

Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*

(Entomophthorales/epizootic/Lymantriidae)

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ABSTRACT An entomopathogenic fungus, *Entomophaga maimaiga*, was found causing an extensive epizootic in outbreak populations of the gypsy moth, *Lymantria dispar*, throughout many forested and residential areas of the northeastern United States. This is the first recognized occurrence of this or any entomophthoralean fungus in North American gypsy moths, and its appearance was coincident with an abnormally wet spring. Most fungal-infected gypsy moth larvae were killed in mass during the fourth and fifth stadium and were characteristically found clinging to the trunks of trees with their heads pointed downward. The fungus produces thick-walled resistant resting spores within dried gypsy moth cadavers and infectious conidia when freshly killed larvae are held in a wet environment. The morphology and development of the fungus are described. The fungus appears to have had its origin in Japan, and the current epizootic may have resulted from the survival and inapparent spread of an early introduction in 1910-1911.

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is the most important defoliator of hardwood forests in the northeastern United States. It is an imported insect from France that was accidentally introduced in Massachusetts around the Boston area in 1869. The pest is naturally present throughout most of Europe and Asia.

Entomophaga maimaiga Humber, Shimazu, and Soper (Entomophthorales: Entomophthoraceae) is a highly virulent fungal pathogen of the gypsy moth. It is considered to be one of the most important natural mortality factors affecting gypsy moth populations in Japan, where it periodically causes extensive epizootics that in some instances can completely destroy an outbreak population (1-8). The fungus has a very limited host range (9), and its morphology, taxonomy, and pathogenicity have been fully characterized (7, 9, 10).

In 1910-1911, a fungus now believed to be *E. maimaiga* (9) was imported from Japan via infected gypsy moths and subsequently was released at several locations near Boston (11). This Japanese "gypsy fungus," as it was referred to by the authors, was thought to have failed to establish itself, however, because of an outbreak of nuclear polyhedrosis virus (NPV), which apparently caused a collapse in the host gypsy moth population (11). The fungus was never recovered, and despite numerous surveys (12-18), neither *E. maimaiga* nor any other entomophthoralean fungus has ever been observed in North American or European gypsy moth populations.

In early June 1989, large numbers of dead and dying gypsy moth larvae were found clinging to the trunks of trees throughout many forested and residential areas of the northeastern United States. Microscopic examination of these larvae revealed the presence of a fungal pathogen morphologically identical to *E. maimaiga*, thus representing the first

reported occurrence of this fungus in North American gypsy moths. In this report we present a full description of the fungus and its disease in native gypsy moths and further recount its distribution, epizootiology, and impact on the population.

MATERIALS AND METHODS

Identification and Characterization of the Fungus. Characterization of the fungus was made from microscopic examination of naturally infected *L. dispar* larvae collected from several different locations in Connecticut during June and July 1989. Observations were made from living host larvae and from cadavers in various states of decay, and all stages of *E. maimaiga* were described. Measurements of spores and vegetative stages were obtained from wet-mount preparations of live fungus by using an ocular micrometer with phase-contrast microscopy ($\times 400$). Nuclear counts were additionally made from fungal samples stained with 2% lactoaceto-orcein (Fisher).

To facilitate observations on conidia formation and transmission of the fungus, moribund and recently killed larvae were individually placed in 60 \times 15 mm Petri dishes containing moistened filter paper discs and held in the dark at 20°C. Under these saturated conditions, conidiophore eruption and subsequent discharge of conidia would usually occur within 24-72 hr. Sporulating cadavers were additionally allowed to "shower" onto healthy second-to-fourth instar gypsy moth larvae that were placed atop the cadavers and separated from them by a 20-gauge mesh screen. These healthy larvae were exposed for 24 hr and then individually reared on an artificial diet until they died.

Distribution and Surveillance. Immediately after the initial discovery of fungal-infected larvae in June, a systematic survey of all gypsy moth-infested areas of Connecticut was initiated to obtain qualitative information on the distribution and prevalence of the fungus within the gypsy moth population. The survey was conducted during late June and throughout July and involved the collection and microscopic examination of live, moribund, and dead larvae. Larval and pupal specimens were also field-collected from various locations in New Hampshire, New Jersey, New York, Massachusetts, Pennsylvania, and Vermont from July through November, and these were similarly examined.

Impact on Gypsy Moth Populations. To obtain some quantitative assessment of the impact the fungal epizootic had on the gypsy moth population, egg-mass counts were done in 37 locations in western Connecticut. Densities were determined in the preceding spring (March and April) and after the active gypsy moth season in the fall (October). In most locations, the same 0.025-hectare (ha; 1 ha = 10,000 m²) area plot was visited both times, and the number of viable egg masses was determined on all objects as well as trees. In two locations in

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Abbreviations: ha, hectare; NPV, nuclear polyhedrosis virus.

Wilton and one in Weston in Connecticut's Fairfield County, egg-mass densities were determined by using the fixed- and variable-radius technique of Wilson and Fontaine (19), with 8–12 points per location.

Defoliation estimates were additionally obtained in July from aerial surveillance of all gypsy moth infested areas of Connecticut.

RESULTS

Field Observations. Fungal-infected gypsy moth caterpillars were first noticed on June 9, 1989, in a mixed hardwood forest (comprised of oak, *Quercus* spp.; maple, *Acer* spp.; birch, *Betula* spp.; and beech, *Fagus* sp.) in Wilton, Connecticut. The gypsy moth population had increased rapidly from the previous year, and most larvae at this time were in the fourth stadium. By June 19, thousands of dead and dying fourth- and fifth-instar larvae were seen throughout many infested areas of southwestern Connecticut. Larvae killed by the fungus were typically attached by their prolegs to the lower portions of tree trunks with their heads pointed downward (Fig. 1). Some larvae were also seen hanging limply from the bark in an inverted "V" position as is characteristic of infection with NPV; but unlike virus-killed larvae, none were found hanging from small branches or foliage.

Larvae recently killed by the fungus were soft-bodied and flaccid, also as if infected with NPV. However, their integument was much more resilient and less easily ruptured. The body contents of these larvae were liquified and usually filled with a mixture of hyphal bodies, immature azygospores, and a few mature resting spores (Fig. 2). Older cadavers that had dehydrated were laterally compressed and black in color. They were most often infected with resting spores. In some

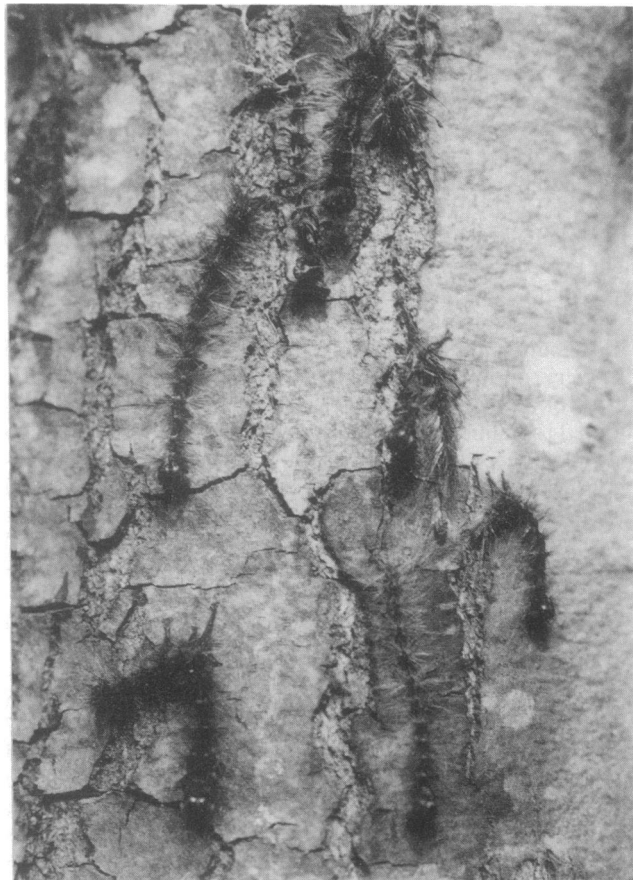


FIG. 1. Dead gypsy moth larvae infected with *E. maimaiga* as observed in the field.

instances, a few cadavers were found covered with a greyish-green velvet-like mat of conidiophores. These cadavers were usually collected from more sheltered microhabitats where humidity and moisture were presumably higher. Conidial eruption was more often obtained, however, by holding fresh soft cadavers that were infected with hyphal bodies and azygospores in Petri dishes with moistened filter paper.

Infected cadavers could be found on tree trunks from June through November, and those collected in the fall (September through November) were typically mummified and filled with mature resting spores. Fungal infections were also seen in a few pupae, but most infected individuals appeared to die in the larval stage.

Fungal Description. The hyphal bodies found in fresh cadavers (Fig. 2A) are unicellular, irregular to sac-like in shape, and multinucleate. They give rise to simple, unbranched conidiophores that grow out through the integument and form a velvet-like coating over the body of the infected larva. A single conidiogenous cell (Fig. 2B) is formed at the tip of the conidiophore. These are multinucleate, clavate to club-shaped, and measure $75\text{--}181\ \mu\text{m} \times 12.5\text{--}25\ \mu\text{m}$. Conidia (Fig. 2C) are obovate to pyriform with a broad papillate base and an evenly rounded apex, have single walls, and are hyaline. They contain *ca.* 25 nuclei (range, 20–31) and a single fat globule and measure $37.5\text{--}50\ \mu\text{m} \times 31\text{--}40.5\ \mu\text{m}$. Secondary conidia are similar in shape but smaller in size. The resting spores (Fig. 2E) are formed as azygospores (Fig. 2D) by budding from a hyphal body. They are spherical (31- to $46\text{-}\mu\text{m}$ diameter), have a thick bilayered wall, contain a large central fat globule, and are hyaline to light-yellow. Cystidia and rhizoids are absent.

Infections are initiated by germinating conidia, which penetrate the integument of the larval host. Infected larvae usually die prior to pupation and characteristically cling by their prolegs to the substrate (tree trunk) with their heads pointing downward. Most infected cadavers show no external fungal growth in the field but are filled internally with resting spores. They become laterally compressed in the absence of moisture and remain attached to trees for several months.

Second- to fourth-instar larvae that were infected in the laboratory via conidial showering usually died within 7–10 days when held under 100% relative humidity at 20°C.

Distribution. Fungal-infected larvae were found throughout all gypsy moth-infested areas of western Connecticut (108 townships in six counties: Fairfield, Hartford, Litchfield, Middlesex, New Haven, and Tolland) (Fig. 3). Although we were not able to quantify infection rates, the highest prevalence of the disease appeared to be in southwestern Connecticut (Fairfield Co.), where the gypsy moth populations were the largest and caused extensive defoliation. However, the fungus was also found along the leading eastern edges of the infestation, where gypsy moth populations were very low (<100 larvae/ha).

Fungal-infected larvae were also detected in many other widely separated areas of the northeastern United States. These included: southern Vermont (Bennington Co. and Windham Co.), New Hampshire (Cheshire Co. and Merrimack Co.), central (Franklin Co. and Worcester Co.) and western (Berkshire Co.) Massachusetts, southern (Putnum Co. and Westchester Co.) and eastern (Washington Co.) New York, northern New Jersey (Bergen Co., Hunterdon Co., Mercer Co., Morris Co., and Sussex Co.), and at one locale in Pennsylvania (Monroe Co.). No fungus was found in larval samples from southeastern (Strafford Co.) and northern (Carroll Co.) New Hampshire or southern (Salem Co.) and far western (Warren Co.) New Jersey.

Gypsy moth infection with NPV was rare and was found in only 2 of the 151 sampled locales, those being New Hamp-

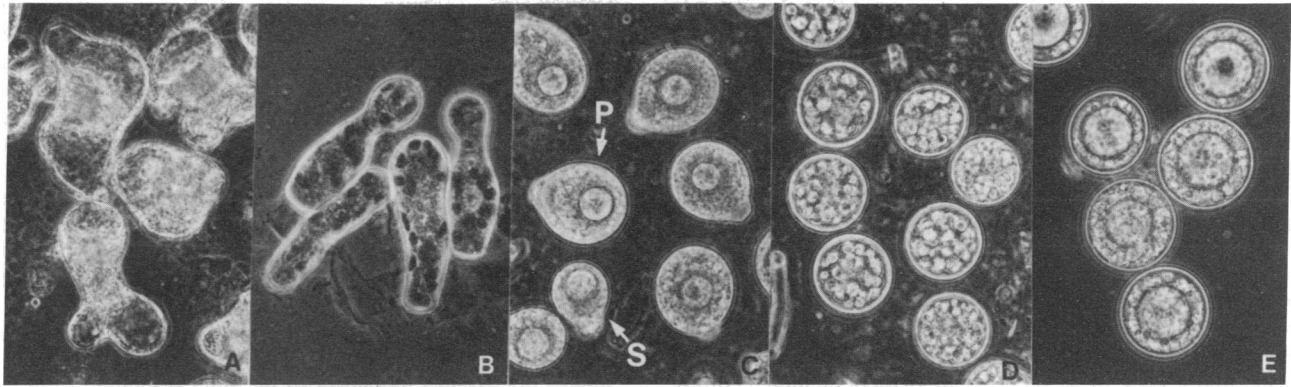


FIG. 2. Developmental stages of *E. maimaiga* from naturally infected gypsy moth larvae. (A) Hyphal bodies. ($\times 240$.) (B) Conidiogenous cells stained with lactoaceto-orcein. ($\times 265$.) (C) Primary (P) and secondary (S) conidia. ($\times 295$.) (D) Immature azygospores. ($\times 275$.) (E) Mature resting spores. ($\times 290$.) All are phase-contrast micrographs, and all specimens are live except in B.

shire's Stafford Co. and New Jersey's Salem Co., where no fungal infections were observed.

Impact on the Gypsy Moth Population. A total of 66,507 ha of residential and forested land, mostly in southwestern Connecticut (Fig. 4) experienced gypsy moth defoliation in excess of 10%. However, only 16,263 ha were severely defoliated (>75%), and more than half of the acreage (34,766 ha) was defoliated <25%.

Significant declines in gypsy moth egg mass densities (from -40 to -2727 egg masses per ha) were observed in most areas of southwestern Connecticut that experienced noticeable defoliation during the season (Fig. 4). Conversely, notable increases in the population (87 to 15,850 egg masses per ha) were generally seen along the leading edge of the infestation, where defoliation was negligible (<10%) in 1989. Where no egg masses were detected in the spring, no egg masses were found in the fall.

DISCUSSION

The discovery of *E. maimaiga* causing an extensive epizootic in larval gypsy moth populations throughout many areas of the northeastern United States marks the first recognized occurrence of this fungal pathogen in North America. Our observations on the morphology, development, and pathology of the fungus in native gypsy moths are consistent with the description of *E. maimaiga* from Japanese gypsy moths

(4, 7, 9). The only exception seems to be the size of resting spores and conidia. These fall within the range reported from Japanese isolates [resting spores, 20–42 μm ; conidia, 20.5–42 $\mu\text{m} \times 15.8$ –34.5 μm (4, 9)] but on average appear to be slightly larger.

The characteristic disease symptomology displayed by infected gypsy moths in the field, with larvae dying as late instars and clinging to the bark of trees with their heads facing downward and laterally compressed bodies, are also virtually identical to those reported in outbreak populations in Japan (1–5, 8), where epizootics of *E. maimaiga* occur regularly. Additional confirmation on the identity of this fungus is reflected in the strong specificity of *E. maimaiga* for *L. dispar*. This has been demonstrated in transmission tests (9, 11) in which native gypsy moth larvae have shown susceptibility only to isolates of *E. maimaiga* originating from Japanese gypsy moths and have never been infected successfully by any North American isolates of *Entomophaga* from other lepidopteran hosts. Further corroboration has come from recently completed isoenzyme comparisons, which have shown that the two fungal isolates from Japan and North America are identical (20).

Based on the high prevalence and widespread distribution of the fungus in gypsy moth populations throughout the northeastern United States, it appears that the epizootic was

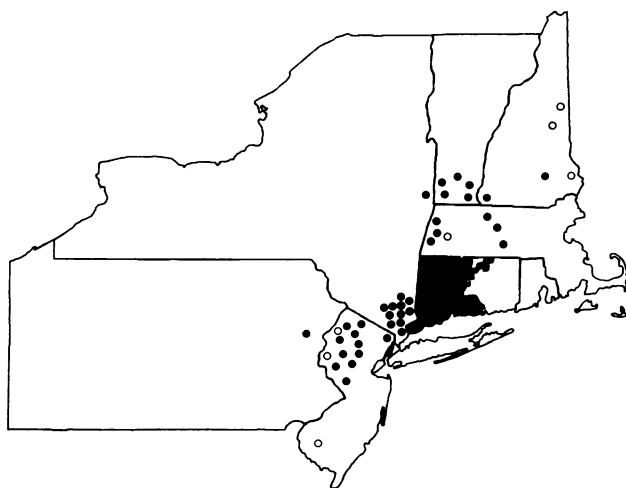


FIG. 3. Distribution of *E. maimaiga* in gypsy moth populations in the northeastern United States. Black circles and areas denote the presence of the fungus and open circles represent larval samples without fungus.

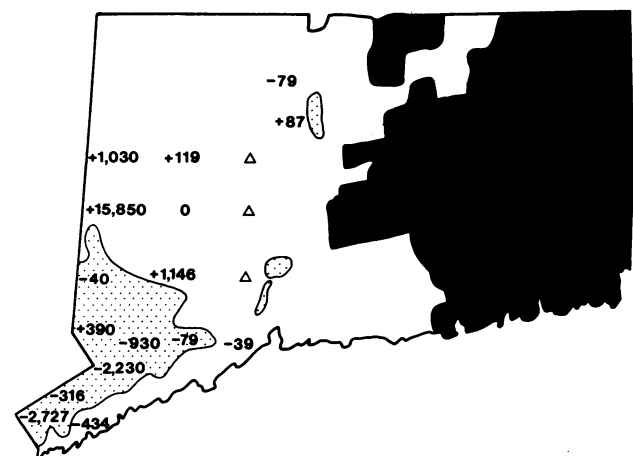


FIG. 4. Net change in gypsy moth egg-mass densities per ha (postseason minus preseason town averages) at various locales in Connecticut during 1989. Unshaded and stippled areas denote regions of infestation and distribution of *E. maimaiga*. Stippled areas denote regions of the state experiencing gypsy moth defoliation in excess of 10%. Δ , Locales where no egg masses were detected on either occasion.

not the result of a recent introduction, and that this fungal pathogen has probably been present in gypsy moth populations for some time. Reasons for its sudden and dramatic occurrence are not entirely clear. The first reported introduction of *E. maimaiga* into North America was by Speare and Colley nearly 80 years ago (11). Although they never recovered the fungus, it is possible that it may have survived via resting spores and spread slowly through the gypsy moth population. In summarizing their experiments, Speare and Colley speculated that "should it (*E. maimaiga*) obtain a foothold in the field, it might be expected to prove continuously effective from season to season, owing to its habit of forming resting spores in great abundance, which experiments have shown are able to survive the New England winter, and a very slight increase in virulence, such as often appears in parasitic fungi in successive seasons, might bring about quite different results." Soper *et al.* (9) also report a recent inoculation of gypsy moth larvae with *E. maimaiga*, obtained from isolates received from Japan in 1984, at field sites in Allegheny State Park, New York, and Shenandoah National Park, Virginia. However, considering the widespread distribution of the current epizootic, it seems unlikely that the fungus could have spread to such a large area in such a short time. We additionally cannot entirely dismiss the possibility that *E. maimaiga* was inadvertently introduced, along with egg parasitoids from Japan, at another time via contaminated egg masses, which have been shown to harbor resting spores (6). In any event, we suspect that *E. maimaiga* has maintained itself at very low levels and gone undetected because environmental conditions, most notably rainfall, have not been adequate to initiate an epizootic when gypsy moth populations have been large. This view is consistent with the observations of Shimazu and Soper (7) who report that 100% relative humidity is necessary to infect gypsy moth larvae with *E. maimaiga*. Tyrrell (21) has further demonstrated that free water in the form of rainfall rather than high humidity is required for sporulation of *Entomophaga aulicae*, a closely related species, on mummified cadavers of the spruce budworm, *Choristoneura fumiferana*. Another possibility is that fungal-killed larvae may have been mistaken for NPV-diseased larvae, because gross symptomology is superficially similar in both cases.

In 1989, near record amounts of rainfall were experienced throughout the northeastern United States during May and June. Data gathered from three official National Weather Service Climatological Stations in Connecticut located in Bridgeport, Hartford, and Mt. Carmel, for example, showed average rainfall of 26.9 cm (18 cm above normal) on 13 days in May and 16.1 cm (7.7 cm above normal) on 16 days in June. We contend that the quantity and frequency of rainfall during these two months were primary factors responsible for initiating and maintaining the fungal epizootic. According to Shimazu *et al.* (8), epizootics of *E. maimaiga* in Japan are initiated by the infection of early-instar larvae in the spring with hibernated resting spores that can be found in the leaf litter and soil. Newly hatched larvae are often found on the ground, and this apparently affords ample opportunity to come into contact with germinating resting spores. These larvae die and, if moisture conditions are adequate, discharge conidia, which infect other larvae. This results in an epizootic which is typically expressed in late instars during June and July as was observed here. Although we have no definitive information on the source of infection, it is logical to presume that the present epizootic in the northeastern United States was initiated via resting spores.

It is difficult to assess the impact that the epizootic had on gypsy moth populations. Most of the fungal-induced mortality occurred among late-instar larvae during June. As a result, defoliation was primarily limited to the oaks, and many less-preferred host trees (i.e., birch, beech, and maple) that

normally would have been defoliated were scarcely attacked. NPV was not an important mortality factor in 1989, as very few NPV-infected larvae were encountered throughout the fungal-affected regions. Although we did not measure mortality caused by other natural enemies, it is well recognized that in building gypsy moth populations, such as the present one, such mortality is typically quite low (22). Furthermore, we note that in 1980, comparable outbreak gypsy moth populations defoliated more than twice the acreage (154,540 ha vs. 66,507) in Connecticut (23), and we feel certain that had the fungal epizootic not occurred in 1989, defoliation would have been far greater.

Significant declines in egg-mass densities were also observed in many infested areas of southwestern Connecticut, where the disease appeared to be most prevalent, and contrary to the last outbreak in 1980–1983, there was very little eastward expansion of the infestation. However, the population did not collapse everywhere, and increased egg-mass densities were recorded along the leading edge of the infestation where many infected larvae were also found.

We can only speculate on the long-range impact that the fungus may have on North American gypsy moth populations. The fungus now appears to be firmly established in the northeastern United States, and probably, there is more inocula in the environment than at any other time. However, given its apparent dependence on moisture in the form of rainfall, the degree to which *E. maimaiga* will suppress gypsy moth populations as it does in Japan and help to prevent outbreaks in the future are unknown.

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