notably, *P. syringae* was not synergistic at any concentration of either ICP. With the cryIA(a) ICP, *Corynebacterium* spp., *B. cereus*, and *X. campestris* were synergistic only at the highest concentration of the ICP-bacteria combination. *B. subtilis* and *Corynebacterium* spp. were not synergistic with cryIA(c) at any concentration. At similar independent ICP insecticidal activity of 23% and 26% mortality, *X. campestris*, *B. subtilis*, and *Corynebacterium* spp. did not increase significantly the lethal effects of the cryIA ICPs. *E. coli* was synergistic only with the cryIA(c) ICP and all other *Enterobacteriaceae* were equally synergistic with both ICPs. The *Actinomyces* spp. was synergistic only with the cryIA(a) ICP. Curiously, *B. cereus* was synergistic only at low spore concentration and synergism with HD73cry^- was limited by low spore concentration when combined with cryIA(c) ICP. Both Klebsiella and Erwinia species and the *Flavobacterium* spp. were very synergistic at most dose combinations with both ICPs. It is unlikely that there is true specificity between the bacteria and the ICPs; rather, the interaction may be a non-specific facilitated invasion into the hemolymph through a perforated midgut.

MODELLING THE WITHIN-SEASON DYNAMICS OF GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS

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ABSTRACT

Qualitative understanding of the dynamics of epizootics of the nuclear polyhedrosis virus of gypsy moth has become strong enough to justify attempts to quantitatively predict the timing and intensity of epizootics within a season. In earlier work (Dwyer and Elkinton 1993), we compared the predictions of a simple differential equation model derived from Anderson and May (1981) to time series of virus mortality in each of eight gypsy moth populations (Woods and Elkinton 1987). The model's predictions were very accurate for high density populations, but seriously under-estimated virus mortality in low density populations. Here we compare the predictions of the simple model to those of the gypsy moth life system model (GMLSM, Colbert *et al.* unpublished), a highly complex computer simulation of gypsy moth population dynamics and forest stand growth that incorporates much of existing knowledge of the many factors influencing gypsy moth populations. In particular, we looked at two different versions of the GMLSM that incorporate two different models of virus transmission. One was identical to that of the simple model (Dwyer and Elkinton 1993, Anderson and May 1981), in which the rate of transmission was a constant (the transmission coefficient) multiplied by the densities of healthy larvae and the densities of infectious virus particles on foliage . The other approach, developed by Valentine and Podgwaite (1982), took into account age-related changes in the amount of foliage consumed and the susceptibility of larvae to virus (LD_{50}). The Anderson and May version of the GMLSM performed about as well as the simple differential equation model, but only for values of the transmission coefficient about 250 times higher than those we had determined experimentally (Dwyer and Elkinton 1993). The Valentine and Podgwaite (1982) version of the GMLSM gave a much better fit, but only for values of LD_{50} that were 100 times higher than those determined experimentally. Future research will focus on efforts to refine our understanding of virus transmission in order to explain and reduce the discrepancies between model predictions and observed mortality from virus in field populations.

EVALUATION OF GYPSY MOTH BIOCONTROL EFFORTS

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ABSTRACT

Many gypsy moth researchers appear to view the introduction of natural enemies as a failed strategy. On the contrary, I would argue that the combined action of naturally occurring predators and introduced parasitoids and pathogens has been very successful in keeping gypsy moths at low density in most places in most years. Many stands in the Northeast go 20 years or more between outbreaks because of these agents. The extensive worldwide effort to introduce natural enemies in previous years suggests that the chances of establishing new agents are small. Nevertheless, it should be pursued because biological control is the **only** viable long-term management strategy for gypsy moth. Other strategies, such as silvicultural management, are economically impractical on a large scale. With some agents, such as microsporidia, we have only scratched the surface of potential introductions. Perhaps some of the failed introductions of the past would succeed in southern regions newly infested by gypsy moth. Perhaps we would succeed if we introduced different biotypes of some of these species. The examples of *Entomophaga maimaiga* and *Brachymeria intermedia* illustrate that it may take decades for agents to adapt to gypsy moth in a new habitat. Possibly, such a lack of adaptation of already established parasitoids explains the eruptive nature of gypsy moth populations in the southern Appalachians. New technologies involving gypsy moth pathogens hold great promise for more effective and specific bio-pesticides in the future.

GYPSY MOTH POPULATION DYNAMICS: OLD THEORIES AND NEW EVIDENCE

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ABSTRACT

Long-term studies and experimental manipulations of gypsy moth populations support the conclusion that the dominant source of mortality in low density populations of gypsy moth is predation by the white-footed mouse, *Peromyscus leucopus*. Increases in the density of gypsy moths are associated with declines in mouse density. Predation rates by mice on gypsy moth

pupae are inversely density dependent at higher gypsy moth density, as one would expect for a generalist predator. However, we have not been able to demonstrate that mouse predation is positively density dependent at lower gypsy moth density. Nevertheless, we do have evidence that overall mortality of late instars is density dependent, suggesting the existence of a low density equilibrium. Further experiments reveal that parasitism by tachinid flies may be an important component of this density-dependent mortality.

FOREST STAND CONDITIONS AFTER 13 YEARS OF GYPSY MOTH INFESTATION

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ABSTRACT

Of 603 plots that were established in 1978 in central Pennsylvania to measure the impact of repeated gypsy moth (*Lymantria dispar* L.) defoliation, 228 were selected in 1985 to further monitor long-term impacts on the overstory. Defoliation and vigor were measured for each overstory tree through 1990 on this subsample. Ingrowth data were collected in 1985 and 1990; regeneration surveys were conducted in both 1989 and 1992 to measure understory response following gypsy moth infestation. From 1978 to 1985, defoliation peaked in 1981 when 34% of the plots were heavily defoliated. From 1985 to 1990, defoliation peaked in 1986 when 18% of the plots were heavily defoliated. Had 26 of the plots not been sprayed for gypsy moth control that year, severe defoliation likely would have reached 30%.

Net change in overstory basal area per acre from 1978 to 1990 (including the effects of both accretion and ingrowth) ranged from +70 to -92% depending on defoliation severity and frequency. Oak species were defoliated disproportionately and had higher mortality rates than non-oak species. Ingrowth data show that 88.5% of ingrowth trees are of commercial hardwood species (3.3% oak), 4.3% are of commercial softwoods, and 7.1% are of noncommercial species. Regeneration surveys conducted in 1989 and 1992 showed that 77 and 65%, respectively, of the regeneration plots within stands with severe mortality (> 30% basal area/acre) are adequately stocked with commercial hardwood species; 16 and 25% of the regeneration plots in 1989 and 1992, respectively, were fully stocked with oak species. As of 1990, 77.6% of the plots contained fully stocked or overstocked overstories despite two waves of gypsy moth infestation and resultant mortality. The residual stands still contain a viable oak component (ave. = 58% basal area/acre in oak), though ingrowth and advanced regeneration data indicate that future stands may have more red maple, birch, and black gum stems, and thus be better able to withstand future gypsy moth infestations.

CURRENT FOREIGN EXPLORATION ACTIVITIES FOR PARASITES AND CURRENT RELATED RESEARCH

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ABSTRACT

Overall progress in gypsy moth natural enemy importation and parasite research was reviewed for the period 1980-1992. It was concluded that although imported natural enemies play a role in the population dynamics of gypsy moth, gaps in the parasite complex and limitations of established species render biological control substantially successful, but not completely successful. Prospects for using importation, conservation, and augmentation approaches to improve biological control were explored. Importation of natural enemies remains the most promising approach for long-term mitigation of the gypsy moth problem. During the 1980's and early 1990's, foreign exploration shifted to Asia, and three predators and 17 parasite species were received at Newark. Most of these were from Korea, Japan, and India (parasites of Indian gypsy moth, *Lymantria obfuscata* Walker), whereas little material was obtained from the most promising regions, China and the Russian Far East. Releases of 15 species were made, but establishment of only one species, the pupal parasite *Coccygomimus disparis* (Viereck) is certain. This species appears to be dispersing well over the generally infested area, and evaluation of its effectiveness is in progress. Current importation efforts are focused on promising species that haven't been tried, particularly univoltine species or those having a high incidence of diapause such as *Blepharipa schineri* (Mesnil) and *Ceranthia samarensis* (Villeneuve). Polyvoltine species known to attack late summer defoliators of North American origin [e.g. fall webworm, *Hyphantria cunea* (Drury)], are also being considered, but extensive host range testing will probably be required to obtain approval for release. Dominant species from southern Europe which failed to become established in New England or the Middle Atlantic States [e.g. *Glyptapanteles porthetriae* (Muesebeck)] are being imported and reared for trial in the southern states. Manipulative experiments conducted in New England by other investigators suggest that artificial elevation of host populations might be useful for conserving populations of the larval parasites *Compsilura concinnata* (Meigen), *Parasetigena silvestris* (Robineau-Desvoidy), and *Cotesia melanoscela* (Ratzeburg). Research on the chemical ecology of hyperparasites might offer possibilities for lessening their adverse effects on overwintering *C. melanoscela*. Because of the large acreages involved, parasite augmentation appears to be the least viable biocontrol strategy for gypsy moth. Progress in *in-vitro* rearing of parasites could improve the outlook for this approach, but the species selected would require very high searching capacity.

EVALUATING BIOLOGICAL CONTROL POTENTIAL OF ESTABLISHED AND EXOTIC PARASITES

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DETERMINE REASONS FOR INEFFECTIVENESS OF APPLIED BIOLOGICAL CONTROL

Gaps in the Parasite Complex

Some big guns are missing, for example:

Glyptapanteles liparidis - dominant species in Far East (Schaefer and ODell 1983, Schaefer *et al.* 1984)

G. porthetriae - dominant species in southern Europe (Burgess and Crossman 1929, Fuester *et al.* 1988)

Another problem -- low species diversity: Results in lack of compensatory action when one species "fails". Generally takes one of three forms:

1. Low mortality of small larvae (Ticehurst 1984)
2. Low attack rates in certain habitats (Bess 1961)
3. Poor parasitism in some regions [e.g. ***Phobocampe*** in NJ vs. PA] (Williams *et al.* 1992)

Caveat: Problem can be alleviated but not totally eliminated

Shortcomings in Individual Species

Egg parasites:

Ooencyrtus kuvanae - Dominant species, reviewed by Brown (1984). Can't reach all eggs in mass - % parasitism negatively correlated with egg mass size

Parasitism low on oaks (Bellinger *et al.* 1988)

Adults avoid dark places (ODell *et al.* 1989)

Anastatus disparis - Not very important, disperses very slowly (Schaefer 1991)

Larval parasites:

Cotesia melanoscela - Bivoltine, oligophagous species; overwinters in cocoon

Second generation poorly synchronized, many 4th instars escape parasitization (Weseloh 1976)

Overwintering generation sustains high rates of hyper-parasitism (Weseloh 1980); density-dependent mortality by some species (Weseloh 1983, 1986)

Mixed reports on density dependent action: both negative (Reardon 1976, Ticehurst *et al.* 1978) and positive (Williams *et al.* 1992)

Phobocampe unicincta - Univoltine, oligophagous species; overwinters as cocoon; eggs encapsulated (Muesebeck and Parker 1933)

Mixed reviews on effectiveness: Low density specialist in PA (Ticehurst *et al.* 1978, 1981); does poorly in NJ (Williams *et al.* 1992)

Density Dependent Action?? Negative in PA (Ticehurst *et al.* 1978) & MA (Gould *et al.* 1990); positive in NJ (Williams *et al.* 1992)

Prefers mesic habitats (Skinner *et al.*, in press)

Compsilura concinnata - Polyphagous, multivoltine tachinid; overwinters as maggot in alternate hosts (Webber and Schaffner 1926)

Parasitism usually highest in low (Sisojevic 1970, Barbosa *et al.* 1975) and building host populations (Ticehurst *et al.* 1978, Williams *et al.* 1992)

Alternate host requirements prevent delayed density dependent response to increases in gypsy moth abundance (Elkinton *et al.* 1990, Williams *et al.* 1992)

Does poorly at xerophytic sites in SE MA (Bess 1961) and southern NJ (Fuester, unpublished)

Parasetigena silvestris - Univoltine, oligophagous species, overwinters as puparium in litter

Best species overall with both negative (Ticehurst *et al.* 1981) and positive (Williams *et al.* 1992) correlations between host density and parasitism reported

Also very important in Europe (Sisojevic 1975, Fuester *et al.* 1981, Bogenschutz *et al.* 1989)

Generally peaks 1-3 years after collapse of gypsy moth population (Reardon *et al.* 1976, Ticehurst *et al.* 1981)

Foraging behavior allows it to capitalize on low density populations of *L. dispar* (ODell and Godwin 1979); probably best oligophagous species at low host density

Late season attacks resulted in positive density-dependent action (Elkinton *et al.* 1990); this type of action directed mostly at female larvae (6th instars)

No evidence for spatial delayed density-dependent action in small plots (Gould *et al.* 1990); year-to-year increases in parasitism suggest temporal delayed density-dependence on larger spatial scale (Godwin and ODell 1979, Elkinton *et al.* 1990)

Study by Gould *et al.* (1992) on superparasitism:

Superparasitism aggregated, not random, and certain hosts more likely to be parasitized than average

Superparasitism more likely to result in host mortality, but had negative effect on parasite survival

Study by Kotenko (1980) in Ukraine: "**The increased number of less mobile (parasitized) larvae in the crown ... during daylight ... as a result of migration led to increased competition, and the effectiveness of ... *Parasetigena silvestris* was reduced.**"

Blepharipa pratensis - Univoltine and oligophagous; overwinters as puparium; aggregates to and lays eggs on leaves damaged by gypsy moth (ODell and Godwin 1984)

Parasitization often elevated in building (Ticehurst *et al.* 1978, Williams *et al.* 1992) and declining populations (Hedlund and Angalet 1979)

Apparently not important in low or artificially elevated populations (numerous studies)

Does poorly at xerophytic sites in SE MA (Bess 1961) and southern NJ
(Fuester, unpublished)

Pupal parasites:

Brachymeria intermedia - Dominant pupal parasite; polyphagous, bivoltine species; overwinters as an adult; elevated parasitism only in dense populations (Reardon 1976, Ticehurst *et al.* 1978); prefers open stands (Fuester 1988, Williams *et al.*, in *ms*)

Effectiveness reduced in 5-9 day-old pupae (Lashomb *et al.* 1983); host defenses (Rotheray and Barbosa 1984)

Parasitism of female gypsy moth pupae much lower than male pupae (Fuester and Taylor, in *ms*)

Coccygomimus disparis - Asiatic species, polyphagous, polyvoltine; overwinters as immature in host pupa (Weseloh and Anderson 1982). Most recent addition to U.S. parasite complex (Schaefer *et al.* 1989); appears to be dispersing well

Wide attack window: develops on prepupae and pupae up to 9 days old (Fuester *et al.* 1989)

Substantial incidental mortality due to stinging; pupae of both sexes equally likely to be attacked (Fuester and Taylor, in press)

CONSERVATION

Determine Species to be Conserved & Approaches to Conservation

Host augmentation during latent (innocuous) period:
Cotesia melanoscela, Compsilura concinnata, Parasetigena silvestris

Provision of alternate hosts: *C. concinnata*

Provision of "other" resources (e.g., flowering plants):
C. concinnata, P. silvestris, Blepharipa pratensis

Elimination of natural enemies (i.e., hyperparasites): *C. melanoscela*

Rationale for Host Augmentation

Theoretical considerations: Cyclic oscillations in abundance of gypsy moth (Turchin 1990) and selected natural enemies (Sisojevic 1975, Fuester 1993) suggest delayed density dependent action

Manipulative experiments: Elevated, density-dependent parasitism observed in populations of ***L. dispar*** artificially elevated from low densities: Maksimovic and Sivcev (1984) in Yugoslavia, Elkinton *et al.* (1989) in Massachusetts, Wilmot *et al.* (in *ms*) in Vermont and ODell (unpublished) in Pennsylvania

Mitigation of Hyperparasitism

More research needed--Little is known about chemical ecology of hyperparasites. Weseloh (1988) recovered materials in cocoons of ***Cotesia melanoscela*** attractive to ***Eurytoma appendigaster***, an important species

Decoy strategy--Hyperparasites are extremely polyphagous (e.g., Hu and Barbosa 1986). Possibility of broadcasting empty cocoons of species being mass reared for colonization or augmentation

Determine Limitations of Biocontrol Agents & Develop Compatible Management System

Cotesia melanoscela:
Synchronization problem - **Bimodal egg releases**
Hyperparasitism - **Decoy or confusion strategy**

Compsilura concinnata:
Lack of carryover - **Augment key alternate hosts**
(Is this a problem?)

Parasetigena silvestris:
Apparent lack of carryover - **Artificial elevation of host populations on large scale**

IMPORTATION

Foreign Exploration: 1981-Present

Greatest emphasis in Far East:

China: USDA Interagency teams (Schaefer and colleagues 1982, 1983, 1984); USFS team (ODell *et al.* 1987); ARS extramural project (Yan *et al.* 1992)

Korea: ARS Asian Parasite Lab, R. Carlson (1981-84), D. Reed (1985-88) and R. Pemberton (1989-present)

Russian Far East: ARS, R. Pemberton [1992]

India: PL-480 Project on parasites of ***Lymantria obfuscata*** (Fuester and Ramaseshiah 1989)

Parasites Imported from Asia: 1981-92

Egg Parasites	
Anastatus bifasciatus	Japan
Larval Parasites	
Blepharipa schineri	Korea
Casinaria arjuna	Korea
Cotesia melanoscela	Korea
Cotesia schaeferi	Korea
Dolichogenidea lacteicolor	India
Glyptapanteles flavicoxis	India
Glyptapanteles indiensis	India
Glyptapanteles liparidis	China, Korea
Hyposoter lymantriae	India
Meteorus pulchricornis	Korea
Parasetigena silvestris	Korea
Phobocampe lymantriae	Korea
Phobocampe unicincta	Korea
Pupal Parasites	
Brachymeria lasus	Korea
Coccygomimus disparis	Korea
Coccygomimus luctuosus	Korea

Parasite Releases in U.S.: 1978-92

Species	Numbers Released
Anastatus disparis	5,876
Brachymeria lasus	169,235
Casinaria arjuna	83,749
Coccygomimus disparis	86,560
Cotesia melanoscela (mostly "halo")	357,251
Cotesia schaeferi	247,437
Glyptapanteles flavicoxis	2,594,171
Glyptapanteles indiensis	265,231
Glyptapanteles liparidis	376,110
Glyptapanteles porthetriae	493
Hyposoter lymantriae	143,190
Meteorus pulchricornis	14,429
Parasetigena silvestris	132
Rogas lymantriae	(incomplete) >603

Promising Species That Haven't Been Tried

Univoltine species (or high incidence of diapause):

Blepharipa schineri - Bogenschutz *et al.* (1989)
Ceranthia samarensis - Mills and Nealis (1992)

Polyvoltine species known to attack late summer pests:

Casinaria nigripes - Fall webworm (Shu and Yu 1985)
Probably others

Dominant species from southern Europe in South:

Glyptapanteles porthetriae - (Fuester *et al.* 1988)
Palexorista inconspicua - (Fuester *et al.* 1988)

Quarantine & Identification & Release

Problem #1:	Identifications - some genera from Far East poorly known. Gupta (1983) review of Ichneumonid parasites of gypsy moth filled a big gap
Problem #2:	Current regulations are slowing research, development, and implementation of importation approach
Main Issue:	**Increased host range testing**

Mass Production

In-vitro production probably a way off for many species: Greany (198?) with *Compsilura concinnata*

Poor sex ratio (% females):

Casinaria arjuna	29% (Fuester and Taylor 1991)
G. flavicoxis	32% (Fuester *et al.* 1987)
Hyposoter lymantriae	27% (Fuester and Taylor 1991)
Rogas lymantriae	23% (Grinberg and Wallner 1991)

Whiting Principle?

Univoltine tachinids generally difficult to rear:
Blepharipa pratensis (ODell and Godwin)
Ceranthia samarensis (Mills and Nealis 1992)

Distribution

Most species are present throughout New England and Middle Atlantic States, but some species lag considerably behind the leading edge:

Anastatus disparis	300-350 miles
Calosoma sycophanta	100-200 miles

Are there others? Surveys needed in South and Great Lakes States

Parasite Evaluation

Estimation of Impact of Successful Parasitism:
- Recognition of problem (Van Driesche *et al.* 1983)
- Suitability of approaches (Gould *et al.* 1992)
  - **Problem:** *Parasetigena silvestris*

Incidental Parasitism Still a Problem:
- *Cotesia melanoscela* (Thorpe *et al.* 1990)
- *Coccygomimus disparis* (Fuester and Taylor, in *ms*)

Bias in Attacks on Male & Female Hosts:
- *Brachymeria intermedia* - Male bias (Fuester, in *ms*)
- *Blepharipa pratensis* - Female bias (B & C 1929)

Use of Manipulative Field Experiments: Elkinton and colleagues effectively demonstrated spatially density-dependent mortality in MA (Liebhold *et al.* 1989, etc.)

AUGMENTATION

Step 1--Select Candidate Species

Cotesia melanoscelus: Increased parasitism in prior augmentation studies (Kolodny-Hirsch 1988, *etc.*)
Glyptapanteles flavicoxis: Modest success in IPM test in Virginia (Ticehurst and Finley 1988)
Phobocampe unicincta: Augmentation never tried; low density specialist (Ticehurst *et al.* 1978)
Compsilura concinnata: Low density specialist (Ticehurst *et al.* 1978, Williams *et al.* 1992)
Parasetigena silvestris: Augmentation never tried; best overall parasite (*vide supra)*
Blepharipa pratensis: Proposed by Knipling (1992); high fecundity (Godwin and ODell 1981); important in building populations (Ticehurst *et al.*, Williams *et al.)*
Other species?

Step 2--Determine Performance Potential

High aggregative response: *Cotesia melanoscela, Compsilura concinnata, Parasetigena silvestris, Glyptapanteles porthetriae*

High functional response: Poorly known for most gypsy moth parasites

High reproductive response: *Blepharipa pratensis, Cotesia melanoscela*

High searching capacity: *Phobocampe unicincta, Parasetigena silvestris*

Step 3--Develop Mass Production Technology

Probable continuum of difficulty:

Least Difficult ===========> Most Difficult

Glyptapanteles flavicoxis (or *liparidis*)
Cotesia melanoscelus
Compsilura concinnata
Phobocampe unicincta
Parasetigena silvestris
Blepharipa pratensis

Step 4--Develop Formulation and Application Systems

Augmentation most promising at low host densities
- *Cotesia melanoscela* (Hoy 1975, Weseloh and Anderson 1975)
- *Glyptapanteles flavicoxis* (Krause 1991)

Releases of cocoons better than releasing adults?
- Host recognition cues in insect frass (Lewis and Tumlinson 1988)
- Most promising trials with *C. melanoscela* involve releases of cocoons (Hoy 1975, Weseloh and Anderson 1975)

Patterns
- Point release vs. broadcasting
- Concentration on "foci"
- Serial vs. one-time

Step 5--Develop Decision Aids for Proper Use

Step 6--Train Educators & Users

WHERE DO WE STAND?

Better Tools for Evaluation Available

More Information Needed on Sex Determination in Parasitic Hymenoptera

Better Methodologies for Handling Univoltine Tachinids Needed

More Information Needed on Functional Responses

Regulatory Issues Will Play a Greater Role in Both Research and Action Programs

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STATUS OF THE INTRODUCED GYPSY MOTH PREDATOR, *CALOSOMA SYCOPHANTA* L. (COLEOPTERA: CARABIDAE), IN THE MIDDLE ATLANTIC STATES

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ABSTRACT

The abundance of *Calosoma sycophanta* L. and the gypsy moth, *Lymantria dispar* L., was monitored in oak stands in southern New Jersey from 1982 to 1992. Over time, the predator population responded to changes in prey abundance in a density-dependent fashion; the relationship was direct for larvae and delayed for adults. These density dependent responses were both temporal and spatial. In the case of larvae, this response appeared to be caused by enhanced reproduction where prey were numerous. The long-lived adults of the predator remained abundant for four years following the year of peak prey abundance. Attempts to measure the impact of adult beetles on larval populations of *L. dispar* with exclusion methods were unsuccessful. Predation of gypsy moth pupae by invertebrates averaged 27% over the 11-year study and was correlated positively with abundance of gypsy moth as well as with abundance of *C. sycophanta* larvae. Trapping surveys for *C. sycophanta* adults suggest that this species is missing from much of Pennsylvania, Maryland, and Delaware, and that its distribution lags 100-200 miles behind the leading edge of the gypsy moth infestation. Experimental releases of *C. sycophanta* adults in states along the leading edge would enable us to obtain better estimates of its impact on larval populations of the gypsy moth and hasten the spread of this important predator.

SUSCEPTIBILITY MAPS FOR GYPSY MOTH

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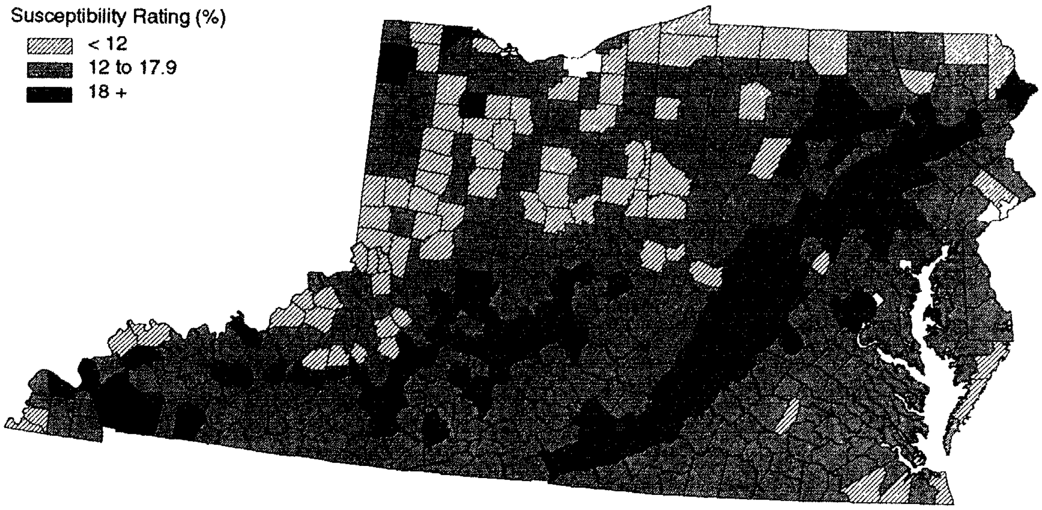
ABSTRACT

Levels of defoliation vary greatly within areas infested by the gypsy moth (*Lymantria dispar* L.). So, practical methods for identifying highly susceptible locations (that is, those most likely to suffer heavy defoliation during an infestation) greatly aid forest resource and pest managers. A model that uses forest stand characteristics to estimate the likelihood of gypsy moth defoliation has been developed. Its application to recent forest inventory plot data has resulted in susceptibility ratings and a map showing defoliation potential for counties in a seven-state area where gypsy moth is an immediate concern.



DEVELOPMENT OF NUCLEAR DNA MARKERS FOR IDENTIFICATION OF THE ASIAN GYPSY MOTH AND EUROPEAN GYPSY MOTH

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ABSTRACT

Gypsy moths are often detected in the field through the use of pheromone sticky traps. Because of the poor physical condition of moths caught in these traps and the similarity of appearance of the Asian gypsy moth (AGM) and European gypsy moth (EGM), it is difficult to distinguish the races by morphological features. DNA analysis provides a means of positive identification. A mitochondrial DNA diagnostic method exists which can identify progeny derived from AGM females, but will not allow identification of progeny from the mating of an Asian male and European female. Nuclear gene markers provide a means to detect progeny from all types of crosses between AGM and EGM. Identification of nucleic acid sequence differences within single copy genes, middle repetitive genes, middle and highly repetitive sequences, satellite sequences, transposable elements, and randomly amplified polymorphic DNAs (RAPD) are approaches to development of a nuclear sequence assay for identification of the AGM, EGM, and other gypsy moths from different geographic locations. Our current efforts focus on the middle repetitive ribosomal RNA genes and the identification of RAPD sequences. The ribosomal RNA genes of the AGM and EGM were analyzed for the presence of restriction fragment length polymorphisms (RFLPs). Genomic DNA was isolated from female moths, digested with restriction endonucleases, the fragments were separated by agarose gel electrophoresis, and the gels were blotted and then probed with 32-P labelled *Drosophila busckii* ribosomal RNA gene clones. This analysis has shown that the predominant class of ribosomal RNA repeats, of approximately 10.7 kb in length, present in AGM and EGM are of the same length. However, RFLPs were found in minor repeat classes. For example, the restriction enzyme AccI generates ribosomal RNA gene fragments of 4.8, 2.2, and 1.15 kb in length that are specific to the EGM, and fragments of 1.8 and 1.25 kb specific to the AGM. These results indicate that a diagnostic assay based on ribosomal RNA gene RFLPs can be developed once these genes are cloned and sequenced. The existence of genomic RAPD sequences allows the generation of genomic linkage maps and a specific "fingerprint" of an individual, strain, or species. We have applied the RAPD technique to identification of sequence polymorphisms that exist between the AGM and EGM. Preliminary analysis with approximately 30 RAPD primers have identified three polymorphic DNA sequences that appear to be specific to the EGM, and one sequence specific to the AGM. This analysis was performed with five individuals from each population. The sample size of AGM and EGM is being expanded to assess whether these

sequences can be used for a diagnostic assay. In addition, several hundred additional RAPD primers are being screened to identify additional sequence polymorphisms.

CENTRAL PENNSYLVANIA INDIVIDUAL TREE MORTALITY RISKS REVISITED

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ABSTRACT

Work by Herrick and Gansner (1987) provided individual tree mortality risks for gypsy moth invasion of Central Pennsylvania stands. The mortality risks are valuable for use in predicting potential mortality from gypsy moth and for selecting trees to remove in silvicultural treatments. However, they did not use defoliation as part of their analysis. Because defoliation is so often related to subsequent mortality, and the risks can't be separated for different defoliation scenarios, a new analysis was done using defoliation classes as a variable. Inclusion of defoliation intensity increased mortality risks for heavily defoliated trees and decreased them for moderately defoliated and undefoliated trees. Increasing defoliation level within crown condition classes increased mortality risk for good and fair crowned trees, but not for poor crowned trees. Increasing defoliation level within crown position classes increased mortality risk for all crown positions. Increasing defoliation level within species groups increased mortality risk for other oaks and other species groups, but undefoliated trees in the black/chestnut oak group did not follow this pattern and had very high risks. The ten groups identified by Herrick and Gansner's decision tree analysis were also separated by defoliation classes. Groups that contained black and chestnut oaks (numbers 1, 5, 8, & 9) and dogwood (number 3) had higher mortalities when undefoliated than when moderately or heavily defoliated. In most other groups, undefoliated groups had lower mortality risk than moderately defoliated groups which in turn were lower than heavily defoliated groups. Within a defoliation class, many groups from the pooled analysis were the same. This would result in fewer statistically different groups. The defoliated groups followed the same general pattern of mortality risk as the pooled groups; increased mortality with decreased crown position, decreased crown condition, and increased feeding preference (species group). Managers may now select defoliation levels and compare predicted mortality to undefoliated levels. Selection of trees to remove in silvicultural treatments can be based on projected defoliation levels. Undefoliated black and chestnut oaks had high mortality rates, probably due to factors like drought, site and stand conditions, and age.

Herrick, O. W. and D. A. Gansner. 1987. Mortality risks for forest trees threatened with gypsy moth infestation. USDA For. Serv., Northeastern For. Expt. Stn., Res. Note NE-338. 4 p.

A COMPARISON OF THREE EGG HATCH MODELS IN EASTERN NORTH AMERICA

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ABSTRACT

Large sections of the continental United States face the threat of invasion of the European strain of gypsy moth, and now possibly also the Asian gypsy moth. A geographically robust model of gypsy moth egg hatch would assist in gypsy moth management by: (1) eliminating, due to an insignificant probability of egg hatch, some areas from survey for European gypsy moth; (2) assisting in timing of control tactics over broad landscapes where insect development may not be uniform, or in areas where population levels are too low to locate egg masses and monitor development; and (3) predicting the probability of hatch of undetected eggs on ocean freighters that originated in an area infested with the Asian gypsy moth, thereby reducing the duration of ship exclusion that is designed to prevent reintroduction.

Three models of egg hatch were compared in eastern North America. See Johnson *et al.* (1983) and Lyons and Lysyk (1989) for a description of the first two models. A modification to the Johnson model predicts egg hatch distribution based on the number of cold days experienced by the eggs (from Masaki 1956). The third model is the three phase model of Gray (Gray *et al.* 1991) which recognizes three distinct phases of gypsy moth egg development. Progression through each phase is governed by its own temperature-dependent rate function and there are no requisite temperature conditions for phase transition. Oviposition is simulated by a cumulative logistic function and peak oviposition occurs on a user chosen date.

A validation test of the models was made using observed egg hatch and hourly temperature records from four sites in Virginia. In all four sites the three phase model of Gray performed equally to or better than either the model of Johnson *et. al.* or Lyons and Lysyk. Using Gray's model, predictions of first hatch were more accurate in two of four sites; predictions of 50% egg hatch were more accurate in three of four sites; and predictions of total hatch distribution were more accurate in all four sites.

A comparison of models was made using simulated weather patterns for locations from Montreal, PQ to Tallahassee, FL. Simulated hatch did not differ greatly among the three models in the northern region of our comparison (Quebec to Virginia) when a peak oviposition of July 9 is chosen for the Gray model. However, in northern regions such as Montreal, PQ, a late peak oviposition date (September 7) and cool autumn temperatures combine to reduce hatch success to 75% by limiting prediapause completion in the Gray model. In contrast to the northern regions, simulated hatch success in southern regions is limited by insufficient cool temperatures. Only 40% of the population completes diapause (and initiates postdiapause) by June in Albany, GA. In Tallahassee, only 10% of the simulated population experiences sufficient cool temperatures to complete diapause and initiate postdiapause by June. Due to the assumption of diapause completion by January or March 1, hatch success is not limited by the two other models in any location.

Timing of egg hatch is virtually unaffected by oviposition date in the Gray model. Thus, the Gray model mimics the effect of diapause on the maintenance of gypsy moth seasonality. Throughout the range of comparison the model of Lyons and Lysyk predicts the narrowest hatch distribution. Even in southern regions, hatch simulated by this model is very synchronous. It has been commonly observed that inadequate periods of cool temperatures broaden hatch distribution. Hatch distributions predicted by the Johnson model and the Gray model become broader in the southern regions. Egg hatch predicted by the Johnson or Lyons and Lysyk model becomes increasingly dependent in more southern regions on the arbitrarily chosen date of diapause completion. The Gray model predicts later egg hatch in southern regions due to slower diapause development.

Gray, D. R., J. A. Logan, F. W. Ravlin, and J. A. Carlson. 1991. Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of pre-diapause development. Environ. Entom. 20(6): 1645-1652.

Johnson, P. C., D. P. Mason, S. L. Radke, and K. T. Tracewski. 1983. Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), egg eclosion: degree-day accumulation. Environ. Entom. 12: 929-932.

Lyons, D. B. and T. J. Lysyk. 1989. Development and phenology of eggs of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), in Ontario. pp. 351-365 In Proc. Lymantriidae: a comparison of features of New and Old World tussock moths (W. E. Wallner and K. A. McManus, eds.), June 28 - July 1, 1998, New Haven, CT. USDA For. Serv. Gen. Tech. Rep. NE-123.

Masaki, S. 1956. The effects of temperature on the termination of diapause in the eggs of *Lymantria dispar* Linne. Jap. Appl. Zool. 21: 148-157.

PREDATION OF GYPSY MOTH PUPAE NEAR THE SOUTHERN LEADING EDGE

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ABSTRACT

Small mammal predators, especially the white-footed mouse (*Peromyscus leucopus*), may be the forest's first line of defense against invading gypsy moth populations. However, small mammal populations do fluctuate and, at times, are extremely scarce. During periods of small mammal scarcity, invertebrate predation may compensate to some degree for the lack of vertebrate predation. The objective of this poster is to show that the amount of gypsy moth pupal mortality occurs at a faster rate with increasing densities of the white-footed mouse, but that the total amount of mortality during the study period increased only slightly due to increased predation by invertebrates at low mouse densities.

Two small mammal surveys were conducted in two sites of the coastal plain, piedmont and mountains of Virginia/North Carolina. Live traps baited with a peanut butter-oatmeal mixture were used. Between the live-trap surveys, four freeze-dried gypsy moth pupae were placed at each trap station to determine predator impact. The pupae were placed on host trees at the litter layer, 0.25, 1.0, and 2.0 m up the tree bole. The coastal plain sites had a low mouse population for both surveys (2.2-3.0 mice/100 trap days). The populations at the piedmont sites were considerably higher than the coastal plain and increased significantly between surveys (11.3-26.0 mice/100 trap days). The mountain sites had the highest populations and they also increased between surveys (29.1-40.6 mice/100 trap days). The survival of the freeze-dried pupae one to three days after set-up showed a clear indication that predation is more rapid in the mountains where the mouse population was the highest. For example, 100% of the pupae were destroyed in the litter layer after just two days in both mountain sites, while 97% were destroyed in the piedmont sites after three days, and 88% were destroyed in the coastal plain after three days. On the other hand, the total amount of predation in the three regions was similar (63.8% for the coastal plain, 73.8% for the piedmont, and 71.2% for the mountains) despite the differences in the mouse populations. At least some of this can be attributed to invertebrate predation. In the mountains, invertebrate predation was 0.8% of the total, in the piedmont it was 11.8%, and in the coastal plain it was 22.2%.

EFFECTS OF THE FUNGAL PATHOGEN *ENTOMOPHAGA MAIMAIGA* ON NON-TARGET LEPIDOPTERA

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ABSTRACT

In recent years, concern has been expressed over the impact of gypsy moth suppression programs on non-target arthropods. The gypsy moth fungal pathogen, *Entomophaga maimaiga* Humber, Shimazu & Soper, has caused epizootics in North American gypsy moth populations since its discovery in 1989. Previous work with a Japanese strain of *E. maimaiga* demonstrated that the fungus only infects Lepidoptera. What impact *E. maimaiga* will have on the endemic North American lepidopteran fauna? We challenged lepidopteran larvae with *E. maimaiga*, emphasizing field-collected larvae from WV where *E. maimaiga* is not well established. Lepidopteran species for experiments were reared from eggs, field collected as larvae, or obtained from a laboratory colony. All larvae were reared on their preferred host plant species. Larvae were challenged with *E. maimaiga* by dipping them into a conidial suspension of known dose. Bioassays were conducted using 43 species in 5 lepidopteran suborders: Papilionoidea (1 species), Pyralidoidea (1), Geometroidea (12), Bombycoidea (5), and Noctuoidea (24). We emphasized the Noctuoidea because this is the suborder that includes gypsy moth and the group that demonstrated infection in trials with the Japanese strain of *E. maimaiga*. *E. maimaiga* infected larvae in three of the five suborders tested: Noctuoidea, Bombycoidea, and Geometroidea. In all families besides Lymantriidae, infection was not uniform and infection levels were low. The four species of lymantriids treated became infected at levels from 60.0-100%. As a caveat, our results at this time reflect only bioassays conducted in the laboratory where infection by *E. maimaiga* was optimized. However, we know that the activity of *E. maimaiga* is partially dependent upon weather conditions and we hypothesize that the gypsy moth-specific larval movements and aggregations promote infection. Therefore, it is not known whether our bioassay results are any indication of levels of infection of alternate hosts under field conditions. To determine the seasonality of resting spore germination, resting spores were placed in replicated bioassay boxes in the field and, beginning April 7, each week fourth-instar larvae were challenged until the end of August. Results from field bioassays indicate that resting spores began causing infections approximately two weeks before gypsy moth eggs began hatching. Larvae became infected until the end of July, when all gypsy moths had pupated and many adults had emerged. Therefore, the activity of this fungus appears to be quite finely tuned to the seasonal appearance of gypsy moth larvae and pupae, although it appears to be active for a relatively brief period before egg hatch and for a time after most larvae had pupated.

SPREAD OF *ENTOMOPHAGA MAIMAIGA*

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ABSTRACT

During 1989 and 1990, epizootics of the fungal pathogen, *Entomophaga maimaiga* Humber, Shimazu & Soper, occurred in gypsy moth populations throughout New England. In 1991, we released *E. maimaiga* resting spores in PA, VA, WV, and MD, along the leading edge of the gypsy moth infestation. May and June, 1991, were exceptionally dry, yet *E. maimaiga* successfully became established at 27 of 34 sites; high levels of infection occurred at several sites, and gypsy moth cadavers containing *E. maimaiga* spores were collected up to 350 m from release sites. During 1992, *E. maimaiga* was released at seven additional sites in WV and VA, three new control sites were established, and 1991 sites were resampled. During 1992, *E. maimaiga* was recovered at 96.4% of 1991 fungal release sites, but was also found at 83.3% of 1991 control sites. *E. maimaiga* was found at all 1992 release sites and at two of three control sites. At the majority of sites, percentages of cadavers with *E. maimaiga* spores were extremely high. Based on egg mass counts, gypsy moth populations at almost all sites declined, but this decline was greater at 1991 fungal release sites than 1991 control sites. High percentages of cadavers containing *E. maimaiga* spores were collected 1 km away from 1991 and 1992 fungal release sites. This spread by *E. maimaiga* to 1 km from 1991 and 1992 release sites had not been anticipated based on spread during 1991. A distributional survey demonstrated that *E. maimaiga* now occurred throughout VA and was scattered through WV and MD. Based on data taken from 1986-1991, the distribution of *E. maimaiga* had increased dramatically. After the unexpected widespread occurrence of *E. maimaiga* epizootics in 1989, conventional wisdom suggested that such high levels of infection only occurred during very wet, cool springs. In 1992, rainfall was not exceptional during May and June and temperatures were not unusually low. At this time, we cannot say whether the source of this spread by *E. maimaiga* was fungal release sites, areas to the north where *E. maimaiga* is naturally established, or both. *E. maimaiga* has also spread in NY and PA since 1989. In NY and PA, once again, there is no clear source for this spread. Generalized changes in the distribution of *E. maimaiga* from 1989-1992 demonstrate a pattern of long distance movement to the south and west. *E. maimaiga* could have spread in many different ways but, based on the epidemiology of plant pathogenic fungi, we conducted preliminary studies of movement by airborne conidia (spores). A spore trap was operated in the forest for 16 weeks to determine hourly densities of *E. maimaiga* conidia in the air. Fourth instar gypsy moth larvae were placed in screen cages at the same height as the spore trap for three-day periods. Airborne conidia were very abundant at times and infections among caged larvae demonstrated that conidia were alive and infective.

RAPID ACCUMULATION OF FEW POLYHEDRA MUTANTS (FP) DURING SERIAL PASSAGE OF *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS IN *L. DISPAR* 652Y CELLS

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ABSTRACT

During propagation of baculoviruses in cell culture, a class of viruses with an altered plaque phenotype arise at high frequency and are termed few polyhedra (FP) mutants. FP mutants exhibit the characteristics of formation of few polyhedra per cell (approximately 4-10), the occlusion of few or no virions in polyhedra, synthesis of greater amounts of budded virus compared to wild type (many polyhedra, MP) virus, and become the predominant viral type during serial passage. The genesis of FP mutants during serial passage is a significant impediment to commercial production of polyhedra *in vitro*.

To gain a better understanding of the processes involved in LdMNPV polyhedra formation, a study of the genesis of LdMNPV FP mutants was undertaken. LdMNPV isolates A21, B21, 122, and 163 were serially passaged in *L. dispar* 652Y cells. The number of polyhedra produced was found to rapidly decrease during passage. A 4.0-, 2.3-, 4.8-, and 3.6-fold reduction in the number of polyhedra produced per cell was observed at the third passage in comparison to the first passage in isolates A21, B21, 122, and 163, respectively. The isolates also exhibited an average decrease of 35.6% in the number of cells that produced polyhedra during serial passage. The amount of budded virus produced by the isolates increased from 169 to 891 fold at the second passage relative to the amount of budded virus produced at the first passage. The proportion of virus exhibiting a FP phenotype increased during serial passage. Greater than 90% of the virus present after the third passage exhibited a FP phenotype in three of the four LdMNPV isolates examined. Cross sections of polyhedra produced at the fifth passage by the isolates were found to exhibit approximately a seven-fold reduction in the number of nucleocapsids present relative to the number present in polyhedra synthesized at the first passage.

Viral isolate genomic DNA restriction endonuclease digestion profiles were analyzed to determine if restriction fragment length polymorphisms (RFLP) were generated during serial passage. Genomic DNA BglII digestion profiles of 10 FP lines of each isolate were also analyzed. The DNA digestion profiles of A21, B21, and 163 MP viral lines, serially passaged virus, and FP viral lines contained no RFLPs. RFLP analysis of serially passaged isolate 122

detected the appearance of an insertion. Isolate 122 plaque purified lines of viruses with FP and MP phenotypes were generated and analyzed. The genomic digestion profiles of these isolates were the same, suggesting that the insertion was not the basis for the FP phenotype.

The results of this study indicate that FP mutants of LdMNPV rapidly accumulate during serial passage in *L. dispar* 652Y cells. The majority of FP mutants that arose lacked detectable DNA insertions, and when insertions were detected they appeared not to cause the FP phenotype. The basis for the FP phenotype of the LdMNPV FP mutants described in this study is under investigation.

PREDICTING GYPSY MOTH FRASS INPUT FROM FOREST DEFOLIATION[1]

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ABSTRACT

Gypsy moth frass was collected weekly from May until mid July from 43 plots in 1990 and 1991 and from 56 plots in 1992. Plots were located along the leading edge of gypsy moth spread into north central West Virginia. Frass was sorted, weighed and summed over the season. In mid July of each year, we estimated percent defoliation for all trees within 0.1-acre plots that corresponded to the frass collection sites and computed mean defoliation for the plots.

Using frass input (kg/acre) as the dependent variable and average percent defoliation as the independent variable, a regression was developed. The r^2 for the regression was 0.417 and the prediction equation is as follows:

Frass weight (kg/acre) = 1.784 + 4.923 * % defoliation

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[1] This work was performed at the West Virginia University Forest and was partially funded through the USDA Forest Service, Northeastern Forest Experiment Station, Morgantown, WV

USING AERIAL PHOTOGRAPHS TO ASSESS FOREST COVER FOR GYPSY MOTH HAZARD RATING

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ABSTRACT

It is a well known fact that gypsy moths prefer certain host species over others, notably oaks in the Appalachian forests. We attempted to use color infra-red photography taken at the time of peak fall color change to delineate cover types at the West Virginia University Forest. As a test case, we selected one compartment (approximately 450 acres). Our approach was to delineate areas on the photograph that appeared similar. We then looked at reference trees (near identifiable landmarks) on the photo and on the ground to get an idea of how individual species appeared on the photograph. The interpreter then sampled stands on the photograph (approximately 10% of the area). Cover types were assigned using the Society of American Foresters and Northeast Decision Model forest cover type codes. Type verification was done by point sampling all the stands in the field.

Our results showed that we need to refine our photo identification procedures. We correctly identified about 33% of the stands with either code system. It appears that yellow-poplar and red maple were consistently underestimated from the photograph and that northern red oak was overestimated. Perhaps with this knowledge and incorporation of topographic position, etc., we can make the necessary adjustments in future typing.

ISOLATION AND CHARACTERIZATION OF GENE ENCODING LARGE SUBUNIT OF VITELLOGENIN FROM THE GYPSY MOTH

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ABSTRACT

Juvenile hormone plays an important role in the growth, development and reproduction of insects. The hormone action is effected through regulation of several developmental-specific genes. The hormone causes both stimulatory as well as inhibitory (suppressive) effects on these genes in a coordinated manner leading to normal development and metamorphosis. Knowledge of the mechanism of action of juvenile hormone will be very beneficial in developing biorational and/or biological insect control agents designed to disrupt the hormone action.

In the gypsy moth larvae, juvenile hormone regulates the synthesis and secretion of vitellogenin by the fat body. Recent work has shown that the hormone exerts its effect at the transcriptional level. The vitellogenin gene, thus, could serve as a good candidate to study the mechanism of juvenile hormone action in the gypsy moth.

In order to determine the pathways involved in the regulation of vitellogenin gene expression, it will be necessary to isolate and characterize the vitellogenin gene(s). A recombinant probe (pVL-80) encoding a portion of the largest subunit of gypsy moth vitellogenin (Vg190) was obtained by cloning DNA complementary to Vg190 mRNA. Nucleotide sequencing of the probe indicated that it represented internal coding region of Vg190 mRNA. Comparison of the sequence with those in the Genbank indicated no significant homology to any known sequences including sequences of Vg from vertebrates and other invertebrates.

The pVL-80 was used to identify and characterize the Vg190 gene in the gypsy moth genome. Genomic Southern blot hybridization analyses suggested that there is a single copy of the Vg190 gene in the gypsy moth genome. Screening of a gypsy moth genomic library using pVL-80 as a probe resulted in the isolation of a phage clone, EMVg6.6, containing sequences related to Vg190. The phage clone, which has an insert of ~18 kbp, is being further characterized to determine whether the insert contains the entire Vg190 gene.

FACTORS AFFECTING PUBLIC OPINION TOWARDS GYPSY MOTH SUPPRESSION

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ABSTRACT

Visitors to national parks, state parks, and state forests in the Mid-Atlantic Region (n=202) were surveyed to: 1) quantify attitude (approve, neutral, oppose) towards commonly used gypsy moth suppression tactics, and 2) to examine these findings in relation to various situational and demographic variables. Potential situational variables included type of suppression tactic used and land use type or ownership (e.g. national parks, state forest, private land, etc.) on which suppression would take place. Demographic variables included age, gender, income, education, environmental attitude, and place of residence. Overall, 62% of responses were supportive of some type of suppression while only 6% were opposed to any form of suppression. Opposition to suppression was highest for Dimilin (41%), followed by *Bt* (22%), and Gypchek (14%). Respondents who previously heard about and/or had personal experience with gypsy moth were less likely to oppose suppression. However, previous experience with gypsy moth also caused visitors to be more uncertain about when and where to use a particular suppression method. Results of the Logistic Regression Analysis (categorical modeling using maximum likelihood estimates) suggest that the type of suppression method used (Dimilin, *Bt*, Gypchek) was the strongest overall predictor of attitude towards suppression (df = 9, $X^2 = 703.3$, $p < 0.0001$). This finding suggests that the public support managers receive for suppression programs will likely depend primarily on the methods used. The effect of land use type on which suppression would take place was negligible (df = 21, $X^2 = 30.6$, p = .08), suggesting that the public is unable or unwilling to distinguish types of public and private land. From a public perception perspective, the implication is that gypsy moth suppression need not be tailored to particular land uses. Primary recreation interest, followed by environmental attitude, place of residence, and past experience with gypsy moth were the most important demographic variables influencing attitudes towards suppression. This finding suggests that managers will encounter varying support for their suppression programs, depending on the demographic make-up of affected publics.

GYPSY MOTH BIOCONTROL: SYNTHESIS AND EVALUATION FOR DEVELOPING RESEARCH PRIORITIES

Comments and Conclusions

Moderator:
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Dick Reardon reviewed the work with parasites (= parasitoids) from 1900-1984. Many species were collected (all from dense gypsy moth populations), reared, and released; nine exotic species established. Despite a large effort in rearing and redistributing parasites in the 1960s and 1970s, only one species (*Coccygomimus disparis*) established. He recommended that additional foreign collections in Asia concentrate on a few species, which should be collected and released in larger numbers. Past augmentation efforts have resulted in parasitism levels far below those predicted by Knipling's model.

Roger Fuester outlined the biological traits of foreign parasites released from 1984 to the present and their ecological roles in gypsy moth population dynamics in the U.S. versus Eurasia. Two dominant parasites in the Far East and in southern Europe which are missing from the U.S. are *Glyptapanteles liparadis* and *G. porthetriae*, respectively. Also, because there is lower parasite species diversity in the U.S. than in Eurasia, when one species "fails" the needed compensatory action by other species often is absent. Parasites recommended for new importations include: (1) *Blepharipa schineri* and *Ceranthia samarensis*, species which are well-synchronized with endemic, low density gypsy moth populations; (2) *Casinaria nigripes*, a polyvoltine species known to attack late summer hosts; and (3) *Glyptapanteles porthetriae* and *Plaexorista inconspicua*, dominant species from southern Europe. Recommended approaches for parasite conservation include host augmentation, provision of alternate hosts and habitat enhancement. Manipulative field experiments reported recently suggest density dependent parasitism occurs in artificially elevated gypsy moth populations, and these studies should be continued. Surveys are needed in southern and Great Lake states to determine parasite species occurrence (e.g., indication of lag of some species at the leading edge). Gypsy moth biocontrol is in need of: (1) better evaluation tools and information on functional responses of parasites; (2) information on parasite sex determination; (3) improved methodologies for handling univoltine tachinids; and (4) clarification of regulatory issues for biocontrol research and implementation (also see article by R. Fuester).

Harvey Smith reviewed the vertebrate and invertebrate predators and their roles in gypsy moth population dynamics. Predator diversity and abundance is significantly affected by habitat qualities (e.g., alternate food for mice). Predation is highly variable but is highly predictable. Small mammals are often the major mortality factor at low gypsy moth densities. Predation is usually not identified as a significant mortality agent of the gypsy moth in Eurasia, but predators appear to have been overlooked (as he observed with an arboreal forest mouse in Russia).

Joe Maddox concluded that NPV and *Entomophaga maimaiga* are the only exotic pathogens of importance to gypsy moth in the U.S. Although gypsy moths are susceptible to many microsporidia, they have low prevalence in the field. In Eurasia, many more pathogens are present. He cautioned that laboratory host range does not equate with the more narrow ecological host range experienced in the field. The challenge is how to manage pathogens so that epizootics occur prior to host populations reaching thresholds.

Jim Krysan (ARS), Joe Elkinton (Univ. Mass.), Bill Metterhouse (NJ Dep. Agric.), and Ernest DelFosse (NBCI) comprised an expert panel which made concluding statements, then addressed questions from the audience. Jim Krysan suggested that more knowledge of parasitoid biology is needed. Joe Elkinton proposed that, indeed, biological control is already working well in North America especially in regions that have been infested for many decades. Furthermore, biocontrol is the only feasible long-term management. However, without understanding the dynamics of natural populations of gypsy moth, long-term management decisions are impossible. Joe suggested the need for long-term study in permanent plots to characterize populations over time and the value of using manipulative field experiments to measure natural enemy activity. Bill Metterhouse concluded that sufficient rearing, release and monitoring of parasites was done during 1971-1978, but that genetic deterioration of colonies was likely. The lack of alternate hosts prevented the success of many species. New thrusts in classical biocontrol should emphasize careful selection of exotic species that possess the desirable biological attributes. Ernest DelFosse suggested that we should view gypsy moth as we do weed pests. That is, the goal is to find beneficial species that are host-specific to the gypsy moth. Studies in the home range should occur before enemy release to identify key ecological factors. Systematics is desperately needed to correct the lack of definitive identifications in many foreign collections. Evaluation must be committed to as a long-term basic research agenda for all biocontrol projects, including the study of non-target effects (i.e., potentially *vs* actual non-targets). Much gypsy moth mortality is compensatory; therefore, interactions among enemy species must be considered. The changes in natural enemy complexes, alternate hosts, and risks of losing habitats as gypsy moth expands in the U.S. should be studied. As long as North American forests are dominated by oaks (*vs* Eurasia) gypsy moth outbreaks will continue; however, natural enemies ameliorate the stability of forest ecosystems. A draft of new regulations for the use of biocontrol agents is expected soon from NBCI, and Del invites your review.

CHARACTERIZATION OF PROTEOLYTIC ACTIVITY IN *LYMANTRIA DISPAR* MIDGUT

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ABSTRACT

Increased concern over the environmental impact of chemicals has led to an interest in the development of biological pesticides for use in the control of *L. dispar.* These include *Bacillus thuringiensis* toxins, baculoviruses, and azadirachtin, a natural insecticide found in neem seeds and natural inhibitors of insect digestive enzymes.

Protease inhibitors have been used successfully in the control of agricultural pests (Hilder *et al.*, Nature, 1987, and Johnson *et al.*, PNAS, 1989) and are an attractive method of control for several reasons. Inhibitors are produced in seed and plant tissues as a natural defense mechanism. Protease inhibitors are single gene products and not the end result of a complex biochemical pathway; therefore, they can be easily cloned, produced in large amounts, and subsequently genetically engineered to alter substrate binding affinity or specificity.

We have used an *in vitro* assay system to identify the proteases present in the soluble gypsy moth digestive fluids and to subsequently screen a variety of inhibitors to determine which kind of inhibitor could be most effective against gypsy moth *in vivo.* The types of proteases present in the midgut of *L. dispar* larvae were characterized by their substrate specificities using synthetic substrates, pH optima and their interaction with different kinds of inhibitors.

Several inhibitors for each class of protease have been assayed for their effectiveness in controlling midgut proteolytic activity. The serine class of proteases appear to be predominant in gypsy moth, and one of these proteases has been purified from the midgut. Several serine protease inhibitors effectively inhibit midgut proteolytic activity *in vitro*, and a preliminary feeding study with neonate larvae suggests that cowpea trypsin inhibitor may be effective *in vivo.*

These studies have indicated that the proteolytic activity in gypsy moth larval digestive fluids was due to the presence of several alkaline proteases and that it will be necessary to fully characterize these activities and their interaction with inhibitors if one is going to develop a strategy to employ proteinase inhibitors for gypsy moth control.

MEIOSIS IN THE GYPSY MOTH

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ABSTRACT

The economic and esthetic impacts of gypsy moths have fostered interest in genetic selection techniques as part of a general control strategy. Since F_1 sterility protocols are more directed genetic approaches are aided by knowledge of chromosome structure and behavior, we have examined the meiotic divisions of gypsy moths. The chromosomes of many Lepidoptera are small and are asserted to lack features which can identify individual elements of the diploid set. Gypsy moth chromosomes have been predominantly described from the divisions of meiosis II in structures in either sex. Our preliminary observations on the chromosomes of *Lymantria dispar* germ cells from both testis and ovary are presented. Our preliminary results suggest that:

1. *Lymantria dispar*'s haploid set contains 31 chromosomes.
2. Metaphase I and II stages which deviate from 31 are frequent in the NJ strain, approaching 10% in both sexes.
3. The meiotic figures of males and females differ significantly in the degree of compaction achieved at metaphase I and formation of chiasmata. Associations between the ends of chromosomes are very evident in the female through meiosis I.
4. Recombination figures are found exclusively in male material. Chromosomes of all sizes can form a chiasma. These observations suggest that classical reciprocal exchange is restricted to the male in *L. dispar*.
5. The prophase and metaphase figures of females contain elements which are less dense than the rest of the karyotype. At least one element does not appear in male meiotic figures. *L. dispar* is argued to have a ZZ/ZW sex determining system in which the female is heterogametic. These may be elements of the sex chromatin and/or supernumerary chromatin.

CHORIOGENESIS IN GYPSY MOTH: ACCUMULATION OF CHORION-SPECIFIC GENE TRANSCRIPTS

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ABSTRACT

The 3' untranslated region of 15 gypsy moth chorion cDNAs was analyzed to identify clone-specific oligonucleotide sequences which could be used as probes to detect cellular transcripts corresponding to their respective cDNAs. Pairwise nucleotide comparisons of this region clustered the cDNAs into six groups of one to four members each. Sequences within some groups exhibited 100% identity, precluding the isolation of clone-specific probes. Six group-specific probes could be identified and were tested for their specificity. No cross-hybridization was detected; hybridization was specific for the cDNA from which the probe was derived.

Hybridization of these group-specific probes to RNA isolated from progressively more mature choriogenic follicles (isolated from sets of five contiguous follicles) detected similar patterns of transcript accumulation for each probe. Transcripts were first detected in choriogenic follicles 1-5, reached their maximal levels in follicles 11-15, then declined steadily until the end of choriogenesis, follicles 16-20 and 21-25. Transcripts were not detected in prechoriogenic follicles nor in control RNA isolated from male pupae. Signal intensity differences were observed for the various probes and can be explained by the abundance or the diversity of transcripts recognized by that probe.

The RNA transcript accumulation profiles, the protein synthetic patterns within follicle cells, and the coding potential of the cDNAs suggest that these cDNAs may encode the ca. 14 kd proteins which are abundantly expressed throughout choriogenesis.

REGIONAL PREDICTION OF GYPSY MOTH DEFOLIATION FROM COUNTS OF EGG MASSES, PUPAE, AND MALE MOTHS: A GEOSTATISTICAL ANALYSIS

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ABSTRACT

We compared three different sampling techniques for their ability to predict regional defoliation maps. A network of 150 plots irregularly distributed throughout Massachusetts was established by the Massachusetts Department of Environmental Management. Plots were located in forest stands considered susceptible to gypsy moth defoliation. At each plot, burlap bands were placed around the stem of the 20 closest oaks over 6 inches at DBH. In early summer of each year from 1984 to 1991, a standard disparlure-baited milk carton pheromone trap was hung at the plot center. In late summer, pheromone traps were returned to a laboratory where male moths were counted; counts were also made of all egg masses and pupal remains under burlap bands. Historical sketch maps of gypsy moth defoliation collected from 1984 to 1991 were digitized into 2 x 2 km cells in a geographic information system. We examined the correlation between counts of the three gypsy moth life stages and defoliation using indicator pseudo cross-correlograms. A cross-correlogram is formed by calculating the cross-correlation statistic for a series of interpoint distances, *h*. In a typical cross-correlogram, values are near unity at low values of *h* but decline toward zero as *h* increases. This indicates that the two variables are correlated at nearby locations but dissimilar as points become more distant.

Cross-correlograms indicated that there was substantial correlation (in certain years) between egg mass counts and subsequent defoliation. These cross-correlations extended for up to five cells (10 km) in some cases. Similar patterns were observed for counts of pupae. By contrast, there was little evidence for cross-correlation between defoliation and counts of male moths. These results indicate that counts of egg masses (or pupae) under burlap bands probably are the most suitable variable to measure for prediction of defoliation on a regional

scale. It was surprising that counts of adult males did not perform better considering that grids of pheromone traps have been used widely with some success to characterize regional gypsy moth population conditions along the leading edge of the infestation. Presumably, the failure here of pheromone traps as a tool for regional characterization of populations is related to the extensive mixing of male populations once populations are permanently established.

WHAT CAN HISTORICAL DATA TELL US ABOUT GYPSY MOTH POPULATION DYNAMICS?

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ABSTRACT

Despite many years of research, we still have an incomplete understanding of the major processes affecting the dynamics of gypsy moth populations. Many dynamical patterns may not be recognized unless several years (> 20 years) of population data are analyzed. Unfortunately, such data sets are not common. Further, extreme caution must be exercised in the interpretation of historical population data. This is illustrated by the case of a recently published study in which the historical Melrose Highlands data were used to infer that gypsy moth populations exhibit delayed density-dependent mortality and that this must be caused by the numerical response of a natural enemy that is regulating gypsy moth populations. We demonstrated in hypothetical simulations that commonly used analyses that indicate the presence of delayed density dependence can sometimes be caused by a forcing variable that is autocorrelated in time. This forcing variable may represent a natural enemy that exhibits first-order density dependence, or it could be a weather variable. We also illustrated how historical data on the occurrence of isolated gypsy moth populations outside of the generally infested area can be used to understand the dynamics of population establishment. These data indicate that most isolated populations disappear after one year. Simple biogeographic models can be used to predict this phenomenon and other processes observed during population establishment. One interesting prediction of these models is that an organism with a greater dispersal magnitude (such as the Asian strain of the gypsy moth) can be expected to have a lower probability of establishment.

EFFECTS OF ASPEN DEFENSIVE CHEMISTRY ON EFFICACY OF *BT* AGAINST GYPSY MOTHS. I. ECOLOGICAL CONTEXT

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ABSTRACT

In recent years the gypsy moth has become established in the eastern and central Great Lakes region and has continued to spread westward into areas such as Wisconsin and the upper peninsula of Michigan. Concern has mounted over the potential impact of gypsy moths on aspen forests, as aspen is a major forest product of the Great Lakes area. Our research has focused on the biochemical and phytochemical mechanisms mediating ecological interactions between gypsy moths and aspen, and how the efficacy of *Bt* may be altered by aspen defensive chemistry.

Aspen (*Populus tremuloides*) defensive chemistry is comprised almost entirely of phenolic compounds, including phenolic glycosides and condensed tannins. The putative mode of action of these compounds is to cause degenerative midgut lesions. Insects adapted to feeding on aspen foliage detoxify phenolic glycosides via midgut esterases. Our research has shown that the gypsy moth is moderately adapted to phenolic glycosides. At intermediate to high concentrations of the compounds (> ~3% fresh weight), larval survival and growth decline, whereas development times are prolonged. Under field conditions, aspen clones exhibit substantial variation in both phenolic glycoside concentrations and resistance to defoliation by gypsy moths. We are currently testing the hypothesis that differential defoliation is a consequence of variation in aspen defensive chemistry. Thus far, our work has shown that gypsy moths exhibit three-fold variation in pupal weight on different aspen trees, which exhibit 14-fold variation in phenolic glycoside concentrations. Both growth rates and pupal weights are strongly and inversely correlated with phenolic glycoside concentrations.

Because both phenolic glycosides and *Bt* cause gut lesions, we have been interested in evaluating the potential for interactive effects of the two toxins on gypsy moths. During 1992, we evaluated the effects of aspen foliar chemistry on efficacy of *Bt*, and the interactive effects of dietary phenolic glycosides and *Bt* on gypsy moth performance.

We conducted *Bt* toxicity trials with new third instars that had been reared on foliage from one of five aspen trees. To determine LD_{50}s, we assayed six doses of *Bt* (Foray 48B) with four replicates per dose and 40 larvae per replicate. Leaf tissue was analyzed for levels of

nitrogen, phenolic glycosides, and condensed tannins. We found 100-fold variation in LD_{50} values among insects reared on different aspen hosts. Toxicity of *Bt* was positively correlated with foliar phenolic glycoside concentrations, but negatively correlated with foliar tannin concentrations. A multiple regression analysis showed that foliar phenolic glycoside and tannin concentrations explained over 95% of the variation in (log) LD_{50} values. We assessed the direct and interactive effects of *Bt* and phenolic glycosides on larval performance. *Bt* and phenolic glycosides were incorporated into high wheat germ artificial diets at levels of 0, 100, and 200 IU/ml and 0, 2, and 4% (wet weight), respectively. Each diet was assayed with eight replicates of 20 second instars. Mortality was substantially higher in combined *Bt*/phenolic glycoside diets than in either the *Bt* or phenolic glycoside diets alone. These results concur with those from the LD_{50} trials, indicating that aspen phenolic glycosides potentiate the toxicity of *Bt* to gypsy moths. Our work suggests that variation in the success of *Bt* sprays in the field may be due in part to variation in host plant chemistry. This possibility will be pursued in future research.

SPRAY DROPLET EVAPORATION RATES: *BT*, DIMILIN AND WATER

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ABSTRACT

An experimental evaporation facility, consisting of a droplet generation system, an environmental control system, and a video-based droplet size measurement system, was constructed and used to measure the effects of environmental conditions on droplet evaporation of different pesticides. Water droplets were measured as a comparison standard for the pesticide formulations. In this paper, evaporation rates of a *Bt* formulation, Foray 48B diluted to 16B, and Dimilin 25W were measured and compared. The *Bt* formulation evaporated at a similar rate to water until the droplet reached about half of its original size when it quit evaporating. The Dimilin formulation evaporation was similar except that it lost water for a longer period, to approximately 85% of its original size. The similarity of the evaporation rate to that of pure water is evidence that the active ingredient is conserved in the droplet and only the water portion of the suspension is being evaporated.

NATURALLY OCCURRING PATHOGENS OF THE GYPSY MOTH

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ABSTRACT

Pathogens Causing Epizootics in North American Gypsy Moth Populations

Numerous studies have been conducted over the past 20 years to determine the extent of disease in North American gypsy moth populations. Three primary questions were addressed in these studies. What diseases were present? How did they affect gypsy moth populations? Could they be developed as microbial insecticides?

In addition to the gypsy moth nuclear polyhedrosis virus (NPV), which was present in North American gypsy moth populations before 1900, several species of fungi, nematodes, and bacteria have been recovered from diseased gypsy moths. Microsporidia have never been recovered from gypsy moths field collected in North America. Although insect pathologists have a basic interest in many of the pathogens recovered from gypsy moths, only the NPV and the entomopathogenic fungus, *Entomophaga maimaiga*, cause epizootics in North American gypsy moth populations. The NPV was introduced into North America before 1900. The method of introduction, probably unintentional, is unknown. The fungus *E. maimaiga* was intentionally introduced from Japan in 1911 and since its rediscovery in 1989 has spread dramatically through gypsy moth populations in the Northeast. The reasons for the sudden resurgence of *E. maimaiga* in 1989 and its expanding geographical range is a fundamental research question.

Most other current research on both the NPV and *E. maimaiga* as naturally occurring pathogens is directly or indirectly aimed at developing a better understanding of the epizootics produced by these diseases. The major questions concern the variables that influence (1) pathogen transmission, (2) the effect of the disease on gypsy moth populations, and (3) interactions of the disease with other biological control agents. In order to make maximum use of these two diseases, we must identify and to some extent be able to manipulate the variables that influence the development of an epizootic. Some of the variables currently being investigated are the spatial and temporal dynamics of the diseases, genetic variability of both host and pathogens, and dynamic functions of specific processes such as degradation of polyhedra, production of primary conidia, etc. The interactions between these variables are very complex and can best be manipulated using various types of mathematical or simulation models. As the data sets of epizootiological variables become more inclusive and our ability

to deal with interactions between these variables becomes more sophisticated, we will develop a much better perception of how epizootics develop.

Pathogens Causing Epizootics in Eurasian Gypsy Moth Populations

At least seven species of microsporidia, a granulosis virus, a cytoplasmic polyhedrosis virus, several species of fungi, and an NPV reportedly cause epizootics in Eurasian gypsy moth populations. Many of these pathogens have importance for introduction into North American gypsy moth populations as classical biological control agents. The concept of introducing exotic gypsy moth pathogens as classical biological control agents is supported by the delayed, but spectacular, success of the introduced fungal pathogen, *E. maimaiga.*

The only gypsy moth pathogens currently being proposed for introduction as classical biological control agents are five species of microsporidia, but additional microsporidia and other pathogens should be considered. Permission has been obtained from regulatory authorities to experimentally introduce five species of exotic gypsy moth microsporidia. Experimental introductions are restricted to isolated woodlots of less than 10 acres. Permission for wide-scale releases and permanent establishment is being sought for these five species of microsporidia. If exotic gypsy moth pathogens are to be used in a manner similar to exotic parasitoids, additional foreign exploration must be conducted in order to obtain additional species and biotypes. Many exotic gypsy moth pathogens are poorly known. They must be adequately characterized for proper identification and classification. Before an exotic organism is introduced, it must be sufficiently characterized so pathogens recovered after the introduction can be unequivocally identified as the pathogen released.

Understandably, a primary regulatory concern is the effect of the introduced pathogen on non-target hosts. The host range of exotic gypsy moth pathogens should be estimated before the pathogen is introduced. Although the ecological host range is the real concern, only laboratory or physiological host range can be absolutely determined before introduction. Most insect pathogens will infect some non-target hosts under laboratory conditions, but few cause epizootics in non-target host populations. Discriminating between physiological and ecological host range is a regulatory dilemma. Every exotic gypsy moth pathogen can not, and probably should not, be introduced into North America as a biological control agent. The selection of appropriate candidates for introduction as classical biological control agents must be considered. Selection criteria have been proposed for microsporidia, but these criteria will undoubtedly be modified as we learn more about their epizootiological characteristics.

Finally, it is very important that every exotic pathogen introduced into North America be properly evaluated after the introduction. Establishment, spread from point(s) of release, effect on gypsy moth populations, effect on non-target host populations, and interactions with other biological control agents should be emphasized. After permanent establishment, studies similar to the studies now being conducted on the gypsy moth NPV and on *E. maimaiga* should be initiated.

REGIONAL, AMONG-POPULATION, AND WITHIN-POPULATION GENETIC VARIATION IN RED OAK FOLIAGE QUALITY FOR GYPSY MOTH LARVAL FEEDING AND GROWTH

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ABSTRACT

Trees typically exhibit genetic variation within and among populations as well as across broad regions. We examined genetic variation in foliage quality for gypsy moth larval feeding and growth in two experiments carried out at a red oak provenance plantation in Wooster, Ohio. The first experiment was designed to detect regional and inter-population variation, while the second was designed to detect variation among open-pollinated families and among-tree variation.

Regional and population-level variation in foliage quality was not significant. There were red oak family-level differences in leaf area consumption, however leaf weight consumption was found not to differ among families, suggesting that the first result is explained by variation in specific leaf weight. In conclusion, from the perspective of feeding gypsy moth larvae, the red oak genetic landscape appears relatively uniform. However, from the perspective of the host trees, other traits must be considered before dismissing genetic variation as unimportant.

METEOROLOGICAL FACTORS INFLUENCING THE DEPOSITION OF AERIAL SPRAYS OF *BACILLUS THURINGIENSIS*

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ABSTRACT

Rapid changes of the atmospheric boundary layer structure during the normal aerial spray period results in concurrent changes of spray movement and behavior. The rates and stages of boundary layer development throughout a 24-hour day were reviewed and related to their effects on spray operations. The effects of various combinations of atmospheric conditions during the day were related to spray penetration into the canopy using data from the NEFAAT Group Blackmo 88 and 90 field experiments. General criteria for spray movement were then given for various combinations of atmospheric conditions. It was suggested that the high degree of uncertainty in the coverage and drift of aerially applied *Bt* can be significantly reduced if the spray operator can react quickly to changing environmental conditions.

A VIEW FROM THE HOMELAND, EURASIA

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ABSTRACT

The gypsy moth, *Lymantria dispar* L., is a serious pest of deciduous trees over much of the northern temperate regions of the world. It likely originated in Eastern Asia, spread naturally to Europe, and from there was introduced into the United States in 1869. It undergoes periodic fluctuations in its population density both where it is native and introduced.

In Europe, the population fluctuations are fairly regular occurring at about eight-year intervals. A population in Yugoslavia, followed for 33 years, exhibited delayed density

dependence. There is good evidence that univoltine, host-specific tachinid parasitoids respond to, but lag behind, the increase in these populations. The parasitoids are very numerous during the collapse of outbreaks. Recently, gypsy moth populations have become more stable in Yugoslavia and widespread outbreaks have not occurred in the last 20 years. Concurrently, the browntail moth, *Euproctis chrysorrhoea*, has become a major defoliator of deciduous forests. There are several multivoltine parasites that attack both *L. dispar* and *E. chrysorrhoea.*

In Russia, egg mass densities are expressed as the number of egg masses per tree or as eggs per 100 shoots. In the European part of Russia, the population densities during an eruption, decline latent phase varied from 0.03 to 12 eggs per 100 shoots with a maximum per capita increase of three. In the Far East, the gypsy moth population went through an eruption-decline phase during a 10-year period and the number of egg masses per tree ranged from 0.06 to 10.57 and the net per capita rate of change varied from five to eight. In both regions, univoltine, host-specific parasitoids were the prominent mortality agents during the increase phase and virus disease dominated during the collapse.

The most striking difference of the numerical dynamics in Eurasia compared to North America is the regularity or predictability of the year-to-year changes in density. One year lag phase models can be fitted to the Eurasian data with an R^2 of 50-75%, whereas somewhere around 25% is typical with North American data sets. It is difficult to determine, though, if North American populations are inherently less stable than indigenous populations since the North American data are derived from much smaller sample units.

USDA FOREST SERVICE QUARANTINE LABORATORY

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ABSTRACT

The USDA Forest Service Quarantine Laboratory is a state-of-the-art facility for biological control research. It is certified to confine and colonize entomophagous and phytophagous arthropods and entomopathogens for biological control research. The area under quarantine is 3,100 square feet and is entered through a double air lock system equipped with light traps. Provision is made for personnel to change into outer garments and shoes or shoe covers that are worn only inside the facility. The internal and make-up air of the three negative pressure zones is passed through independent HEPA filters and air conditioning systems and is exhausted through 100-mesh screening. An automatic generator maintains the negative pressure system and several environmental chambers during power failure. Environmentally controlled chambers with the capacity to program temperature, humidity, and daylength provide space for rearing large numbers of arthropods. All insect handling involving the opening of containers is performed inside biological safety cabinets to contain the insects and protect workers' health. A pass-through autoclave ensures that all trash can be disposed of safely. In addition to normal security measures, the negative air pressure system, environmental chambers, and city power are monitored by a professional company, which alerts personnel of equipment failure, fire, and breach in building security.

This facility provides the opportunity to conduct research on biological control tactics to prevent the introduction of forest pests and to eliminate or manage those that are introduced. The current research at the facility involves genetic and developmental studies, research with host plants, behavior work, and microbial bioassays.

MODELLING HORIZONTAL TRANSMISSION OF MICROSPORIDIA IN GYPSY MOTH POPULATIONS

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ABSTRACT

The overall objective is to develop, test, and evaluate two models of within-season gypsy moth larval population dynamics and transmission of five species of microsporidia: *Microsporidium* spp. and *Endoreticulatus* spp. from Portugal, *Vairimorpha lymantriae* and *Vairimorpha* spp. from Czechoslovakia, and *Vairimorpha* spp. from Bulgaria. The emphasis is on non-outbreak populations in which other biocontrol agents are insignificant or absent. Two types of models are evaluated because simpler Anderson and May models used successfully for virus epizootiology by Foster *et al.* and Dwyer *et al.* may not be as helpful for understanding microsporidian epizootiology compared to more complex models. The complex model has age structure and explicitly defines space as a grid of 10 m^2 units of ground area that can be empty, occupied by a non-host, or occupied by a host tree. At each site, the complex model requires information about surface area occupied by gypsy moth larvae. When both sides of leaves are counted, published data for oak woodlands indicate that plant surface area for a 10 m^2 site with a host tree is 100 m^2, 10 m^2 for a site with host shrubs only, and zero for sites without hosts.

Horizontal transmission measured on pin oak branches will allow calibration of the two models. Sleeve cages enveloped the 20 healthy and five infectious larvae placed on each limb. The 50 cm long sleeve surrounded approximately 9,800 cm^2 of surface area. Thirty days later, approximately 10% of the susceptible larvae had become infected. Data collected in field plots in Maryland from 1986-1989 will be used to test the models.

RESULTS OF *BT* (FORAY 48B) BIOASSAYS ON NON-TARGET LEPIDOPTERA

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ABSTRACT

The larvae of 35 species of native, non-target Lepidoptera were evaluated in laboratory bioassays to determine their susceptibility to infection by *Bacillus thuringiensis* Berliner var. *kurstaki* (*Bt*). The species evaluated were: *Papilio glaucus* (Papilionidae), *Speyeria diana* (Nymphalidae), *Hemileuca maia*, *Antheraea polyphemus* and *Actias luna* (Saturniidae); *Phigalia titea*, *Euchlaena obtusaria*, *Ennomos magnaria*, *Lambdina fervidaria*, *Eutralepa clemetaria* and *Prochoerodes transversata* (Geometridae); *Catocala obscura*, *C. vidua*, *C. ilia*, *C. sordida*, *C. coccinata*, *C. praeclara*, *C. similis*, *C. lineela*, *Amphipyra pyramidoides*, *Lithophane petulca*, *L. grotei*, *Eupsilia vinulenta*, *Sericaglaea signata*, *Jodia rufago*, *Metaxaglaea semitaria*, *Chaetaglaea sericea*, *Sunira bicolorago*, *Xylotype capax*, *Eutolype rolandi*, *Psaphida resumens*, *Orthosia alurina*, *O. hibisci*, *Egira alternans*, and *Abagrotis alternata* (Noctuidae).

Appropriate host foliage was treated in a spray tower using a neat application of Foray 48B that approximated the field application of 36 BIU's per acre. Larvae of each species were evaluated using the instar in which they occur when *Bt* is applied aerially in southern New Jersey. Larvae were confined in sleeve cages on potted seedlings, and larval mortality was monitored daily for five days; survival to the pupal stage was also recorded. Significant mortality was recorded for 18 of the 35 species tested (including both butterfly species, all three saturniid moths, three of six geometrid moths, and 10 of 24 noctuid moths). For those species treated in the early (first to third) instar, over 80% (14 of 17) were very susceptible to *Bt* infection; only about 20% (4 of 18) of the species treated in later instars were susceptible. In addition to the above findings, our data also show interspecies differences in larval susceptibility in the noctuid genera *Catocala* and *Lithophane.*

Based on these data, we conclude that one should not generalize about the potential effect of *Bt* on non-target Lepidoptera, whether in terms of predicting susceptibility based on larval instar at the time of *Bt* application, or in terms of predicting susceptibility at the family, genus, or species level. It is clear from our findings that the susceptibility of non-target Lepidoptera to *Bt* must be dealt with on a species-to-species basis. This will be of particular concern where threatened or endangered Lepidoptera must be considered in management decisions concerning gypsy moth suppression or eradication.

MOLECULAR MARKERS FOR THE IDENTIFICATION OF GYPSY MOTH SPECIES

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ABSTRACT

The morphological differentiation between the Asian and European races of *L. dispar* male moths caught in pheromone traps is currently not possible. In addition, egg masses found aboard foreign vessels require rearing of the insect for proper identification. We have developed molecular markers for the differentiation between gypsy moth races and species. Currently there is available a mtDNA polymorphism for distinguishing between the Asian (AGM) and European (NAGM) gypsy moth as developed by Harrison and coworkers. We have constructed PCR primers based on *D. yabuka* and *G. mellonella* mtDNA COII and ATPase6 gene sequences to allow for amplification of the intergenic regions. An 800 bp product for NAGM and a 900 bp product for AGM was seen after analysis of the PCR reaction by agarose gel electrophoresis. Sequencing is presently in progress to verify the amplification product. Although mtDNA markers allow differentiation between the two *L. dispar* races, nuclear markers are more useful for identification of hybrids of the two races. PCR primers for the nuclear rRNA region were used to analyze for DNA restriction enzyme polymorphisms between the DNA products of AGM and NAGM. Three restrictions enzymes of the 23 tested allowed differentiation between the two races. Subsequent sequencing of the PCR product allowed construction of homologous primers which amplified a 500 bp DNA product from either race. Cleavage by one of three restriction enzymes allowed for differentiation between the two races. These molecular markers have been applied to 39 field samples and all were correctly identified. Additionally, a single hybrid was identified based on the restriction fragment pattern seen on ethidium bromide stained polyacrylamide gels. The constructed PCR primers also amplified a 500 bp segment of DNA from *L. mathura* and *L. monacha*. When these products were cleaved with the appropriate restriction enzyme, they can be distinguished from the patterns of either *L. dispar* race. Further analysis of other potential nuclear markers is in progress as well as the analysis of other Lymantria species.

FIELD EVALUATIONS OF GYPCHEK FORMULATIONS

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ABSTRACT

The integration of Gypchek into gypsy moth (*Lymantria dispar* L.) management programs has been slowed by the lack of a commercially available, ready-to-use product. Further, the current production and application cost for an acre-treatment [5 X 10^{11} occlusion bodies (OB) in 2 gallons per acre, twice] of the Forest Service manufactured product exceeds $30. With the objective of cost reduction, a low dosage, low volume Gypchek prescription (2 X 10^{11} OB in 0.5 gallons per acre, twice) was pilot-tested in Pennsylvania and Ontario in 1992. In Pennsylvania, three 200-acre blocks, each paired with a control area, were treated. Despite an area-wide viral epizootic, treatment effects were noted in all three blocks. T-tests of mean posttreatment egg mass counts between each treated block and its associated control were significant ($P < 0.001$, $P < 0.01$, $P < 0.03$). Similar tests of mean defoliation estimates were significant between each of two block-pairs ($P < 0.001$) and just shy of significance in the third ($P = 0.057$). These results and similar findings in Ontario are the basis for recommending the reduced dosage and volume prescription for operational use.

The Forest Service is cooperating with American Cyanamid Company in the development and evaluation of a commercial formulation of Gypchek and with Entotech, Inc., in the development and evaluation of a spray-adjuvant to replace the current tank mix. In 1992, products from both companies were field tested against the standard Gypchek tank mix. Clear treatment effects for all formulations were noted from early-larval mortality data and defoliation estimates. All experimental products protected foliage at least as well as the standard and plots treated with any formulation suffered significantly less defoliation ($P < 0.05$) than the controls. Virus-induced mortality was also significantly higher ($P < 0.05$) in treated plots than in the controls. The Forest Service will continue to cooperate closely with American Cyanamid and Entotech as they develop improved, ready-to-use formulations.

SEARCHING FOR ASIAN GYPSY MOTH USING MOLECULAR DIAGNOSTICS

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ABSTRACT

A new molecular diagnostics laboratory has been established within APHIS whose function is to develop molecular methods for agricultural pests that are difficult to identify. The laboratory's first problem was analyzing gypsy moth specimens trapped during the '92 field season for the presence of the Asian strain. The diagnostic method used, which is based on the work of Rick Harrison's group at Cornell University, involves indirectly looking for a particular nucleotide sequence within the mitochondrial DNA. A crude extract of each moth is prepared and used to amplify enzymatically (i.e. PCR) the COI locus within the mitochondrial genome. The DNA fragment generated during the PCR is analyzed for the presence or absence of a *Bam*HI restriction site. DNA produced using extracts from an Asian individual contain the site where DNA produced from an individual native to North America lack the restriction site. Thus, after digestion, Asian DNA will show a smaller molecular size than that from a native gypsy moth when analyzed by agarose gel electrophoresis. Out of nearly 2,000 specimens analyzed by the end of 1992, none possessed the Asian mitochondrial genotype (Table 1). A variety of limitations and improvements on the current technology were discussed. One limitation on using a genetic marker in the mitochondrial DNA is that theoretically only 50% of the F1 hybrids will be detected since mitochondrial DNA is maternally inherited.

Table 1. mtDNA diagnostics of gypsy moths trapped in the 1992 field season.

	EGM	**AGM**	**NR**
British Columbia	81	1 (1991)[1]	2
Oregon	47	0	0
Washington	430	1 (nt)	21
Connecticut	1,323		
AL, AK, AR, CA, IL, LA, MS, NJ, NY, TX	1-15 each		

EGM = European gypsy moth
AGM = Asian gypsy moth
NR = no result
nt = non-target specimen

[1] The single AGM from BC was actually trapped during the 1991 field season.

DIFLUBENZURON RESIDUES AND PERSISTENCE ON AN OAK FOREST FOLLOWING AERIAL APPLICATION

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ABSTRACT

A 50-acre forest block in central Pennsylvania was sprayed with Diflubenzuron (Dimilin 25W) at the rate of 0.5 oz. A.I. per acre. Leaf samples were collected from the upper and lower canopy, and understory of 27 oak trees within this block on the day of spray, May 29, 1991. Canopy leaves were also collected on May 31, June 10, July 29, and September 26, 1991. On the day of spray, Diflubenzuron residues on the upper canopy, lower canopy and understory averaged 70.95, 34.65, and 7.30 ng/cm^2, respectively. Differences between residues on all canopy levels were significant (experiment wide alpha = 0.05). Diflubenzuron residues, on the entire canopy, collected immediately after spray (May 29, 1991), averaged 49.72 ng/cm^2 (SE = 36.44). Diflubenzuron residues on canopy leaf samples collected 2, 12, 61, and 120 days post-spray averaged 11.11 (SE = 8.64), 11.41 (SE = 6.7), 7.86 (SE = 5.0), and 6.85 (SE = 4.60) ng/cm^2, respectively. Analysis of Diflubenzuron residues per gram of leaf material suggests that this toxin will be present in concentrations that are toxic to macrolepidopteran caterpillars 120 days post-spray.

RECENT HYBRIDIZATION STUDIES

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ABSTRACT

Preliminary studies indicated complete compatibility between the North American and the Asian strain of gypsy moth, *Lymantria dispar* L.. The Asian strain was colonized from egg masses collected on Russian vessels harbored near Vancouver, WA. The larvae were reared in the quarantine facility at the gypsy moth rearing facility, United States Department of Agriculture, Animal and Plant Health Inspection Service, located at the Otis Air National Guard Base, Cape Cod, MA. Adults from these eggs were crossed or outcrossed with adults from the standard laboratory New Jersey strain (NJSS) of the North American gypsy moth. The reproductive compatibility of progeny from these crosses was studied. When NJSS females were crossed with Asian, NJSS, or either hybrid, no difference was observed in the quantity of sperm in the spermatheca. However, when Asian or either hybrid female was mated, Asian males transferred more sperm than NJSS males. Spermathecae of Asian or hybrid females crossed with hybrid males contained quantities of sperm that were intermediate between those mated with Asian males and ones mated with NJSS males. Still, nearly all females contained an abundant supply of sperm regardless of male. Female pupae of the Asian strain were heavier than those of NJSS. Hybrid females were heavier than either parent, indicating overdominance. Similarly, egg mass weights were greater for hybrid females than those of either parent. Egg masses from Asian females were heavier than those of NJSS. For each female type, there was a highly significant correlation between pupal and egg mass weight ($r > 0.56$). When egg mass weights were analyzed by covariance analysis, using pupal weight as the covariate, least square means of eggs from NJSS were significantly greater than those from the Asian strain. Egg mass weights from hybrid females from NJSS fathers were heavier than those of any other female; least square means of eggs of females from the reciprocal cross were similar to those of Asian females.

ACCESSING GYPSY MOTH LIFE SYSTEM MODELS

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ABSTRACT

Describes interface management system for accessing Gypsy Moth Life System Models. The models include the full-sized Gypsy Moth Life System Model, the medium-sized Gypsy Moth Life System Model, the smaller Differential Equations Model, and the Stand Damage Model. The models simulate forest habitat and habitat suitability; gypsy moth population dynamics, trends, and defoliation levels; natural control through predators, parasites, and pathogens; and several control methods: direct suppression of gypsy moth and stand management alternatives. The interface provides a user-friendly means for accessing and controlling the models. The software allows the user to manipulate files, view and manage outputs, and edit input data. Management of gypsy moth data made it necessary to define structures to store data and provide the mechanisms to manipulate this information. The interface includes pull-down menus, pop-up windows, text editing with validation, color graphics, mouse support, dynamic memory management, and context-sensitive help. It is written in the C language for DOS microcomputers.

INTRODUCTION AND AUGMENTATIVE RELEASE OF GYPSY MOTH PARASITES:

A HISTORICAL REVIEW (1900-1980)

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ABSTRACT

Biological control is the regulation by natural enemies (parasites, predators, pathogens) of another organism's population density at a lower average than would otherwise occur. Applied biological control implies manipulation by man; the management of parasites can be divided into three categories: classical (import, release, and attempted establishment), augmentation (manipulation of the parasite), and conservation (manipulation of the environment). The goal is to maximize parasite stabilizing pressures in a wide range of environments and thereby reduce the number and severity of outbreaks.

The classical approach for gypsy moth has been one of the most massive programs in biological control history. From 1900 to 1980, approximately 78 species were sent to the ARS quarantine in the U.S. and subsequently approximately 53 species were shipped to cooperating agencies for initiation of laboratory colonies or release. Only 10 exotic species of parasites established due to numerous problems associated with rearing (e.g. lack of rearing techniques, inadequate taxonomic identification and keys, poor and variable host quality and quantity) and releasing (e.g. inadequate numbers, "laboratory" strains, lack of alternate/overwintering hosts, lack of host density and habitat requirements) the majority of species. Recommendations for future (1980+) importation and attempted establishment of exotic species included: (1) continue to explore in Asia, not Europe; (2) favor host specific or very polyphagous species; (3) modify rearing strategies; and (4) release large numbers in few areas.

The augmentation approach either as inundative releases (released individual regulate) or inoculative releases (progeny regulate subsequent generations) has been attempted with numerous species against artificial and natural gypsy moth populations. Also, the use of combinations of natural enemies (e.g. *Bt* and *Cotesia melanoscela*, NPV and *C. melanoscela*) has been attempted with some success. There is an obvious need for continued long-term intensive laboratory and field evaluations of individual parasite species as well as their complexes before effective utilization can be achieved for both established and exotic species. Information on dispersal, alternate host requirements, and host and niche preferences, should provide the foundation for effective manipulation of parasites.

SEQUENCE CHARACTERIZATION AND TEMPORAL EXPRESSION OF THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS ECDYSTERIOD UDP-GLUCOSYL TRANSFERASE (EGT) GENE: GENERATION OF AN EGT TRANSPLACEMENT VECTOR CONTAINING A B-GALACTOSIDASE GENE

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ABSTRACT

Biological control is an important weapon for the control of the gypsy moth (*Lymantria dispar*). The baculovirus, LdNPV, is one biological control agent which is being used to fight gypsy moths. Slow killing speed is one of the problems of LdNPV. The related baculovirus *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV) contains the ecdysteroid UDP-glucosyl transferase gene (EGT). This gene inactivates ecdysone, the larval molting hormone. Larvae which do not molt consume more food and survive viral infection longer than those which undergo molting. Enhanced killing speed and lower food consumption result when the EGT gene is knocked out of AcNPV (O'Reilly and Miller 1991). We have cloned, sequenced, and characterized a homologous gene from the gypsy moth virus. The gene is located at 80 map units on the viral genome. This gene encodes a 488 amino acid polypeptide. The gene has a 49% nucleotide identity with the AcNPV EGT gene (O'Reilly and Miller 1990). The encoded polypeptide has a 42% amino acid identity with the AcNPV protein. There are five regions of particularly high identity: from amino acids 19 to 57 (with respect to the AcNPV protein), 63% amino acid identity; from 92 to 108, 88% identity; from 135 to 192, 60% identity; from 201 to 268, 61% identity; and from 341 to 392, 59% identity. Transcription initiation occurs from a G residue 54 nucleotides upstream from the translation initiation site. There is a TATA box located 11 nucleotides upstream of the transcription start site. There is a polyadenylation signal 62 nucleotides downstream of the translational stop codon. A 1700 bp EGT RNA is first expressed at 16 hours post-infection and continues to be expressed at 72 hours post-infection, after the onset of polyhedrin synthesis. In contrast, the AcNPV gene is turned off when polyhedrin synthesis begins (O'Reilly and Miller 1990). We have also generated a knock out transplacement vector which replaces 872 nucleotides of the EGT coding sequence with B-galactosidase. The resulting gene encodes a fusion protein with the amino terminal 21 amino acids from EGT fused to the B-galactosidase protein. The fusion protein is expressed under control of the EGT promoter. Efforts to generate a recombinant LdNPV lacking a functional EGT gene are in progress.

O'Reilly and Miller. 1990. J. Virol. 64: 1321-1328.
O'Reilly and Miller. 1991. Bio/Technology 9: 1086-1089.

EFFECTS OF *BACILLUS THURINGIENSIS* AND GYPSY MOTH DEFOLIATION ON NON-TARGET LEPIDOPTERA IN AN EASTERN DECIDUOUS FOREST

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ABSTRACT

Impacts of *Bacillus thuringiensis* (*Bt*) and forest defoliation by gypsy moth (*Lymantria dispar* L.) larvae on native, non-target Lepidoptera were evaluated in eastern West Virginia from 1990 to 1992. Adult and larval Lepidoptera were collected at 24 20-ha plots, representing six replicates of four treatments: unsprayed without gypsy moths, unsprayed with gypsy moths, sprayed without gypsy moths, and sprayed with gypsy moths. Pre-treatment data were collected in 1990. In May 1991, *Bt* was applied to 12 plots at a dosage of 36 BIU/acre. Post-treatment data were collected in 1991 and 1992. *Bt* application reduced species richness and abundance of larval and adult non-target Lepidoptera. While effects of *Bt* application became evident among larval Lepidoptera in 1991 (the treatment year), effects among adults were not observed until the year following treatment (1992). Despite few plots receiving gypsy moth defoliation (none in 1990, two in 1991 and four in 1992), larval and adult richness and abundance were reduced at defoliation plots. Impacts were greatest among direct competitors of the gypsy moth, e.g. larvae on oaks, notodontids, and lasiocampids. Because larval and adult Lepidoptera are important prey items for many birds and bats, reductions in Lepidoptera abundance may adversely affect these vertebrate populations.

ISOZYMES AND GENETIC VARIABILITY

IN *COMPSILURA CONCINNATA* (DIPTERA: TACHINIDAE)

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ABSTRACT

The genetic patterns and processes in populations of introduced species reflect the amount of gene flow among interbreeding groups and the demes range. Soon after the population outbreaks of Lymantriid pests in the Northeast in the early 1900's, various parasitoids were introduced from Europe to mitigate the widespread defoliation. Of the tachinids introduced at the time, none seems to have naturally spread farther in the Northeast than *Compsilura concinnata* Meigen. This study continues work on the population genetics of this tachinid in the Northeast.

Horizontal zone electrophoresis of soluble proteins in a laboratory strain of *C. concinnata* provided baseline data on their isozyme patterns. Among the 35 enzyme systems analyzed, 72% gave adequate resolution for further analysis. Putative allele frequencies at each presumptive locus were calculated with the isozyme information. The frequency data was then used in calculating gene diversity estimates for the sample population. Polymorphism occurred in four of the proteins sampled, and the average heterozygosity ($\hat{H}$=0.049) was low. Although the later measure is less subjective, both estimates probably reflect a loss in variability through laboratory inbreeding. Currently work on the heritability of these isozyme systems is underway.

The pattern of isozymes in *C. concinnata* populations may provide useful markers with which to delimit their spread in Northeastern pest-host populations. When these patterns are heritable, information about the variability within and between individuals can be used indirectly to estimate deme size. Research on the population genetics of forest insects contributes in understanding evolutionary biology, particularly in the application of ecological genetics toward the reduction of forest insect outbreaks.

CHEMICAL BASES OF THE DIFFERENTIAL IMPACT OF RED AND CHESTNUT OAK LEAVES ON GYPSY MOTH GROWTH AND SUSCEPTIBILITY TO MICROBIAL PESTICIDES

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ABSTRACT

Gypsy moth larvae eat more, grow larger, and are more susceptible to NPV, Gypchek, and Thuricide on chestnut oak (*Quercus prinus* L.) than on red oak (*Q. rubra* L.). Several differences between the two species in foliar chemistry may explain this differential host plant impact. We have found that chestnut oak leaves lack some hydrolyzable tannin (HT) polymer size classes that are common in red oak leaves. Chestnut oak leaves also lack gallotannins, containing instead only ellagitannins, while red oak produces both types. Finally, red oak leaves contain high levels of polyphenoloxidase (PPO) activity, while chestnut oak has none. Thuricide is less effective on individual red oak trees having high PPO activity. We hypothesize that the combined differences in tannin type, polymer size, and PPO activity are responsible for differences between the oaks in their impact on gypsy moth growth and resistance to microbial pesticides.

MEDIUM-SIZE MODEL OF GYPSY MOTH:

A TRADE-OFF BETWEEN SIMPLICITY AND BIOLOGICAL INTERPRETATION

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ABSTRACT

The goal was to develop a model that works much faster than the full Gypsy Moth Life System Model (GMLSM) and at the same time keeps as many biological details in it as possible. In the model, all ecological processes related to gypsy moth are stage-specific. Five stages are considered in the life cycle: eggs, small larvae (1st - 3rd instars), big larvae (4th - 6th instars), pupae, and adults. Generation k-value (negative logarithm of survival) is estimated as a sum of k-values for all mortality processes at all stages. Ecological processes related to parasitism, predation, starvation, and infection by pathogens are density dependent. Population dynamics of three parasite guilds (*Cotesia melanoscela*, tachinids, and pupal parasites) are simulated as well. The medium-size gypsy moth model is linked with the Stand submodel and with the graphic interface which are the same as in the full GMLSM.

Advantages of the medium-size model as compared with the full GMLSM are the following: 1) it simulates long-term population dynamics about 100 times faster, 2) it is better for optimization of long-term population management strategy, 3) it is better for explanation of population dynamics (using sensitivity and stability analysis), and 4) it can be used in spatio-temporal models. Disadvantages of the model are: 1) many model parameters are aggregated, and thus, they have no direct biological interpretation, and 2) phenology of populations is not considered. Thus, it is impossible to simulate phenology-related effects like coincidence of bud break and gypsy moth egg hatch, or the effects of spray timing. Preliminary results of gypsy moth population management simulation are the following: release of partially sterile males (with inherited sterility) is the most efficient among all tested methods of gypsy moth genetical control. Pheromone traps cannot prevent gypsy moth outbreaks but they can slow the population growth rate and postpone outbreaks by three to four years. Eradication of isolated gypsy moth populations in a favorable environment takes from 6 to 11 years; best results are obtained combining genetical control or mating disruption with spraying *Bt* or Dimilin. Gypsy moth population does not grow after *Bt* spray as fast as after Dimilin spray because it does not kill natural enemies. Thus, *Bt* can be applied not as often as Dimilin.

EFFECTS OF ASPEN DEFENSIVE CHEMISTRY ON EFFICACY OF *BT* AGAINST GYPSY MOTHS. II. ULTRASTRUCTURAL EFFECTS ON MIDGUT

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ABSTRACT

The mode of action of *Bt* in lepidopterous insects has been extensively studied, but very little is known about how plant allelochemicals might affect the susceptibility of larvae to *Bt*. Preliminary work indicated that gypsy moth larvae feeding on artificial diet containing aspen phenolic glycoside extracts and *Bt* (Foray 48B) experience significantly higher mortality than those fed each component separately. Since both *Bt* and phenolic compounds are reported to cause lesions in midgut, we hypothesized that the effect of these two compounds was additive, and that the midguts of larvae feeding on combined *Bt*/phenolic glycoside diets would show evidence of more extensive degenerative lesions than would the midguts of larvae from other test groups.

Foray 48B and phenolic glycoside extracts were mixed into artificial diet at levels of 0, 100, and 200 IU/ml and 0, 2, and 4% wet weight, respectively. Newly molted second instar larvae were allowed to feed freely on the diets, and were dissected at intervals of 1, 2, 4, 8, 12, 16, 24, 48, 72, 96, and 120 hours after initial ingestion of diet. Midgut tissue was fixed, dehydrated, and either embedded or critical point dried. Tissue was examined using light microscopy, scanning electron microscopy, and transmission electron microscopy.

Midgut columnar cells from larvae feeding on *Bt* diets exhibited early pathological changes similar to those reported in other Lepidoptera. These include swelling and loss of apical microvilli, swelling of apical cytoplasm, budding of cytoplasmic matrix, and rupture of the plasma membrane. However, 8 to 16 hours post-ingestion, there is evidence of regenerative cells being activated, and some cell repair and regeneration has taken place by 24 hours post-ingestion. But by 48 hours post-ingestion, continued degradative changes are observed.

Midgut cells from larvae feeding on phenolic glycoside diets exhibited very different pathology. Goblet cells become gradually depleted and columnar cells appear to be metabolically inactive. The cells become very dense and basophilic, but there is no loss of microvilli, no swelling, and no early evidence of lesions. Regenerative cells, when observed, show no evidence of differentiation.

Larvae feeding on the combined *Bt*/phenolic glycoside diets ultimately experienced the most extensive pathology. Early changes include swelling of the apical cytoplasm and extrusion of cytoplasmic constituents into the lumen, but there seems to be no effect on the brush border. Microvilli are in abundance, and have good microfibrillar structure. Although extensive damage occurs to columnar cells, there is little evidence of any cell repair or regeneration. By 24 hours post-ingestion, cells appear disorganized and there are no signs of new growth. By 96 hours post-ingestion, surviving larvae have few, if any, midgut cells remaining. The midgut is largely comprised of a muscle layer surrounding a basement membrane. The toxic effects of *Bt*, combined with an apparent lack of cell regeneration, may account for the higher mortality experienced by larvae feeding on the combination diets.

VERTEBRATE AND INVERTEBRATE PREDATORS: LOOKING BACK AND LOOKING AHEAD AT GYPSY MOTH PREDATION RESEARCH

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ABSTRACT

Predation is a major suppressive force on natural insect populations. Most biologists believe it to be the third most important biotic factor affecting natural communities with food and intraspecific competition being first and second. Predation of gypsy moth pupae by small mammals has been found to be the main mortality agent when populations are at low densities. Because the magnitude of predation depends on a large number of variables that interact with one another, it is difficult to generalize about the role of predation on a particular gypsy moth population. In spite of nearly a century of research, the actual role of predation has not yet been fully ascertained. The objectives of this paper are twofold:first, to briefly synthesize predation research (Looking Back), and second, to suggest future research (Looking Ahead).

Looking Back

Avian Predators

In 1896, Forbush and Fernald (1896) were the first to write about the predators of the gypsy moth. They considered birds the primary predators of gypsy moth. Spiders, invertebrates, and amphibians also were mentioned as predators; however, small mammals were completely ignored. Campbell and Sloan (1977) concluded that predation by birds on instars IV-VI was important to year-to-year stability in low density gypsy moth populations based upon higher survival of larvae that had been protected from birds even though results from their study appeared contrary. More recent studies have shown that late instar gypsy moth larvae (III-VI) are only a minor food item among those bird species known to eat them (Smith 1985, Cooper 1988). Based on results from an aviary study, Whelan *et al.* (1989) suggested that predation on early instars (I & II) by wood warblers migrating in large numbers through eastern deciduous forests may be an important source of mortality. Predation of gypsy moth egg masses by birds has been reported by Cooper and Smith (1993). Their results suggest this source of mortality is quite variable with the effect of snow cover on the availability of alternative foods a possible cause of this variation. Overall research findings to date suggest that avian predation impact is minimal and it is doubtful it plays a major role in maintaining low density gypsy moth populations in North America. This is in marked contrast to Eurasian literature where birds are thought to play a major role in the regulation of gypsy moth populations (Furuta and Koizumi 1975, Higashiura 1989).

Small Mammal Predators

Bess *et al.* (1947) were the first to suggest that predation by small mammals was important to gypsy moth population dynamics in North America. It is now generally accepted that when gypsy moth rest in the litter, survival is low due to ground-foraging generalist vertebrate predators, especially *Peromyscus leucopus*, the white-footed mouse. Campbell and Sloan (1976) suggested that the behavior of resting in the litter evolved in Europe to avoid other natural enemies. Further studies by Campbell *et al.* (1975, 1977) showed that predation rates on pupae were positively correlated with gypsy moth population density. These findings support the hypothesis that predation by small mammals is responsible for the regulation of low density gypsy moth populations. In our studies at Bryant Mtn., VT, and on Cape Cod, MA, year-to-year differences in survivorship of pupae deployed in selected microhabitats (litter, bole, and bark flaps) were highly correlated with *P. leucopus* density. Earlier, it had been shown (Smith 1985) that density and diversity of small mammals differed considerably among forest stands that varied in susceptibility to defoliation by gypsy moth. It was hypothesized that predators at these sites may have a reduced affect on gypsy moth populations. Our studies have shown, however, that in spite of small mammal community differences between those stands in any particular year, similar densities of mice occurred on susceptible and resistant sites. These numbers varied markedly between years, however, with concomitant changes in pupal survivorship (Elkinton *et al.* 1989). This provided important information which suggests that differences in mouse numbers were the key factor to change in survivorship rather than differences between habitats in the species of ground-foraging vertebrates preying on gypsy moth. From 1985 to 1988, we observed a dramatic decline in *P. leucopus* density on our plots in both Massachusetts and Vermont and a concomitant increase in gypsy moth density. The occurrence of such simultaneous trends at widely spaced sites suggests that regional changes in *P. leucopus* density may account for the region-wide onset of outbreak phase populations of gypsy moth. Within-year differences in survivorship of pupae (when *P. leucopus* densities were similar) resulted from differences in vertical stratification of cover which affected the foraging behavior of small mammals and the availability of more palatable alternative foods, especially low-bush blueberry (*Vaccinium* spp.), which reduces the consumption of gypsy moth in their diet.

Invertebrate Predators

In comparison to the vertebrates, relatively little is known regarding the role of invertebrate predators of the gypsy moth. *Calosoma sycophanta* was imported from central Europe and released (1906-1926) in New England. Additional releases in the 1960s and 1970s of adults and larvae collected from infestations in the Northeast helped to extend the range of this predator. Some research continues on this carabid; however, there have been no studies of *C. sycophanta* predation on low-density gypsy moth populations. Because of its high prey density-dependency, the impact of this predator on gypsy moth dynamics is thought to be minor. Weseloh (1988) quantified predation by invertebrates on tethered larvae placed at different heights in trees. He found that most predation by invertebrates occurred in the litter. Smith (1989), working with stocked cohorts of pupae placed in three locations (litter, bole, and bark flap), did not show much spatial or temporal variation between invertebrate predation in resistant and susceptible zones, suggesting that this mortality is not a critical factor. Predation by invertebrates (percent total mortality of pupae in litter, bole, and bark flap) in the resistant and susceptible stands studied in Vermont for 1985-87 was 0.35, 13.07, and 8.37; and 3.82, 15.63, and 9.17, respectively. Averaged over the three-year period, invertebrates, predominantly ants (Formicidae) accounted for 8.02% of the total mortality. In contrast, Cook *et al.* (1993), studying predation of gypsy moth pupae in three selected southeastern habitats in Virginia and North Carolina, reported percent mortality rates attributed to invertebrates of 0.8 (mountains), 11.8 (Piedmont), and 22.2 (coastal plain). These rates were inversely proportional to observed mortality by vertebrates in those sites, possibly suggesting that invertebrate predators in the Southeast may exert some compensatory mortality.

Looking Ahead

Predation is a major source of mortality of low-density gypsy moth populations. Our ability to understand predator-ecosystem interrelationships will largely determine the effectiveness and ecological compatibility of future forest pest management scenarios. Predation pressure is variable. Outbreaks of gypsy moth remind us that predation can fail to maintain the low density phase consistently. Future studies must address the factors that cause predator failure in order to enhance our ability to maintain populations at sparse levels. Predation fails to maintain gypsy moth populations or becomes ineffective in the following ways: (1) loss of predators, because small mammal (*P. leucopus*) populations are food regulated--their population fluctuations are positively correlated to mast (acorn) abundance; (2) increased abundance and availability of preferred alternative foods that will cause a significant reduction in predation rate; and (3) reduced palatability of gypsy moth (larvae and pupae) due to virus or fungi that would elicit a learned aversion behavior whereby even unaffected gypsy moth would be avoided.

Other priority areas of future predation research necessary to ascertain the actual role of predators include:

1. Silvicultural interrelationships influencing predation impact
2. Ecosystem impact associations (i.e., habitat susceptibility classifications)
3. IPM compatibility relationships with other treatments or mortality agents
4. Asian gypsy moth/predator interactions

In conclusion, a few important points to remember regarding predators:

- Vertebrate predator density is independent of gypsy moth density
- Effective predation is dependent on ecosystem interrelationships
- Low predator density can result in a region-wide onset of population buildup
- Understanding the ecological role of predators will allow more effective management schemes
- Ecosystem management/conservation is our mission
- Gypsy moth managers can ill afford to ignore the role of predators

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COMPARISON OF METHODS FOR QUANTIFYING THE EFFICACY OF AERIAL APPLICATIONS OF *BACILLUS THURINGIENSIS* AND DIFLUBENZURON AGAINST THE GYPSY MOTH

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ABSTRACT

Research efforts to determine the most efficacious formulations and use patterns of insecticides against the gypsy moth are frequently hindered by the natural collapse of populations in untreated control plots. While *Bacillus thuringiensis* (*Bt*) and diflubenzuron are generally applied against early instars, high rates of mortality from naturally occurring gypsy moth nucleopolyhedrosis virus (NPV) often does not occur until later in the season. Unfortunately, measurements of defoliation and egg mass reduction, which are frequently used to assess treatment effectiveness, are strongly influenced by late instars. Thus, the direct effects of these insecticides on early instars may not be evident from defoliation and egg mass data. While defoliation and egg mass density data are appropriate measures of the effectiveness of a management program, they may not be the most sensitive measures of the effectiveness of individual tactics and formulations. This study is an attempt to evaluate different methods of assessing treatment effects on gypsy moth mortality.

The study was conducted at four separate wooded parcels located in Talbot County, MD. Three of the parcels received three treatments (diflubenzuron, 1 *Bt* application, and 2 *Bt* applications). The diflubenzuron treatment was omitted from the fourth site. An untreated control plot was included at each site. *Bt* was applied undiluted at 30 BIU/acre per application (Foray™ 48B, 23 8004 nozzles) using an Ag Cat (Al Johnson, Magnolia, DE). Diflubenzuron was applied at 0.025 pounds [AI]/acre (Dimilin™ 4L, 36 8004 nozzles) using the same aircraft. The diflubenzuron and first application of *Bt* were applied on May 11, and the second application of *Bt* was made on May 14. Larvae were predominantly second instars during both applications.

Larval population density was estimated in each plot using the frass drop/frass yield method. Frass drop over a 12- to 16-hour sampling period was estimated from 100 10-liter buckets in random locations beneath oak canopy in each plot. Frass yield (number of frass pellets per larva) was determined at each site during the sampling period by holding 50 larvae individually with one to two oak leaves in 177-ml plastic cups with cardboard lids. The mean

density of larvae per plot was estimated using the equation: $\text{Density} = C \blacksquare (x_d/xy)$, where $C = 1/(\text{area sampled by each frass sampling device})$, x_d = mean drop (frass/sampling device), and xy = mean yield (frass/larva).

The first frass sample, taken 20 days after the first and 17 days after the second application, indicated that population density under all treatments was reduced by more than 50%. Population reduction was greater than 80% in plots receiving diflubenzuron and two applications of *Bt*. The second frass sample, taken 27 days after the first application, showed similar results, although control population density was about 25% lower than at the previous sample. Mortality, apparently due to NPV, was evident in the higher density sites at this time. By the third sample, taken 40 days after the first application, control populations had collapsed, obscuring most of the treatment effects, except that populations treated with diflubenzuron had been reduced to nearly zero.

Defoliation was about 15% higher in the untreated plots, but there were no differences among the insecticide treatments. The relative differences in the number of pupae beneath burlap that survived to adult eclosion under each treatment were qualitatively similar to the differences in frass production. However, variability was high enough among the sites that the differences were not statistically significant. Relative differences in number of gypsy moth egg masses among the treatments were qualitatively similar to differences in frass production, but these differences were not statistically significant. The ability to detect differences was poor because of the high levels of variability associated with egg mass counts. Reduction from pre- to post-season egg mass density was over 80% in the control plots, indicating a substantial natural reduction in population density. No differences occurred among the treatments.

The direct estimation of larval gypsy moth populations using frass collections was the most sensitive method of quantifying treatment effects. Numerical differences among the treatments were greatest at the first sample date, which was 20 days after the diflubenzuron and the first *Bt* application and 17 days after the second *Bt* application. Differences among the treatments were smaller one week later, but the same statistical relationships were detected. By the time of the third sample, which occurred 40 days after the first *Bt* application, the larval density in the control plots had dropped to below those in some of the treatment plots, and treatment effects were no longer evident for all but the diflubenzuron treatment. Based on these results, it appears that a frass sample to estimate larval density in plots treated with *Bt* or diflubenzuron should be conducted within 20 days of the treatment.

EFFECTS OF GYPSY MOTH-CAUSED TREE MORTALITY ON BIRD HABITAT

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ABSTRACT

Forty two 4.9-ha fixed plots were established by the West Virginia University Division of Forestry on Sleepy Creek Public Hunting and Fishing Area in eastern West Virginia before an initial gypsy moth outbreak, which occurred the fourth and fifth year of this eight-year (1984-1991) study. High canopy cover (≥12 m) declined sharply, while low canopy cover (3-5.9 m) increased following the outbreak. Average percent live basal area decreased from 87% to 57%. Percent shrub cover averaged 40% higher after the outbreak, while shrub species richness increased 24%. Of 32 shrub species, 18 increased while four decreased in cover. Defoliation and its effects on forest vegetation and structure were unevenly distributed. Eleven habitat variables that most clearly reflected these changes were used to develop a gypsy moth impact scoring model. Based on a cumulative score, plots were classified into one of three impact classes. High impact plots were concentrated in more mesic areas, while low impact plots were primarily on ridgetops or west facing slopes.

Overall bird density increased from 284 to 337 birds/40 ha. This increase was limited to moderate and low impact plots. Species richness increased from 19 to 23 birds per plot. Among guilds or other groups, declines were limited to tree nesters and flycatchers on high impact plots. Increases in low shrub and ground nesters, cavity nesters, low shrub and ground foragers, bark foragers, forest edge species, short-distance migrants, year-round residents, and woodpeckers were widespread but most pronounced on moderate impact plots. Eastern Wood-pewees, Acadian Flycatchers, and Blue-gray Gnatcatchers showed the strongest declines, particularly on high-impact plots, where Yellow-throated Vireos and Red-eyed Vireos also declined. Population increases were observed throughout the study area, but were generally most pronounced on moderate impact plots and least pronounced on low impact plots. The species with the strongest increases were: Rufous-sided Towhee, Carolina Wren, Indigo Bunting, Blue Jay, Rose-breasted Grosbeak, Northern Cardinal, Red-bellied Woodpecker, Tufted Titmouse, Mourning Dove, Brown Creeper, Chipping Sparrow, Northern Flicker, Black-capped Chickadee, Hooded Warbler, Ovenbird, White-breasted Nuthatch, Black-and-white Warbler, Downy Woodpecker, and Hairy Woodpecker. Changes in Eastern Wood-pewee and Ovenbird populations could more easily be accounted for by regional population trends than by gypsy moth-mediated habitat changes.

Dead wood, standing and down, has historically provided critical habitat for many species of birds and other animals in natural areas. This element is absent or scarce in most second-

growth forests of the eastern United States today. Defoliation also leads to dense vegetation in lower forest strata, providing habitat that is scarce in closed-canopy forests. Unlike the abundant forest edge habitat found in fragmented landscapes, habitat within gypsy moth infested forests remains relatively protected from negative edge effects such as nest predation and brood parasitism. Allowing limited defoliation would likely benefit non-game forest bird populations, while intensive gypsy moth suppression would not.

GYPSES: CURRENT STATUS AND FUTURE PLANS FOR THE 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 in cooperation with the Northeastern Area, State & Private Forestry, other Forest Service units, and several universities (West Virginia University, Virginia Polytechnic Institute & State University, and Penn State) and local governments (Prince William County, VA). The gypsy moth is an important component of ecosystems in the eastern hardwood forests of the U.S. Decision making for gypsy moth control needs to consider many factors to produce the best protection with the least intrusion on the ecosystem. Improvement of decisions involves more incorporation of forest type information, topographic data, and insect development models than is generally the case in gypsy moth control programs. GypsES is now in initial testing on one district of the Monongahela National Forest and on one county gypsy moth control program in Virginia. Further development is needed to include improved models of forest stand development in the presence of gypsy moth but without suppression activity (to provide comparisons of what might happen without suppression) and to adapt the system to areas where elimination of populations is the major goal rather than simply suppression of defoliation (outside the generally infested area).

GypsES provides decision support to gypsy moth managers by identifying areas of concern, recommending areas to monitor for gypsy moth, recommending areas to consider for suppression, and producing maps and tabular summaries of data. The key element that makes it useful to managers is the way that it presents many different pieces of information in a graphical form so that the decision of whether to treat an area may be made with full knowledge of alternatives and their consequences.

There are six major components of the GypsES system in different stages of completion. The hazard rating component is the most developed portion. It uses forest type and/or stand data to classify susceptibility to defoliation, information on stand conditions and disturbance history to estimate vulnerability to damage, management priorities for different parcels to help determine hazard from gypsy moth, and insect population information to determine current risk for use in suppression decisions.

The Monitoring Components use hazard rating information to recommend locations to sample for gypsy moth, user's specifications to recommend egg mass and pheromone trap sampling design and procedures, and elevation and temperature data to predict insect development for use in suppression timing decisions. 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. The Geographic Information System provides an underlying system for management of spatial data, capability to view and print customized maps of areas of interest, and advice on the quality of information needed or produced by the system. The Database Management Component links data from all components in the system, enables generation of reports, and provides historical records for program monitoring. The Simulation Component is a new addition to the system, and will allow a user to obtain estimates of future consequences of different treatment decisions or the relative effects of different levels of infestation.

The Development Plan for 1993 includes emphasis on completion of the database functions, integration of hazard rating into treatment decisions by recommending treatment areas, and simulating outcomes of different decisions. The pheromone system will be redesigned to meet eradication needs outside currently infested areas.

RESPONSE OF BLACK BEARS TO GYPSY MOTH INFESTATION IN SHENANDOAH NATIONAL PARK, VIRGINIA

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ABSTRACT

During 1986-1989, more than 27,000 ha of Shenandoah National Park (SNP) were defoliated by the gypsy moth (*Lymantria dispar*), inflicting widespread habitat alteration and hard mast (acorn) failures. No one has demonstrated the relationships between such defoliation, the resultant mast/cover losses, and wildlife. During this time, 54 radio-collared black bears (*Ursus americanus*) were monitored to determine the impact of gypsy moth infestation on the SNP bear population. Demographic parameters, habitat use, food habits, denning ecology, and the effects of defoliation on bear habitat were examined and compared with similar data collected prior to defoliation (1982-1985). Cub production and survival did not differ between predefoliation and defoliation periods. Likewise, adult ($\geq$ 3 years) and subadult survival rates were similar before and during infestation and, thus, were not affected by defoliation. Bears exhibited different habitat preferences in all seasons between predefoliation and defoliation periods, but did not avoid defoliated habitat despite complete overstory cover loss and increased maximum daily temperatures of 4.8 C during peak defoliation. Prior to infestation, bears preferred oak habitat types in the fall and utilized acorns as their primary fall food. During defoliation, in response to a 96% reduction in acorn production, oak habitat types were used in proportion to their availability and bears switched to soft mast fruits of grape (*Vitis* spp.), pokeweed (*Phytolacca americana*), and spicebush (*Lindera benzoin*) as their primary fall foods . Although acorns are one of the highest quality fall food items available in SNP, analysis of estimated nutritional quality (% crude protein, % crude fiber, and % crude fat) of composite seasonal diets before and during defoliation indicated no decline in nutrient composition. Seventy-one percent of bear dens were in tree cavities, primarily in living oaks (mean DBH = 98 cm). Gypsy moth-induced mortality of tree dens was high, and by the end of the study 54% of living oaks used as dens were dead. The long-term impact of this may be a reduction in the availability of den sites with natural replacement of den structures potentially requiring 50 years.

PRELIMINARY RESULTS OF *BT* FIELD STUDIES IN ROCKBRIDGE COUNTY, VIRGINIA

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ABSTRACT

Effects of *Bacillus thuringiensis* on the immature stages of non-target Lepidoptera were studied in the Goshen Wildlife Management Area, Rockbridge County, VA. Five paired 50-acre plots were established in the Guys Run area; one plot from each pair was sprayed with *Bt* (Foray 48B, 36 BIU's per acre) on 14 May, 1992.

Three foliage samples were collected: the first sample (pre-treatment) was collected two to three days prior to *Bt* application. The second two collections (post-treatment) were taken six days and 12 days following *Bt* application. Foliage samples were clipped from three levels of the forest [canopy (scarlet oak), subcanopy (scarlet oak), and understory (blueberry)] in each of the 10 plots. Approximately 150 branch tips were secured from each layer in a plot for a total of 4,500 tips from each of the three forest layers in the 10 plots on a given sampling date. Tips were then visually inspected for all sawfly and lepidopteran larvae. Additional macrolepidopteran larvae were obtained by securing burlap bands to boles of scarlet and chestnut oak at approximately chest height along 20-tree transects in each of the 10 plots. Burlap collections were made two to three times a week following *Bt* application until the end of June. Each larva was placed in a separate container and sent to the University of Connecticut where it was assigned an individual number and reared.

A total of 12,312 larvae were sent from Goshen to the rearing facility at the University of Connecticut. Foliage samples included approximately 483 (4.3%) Macrolepidoptera, 9,655 (85.9%) Microlepidoptera, and 1,097 (9.8%) sawfly larvae; burlap samples included approximately 691 (64.2%) Macrolepidoptera, 153 (14.2%) Microlepidoptera, and 233 (21.6%) sawfly larvae. As of January, 1993, 49 species representing six families of Microlepidoptera and 39 species representing seven families of Macrolepidoptera had been reared to adults. Excluding parasite emergences, 3,413 (27.72%) of all larval collections have produced adults: 1,278 (37.4%) adults issuing from treatment plots and 2,135 (62.6%) from control plots. As many as 15 additional species are expected to eclose in the spring of 1993.

Treatment effects were noted for microlepidopterans and macrolepidopterans. Sixteen out of 19 of the most common taxa decreased in relative abundance in the foliar samples following treatment, 12 of which were micros. Sample sizes on foliage may turn out to be too small to demonstrate statistically significant treatment effects for individual species using nested ANOVA's which employ all sampling levels in the foliar collections. Eleven of the 12 most common macrolepidopteran taxa under burlap decreased in relative abundance following treatment; four of these appeared to be severely impacted: Malacosoma disstria (Lasiocampidae), misc. combined Geometridae (Geometridae), Orthosia rubescens (Noctuidae), and Satyrium calanus (Lycaenidae).

ASIAN GYPSY MOTH RESEARCH AND DEVELOPMENT PROGRAM

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ABSTRACT

In response to the introduction of the Asian race of gypsy moth (AGM) into North America in 1991, a research/regulatory needs planning meeting was held November 19-20, 1991 in Windsor Locks, CT. The urgency of the problem, potential economic and environmental impacts, and the paucity of information on behavior and biological differences between the European gypsy moth (EGM) and AGM necessitated an immediate research response. Some 30 scientists and regulatory personnel from the United States and Canada identified the following high priority research objectives:

Detection and Delimitation
Prediction of Egg Hatch
Susceptibility of Host Plants and Forests
Diagnostic Methods to Identify the Asian Gypsy Moth
Consequences of Asian/European Hybridization
Preventing AGM from Entering North America
Eradication of AGM in North America

During 1992, research initiated on AGM included:

Pesticides: Bioassays conducted in quarantine facilities (N. Dubois, USDA-FS and W. McLane, USDA-APHIS) confirmed that *Bt* was highly effective in controlling AGM. In fact, their studies demonstrated that AGM was more susceptible to *Bt* than two EGM laboratory

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AGM. Results of quarantine rearings demonstrate that in AGM x EGM reciprocal crosses sperm transfer occurs (D. Prasher, USDA-APHIS), no apparent incompatibility exists, and viable progeny result (M. Keena, USDA-FS, V. Mastro, USDA-APHIS, and L. Humble, Forestry Canada). Backcrosses are underway and, as with the original races, development (M.Keena, USDA-FS and V. Mastro, USDA-APHIS) and flight retention and propensity (W. Wallner, USDA-FS and R. Cardé, University of Massachusetts) are being determined.

Flight Behavior: The capacity for directed, long-distance flight by AGM females portends more rapid population spread and makes current detection and delimitation techniques inadequate. Thus, understanding female flight propensity, distance and proportionality within populations is being investigated (R. Cardé, University of Massachusetts and W. Wallner, USDA-FS). Modification of light spectral patterns of lights in ports and on ships to inhibit female attractancy to them was tested in the Russian Far East and is feasible and under development (W. Wallner, USDA-FS and R. Levin, GTE/Sylvania).

Pheromone trials in the Russian Far East, where a variety of concentrations and mixes of disparlure were tested, indicated that the methodology employed for EGM was comparable (B. Leonhardt, USDA-ARS, R. Cardé, University of Massachusetts, and V. Mastro, USDA-APHIS). The addition of an antennally active component isolated from female AGM abdominal tips significantly reduced male attractancy to both plus and racemic disparlure in the Russian Far East but not for EGM in Canada (G. Gries and J. Borden, Simon Fraser University). While AGM male pheromone attractancy was similar to EGM, the periodicity of male activity differed; AGM males were active from noon to 6:00 p.m. and, unlike EGM, from 11:00 p.m. to 3:00 a.m. the next morning.

The eradication program against AGM in both the United States and Canada appears to have been very successful. However, complacency should not dictate that continuing research be forestalled. The fact is, there is a threat of reintroduction, possibility of new detections in eradication areas, and high probability that other pest Lymantriids (nun moth and pink gypsy moth) with similar habits to AGM also may be introduced.

SECONDARY ORGANISMS AND OAK MORTALITY AFTER DEFOLIATION BY THE GYPSY MOTH

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ABSTRACT

Mortality of trees after defoliation is quite variable and difficult to predict. Secondary organisms play a major role in tree mortality but little is known about their variability and its relationship to mortality. These studies were designed to 1) determine the role of *Armillaria* species and the twolined chestnut borer (2LCB) (*Agrilus bilineatus* Web) in oak (*Quercus* spp.) mortality, 2) to evaluate the effects of thinning and defoliation on the population dynamics of these two secondary organisms, and 3) determine the relationship of the variability of these two organisms to subsequent mortality in mixed oak stands in West Virginia defoliated or not defoliated by the gypsy moth (*Lymantria dispar* L.). Stands were thinned in 1989 and some were defoliated heavily by the gypsy moth in 1990 and 1991. Defoliation in 1992 in these stands was scarce to light.

Preliminary results based on sticky band catches indicate a marked increase in population levels of 2LCB from 1989 to 1991 especially in the thinned stands. In all stands, catches in 1991 on trees with poor crowns were higher than on trees with good crowns. Catches also were higher in 1991 in stands that were defoliated heavily in 1990. Effects of these two parameters on *Armillaria* inoculum (rhizomorphs) will be assessed in 1994. Mortality occurred in 1991 in 11 of the 16 stands but was high only in those stands that were heavily defoliated in 1990 and or 1991. In 1991, 121 trees died and 96 trees died in 1992. Of the 121 trees autopsied in 1991, 82 were colonized by *Armillaria*, 36 lightly and 46 moderately to heavily. Most trees (50) were colonized on both the root collar and lateral roots; nine trees were colonized only on the root collar and 23 trees only on the lateral roots. All but seven of the 121 dead trees were infested by the 2LCB: 33 lightly, 30 moderately, 39 heavily, and 11 very heavily. All 39 trees that were not colonized by *Armillaria* were infested moderately to heavily by 2LCB. There was no relationship of rhizomorph weight and tree mortality on a plot or stand basis. Likewise there were no relationships of mortality with number of stumps, number of stumps with *Armillaria*, or 2LCB sticky band catches on a plot basis. However on a stand basis mortality increased with number of stumps, number of stumps with *Armillaria*, and number of 2LCB's.

CALOSOMA SYCOPHANTA: THE CASE FOR AUGMENTATIVE RELEASE

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ABSTRACT

Calosoma sycophanta is an important, specific predator of high-density gypsy moth populations. Mark-recapture studies on beetles have shown that rather few adults are present in areas where gypsy moth populations are high. However, in the same areas, the percentage of pupae on tree trunks that are killed by beetle larvae may reach 70%. Also, mortality to gypsy moth pupae can be enhanced by releasing only 10 pairs of beetles. Because *Calosoma sycophanta* does not disperse as rapidly as does the gypsy moth, there are many areas near the leading edge that have no beetles. Augmentative releases in these areas could be used to establish the predators. Also, because there are no natural beetle populations in these areas, effectiveness of released beetles can be assessed by comparing release sites with nearby check sites. Beetle larvae are cannibalistic, and this has hampered their effective rearing. They can be easily reared in groups, however, if provided with a thick layer of peat moss in the bottom of a plastic crisper. If only 50 pairs of adults are released per plot, 1,000 beetles would be enough for 10 plots. Numerous cooperators in several states are ready to release and evaluate these beetles. If enough can be reared in 1993, releases can occur in 1994.

GYPSY MOTH POPULATION DYNAMICS: A DIFFERENTIAL EQUATION APPROACH

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ABSTRACT

As scientists, we are accustomed to approaching the study of natural phenomena through a process of observation, formulation of hypotheses, testing of these hypotheses, and finally, the formulation of rules which we believe the phenomena to obey based on the results of the previous steps. Modelling is the mathematical analogue of this process. Here, the "rules" are in the form of equations which are hypothesized to predict at least some portion of the

behavior of the system being studied. As with all hypotheses, it is important to realize that they must be constantly studied and revised as their shortcomings are identified.

Modelling of biological systems is very different from the modelling of what one might call physical systems (those obeying the laws of physics). Constructing physical systems can be much easier since one has derivable laws which the system must obey, the system can often be considered as isolated from its surroundings, and one can normally study the system in small parts without loosing the validity of the model. For biological systems, there are no "laws" which can be derived from some first principles which the system must obey. In addition, it is almost always the case that we can not consider isolated systems (we are often concerned with the interactions of whole ecosystems), and it is not possible to isolate single components (one can not model the behavior of gypsy moths and neglect their food source, for example). This, in general, leads to biological models which are not capable of quantitative, long-term predictions, while the physical models do not have this limitation.

Based on the above discussion, it is logical to question whether biological models can be of any real help in the study of natural phenomena. The answer to this question is that they can indeed be a very great help, but only if we use them understanding their abilities and limitations. These models can be highly effective at qualitative predictions. They can also be used successfully to make quantitative predictions over short-term studies. However, due to the complexity of biological systems, they can not give accurate quantitative predictions over long time frames. While this is a limitation of the models, it is not a fatal one. Perhaps the most effective use which can be made of these models is to use them to make predictions which can then be tested by experimental studies. This not only allows us to test how well we understand the dynamics of the system (and therefore how well the model emulates nature), but also gives us a basis on which to modify the model and bring it in line with the biological reality. If modelling and observation are used hand in hand, the advancement of the understanding of the phenomena is much more rapid than if they are used separately.

With this as our goal, we have developed a three variable model for gypsy moth population dynamics which involves gypsy moth, foliage, and natural enemy biomass densities. We chose to work in terms of biomass instead of number densities so that we did not have to include separate variables for the various stages of the gypsy moth life cycle. We wanted to keep the dynamics as simple as possible, while still having a model robust enough to qualitatively study such phenomena as artificial stimulation of gypsy moth outbreaks, the spread of gypsy moths, and the effectiveness of various treatment plans. We have found this model to show a range of behavior from periodic to chaotic population dynamics. We have developed versions of this model which assume a homogeneous stand, as well as ones which include spatial effects and seasonal effects.

One now needs to consider what is needed to use these models. The answer to this question depends on one's goals. It is very possible that one could use the models with very little information on the true values of the parameters in the model as "what if" scenarios. In this capacity, the user can run the models under various conditions to explore possible behavior under a wide range of conditions. Alternatively, one can use known information about the behavior of gypsy moths to attempt to fit the behavior of the model to the observed behavior by changing the model parameters until the desired correspondence between the two is obtained. Obviously, the more information one has about the system, the better the model can be made. However, it must also be realized that the more one wants to make the model quantitative, the more complex it must become. It thus becomes very important that we identify those aspects of the biological system which are essential to the phenomena we are interested in studying and build our model around these features, and not attempt to construct a model which will include all of the true dynamics of the system unless we are willing to pay the price of a very complex model which requires very detailed information about the system before it can be of any use. We believe that our simple three variable model is a good first attempt at doing this, and we are now in the process of attempting to refine it based on comparisons with the biological system.

MODELLING OF 1-DIMENSIONAL SPATIAL EFFECTS ON THE SPREAD OF GYPSY MOTHS

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ABSTRACT

This work is concerned with the development of a differential equation model capable of studying the effects of spatial dynamics on gypsy moth populations. This was done by modifying the existing three variable ordinary differential equation model (involving gypsy moth, foliage, and natural enemy biomasses) to include a diffusive term in the equations for the gypsy moths as well as natural enemies, thus changing the model to one involving partial differential equations.

In studying this model, it was found that under certain conditions the model behavior was very surprising. For example, under conditions such that the ordinary differential equations gave chaotic behavior, the solutions to the partial differential equation model were found to have a very regular structure in the form of a traveling wave if a perturbation was applied to the gypsy moth density above a certain threshold. Below this threshold, the behavior was chaotic as in the ordinary differential equation model. This is a very surprising result, and if the true system were to behave in a similar manner, this could have important applications.

In addition, various pesticide treatment plans were tested using the model to begin to assess the relative effectiveness of different plans. While stronger pesticides were better at controlling the populations as one would expect, it was observed that ones which also affected the natural enemies were significantly less effective than weaker ones which affected only the gypsy moths.

THE EFFECTS OF SEASONAL DYNAMICS ON A THREE VARIABLE, SPATIALLY HOMOGENEOUS MODEL FOR GYPSY MOTH POPULATION DYNAMICS

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ABSTRACT

This work considers effects of adding some basic seasonal dynamics to a three variable differential equation model for gypsy moth population dynamics which was developed previously. This model involving the gypsy moth, foliage, and natural enemy biomasses includes the following rudimentary seasonal effects: bud break is assumed to take place at a user specified amount of time prior to egg hatch. From the time of egg hatch to the time when eggs are laid, the previously described differential equation model is used. After this time, there is a gypsy moth biomass loss due to death of adults. To include the effects of winter, the foliage biomass is reduced to zero, and a user specified winter mortality for the gypsy moths is applied.

Using this model, various field data collection strategies were examined. It was found that collecting data at only one time during the gypsy moth life cycle can lead to some erroneous conclusions. For example, it is possible to see different population behavior merely by varying the time at which the data is collected. This behavior can vary from what looks very periodic to some which looks chaotic. The implications of these results are currently being studied.

A THREE VARIABLE, SPATIALLY HOMOGENEOUS MODEL FOR GYPSY MOTH POPULATION DYNAMICS

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ABSTRACT

This work considers a three variable ordinary differential equation model for gypsy moth population dynamics. The variables are the gypsy moth biomass density, foliage biomass density, and natural enemy biomass density. It also consists of twelve parameters which involve such things as the search rates, consumption rates, conversion rates, immigration rates, and emigration rates of the species involved.

This model evidences a wide range of behavior from periodic solutions corresponding to limit cycles to chaotic behavior. In addition, we have studied the behavior of the model under conditions similar to those for field studies in which the investigators have attempted to stimulate outbreaks. As in the field studies, we found that the artificially elevated population very quickly "crashed" back to a very low level. However, it was also observed that the time when the population was elevated made a difference as to the behavior of the system, and in fact it was found that if the perturbation to the gypsy moth density was performed at the correct time, a short outbreak could be stimulated.

MECHANISMS FOR AMINO ACID ABSORPTION FROM THE MIDGUT OF GYPSY MOTH LARVAE

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ABSTRACT

Extensive studies by a group of researchers at the University of Milan have shown that amino acid absorption from the lumen into the columnar cells of larval *Philosamia cynthia* midgut occurs mainly by cotransport with potassium ions. At least four different systems for the cotransport of various L-amino acids have been identified in larval *P. cynthia* midgut. These systems allow the larvae to utilize the energy of the electrochemical gradient established by active secretion of potassium ions from the cells into the midgut lumen to accumulate amino acids within the cells. One of these systems accepts most neutral amino acids and either sodium or potassium ions as substrates. Another system shows a strong preference for lysine and potassium ion as substrates. A third system exhibits a preference for glutamic acid and sodium ion as substrates.

The absorption of amino acids by larval *Lymantria dispar* midgut was investigated using brush border membrane vesicles prepared from both fresh and frozen midguts by Mg/EGTA precipitation and differential centrifugation. The vesicles were enriched 10 to 13 fold, relative to the midgut homogenate, in the activity of the brush border membrane marker enzymes aminopeptidase and gamma-glutamyl transferase. There was no significant difference in enzyme enrichment between vesicles prepared from fresh and frozen midguts. Rapid filtration experiments yielded evidence for the presence of mechanisms for the cotransport of the neutral amino acids phenylalanine and leucine as well as the basic amino acid lysine. Potassium ion was the preferred second substrate for both neutral amino acids and lysine. Differences in ion selectivity favored leucine and lysine symport being mediated by different cotransporters. Unlike that of the neutral and basic amino acids, glutamic acid absorption was not stimulated by potassium or any other alkali metal ion. Therefore, it appears that larval gypsy moth midguts may lack a mechanism for utilizing the potential energy of the inwardly directed electrochemical potassium gradient in the absorption of glutamic acid.

BIOLOGICALLY ACTIVE RADIATION ENVIRONMENT IN A HARDWOOD FOREST

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ABSTRACT

The biologically active radiation environment in a partially refoliated hardwood forest after gypsy moth defoliation was experimentally characterized in terms of vertical profiles of solar radiation in wavebands of ultraviolet-B (UVB 280-320 nm), photosynthetically active (PAR, 400-700 nm), and the whole spectrum (total). The measurements were taken at 10 randomly chosen locations in the Black Moshanon State Forest near State College, PA, using broadband radiation sensors mounted on the auto-leveling platform of an up-down lifting tower. Profiles of foliage area distribution were simultaneously measured using canopy analyzers. Data were averaged over the locations and normalized with the above-canopy readings before analysis. The major results of the study are:

1. The canopy foliage distribution was characterized by downward cumulative leaf area index which was fit to the Weibull cumulative distribution function. Model parameters of the Weibull cumulative distribution function were significantly correlated with the canopy leaf area index.

2. The irradiance measurements of UVB, PAR, and total radiation were all attenuated with depth or the downward cumulative leaf area index. The attenuation rate was the greatest for UVB, smallest for total, and intermediate for PAR.

3. Beer's law was found to describe the vertical radiation profiles in all three wavebands fairly well in this relatively homogeneous forest canopy. The extinction coefficients, defined by Beer's law and determined via regression analysis, were significant for all three bands of wavelength.

4. Ratios of UVB to PAR, UVB to total, and PAR to total were all shown to decrease with cumulative leaf area index, indicating that top leaves, while intercepting and absorbing most of the PAR for photosynthesis, would be most vulnerable to damages caused by UVB.

USDA Interagency Gypsy Moth Research Forum
January 19-22, 1993
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