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The Gypsy Moth Fungus *Entomophaga maimaiga* in North America

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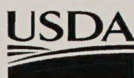

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About the Cover

Photo shows (clockwise from top left): (1) Double wall overwintering resting spores, (2) pear-shaped conidia, (3) *Entomophaga maimaiga* epizootic, and (4) late instar gypsy moth larva killed by *Entomophaga maimaiga*.

Authors' Note

This handbook is an update of NA-TP-15-93, "*Entomophaga maimaiga* in North America: A Review" (September 1993). This update includes new information on the biology, population dynamics, recently completed field and laboratory nontarget impact studies, and use of *E. maimaiga* as a mycoinsecticide.

For additional copies of this publication or information concerning the gypsy moth fungus, Entomophaga maimaiga, please contact either Dr. Richard C. Reardon at (304) 285-1566, or Dr. Ann E. Hajek at (607) 254-4902.

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Contents

Introduction **1**
Gypsy Moth Diseases 2
Biology **3**
Larval Transmission 4
Instars and Conidia 6
Disease Symptoms **7**
Larvae Killed by *E. maimaiga* and NPV 7
Population Dynamics **9**
Cycles of Infection 10
Geographical Distribution **11**
Still Spreading 13
Host Range **14**
Effects on Nontargets 15
Potential as a Mycoinsecticide **16**
Mass Production for Inoculation 17
Constraints on Operational Use 17
Research and Methods Development **18**
Summary **18**
References **19**

List of Figures

Figure 1. Overwintering resting spores (azygospores) of *Entomophaga maimaiga* 4

Figure 2. Protoplasts of *Entomophaga maimaiga* that occur within the hemolymph of infected insects 5

Figure 3. Continuous life stages of *Entomophaga maimaiga* depicting germination, infection, death, and sporulation 5

Figure 4. Conidia of *Entomophaga maimaiga* actively ejected from cadavers to cause infection during the same season 6

Figure 5. Cadaver of a late instar gypsy moth filled with *Entomophaga maimaiga* resting spores 8

Figure 6. Cadaver of a late instar gypsy moth killed by NPV 8

Figure 7a. Fungal hyphal bodies and immature resting spores within a recently killed gypsy moth larva 8

Figure 7b. Cadavers of larvae dying from this disease are often covered with fungal growth but only for a brief time after larval death 9

Figure 8. *Entomophaga maimaiga* can undergo 4 to 9 multiplicative cycles within one generation of the gypsy moth under optimal spring temperature (between 14°C to 26°C) and moisture conditions 11

The Gypsy Moth Fungus *Entomophaga maimaiga* in North America

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Introduction

Lymantria dispar (L.), commonly known as gypsy moth, causes extensive defoliation of broadleaved forests in the northeastern part of the U.S. and Canada. The gypsy moth is prevalent throughout temperate Eurasia. It was introduced into the Boston area of Massachusetts in 1869 and has spread rapidly in the southwest direction at 6 to 9 kilometers (3.6 to 5.4 miles) per year.

In the northeast, the gypsy moth has defoliated an average of 2 million forested hectares (4.8 million acres) per year. In 1989, after 7 to 8 years at low densities, the northeastern gypsy moth populations started to increase. Since 1991, however, defoliation that can be attributed to the gypsy moth has progressively declined. In 1996 and 1997, for example, a total of 81,378 hectares (199,377 acres) and 17,142 hectares (42,000 acres) of forests were defoliated by the gypsy moth, respectively. The decline in gypsy moth populations and the damage this insect pest causes may be due to the activity of the "gypsy moth fungus" in areas where gypsy moths were prevalent.

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This booklet discusses current knowledge about the gypsy moth fungus (biology, disease symptoms, population dynamics, host range, spread) and its potential use as a natural control agent of gypsy moth.

Gypsy Moth Diseases

Entomopathogens are microorganisms that cause diseases in insects. The gypsy moth is susceptible to a variety of infectious diseases that occur naturally caused by bacteria, fungi, and a nucleopolyhedrosis virus (NPV) (Campbell and Podgwaite 1971).

Six endemic species of entomopathogenic fungi are known to infect the gypsy moth. However, for the most abundant species of the native entomopathogenic fungi, the levels of infection averaged less than 13 percent (Hajek, Elkinton, Humber 1997).

Many entomophthoralean fungal pathogens (members of the Zygomycetes or bread molds) are known to cause dramatic disease epidemics in insect populations. One of these fungal pathogens is *Entomophaga aulicae* (Reichert) Humber, a complex of fungal species, all of which attack moths and butterflies (Lepidoptera). North American strains of the *E. aulicae* complex are known to infect species in nine families within the Lepidoptera, including the tussock moths (Lymantriidae) (Hajek, Humber, Walsh et al. 1991). Yet, native *E. aulicae* species have never been reported from North American populations of two important lymantriids: the gypsy moth and the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). In Japan, epizootics (epidemics) of an entomophthoralean fungus frequently have been reported from high density populations of gypsy moth (Koyama 1954).

In 1908, pest managers given responsibility for controlling the spread of gypsy moth populations in the northeast first heard about the effectiveness of a Japanese fungal pathogen. In 1909, gypsy moth larvae infected with this fungus were collected near Tokyo, Japan, and brought to the U.S. The fungus appeared to be a member of the *E. aulicae* species complex.

In 1910-1911, larvae infected with the "gypsy fungus" were released at six sites near Boston. However, due to unfavorable weather conditions and the occurrence of an NPV outbreak, no transmission of this disease occurred.

When the local gypsy moth population collapsed in 1911, the project was considered unsuccessful and discontinued (Hajek, Humber, Elkinton 1995).

In 1984, Soper and Shimazu isolated a fungus from the Japanese gypsy moth and brought the isolates to the United States. The morphological characteristics of the Japanese isolates were identical to *E. aulicae* strains, yet only the Japanese fungus could infect the gypsy moth. Since the protein (isozyme) patterns, distribution, and host range of this fungus differed from those of other isolates within the *E. aulicae* species complex, the Japanese isolates were given the name *Entomophaga maimaiga* Humber, Shimazu and Soper (Soper, Shimazu, Humber et al. 1988). "*Maimaiga*" is derived from the common name for gypsy moth in Japanese.

Japanese isolates of *E. maimaiga* were evaluated in the laboratory. One isolate was selected for field release because of its ability to cause a high percentage of mortality in gypsy moth larvae between 15°C and 25°C (59°F and 77°F, respectively). Compared with other isolates, the isolate chosen for field release also had a shorter infection-to-death-of-hosts time span (Shimazu and Soper 1986). Gypsy moth larvae infected with the 1984 isolate of *E. maimaiga* were released on a small-scale in Allegany State Park, New York (1985), and Shenandoah National Park, Virginia (1986).

The release programs were beset by problems with drought and with releasing fungus-infected insects, which probably contributed to the extremely low infection rate achieved in the year of release. *E. maimaiga* was never found in the release sites during field collections conducted in 1987, and 1989 to 1991 (Hajek, Humber, Elkinton 1995).

Biology

The resistant form of *E. maimaiga* is an azygospore (resting spore) (Figure 1) that persists in the environment in adverse conditions (e.g., lack of larval hosts). These spores have an obligate dormant period after production and asynchronously germinate throughout springtime (i.e., germinate to infect early through late stage larvae). Maximum infection in larvae exposed to resting spores in the soil usually occurs 1 to 2 days after significant precipitation (Weseloh and Andreadis 1992).

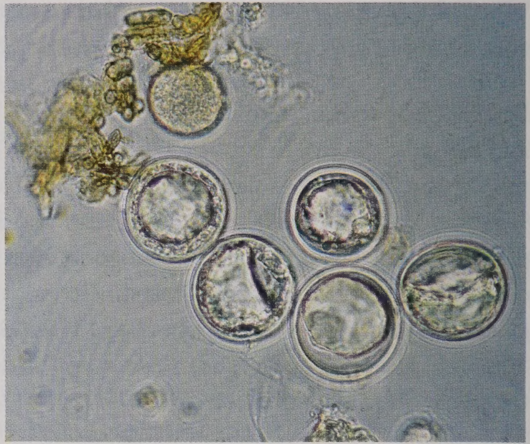

Figure 1. Overwintering resting spores (azygospores) of ***Entomophaga maimaiga****.*

When resting spores germinate, germ conidia are actively discharged and infect when they or their progeny land on the skin of larvae (primary transmission). Enzymes assist the fungus in penetrating into the caterpillar's body. In the protoplast stage, the fungus uses nutrients in the blood to reproduce (Figure 2). Shortly before the infected larvae die, the fungus invades the vital organs.

Larval Transmission

Under constant temperatures between 20°C and 25°C (68°F - 77°F), caterpillars die in less than a week (Shimazu and Soper 1986). Feeding decreases a few days before death. After the death of the larvae from germ conidial infections, hyphal bodies form in the hemolymph, leading to the production of conidiophores. Conidiophores are hair-like filaments that grow out through the integument of the cadavers and which actively discharge pear-shaped conidia (Figures 3, 4). The relatively short-lived conidia infect hosts as do germ conidia. If a conidium does not land on a host, it can germinate to actively discharge an infective secondary conidium that is morphologically identical but slightly smaller. This process can also extend to production of infective tertiary conidia.

Transmission may be primary or secondary. Primary transmission occurs when larvae become infected by germ conidia produced by resting spores. Regard-

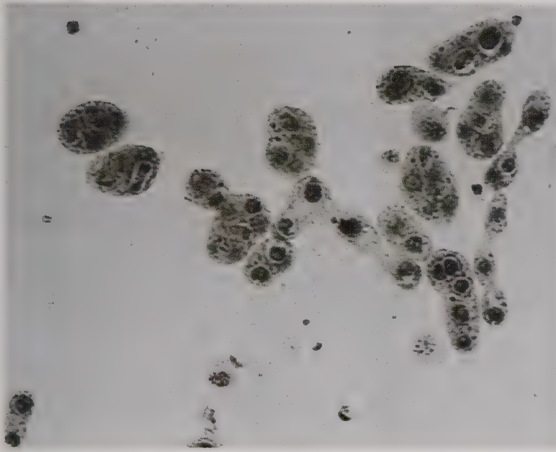

Figure 2. Protoplasts of ***Entomophaga maimaiga*** *that occur within the hemolymph of infected insects.*

SPRING
EARLY SPRING
LATE SPRING
Infection
Germination
Young larvae
Older larvae
Conidia
Death
Sporulation
Conidiophores on dead larva
Azygospores germinate in May and June
Azygospores (Resting spores) deposited in bark and soil
LATE WINTER
SUMMER
(Azygospores dormant)

Figure 3. Continuous life stages of ***Entomophaga maimaiga*** *depicting germination, infection, death, and sporulation. Both germ conidia (from azygospores) and conidia (discharged from cadavers) are labeled as "conidia" above to simplify this complex life cycle.*

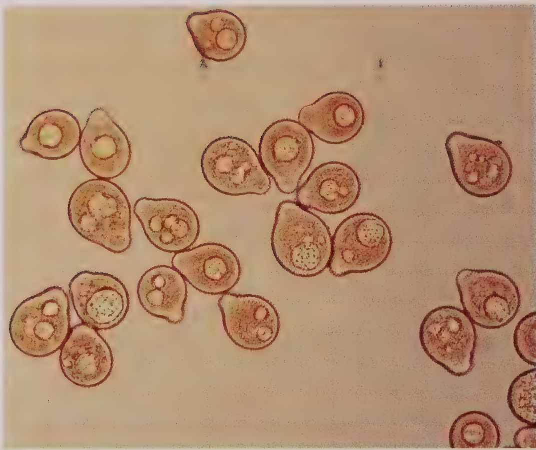

Figure 4. Conidia of ***Entomophaga maimaiga*** *actively ejected from cadavers to cause infection during the same season.*

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less of host instar or relative humidity after death, cadavers of larvae infected through primary transmission produce only conidia. Secondary transmission occurs when infections are initiated by conidia. Infections initiated by conidia yield resting spores only, conidia only, or both spore types. The type of spore produced as a result of conidial infections is primarily influenced by host age but also by fungal attributes (isolate, inoculum density) and environmental factors (temperature, moisture levels) (Hajek and Shimazu 1996).

Instars and Conidia

Infection of early instars by germ conidia from resting spores is thought to initiate disease cycles in spring. After death, early instars predominantly produce conidia, which are actively ejected to disperse and are responsible for the infection of many later stage larvae. Basically, conidia are the type of spore that induces the spread of disease within a season. When later instars die from infection, they can also produce conidia externally but cadavers of older instars almost always produce resting spores internally. Neither type of spore can be seen with the naked eye. In the laboratory, third and fourth instar larvae are most susceptible to the fungus, although high percentages of all instars become infected.

High humidity is necessary for conidial production and discharge. Free water is required for conidial germination. As such, moisture levels act as a switch in conidial germination. Germination changes conidia from disseminating propagules to infectious agents.

Disease Symptoms

E. maimaiga and NPV are the principal natural enemies of gypsy moth. These gypsy moth pathogens are responsible for killing large numbers of gypsy moth larvae (caterpillars). Caterpillars killed by *E. maimaiga* and NPV remain hanging on tree trunks.

Larvae Killed by *E. maimaiga* and NPV

Cadavers of late instar larvae killed by *E. maimaiga* are often oriented vertically with heads down, all prolegs frequently at a 90° angle to the axis of the body, and bodies tightly attached to tree trunks. Larvae recently killed by the fungus have soft bodies while older cadavers appear dry (Figure 5) (Hajek and Roberts 1992). In contrast, most caterpillars killed by NPV hang on trees with their anterior prolegs attached to the trunk, the anterior section of the body unattached and the body bent at an angle of less than 90° (often called an inverted "V")(Figure 6).

Cadavers of NPV-killed larvae usually remain soft and moist and the integument ruptures easily. The body contents of cadavers recently killed by *E. maimaiga* are liquefied and cadavers are usually filled with a mixture of hyphal bodies, and immature as well as some mature resting spores (Figure 7a). For a short time, cadavers that produce conidia are covered with a white to brown velvet-like mat of conidiophores. After producing conidia, the fungal growth on the cadaver surface decomposes, but traces of conidia sometimes can be found attached to hairs (Figure 7b) (Hajek and Snyder 1992). Hajek and Roberts (1992) found that about 4% of the cadavers analyzed were infected with both *E. maimaiga* and NPV. External characteristics are not sufficiently reliable for accurate diagnosis.

*Figure 5. Cadaver of a late instar gypsy moth filled with **Entomophaga maimaiga** resting spores. Note the remains of some conidia attached to larval hairs, the dried appearance of the cadaver, and the vertical position with head down.*

Photo by D. Specter

Figure 6. Cadaver of a late instar gypsy moth killed by NPV. Note the moist appearance of this older cadaver, and the inverted "v" position.

Photo by D. Specter

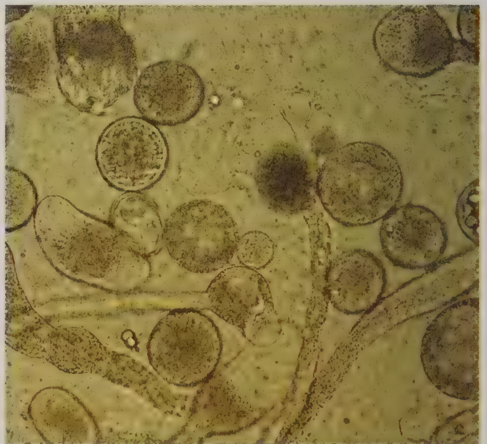

Figure 7a. Fungal hyphal bodies and immature resting spores within a recently killed gypsy moth larva.

Figure 7b. Cadavers of larvae dying from this disease are often covered with fungal growth but only for a brief time after larval death. Occasionally, some spores remain attached to larval hairs.

For greater reliability, larvae should be dissected and body contents examined under the microscope. Infection by *E. maimaiga* can be confirmed in three ways: (1) laboratory analysis of infected living larvae via enzyme-linked immunosorbent assay (ELISA) (Hajek, Butt, Strelow et al. 1991), (2) promoting sporulation from cadavers in humid chambers, and (3) examination of dissected cadavers under a microscope. To observe cadaver contents, soak cadaver in water, remove a small piece, and place it in a drop of water on a microscope slide. Cover with a cover slip. Dissected material is easily observed at 100-400 magnification on a compound microscope. Conidia are pear-shaped and average 20 x 25 micrometers (one quarter as long as width of human hair). Resting spores average 30 micrometers in diameter and have a thick double wall.

Population Dynamics

E. maimaiga is only known as a larval pathogen. In general, cadavers of early instars killed by *E. maimaiga* are found on the foliage; later instar cadavers frequently remain attached to the lower tree trunk by larval prolegs after death. Most

late instar cadavers eventually fall to the ground. Resting spores are leached from the cadavers in abundance into the soil at bases of trees, where they remain in a dormant state through the fall and winter (see Figure 3).

Cycles of Infection

Some resting spores leach from cadavers while they are on tree trunks. These leached spores overwinter in or on the bark of trees. Resting spores germinate the following spring to begin new cycles of infection (Hajek and Humber 1997). Production of resting spores in late instars synchronizes the activity of this pathogen with the life cycle of its univoltine host. Resting spores constitute an important stage of the life cycle because these spores are present in the environment for 9 months until they germinate. Germination occurs throughout the period when gypsy moth larvae are present. Triggers or signals used by *E. maimaiga* resting spores to initiate germination are not known at this time.

Laboratory tests demonstrated that not every resting spore germinates the first year after production. Because resting spores do not necessarily germinate after one year, a reservoir for the pathogen is created in the environment. In fact, in small plots seeded with *E. maimaiga* resting spores, Weseloh and Andreadis (1997) demonstrated that infection can occur six years after the production of resting spores.

Resting spores begin germinating in spring and cadavers of larvae dying from infections subsequently actively discharge conidia. Models demonstrate that it is principally the cycles of infection by conidia that cause the exponential increase in infection characteristic of epizootics. Under optimal spring temperature (between 14°C -26°C) and moisture conditions, *E. maimaiga* can undergo from 4-9 multiplicative cycles within one generation of the gypsy moth host (Figure 8). *E. maimaiga* can cause high levels of infection in both high and low density gypsy moth populations and these epizootics can result in gypsy moth population collapses.

In laboratory studies, Malakar et al. (1997) showed that when larvae are inoculated simultaneously with NPV and *E. maimaiga*, both agents develop. However, larvae usually die first from *E. maimaiga* because of its shorter

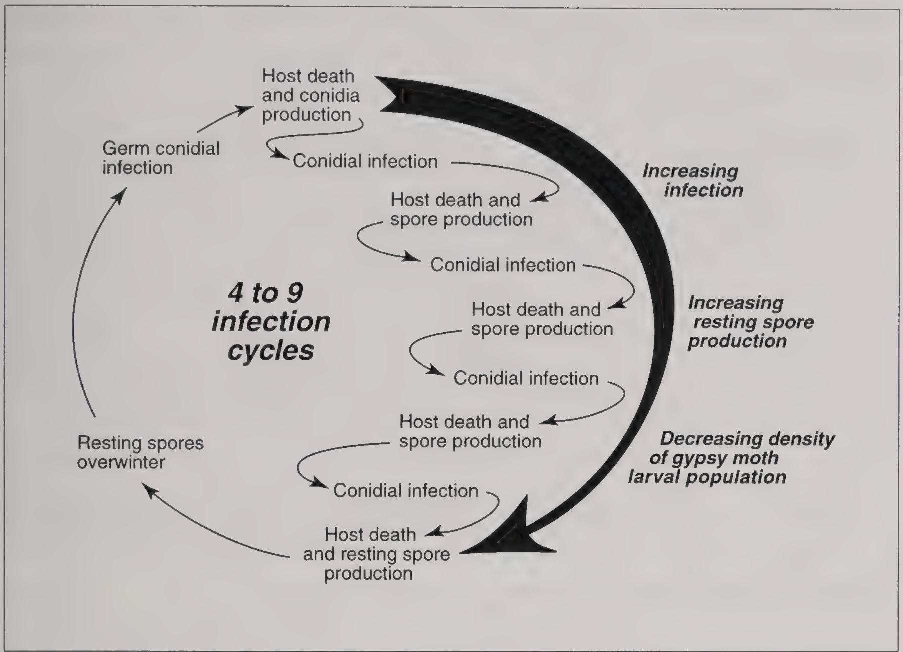

Figure 8. ***Entomophaga maimaiga*** *can undergo 4 to 9 multiplicative cycles within one generation of the gypsy moth under optimal spring temperature (between 14°C-26°C) and moisture conditions.*

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incubation period compared to NPV. There is no evidence, however, that these pathogens interact with or against each other in the host larvae as to cause death.

Geographical Distribution

E. maimaiga was first recovered in North American gypsy moth in June and July 1989. It was identified as responsible for causing extensive epizootics in populations of gypsy moth in Connecticut, Massachusetts, Vermont, New Hampshire, New Jersey, New York, and Pennsylvania (Hajek, Humber, Elkinton et al. 1990). By 1990, *E. maimaiga* was also recovered in three other northeastern states (Maine, Delaware, Maryland) and southern Ontario, Canada (Elkinton, Hajek, Boettner et al.1991). *E. maimaiga* was not recovered from

larvae collected from Virginia, West Virginia, western sections of Maryland and Pennsylvania, despite the fact that these regions experienced dense gypsy moth populations and equally rainy conditions that year.

The prevalence of *E. maimaiga* in 1989 and 1990 was probably due in part to above-average precipitation in the month of May which coincided with increased gypsy moth populations. Despite below-average precipitation in May and June 1991, however, *E. maimaiga* was recovered at numerous sites and caused epizootics in some areas. Disease outbreaks in 1989-1991 cannot be attributed to field releases of the 1984 Japanese isolate of *E. maimaiga* in 1985 and 1986 because high prevalence and widespread distribution of the disease occurred far from the release sites in New York and Virginia, respectively.

In view of the uncertainties in the geographical spread and distribution of *E. maimaiga*, scientists suggest the following alternative hypotheses on how the pathogen may have established in the Northeast:

1. *E. maimaiga* is native to North America.
2. Introductions of *E. maimaiga* in 1910 and 1911 were successful and spread slowly in isolated areas.
3. The strain of *E. maimaiga* released in 1910-1911 was weak but survived in the environment for many years. A more highly pathogenic strain evolved from these releases and began to spread.
4. *E. maimaiga* did not become established from the 1910-1911 releases but was more recently introduced (by accident) into the United States.
5. *E. maimaiga* dispersed independently from Japan to North America.

Hypothesis #3 or #4 seems the most likely explanation for the current occurrence of *E. maimaiga* (Hajek, Humber, Elkinton 1995) due to the fact that *E. maimaiga* was not detected in North America before 1989.

Between 1990 and 1994, the northeastern U.S. strain of *E. maimaiga* was released through inoculations in 147 sites along the leading edge of gypsy moth spread (Hajek and Shimazu 1996). In spring 1991, *E. maimaiga* rest-

ing spores in soil were collected in central Massachusetts and released in thirty-four 0.01 hectare (1/40-acre) plots in Pennsylvania, West Virginia, Virginia and Maryland. By July 1991, infections were found in 28 of the 34 release sites, reaching outbreak levels at some sites. In 1992, *E. maimaiga* resting spores collected in central New York were released in seven plots in Virginia and West Virginia, and high levels of infection were subsequently found at nearly all sites where *E. maimaiga* was released in 1991 and 1992 (Hajek, Elkinton, Witcosky 1996).

By July 1992, *E. maimaiga* infestation of gypsy moth populations in New England was widespread. It had spread extensively and was found throughout the release areas as well as in many adjacent areas. It is unclear whether this increased distribution was the result of *E. maimaiga* spreading from areas where it was established to the north and east, or if it spread from the isolated 1991 and 1992 introduction sites, or both (Hajek, Humber, Elkinton 1995).

From 1991 to 1992, inoculative releases using soil containing resting spores from Massachusetts or larvae infected with *E. maimaiga* and released onto tree trunks were made in Michigan. *E. maimaiga* became established where both inoculation methods were used (Smitley et al. 1995).

Still Spreading

E. maimaiga continues to expand in areas more recently colonized by gypsy moth (e.g., western Ohio, North Carolina, Wisconsin). There is an overlap of areas where *E. maimaiga* has spread naturally and where it is being introduced (e.g., along the southern and western edges of gypsy moth distribution). Spread of greater than 1 kilometer (0.6 mile) for *E. maimaiga* has been recorded between consecutive years at numerous release sites, but at present the rate of spread cannot be predicted.

E. maimaiga is prevalent throughout low-to-high density gypsy moth populations and can be found in each of the 16 midwest and northeastern states (including the District of Columbia) comprising the area generally infested by gypsy moth. Although the fungus is associated with complete collapse of gypsy moth populations, it is highly variable, and as yet unpredictable. In low-density populations and for fungal releases in isolated areas, population collapses do not always take place (Hajek 1997).

Host Range

There is extensive public interest in the host specificity of *E. maimaiga* both because it spreads rapidly and because it may be developed for area-wide control of gypsy moth in the future. In Asia, it has been reported solely from *L. dispar.* The specificity of a 1984 isolate of *E. maimaiga* from Japan was tested against 20 different insect species in the Lepidoptera (moths and butterflies), Coleoptera (beetles), and Orthoptera (grasshoppers) (Soper, Shimazu, Humber et al. 1988) as well as adult honey bees (Vandenberg 1990). The only insects infected with *E. maimaiga* were Lepidoptera belonging to the Noctuoidea, the superfamily containing *L. dispar.* Within the Noctuoidea, only low levels of infection were found in species other than lymantriids (Soper, Shimazu, Humber et al. 1988).

Studies of gypsy moth larvae caged on the soil, on tree trunks, and in the understory vegetation have shown that larvae caged on the soil were always infected at much greater levels than larvae at other locations, suggesting that larval behavior might be playing a big part in determining specificity of this fungus in the field. Thus, species of Lepidoptera exhibiting the specialized behaviors of gypsy moth larvae (aggregately resting in dark locations such as beneath bark flaps, in bark crevices, and on the soil beneath leaf litter at the base of trees) might be more exposed to this fungus. `

Biodiversity studies were conducted by providing hiding places for 418 species of West Virginia forest caterpillars. Only gypsy moth consistently rested in dark locations during the day, suggesting that the behavior of gypsy moth larvae putting them at high risk of infection is unusual for lepidopteran larvae.

E. maimaiga resting spores germinate approximately two weeks before gypsy moth eggs begin to hatch through 1-2 weeks before pupation (approximately 1-1/2 months). This means that only lepidopteran species with larvae active around the time that gypsy moth larvae are active could potentially become infected (Hajek and Humber 1997).

Effects on Nontargets

To evaluate the host specificity of *E. maimaiga,* larvae were inoculated externally with conidia in the laboratory. A total of 78 species of Lepidoptera (aside from gypsy moth) from 10 superfamilies predominantly native to the Appalachian forests, were challenged during bioassays. Cadavers of 36% of the species produced spores after conidial inoculation; infection occurred in seven of the 10 lepidopteran superfamilies tested, although infection levels at these optimal doses were less than 50% for all superfamilies except Bombycoidea, Sphingoidea, and Noctuoidea.

Within the Bombycoidea and Sphingoidea, only one species, the tobacco hornworm *Manduca sexta* (L.), was infected at greater than 50%. In the Noctuoidea, the Lymantriidae was the only family with high levels of infection (Hajek, Butler, Wheeler 1995). *E. maimaiga* could survive in some species when injected into the body; however, it could not penetrate the skin. Conidia produced from cadavers of monarch butterflies [*Danaus plexippus (L.)*] injected with protoplasts were infective to gypsy moth. This suggests that conidia produced in alternate hosts are infective (Hajek, Butler, Wheeler 1995).

Lepidopteran larvae were reared from seven plots in Virginia in which moderate density gypsy moth populations simultaneously exhibited greater than 40% *E. maimaiga* infection. Of a total of 1,511 larvae from 52 species belonging to 7 lepidopteran families in 4 superfamilies, only 2 insects, 1 of 318 forest tent caterpillars, *Malacosoma disstria* Hubner (0.3% infection), and 1 of 96 *Catocala ilia* (Cramer) (0.1% infection) became infected by *E. maimaiga* and no lymantriids were infected (Hajek, Butler, Walsh et al. 1996).

In general, higher rates of infection over a greater diversity of species were achieved in the laboratory (physiological host range) than infections induced in the field (ecological host range). For the one species (*M. disstria*) infected in both the laboratory and field, percentages of infection from the laboratory studies were higher than the findings obtained from the field. Furthermore, 279 nontarget Lepidoptera belonging to 34 species in eight families were collected and reared from areas with low-density native gypsy moth populations. *E. maimaiga* infections were not found in these nontarget hosts, although *E. maimaiga* was active in gypsy moth populations. A survey of lepidopteran cadavers containing entomophthoralean spores collected from 1989

to 1995 documented *E. maimaiga* infections in 3 species of lymantriids (Hajek, Butler, Walsh, et al. 1996). These results demonstrate that data from laboratory bioassays provide poor estimates for predicting nontarget impact and that *E. maimaiga* is predominantly gypsy moth specific under field conditions.

Potential as a Mycoinsecticide

Mycoinsecticides are insecticides developed from fungi. Use of *E. maimaiga* as a mycoinsecticide holds potential, with the following constraints and limitations:

Among the entomophthoralean fungi, conidia are relatively short-lived and not considered an optimal stage for release. Instead, resting spores have been suggested as an excellent life stage for use in pest control. For example, *E. maimaiga* resting spore introductions against gypsy moth larvae were restricted to redistribution of relatively small titers (e.g., 6×10^5 resting spores per 0.01 ha plot) of resting spores collected in the field to establish this fungus in new areas. At present, if *E. maimaiga* resting spores are produced in vivo in the laboratory, it is uncertain whether they would mimic the phenology of field-collected resting spores (Hajek and Humber 1997). Relocation of *E. maimaiga* resting spores and their soil habitat from one location to another requires obtaining the necessary permit from the USDA Animal and Plant Health Inspection Service (APHIS). Precautions must be taken to ensure that plant pathogens (e.g., *Armillaria mellea* rhizomorphs) and arthropod pests are not unintentionally spread with the releases.

The relocation of resting spores using gypsy moth cadavers collected from the field is another method of spreading *E. maimaiga.* Unfortunately, this method can also spread other unwanted microorganisms.

Artificial inoculation of *E. maimaiga* into areas where gypsy moth is present or areas without gypsy moth but where an infestation is anticipated, is not recommended at this time due to the following factors:

- Uncertainty concerning impact to specific nontarget lepidopteran species (especially rare and endangered species).
- Rapid rate of natural spread.

- Inadvertent spread of unwanted microorganisms.
- Lack of understanding concerning conditions necessary for resting spore survival and the factors that initiate germination of resting spores.

As an alternative to artificial distribution of *E. maimaiga* via field collected gypsy moth cadavers and soil containing resting spores, Ann Hajek (co-author of this booklet) has begun efforts to produce the fungal resting spores in the laboratory on artificial media.

Mass Production for Inoculation

Widespread use of entomophthoralean fungi for insect control is not possible at this time because of the high cost of mass producing the fungal stages used for control in the laboratory. The majority of releases of *E. maimaiga* to date used field-collected resting spores that had been leached into the soil. Only recently has it been possible to produce *E. maimaiga* resting spores outside of hosts, but methods for mass production have not yet been developed.

Constraints on Operational Use

For their host-specific characteristics, entomophthoralean fungi may have appeal to a small market. However, serious constraints limit the development and application of entomopathogenic fungi as mycoinsecticides. Among these are:

- Foliar applications of fungi are extremely sensitive to external, abiotic factors (desiccation, degradation by ultraviolet light and solar heat, removal from target habitat by rainfall).
- Synthetic chemical insecticides kill hosts faster than entomopathogenic fungi and thus have less associated damage following treatment.
- Fungi are often short-lived in storage and relatively expensive to produce.

Limited markets may exist for entomophthoralean fungi because of their host specificity and limited impact on nontarget pest species (entomopthoralean fungi are less likely to affect insect pest species for which they are not intended).

Research and Methods Development

Additional research is critically needed in the areas of field ecology, biology, and population dynamics of *E. maimaiga* before this fungus can be developed and used as a mycoinsecticide. Many unanswered issues concerning *E. maimaiga* require data. Two of these issues are as follows:

- Need to identify factors that: (a) trigger germination of resting spores in various microhabitats, (b) influence larval infection and disease incubation period, and (c) affect spatial and temporal patterns of spore dispersal.
- Determine how host and pathogen densities, as well as biological interactions between the fungus and NPV, influence the transmission and spread of the disease.

Evaluations of the effects of *E. maimaiga* on nontarget organisms are ongoing. Commercial and economic feasibility of using *E. maimaiga* as a biocontrol agent depend on the following:

- Identification of fungal strains for commercial laboratory production will require data on survival in various habitats, growth and sporulation characteristics, genetic stability, and pathogenicity and virulence.
- Development of commercially acceptable method of laboratory production and appropriate formulation for mass production.
- Development of a standard quantitative bioassay procedure similar to the standardized *Bacillus thuringiensis* (*Bt*) bioassays in order to optimize any potential use of *E. maimaiga* for controlling gypsy moth.

Summary

In 1909, gypsy moth larvae infected with a fungus were collected from Japan and brought to the U.S. Between 1910-1911, larvae infected with the "gypsy fungus" were released near Boston, Massachusetts. No fungal infections resulted. In 1984, Soper and Shimazu isolated a fungus (*Entomophaga maimaiga)*, from Japanese gypsy moth. In 1985, this Japanese isolate was released in Allegany State Park, New York, and in Shenandoah National Park, Virginia, in 1986. These releases were unsuccessful because transmission to native gypsy moth was almost non-existent.

In June and July 1989, *E. maimaiga* was recovered in North American gypsy moth and found to cause extensive epizootics in seven northeastern states. By the following year, 1990, this fungus was recovered in 10 northeastern states and southern Ontario. The northeastern U.S. strain of *E. maimaiga* has been released since 1991 along the leading edge of gypsy moth spread. Nonetheless, *E. maimaiga* is now so widespread in the northeast that it is difficult to determine whether the fungus in specific areas originated from release sites or from natural migration.

Efforts are ongoing to develop a method for producing *E. maimaiga* in the laboratory toward its use as a mycoinsecticide for area-wide management of gypsy moth. Ecological host range studies are continuing in leading-edge gypsy moth populations in North Carolina, West Virginia, Virginia, and Michigan.

E. maimaiga primarily infects gypsy moth although a limited number of other closely related caterpillars can become infected at low levels. It is not known to pose health risks to people or pets.

The conditions necessary for *E. maimaiga* epizootics to develop are not yet clearly understood. As such, predictions about the frequency or intensity of naturally occurring epizootics cannot be made at this time.

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Caution: Insecticides may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all insecticides selectively and carefully. Follow recommended practices for the disposal of surplus insecticides and insecticide containers.

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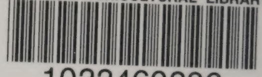
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