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Report NE-146

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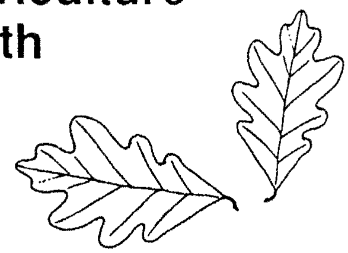

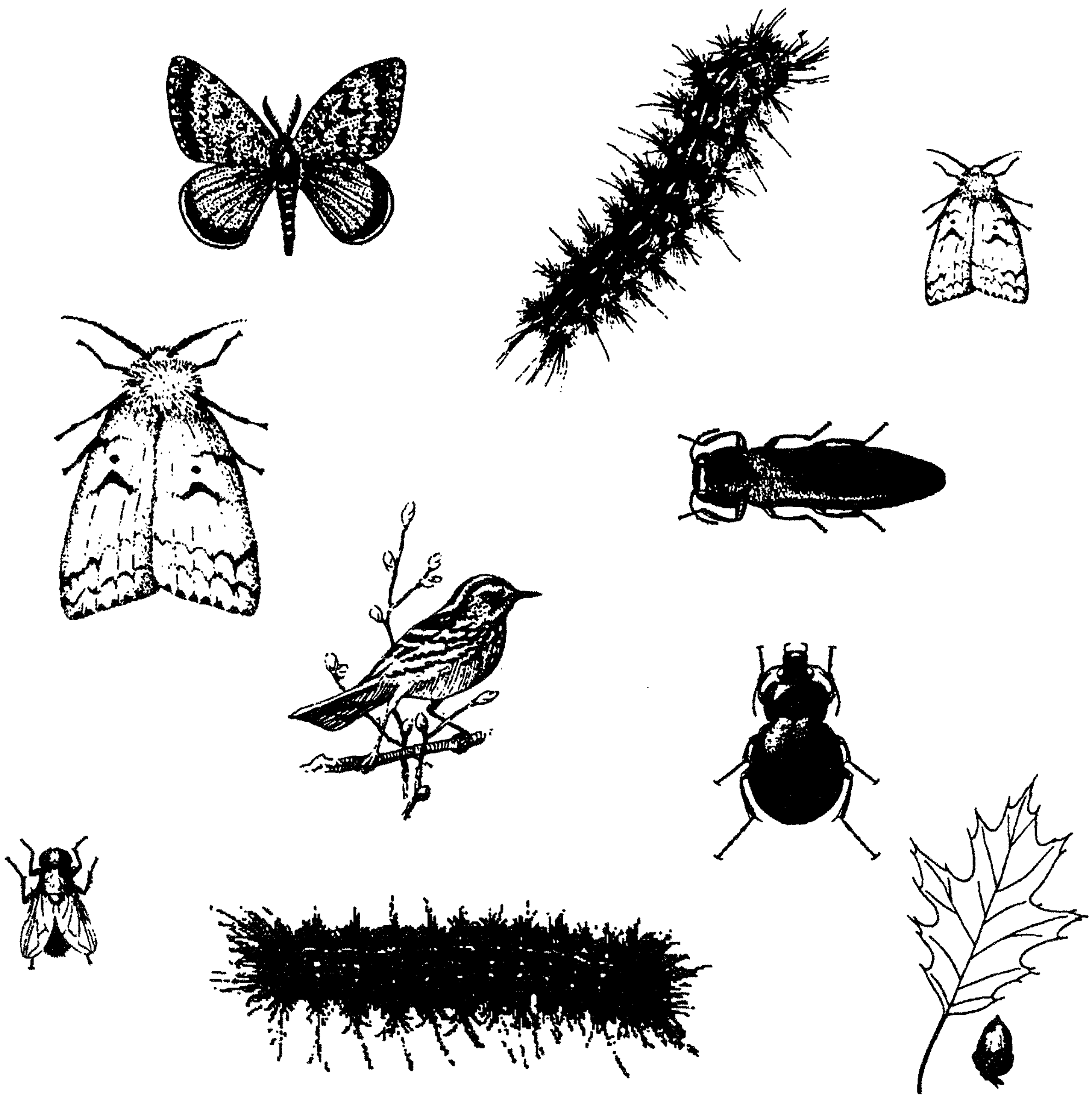

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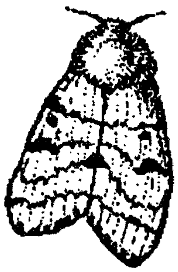
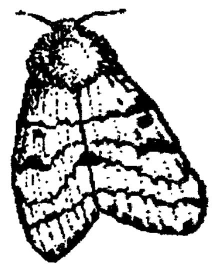


January 22-25, 1990
East Windsor, CT



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FOREWORD

In July of 1989 representatives of Forest Service-Research (FS-R), Animal and Plant Health Inspection Service (APHIS), and Agricultural Research Service (ARS) began regular meetings to discuss opportunities for improving cooperation among the agencies conducting research on gypsy moth. Representatives from the Cooperative State Research Service (CSRS) and Forest Service-State & Private Forestry (FS-S&PF) were added over the next few months. The group is known as the USDA Gypsy Moth Research and Development Coordinating Group and has the following objectives:

a. To monitor the progress of Service programs and any breakthroughs which may influence USDA policies;

b. To keep the Services and the Gypsy Moth Working Group appraised of progress in research and methods development;

c. To identify research and methods development issues and concerns;

d. To set priorities;

e. To maximize use of current resources as well as to provide appropriate rationale to justify increased resources.

The Coordinating Group resolved at its initial meeting that a combined interagency review of gypsy moth research and development activities would add immeasurably to better communication as well as provide a comprehensive overview of ongoing research. Members of the Coordinating Group also agreed that a proceedings should be published following the meeting.

These proceedings document the efforts of many individuals: those who made the meeting possible, those who made presentations, and those who compiled and edited the proceedings. But more than that, the proceedings illustrate the depth and breadth of studies being supported by the agencies and it is satisfying, indeed, that all of this can be accomplished in a cooperative spirit.

USDA Gypsy Moth Research and Development Coordinating Group
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T. Hofacker, FS-S&PF
M. McFadden, FS-R, Chairperson

USDA Interagency Gypsy Moth Research Review
January 22-25, 1990
Ramada Inn
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MICROBIAL PESTICIDES

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ABSTRACT

Interest in the use of microbial pesticides has intensified because of public concern about the safety of chemical pesticides and their impact in the environment. Characteristics of the five groups of entomopathogens that have potential as microbial pesticides are briefly discussed and an update is provided on research and development activities underway to enhance the performance and use of microbial products. Emphasis is placed on viral and bacterial pesticides and their use in managing gypsy moth populations. The status of microbial pesticides registered by the Environmental Protection Agency (EPA) and regulations governing their use are reviewed

INTRODUCTION

The potential for utilizing microorganisms as an applied tactic to control pest insects was recognized in the 1940's when a species of bacteria, *Bacillus popillae,* was used successfully to control the Japanese beetle. Although many entomopathogens of insects have been isolated and described, probably only a few species have real potential as microbial pesticides. Interest in the application of microbial pesticides has accelerated since the 1960's for several reasons:

1) Environmental concerns associated with using chemical pesticides, including pollution of groundwater, the accumulation/biomagnification of residues and metabolites in the environment, contamination of food supplies, and direct effect on non-target organisms.

(2) Development of resistance in insects to chemical pesticides.

(3) Increased emphasis on integrated pest management (IPM) and low input sustainable agriculture (LISA).

(4) Recent developments in biotechnology.

Evidence of the interest in microbial pesticides is elucidated by the history of pesticide use in gypsy moth state/federal suppression programs (Fig. 1).

Since 1982, when about 90% of the acreage was treated with chemical pesticides, the use of Bt, the only commercially available microbial pesticide, has increased substantially. In 1989, Bt was used on over 50% of the 820,000 acres treated in 11 states; however the choice of chemical/microbial pesticides varies widely from state to state and is influenced by management philosophy and environmental pressures. Bt was used exclusively in Michigan and New Jersey whereas Dimilin was used on 74% of the acreage treated in Virginia, and on over 99% of the acreage treated in West Virginia.

The objectives of my presentation are to: provide an overview of the status of entomopathogens being developed as microbial pesticides; to highlight recent developments in those microbials that have been used against the gypsy moth; and to provide an introduction to the topics that will be addressed by the following speakers in this session and in the accompanying poster displays.

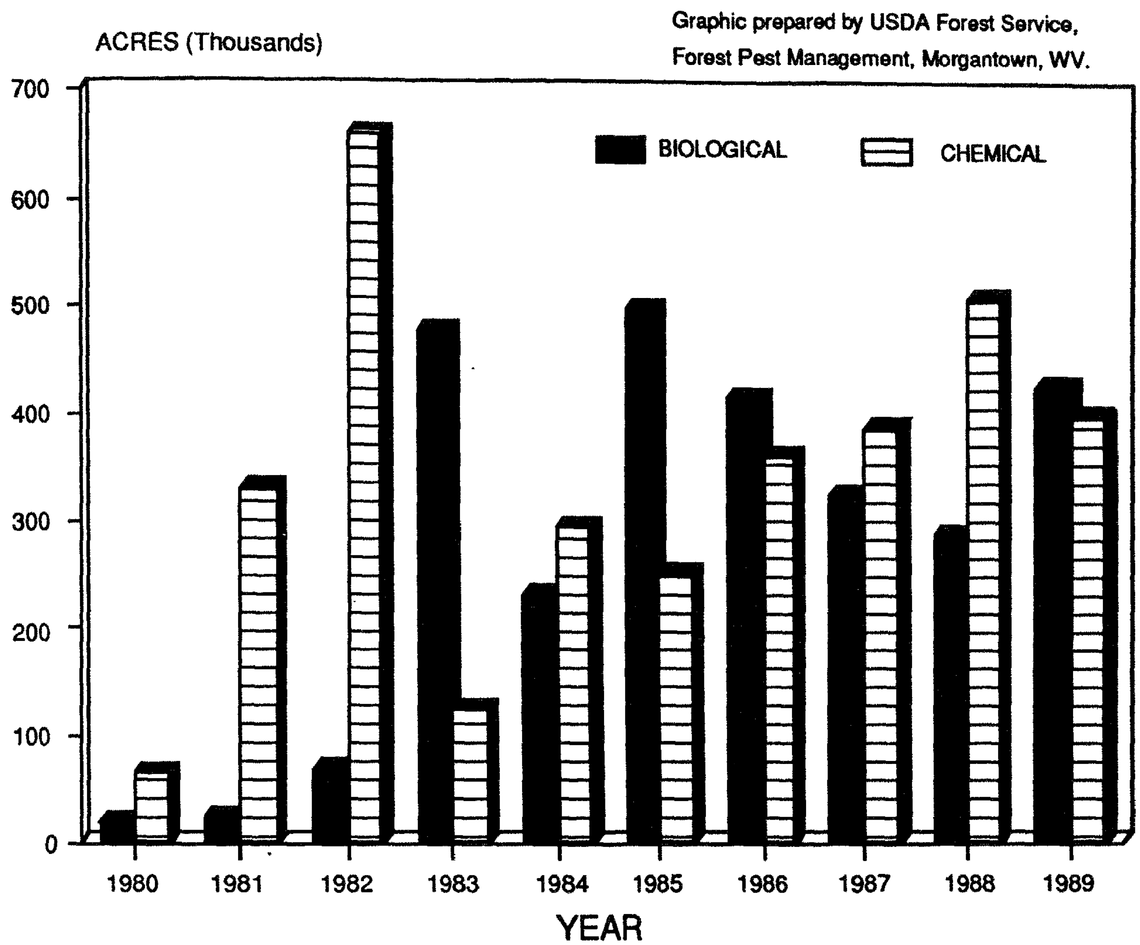

Figure 1. Insecticide use in State/Federal Cooperative Gypsy Moth Suppression projects (1980-1989).

ENTOMOPATHOGENS AND THEIR POTENTIAL AS MICROBIAL PESTICIDES

The Environmental Protection agency (EPA) defines a microbial pesticide as follows: any bacterium, fungus, alga, virus, or protozoan intended for preventing, destroying, repelling, or mitigating any pest, or intended for use as a plant regulator, defoliant, or desiccant. Although this is a very broad definition, it does not include nematodes which insect pathologists normally include within this category. Some of the general characteristics of entomopathogens that are used as microbial pesticides are listed in Table 1 and will be discussed in their respective sections. Emphasis will be directed at those organisms that have been or are being used against the gypsy moth.

Table 1. Some characteristics of entomopathogens used as microbial pesticides.

Pathogen	Mode of infection	Production requirement	Speed of Kill
Viruses	Ingestion	Cell culture Insect larvae	Slow
Bacteria (Bt)	Ingestion	Liquid culture	Stops feeding due to toxin
Fungi	Contact		Liquid/solid culture
Protozoa	Ingestion	Insects	Very slow
Nematodes	Search & penetration	Artificial substrate	Fast

BACTERIA (Bt)

Although many genera of bacteria infect insects, only species in the genus *Bacillus* have been commercialized and are being used as microbial pesticides. *Bacillus thuringiensis* Berliner (Bt) persists in nature worldwide and hundreds of isolates have been recorded (Kurstak and Tijssen 1982); Each strain of Bt appears to have its own specific spectrum of activity against insects.

Bt is a spore-forming bacterium that, when cultured under appropriate conditions, forms a crystalline parasporal inclusion body which contains the delta endotoxin. It is an ideal organism to produce in large scale commercial submerged culture systems using standard methods and fermentation equipment. The commercialization of Bt expanded dramatically in the late 1960's with the isolation and development of the HD-1 strain and its acceptance as the International Standard. This strain was found to have 15X the pesticidal activity of the earlier referenced standard.

In the past 10 years, the quantity of Bt applied annually against the gypsy moth and other forest defoliators in operational programs has varied considerably because of its inconsistent performance. In general, Bt applied once or twice against the gypsy moth provides good foliage protection but has not caused a significant reduction in larval populations; consequently, many areas need to be re-treated in subsequent years. However, substantial improvements have been made in commercial Bt formulations and methods improvement studies have demonstrated that the operational performance of Bt can be further improved through better application technology. This will be discussed later in this presentation.

Approaches to improve Bt performance

Interest and developments in Bt-related research have exploded in recent years due in part to: 1) the isolation of new strains that are toxic against a variety of insect pests, 2) the advent of biotechnology, specifically recombinant DNA technology, and 3) advances in formulation and application technology.

Isolation of natural strains. Isolation of new natural Bt strains worldwide is a continuing activity that has had a significant impact on the industry. *Bt* var. *israelensis* was found to exhibit a high level of insecticidal activity against larvae of mosquitoes and black flies. It has been used successfully against both pests in Africa, Germany, and in abatement districts in the United States, and is considered to be a significant component in public health programs. Other strains such as *Bt.* var *tenebrionis* and *Bt* var. *San Diego* are active against species of Coleoptera; these isolates are currently being developed by industry and evaluated against several pests such as the Colorado potato beetle, elm leaf beetle, and the yellow mealworm.

In 1981, a new strain of Bt was isolated from diseased larvae of the spruce budworm that exhibited 3-4 X toxicity against the gypsy moth and other species of Lepidoptera (DuBois and others 1988). This strain, labeled NRD-12, was commercialized by Sandoz Inc. as Javelin® for use against species of *Spodoptera* and more recently has been marketed as SAN 415® for use against the gypsy moth and other forest defoliators. It differs from the HD-1 strain in its DNA sequence of the toxin genes and their expression, and in its spectrum of insecticidal activity. It has been determined that the increased insecticidal activity is attributed to the 135 KDa protein that makes up the bipyramidal crystal. Discovery of these natural strains has stimulated the continued search for new and more potent Bt strains that may be effective against a range of pest insects.

Biotechnology-Genetically Altered Strains. Genetic engineering technology is capable of producing recombinant organisms such as *Pseudomonas fluorescens* and *Escherichia coli* that express the delta-endotoxin of Bt. This is currently a wide open and fiercely competitive field that involves large chemical and pharmaceutical firms and venture capital companies, as well as public and private laboratories. Certain strains can be induced to produce large quantities (25-30X) of delta endotoxin, while other strains may possess toxic proteins that decompose more slowly in the environment. Since 1984, the EPA has reviewed at least 12 genetically modified microbial pesticides that include organisms with genes inserted or deleted, induced mutants, and transconjugants. Many scientists suggest that eventually, molecular biologists will be able to produce a Bt product that will be specific to most of our major insect pests.

Formulation and Application Technology. Microbial pesticides such as Bt consist of small particulates that are formulated as wettable powders or flowable concentrates and applied as water suspensions. Except for fungi, microbials must be ingested by the target pests; therefore they must be applied to a substrate (i.e. foliage) and retain their viability for several days. This is no easy feat since exposure for less than 24 hrs. to wavelengths below 500 nm (UV spectrum) inactivates Bt spores and crystals and degrades the protein structure of viral polyinclusion bodies (PIB's). Since microbials are most active against early larval instars, the window for their application is very narrow and finite: because of these limitations the successful utilization of microbials is considered to be both an art and a science.

Bt formulations have undergone an evolution in the past five years and for the most part have been improved substantially. Oil formulations have given way to aqueous formulations and adjuvants such as stickers, ultraviolet screens, and humectants have been added to increase their persistence in the environment. Several novel formulations have emerged. Mycogen Inc. has developed an insecticidal delivery system for the delta endotoxin, called MCAP®, whereby the toxin is encapsulated within a non-viable cell of *Pseudomonas fluorescens*, a soil inhabiting, plant colonizing, non-pathogenic microbe. This system affords greater field persistence of the toxin. ARS scientists have also developed a system whereby Bt spores and crystals are encapsulated along with a UV screen within a starch matrix. This formulation retained 50% of its toxicity for up to 12 days in the field.

The difficulties associated with applying microbial pesticides were elucidated at an NSF-USDA sponsored workshop that was held in Gainesville, Fla. in 1978. Microbials are still being applied with conventional application systems that were designed for contact chemical pesticides and it was emphasized at that meeting that radical modification of equipment and ideas regarding application is needed. The erratic performance of Bt since that time has been blamed on weather, poor application, or poor timing of application. However, prior to 1985, studies had never been conducted to relate spray deposit on foliage to efficacy. Since then, we have learned much about the effect of droplet diameter, density of droplets, and their distribution in the forest canopy on the efficacy of Bt against the gypsy moth (Bryant and Yendol 1988; Yendol and others 1989).

Much of the credit for generating interest to improve the aerial application of Bt and other microbials can be attributed to the Northeast Forest Aerial Application Technology Group (NEFAAT), an ad hoc group of scientists and practitioners from the Forest Service (FS), Animal and Plant Health Inspection Service (APHIS), Agriculture Research Service (ARS), Pennsylvania State University, and the University of Connecticut. This group meets several times each year to identify and prioritize research needs, and then jointly conducts laboratory and field studies designed to improve the performance of Bt. The NEFAAT group also provides technical assistance and training to user groups by conducting workshops on the operational aspects of aerial spray programs. Another major activity involves acquiring data sets on spray deposit, meteorology, and canopy geometry that are needed to validate and enhance spray models such as FSCBG and AGDISP that have been developed by the Forest Service. These models have utility for standardizing and improving aerial spray technology. The NEFAAT group represents the only concerted effort to address the aerial application problems that were identified over 12 years ago.

Recently two other committees have been established to improve the coordination of activities related to the application of microbial pesticides. A Bt Technical Committee was formed in 1986 consisting of representatives from the FS, APHIS, ARS, Industry, Academia, and affected states. This committee meets annually in the Fall to review the performance of Bt in operational programs and field and pilot studies, and then identifies research needs and methods improvement studies.

In 1988, the Forest Service established a series of National Steering Committees for Aerial Application of Pesticides to address problems associated with eastern forest defoliators, defoliators of western conifers, insect pests of seed orchards, and vegetation management. Each Committee meets annually to review data and progress of field and pilot tests, identifies needs for improving application systems and strategies, and assigns priorities for field and pilot studies to be conducted in the following year. The joint Committees also developed badly needed guidelines for conducting field and pilot studies, the intent being to better standardize the design of experiments and acquisition of data so that results submitted from various investigators can be compared.

Although the role of these coordinating groups may be perceived as duplicative or overlapping, it in fact is not since each group functions differently and satisfies various needs among the agencies and within the user community.

VIRUSES

General

More than 20 groups of viruses are known to be pathogenic for insects, however most interest is directed toward the nuclear polyhedrosis virus (NPV's) and to a lesser degree to the granulosis viruses. Both of these groups are placed in the family Baculoviridae and are called baculoviruses; they are more restricted in their host range than other viral groups.

Diseases caused by viruses are usually fatal, though infected larvae may not die until 6 to 10 days after first symptoms appear. Epizootics causes by viruses are quite dramatic and frequently cause total collapse of gypsy moth and other forest insect populations. The virions or infectious agent are cylindrical, rod-shaped structures that are enclosed within an inclusion body that is polyhedral in shape, protein in nature, and is a late viral gene product. The polyhedral inclusion body (PIB) protects the infectious virions much like a protective spore, is resistant to desiccation, and under optimal conditions can be stored for years. In gypsy moth populations, the PIB's are released from dying and dead larvae and then passively dispersed in the environment and horizontally transmitted to other larvae, thus attributing to the sometimes rapid development of epizootics. The persistence of PIB's on environmental surfaces contributes to the vertical transmission of the virus in subsequent generations (Murray and Elkinton 1989, Woods and others 1989).

There is a tremendous interest in developing viruses as microbial pesticides because of their host specificity, safety to non-target organisms, and lack of persistence in the environment. Conversely, their specificity limits their potential marketability since incentives for industry to embark on commercial production revolve around predictable and expanding markets. Thus, while Sandoz Inc. registered Elcar® as the first viral pesticide for control of *Heliothis* sp. in 1975, the last three viral products that have been registered for control of the Douglas Fir Tussock Moth, gypsy moth, and European pine sawfly, were developed and registered by the Federal Government.

The potential role of biotechnology in developing and enhancing utilization of baculoviruses is unlimited. Recombinant DNA technology offers many avenues to improve the pathogenicity and effectiveness of baculoviruses; scientists are exploring the possibility of inserting toxin or hormone genes into the viral genome to either amplify toxicity, alter behavior, or arrest the development of insects. However, most commercial interest to date has been directed at using baculoviruses as expression vector systems to produce massive amounts of protein in a short period of time. It's estimated that over 150 laboratories are using baculoviruses for this purpose, one important application being for producing AIDS vaccine for human trials in the U.S.

Gypchek - The Gypsy Moth NPV

Gypchek,® the gypsy moth NPV, was registered by the EPA in 1978 and recently re-registered in 1988. It is not commercially available because, like all baculoviruses, it must be produced *in vivo*, which is a labor-intensive, costly process; furthermore, the specificity of the virus to the gypsy moth and the periodic, eruptive nature of its outbreaks somewhat limits the market potential for a viral pesticide.

Since its registration, Forest Service Research has been producing Gypchek® in collaboration with the APHIS Methods Development Center in quantities sufficient to conduct methods improvement studies annually in cooperation with the FS-Forest Pest Management Group in Morgantown, W.VA. Prior to 1986, the field efficacy of Gypchek® against gypsy moth populations had been erratic at best and varied with the dosage applied and the density of the population. In 1986, excellent control was attained when the dosage of Gypchek applied was increased to 5 X 10" PIB's/acre (100 million potency units) and a new sunscreen, Orzan LS® was added to the tank mix. Aerial tests conducted in 1987-88 confirmed that two applications of Gypchek® at that dosage with Orzan LS provided excellent foliage protection and resulted in ca. 90% reduction in egg masses in the treated blocks. These results stimulated a renewed interest by industry in both in-vivo and in-vitro production of Gypchek® as a viral pesticide.

Approaches to Improve Gypchek® Performance

Interest in Gypchek® has been intensified not only because of its improved performance in aerial field studies but because of environmental concerns about the impact of aerially applied Bt on non-target Lepidoptera, and recent developments in biotechnology and in cell culture production of viruses. Consequently, several initiatives are being pursued simultaneously that are designed to ameliorate both the activity and availability of the gypsy moth virus.

Strain Selection. Gypchek® is produced from a wild type isolate (LDP-67) that apparently contains several genotypic variants. Shapiro and others (1984) reported that a natural isolate of the gypsy moth virus, the Abington strain, demonstrated 5-10X activity against gypsy moth larvae in laboratory bioassays. This strain is currently being evaluated along with Gypchek® in a comparative field study on small plots in Maryland. There is also more recent evidence that isolates cloned from Gypchek® also demonstrated increased activity (5-20X) when bioassayed against gypsy moth larvae, suggesting that more virulent isolates do exist in nature and that cloned isolates may provide a better source of inoculum for future production of the virus.

In vivo production. Significant advancements in rearing technology have been realized at the Otis Methods Development Center, resulting in the production of better quality insects at a lower cost per unit. Additionally, improvement in the processing of viral-killed cadavers at the FS Ansonia facility has resulted in more efficient recovery of viral PIB's and production of a better physical product. Undoubtedly, both rearing and processing could be further improved through mechanization. Theoretically, an alternative strain of gypsy moth could be developed that is specifically adapted for producing virus rather than for producing quality adult insects; similarly a less-expensive, modified diet might be developed that would be adequate for rearing larvae for virus production. Many of these options are being pursued by ESPRO Inc. which has a technology transfer agreement with the FS to produce Gypchek® and is pursuing commercialization of the gypsy moth virus.

In vitro production. ARS scientists have made major advancements toward producing gypsy moth virus in cell culture systems. The feasibility of producing virus in cell culture has been improved by utilizing a new fat body cell line along with the Abington strain, and by reducing the cost of culture media. ARS has entered into a cooperative research and development agreement with IGB Products Ltd. to develop the technology for scaling up a cell culture system for producing gypsy moth NPV. The availability of a cell culture system would eliminate the need for an expensive in-vivo production facility and would promote the production of a more standardized viral product.

Genetic Engineering. As stated earlier, scientists believe that there are unlimited opportunities to improve baculoviruses such as the gypsy moth NPV using gene deletion/insertion techniques, or by producing chemically induced mutants. Many of these alternatives are being pursued at the FS laboratory in Delaware, OH., and at the Boyce Thompson Institute (BTI) in Ithaca, NY. Scientists at BTI are creating a polyhedrin-minus mutant thereby providing a genetic marker than can be used to follow the dissemination of aerially applied virus in space and time. This would be the initial step leading up to replacing the polyhedrin gene with foreign genes designed to enhance activity of the virus or disrupt the development of gypsy moth larvae.

Formulation and Application Technology. Gypchek®, like other viral preparations, is readily degraded by ultraviolet light and therefore must be formulated with a good UV protectant along with other adjuvants needed to improve its stability and persistence in the environment. Now that the field efficacy of Gypchek® has been demonstrated, there is a need to develop a Bt-like formulation for Gypchek® so that we can eliminate use of the current tank mix. This mixture, which includes water, Orzan LS, molasses, a sticker, and Gypchek®, is not practical for use in operational programs. Methods improvement studies are being conducted to identify even better sunscreens or additives that magnify either larval feeding or viral activity. Investigators are also evaluating encapsulated formulations for viruses that would effectively extend their field persistence.

To date, little or no effort has been directed toward optimizing the aerial application of Gypchek®, that is determining the best dose, application rate,and delivery system needed to provide adequate deposit and repeatable efficacy. Although there is much to be done, studies of this nature should not be conducted until an acceptable formulation for the virus is developed, since the physical characteristics of a formulation will greatly affect it's atomization, dispersal, and deposit on foliage.

FUNGI

Pathogenic fungi are unique among the entomopathogens used as microbial pesticides because they infect insects directly through their cuticle and therefore need not be ingested. However, the micrometeorological conditions (temperature and relative humidity) that are required for germination of spores and infection are critical and seldom realized in field applications. There are about 750 species of fungi that are known parasites or pathogens of arthropod pests; more than 30 have been tested as biological preparations, and several are commercially available in foreign countries. According to Weiser (1982), the reason for the lack of widespread use of fungi is that there are too many variable conditions which make their application unreliable, thus requiring that proper conditions would have to be established for each combination of fungus and pest.

Fungal epizootics can be very dramatic and occasionally will decimate pest populations. Hajek (this proceedings) reported on a panzootic in gypsy moth populations caused by *Entomophaga maimaiga*. This was the first recorded fungal epizootic affecting the gypsy moth in the U.S., though fungal epizootics apparently are more common among Japanese gypsy moth populations (Aoki, 1974). Although the species was introduced into New England from Japan in 1910 or 1911 (Speare and Colley 1912) it has never been reported in the literature as a mortality agent in gypsy moth populations. It should be noted however that fungal-infected larvae appear similar to virus-killed larvae, therefore it is very likely that incidents of fungal mortality were heretofore overlooked.

There has been a concerted effort by industry in the U.S. and abroad to develop *Beauvaria bassiana* as a microbial pesticide for use against a wide range of insect pests. However, when *B. bassiana* was applied against the gypsy moth in Slovakia, it caused less than 5% mortality in the population. (Novotny 1988). Fungi probably are not candidates for use as a microbial pesticide against the gypsy moth, but should be used in inoculative releases to effect permanent biological control.

PROTOZOA

Among the Protozoa, the only group considered to have potential as microbial pesticides is the microsporidia. Microsporida are known to infect over 100 species of mosquitoes and several major forest defoliators such as the spruce budworm, gypsy moth and forest tent caterpillar. One species, *Nosema locustae*, is registered in the U.S. as a bait formulation for grasshopper control.

Microsporidia generally produce chronic rather than acute disease in insects, consequently, their effect on populations is not as dramatic as the epizootics caused by bacteria, viruses, or fungi. However they do cause debilitating effects on individuals such as prolonged development, reduced fecundity, and, in some cases, behavioral changes. Microsporidia are reported to act as a stressor in insect populations thereby predisposing individuals to attack by other pathogens such as viruses. Some species are vertically transmitted transovarially to subsequent generations, thus exhibiting a desirable attribute not common to other entomopathogens.

At least seven species of microsporidia have been described or reported from European gypsy moth populations and several species are recognized as having a significant effect on the dynamics of populations in Eastern Europe (Weiser and Novotny 1987). Five species were isolated from gypsy moth populations in Europe and returned to the U.S. for evaluation and potential introduction (McManus and others 1989). One species, *Nosema* N. sp., from Portugal, was introduced into an isolated gypsy moth population and persisted for at least three generations at low levels (Jeffords and others 1989). This species is being recommended as a candidate for general inoculative releases into U.S. gypsy moth populations.

Although Czechoslovak scientists have successfully used a species of microsporidia, *Nosema lymantriae*, as a microbial pesticide (Novotny 1988), there is no intent to use these organisms other than for classical biological control in the U.S. Studies are underway to evaluate the interaction of various isolates with the gypsy moth NPV and to screen other isolates for potential introduction.

NEMATODES

Entomogenous Nematodes occur naturally in soils and possess a wide host range. They are relatively easy to mass produce and apply, however their persistence in soil is limited to a few weeks. The soil environment is a complex medium for nematodes; their efficacy as pathogens is affected by soil moisture, pH, texture, and antagonistic organisms.

Since nematodes are classified as macroparasites, they have been exempted from EPA registration requirements. Several species are being evaluated and actively developed by industry as a control alternative for several insect pests, particularly soil inhabiting species. Novel delivery systems are being evaluated that include desiccated nematodes mixed with baits, and nematodes and seeds encapsulated together within an alginate matrix.

Nematodes have been recovered from gypsy moth populations in Japan, Austria and the Soviet Union. Several species were introduced into U.S. gypsy moth populations during the USDA Accelerated R&D Program (1975-78), however infected larvae were never recovered. In 1984-85, several studies were conducted to evaluate application of *Neoaplectana carpocapsae* against late stage gypsy moth larvae; nematodes that were applied directly to burlapped trees or sprayed on foliage within alginate capsules failed to cause infections among resident gypsy moth larvae. It was concluded that applications of nematodes are not feasible to control foliage-feeding insects like the gypsy moth.

REGULATORY ISSUES

Microorganisms intended for use as microbial pesticides are subject to the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) which has oversight of experimental use, sale and distribution of pesticides. Guidelines for the testing of microbial pesticides, referred to as Subdivision M, were initially published in 1982 and updated in 1989. Data requirements for microbial pesticides (40 CFR Part 158) were published in 1984. There are currently 15 microbial pesticides that are registered for use against insects (Table 2.)

Table 2. EPA Registered Microbial Pesticides (As of 4/19/90).

Microorganism	Year Registered	Pest Controlled
Bacteria		
Bacillus popilliae / B. lentimorbus	1948	Japanese beetle larvae
B. thuringiensis Berliner	1961	moth larvae
Agrobacterium radiobacter	1979	*A. tumefaciens* (crown gall)
B. thuringiensis israeliensis	1981	mosquito larvae
Pseudomonas fluorescens	1988	*Pythium, Rhizoctonia*
B. thuringiensis San Diego	1988	coleopterans
B. thuringiensis tenebrionis	1988	coleopterans
B. thuringiensis EG2348	1989	gypsy moth
B. thuringiensis EG2371	1989	lepidopterans
B. thuringiensis EG2424	1990	lepidopterans / coleopterans
Viruses		
Heliothis Nuclear Poly-hedrosis Virus (NPV)	1975	cotton bollworm, budworm
Tussock Moth NPV	1976	Douglas fir tussock moth larvae
Gypsy Moth NPV	1978	gypsy moth larvae
Pine Sawfly NPV	1983	pine sawfly larvae
Fungi		
Hirsutella thompsonii	1981	Mites
Phytophthora palmivora	1981	citrus strangler vine
Colletotrichum gloeosporioides	1982	northern joint vetch
Trichoderma harzianum/ Trichoderma polysporum	1989	wood rot
Protozoa		
Nosema locustae	1980	grasshoppers

A statement of policy on microbial products of biotechnology and non-indigenous microorganisms, was issued in the Federal Register in June, 1986. Microbial pesticides are distinguished from conventional chemical pesticides by their unique mode of action, their low-use volume, and target species specificity. Each new variety or strain of microbial pesticide must be evaluated and may be subject to additional data requirements. Genetically altered organisms used as pesticides will be subject to additional data on a case-by-case basis, depending on the organism, the parent organism, and the proposed use pattern. The EPA has reviewed 12 submissions of genetically modified microbial pesticides since 1984, 6 of which have been insecticides. The EPA is currently reviewing its policy of including non-indigenous organisms in the same category with genetically-altered microorganisms. This action would be welcomed by insect pathologists since the current policy hinders the introduction and evaluation of exotic entomopathogens that may have potential utility in inoculative releases for permanent biological control.

Microbial pesticides can play an important role in pest management systems either as a principal or supplementary control tactic. However, they are not a panacea and should not be considered as such. There is a need to promote foreign exploration for new and more virulent strains of entomopathogens that can be developed as microbial pesticides or used in inoculative releases to augment classical biological control.

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GYPCHEK® USE PATTERN REALITIES

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ABSTRACT

Gypchek® is the gypsy moth *Baculovirus* product developed by the U.S. Forest Service and registered with the U.S. Environmental Protection Agency in 1978. It has since been reregistered (1988) as a minor use pesticide.

The product was originally intended as a gypsy moth suppression tool whose value would be maximized in those situations where environmental concerns precluded the use of either broad-spectrum chemical, or microbial, pesticides.

Recent refinements in formulation have improved product performance and Gypchek® is now viewed by many resource managers as a "substitute" for either *Bacillus thuringiensis* (Bt) or Dimilin products. Under certain conditions Gypchek® may be an adequate substitute, but the innate biological and physical characteristics of the present product, coupled with high costs associated with its production and application, must temper the urge towards "all-purpose" use.

Promising research with new virus strains and with formulations that provide enhanced foliar persistence, portends well for the eventual expansion of Gypchek's® use pattern. While awaiting this, resource managers can rely upon this "specialty" product, available in limited quantities, for use in situations where no environmental insults from broad-spectrum pesticides can be tolerated.

CURRENT RESEARCH EFFORTS WITH *BACILLUS THURINGIENSIS*

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ABSTRACT

The bioassay of 260 strains of *Bacillus thuringiensis* (Bt) and 70 commercial preparations show that regression coefficient estimates may be as critical as LC_{50} estimates when evaluating them for future consideration. Also most of the last group of 81 Bt strains present in the NRRL-HD culture collection, have been bioassayed. The results of this massive evaluation of all Bt strains available from such a large collection will be discussed. The proteins, coded from the cryIA(a), cryIA(b) and cryIA(c) genes of both the HD-1 and NRD-12 strains of Bt differ in their insecticidal activity against second instar gypsy moth. Finally, field efficacy of Bt appears to be optimized by use of high viscosity and specific gravity formulations applied at high dose and volume rates. Results of 1989 field studies indicate that a healthy and increasing pest population can be consistently reduced to less than 50 egg masses per acre.

SUMMARY OF THE BLACKMO 88 SPRAY EXPERIMENT

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ABSTRACT

The Blackmo 88 spray trial experiment was conducted for two primary purposes: To quantify the effects of local micrometeorological processes, in and near the canopy, on the deposition patterns of aerially applied BT in a mature oak forest; To generate a data set containing simultaneous measurements of spray deposition and detailed micrometeorology, in a canopy of known structure, that could be used to verify the FSCBG spray deposition and penetration model in oak forests.

The experiment was a cooperative venture between the Pesticides Research Lab at Penn State, the forest meteorology research project at UCONN, APHIS and the FPM AIPM project. The sampling and measurement of spray deposition patterns were conducted by the Penn State Lab personnel and the micrometeorology measurements were conducted by the UCONN personnel. Eighteen single swath runs were made with an APHIS ag-truck when the spray deposition and canopy penetration and the micrometeorological conditions were monitored.

The experiment was conducted in a fully leafed, 20 m tall, oak forest in the Black Moshannon State Forest near State College, PA. Canopy photographs during the 1988 experiment were used to calculate the leaf area density at that time. Then, during the following summer (1979) extensive follow-up measurements of vertical leaf area profiles were made. Data and preliminary results from the experiment are given in four other papers in this meeting. Verification analyses of the FSCBG model are now being conducted. The results of this experiment have led to plans for a similar follow-up experiment in the spring of 1990 shortly after leaf bud break, during the gypsy moth first instar. This second experiment will be aimed at determining the conditions necessary to deliver spray materials to the early spring understory vegetation.

GYPSY MOTH MANAGEMENT PROGRAM FOR MODERATELY SIZED URBAN PARKS AND OTHER WOODED PUBLIC LANDS

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ABSTRACT

Specialized gypsy moth (*Lymantria dispar* L.) management programs were developed for moderately sized, high-valued forested public lands. Intensive egg mass sampling, monitoring of aerial applications, and modified treatment thresholds were employed, and multiple applications of *Bacillus thuringiensis* (B.t.) were used where appropriate. The programs were implemented on five Maryland county parks and the Beltsville Agriculture Research Center. Larval mortality averaged 69, 86, and 93% under one B.t., two B.t., and diflubenzuron (Dimilin™) applications, respectively. No noticeable defoliation occurred in blocks treated with diflubenzuron or 2 B.t. applications, and defoliation was noticeable on less than 4% of the acreage treated once with B.t. A computer-based decision support system is being developed to facilitate technology transfer.

VERMONT MANAGEMENT IN FOCAL AREAS

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ABSTRACT

Following the 1979 outbreak of gypsy moths *Lymantria dispar* L. in Vermont, state personnel began monitoring a number of focal areas for signs of increase in gypsy moth populations. In 1986 data from this early warning system indicated an incipient outbreak. We took advantage of this increase to test an experimental management technique. Would disruption of increasing gypsy moth populations at focal sites forestall further expansion of those populations? Four foci were selected, and at each one four one kilometer transect lines were established; one in each cardinal direction, with a 7.5 m radius plot every 100 meters. To better understand and delineate population distribution we set up over 150 additional plots. *Bacillus thuringiensis* was applied to approximately 500 acres on two of the four sites in the spring of 1988. Egg mass and larval density surveys have been conducted at all sites since September, 1987.

Egg mass counts from the fall of 1988 showed that eradication of egg laying females from within the spray block at Brigham Hill was completely successful. Egg masses that remained in the site the year of treatment did not appear to serve as reservoirs for the 1989 population, though there was a unilateral increase in population density. No defoliation occurred at Brigham Hill, in contrast to Arrowhead Mtn., the accompanying check, which was 100% defoliated. Rocky Pond, the check in the central region, was also 100% defoliated, while Perch Pond, the treated area, only had spots of heavy defoliation. The egg mass density in the largest spray block at Perch Pond, which was most effectively contained by treatment in 1988, is now as high as the population at Rocky Pond. It remains to be seen whether the populations at both sites will collapse this coming year.

NPV and *Entomophaga maimaiga* have been positively identified from three of the focal areas used in this study. Incidence of disease was highest at Rocky Pond. Comparison of control and treated sites is continuing to determine the following; whether treatment prolongs the outbreak phase by temporally impeding epizootics, and whether increasing populations outside of a treated area will re-invade the original focal area. The research reported will clarify the significance of the focal area concept for management use, and will determine the value of disrupting gypsy moth outbreaks in susceptible forests before widespread outbreaks occur.

Data collected in the fall of 1988 indicated that two of the three spray blocks at Perch Pond had continued to increase in egg mass density, but the largest one was held to pre-spray population levels. This trend was reversed by the fall of 1989; the population in the largest spray block doubled, but declined in the other two.

SUMMARY OF EXPERIMENTAL RELEASES OF EXOTIC MICROSPORIDIA: CONCLUSIONS AND RECOMMENDATIONS

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ABSTRACT

During a 1985 European expedition, 5 species of microsporidia were obtained from gypsy moth collected in Portugal Czechoslovakia, and Bulgaria. From 1986-1989, we released all 5 species of these microsporidia into gypsy moth populations in isolated woodlots in Maryland. This presentation is a summary of the conclusions and recommendations based on the results of our experimental releases.

Nosema sp persisted in gypsy moth populations in the same woodlot for 3 years. *Vavraia* sp spread horizontally through the gypsy moth populations but did not persist from one season to the next. All three *Vairimorpha* species were slow to spread horizontally through gypsy moth populations and only persisted at very low levels from one season to the next.

Based on our experimental releases we have concluded that: 1) the egg mass method is suitable for introducing most species of gypsy moth microsporidia, 2) synchronizing the batch of contaminated and feral egg masses is critical, 3) the number of spores per egg mass is critical and is different for each species, and 4) *Nosema* sp from Portugal appears to be the most suitable biological control candidate.

Our research recommendations are: 1) conduct detailed taxonomic studies on all species of gypsy moth microsporidia, 2) obtain additional gypsy moth microsporidian isolates from Eurasia, 3) clarify the regulatory requirements for permanent establishment of exotic microsporidia, and 4) experimentally introduce gypsy moth microsporidia into high-density gypsy moth populations.

REGULATION OF DISPARLURE TITER IN GYPSY MOTH FEMALES: EFFECTS OF MATING AND SENESCENCE

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ABSTRACT

In the gypsy moth (*Lymantria dispar* L.), mating causes a decrease in the titer of the sex pheromone. This process appears to be regulated by a two step mechanism. A rapid, but transient initial decrease is mediated by a mechanical signal associated with mating. A long term decrease in pheromone production is mediated by the presence of viable sperm in the spermatheca. Despite a prompt decrease in pheromone titer, mated females remain receptive and remate readily when courted by males. This receptivity is terminated when oviposition begins. Interestingly, all three aspects of mated behavior (i.e. decline in pheromone titer, oviposition and loss of sexual receptivity) were also observed to occur in senescing virgin females.