Entomopathogenic hyphomycetes associated with gypsy moth larvae

Ann E. Hajek
Department of Entomology, Cornell University, Ithaca, New York 14853-0901

Joseph S. Elkinton
Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003

Richard A. Humber
U.S.D.A., Agricultural Research Service, U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, New York 14853-2901

Abstract: Gypsy moth, Lymantria dispar, populations were sampled in four eastern North American states during 1991 and 1992 to evaluate levels of hyphomycete infection in association with releases of the Asian gypsy moth pathogen Entomophaga maimaiga. Paecilomyces farinosus was the most abundant hyphomycete species, occurring at the majority of sites, although levels of infection averaged only 4.6% (1991) and 12.2% (1992). In the plots sampled, concurrent levels of infection by E. maimaiga averaged 22.2 ± 5.5 during 1991 and 71.4 ± 12.7% during 1992 but there was no association between prevalence of P. farinosus and E. maimaiga. Beauveria bassiana was the only other hyphomycete killing larvae in the field but occurrence was rare. Verticillium lecanii and Fusarium polyphialidicum were both isolated from cadavers and could cause larval mortality during laboratory bioassays.

Key Words: biological control, Entomophaga maimaiga, fungal entomopathogen, Lymantria dispar, Paecilomyces farinosus

The entomopathogenic fungus Entomophaga maimaiga Humber, Shimazu & Soper originated in Asia and was first noted in North America in 1989 (Andreais and Weseloh, 1990; Hajek et al., 1990). Since that time, epizootics have repeatedly been observed in North American populations of gypsy moth, Lymantria dispar (L.) (Hajek et al., 1990; Hajek et al., 1996a, b; Smitley et al., 1995; A. E. Hajek, unpublished). The long-term impact of this entomophthora-
TABLE I. Entomopathogenic hyphomycetes infecting gypsy moth larvae collected in Maryland, Pennsylvania, Virginia and West Virginia during 1991 and 1992

<table>
<thead>
<tr>
<th></th>
<th>No. sites</th>
<th>In living larvae</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presenta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991 (35 sites)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Paecilomyces farinosus</em></td>
<td>31</td>
<td>26</td>
<td>4.9 ± 0.8</td>
<td>2.0–18.2</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td>4</td>
<td>3</td>
<td>1.6 ± 0.6</td>
<td>0.9–2.7</td>
</tr>
<tr>
<td><em>Verticillium lecanii</em></td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Fusarium polyphialidicum</em></td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1992 (10 sites)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Paecilomyces farinosus</em></td>
<td>8</td>
<td>8</td>
<td>12.2 ± 2.7</td>
<td>4.4–25.5</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a Pathogens found associated with gypsy moth larvae collected alive that subsequently died as well as gypsy moth larval cadavers.

b Calculated only for sites where pathogens were isolated from cadavers of larvae alive when collected.


3–4 wk after larvae reached mid-fourth instar. Each week from each plot, up to 50 living larvae were collected individually in 29-mL plastic cups containing high wheat germ artificial diet (Bell et al., 1981) and monitored in an outdoor insectary for 1 wk to detect mortality. To prevent potential contamination when checking larvae, diet cups were opened only when absolutely necessary. Any larvae that died in the cups were monitored for three additional days at 20 C to detect conidial production by *E. maimaiga* and were then stored at 4 C. During each field collection, up to 50 cadavers were also collected from each plot.

For 35 plots in 1991 (24 *E. maimaiga* release plots and 11 controls), and 10 plots in 1992 (7 *E. maimaiga* release plots and 3 controls), samples with visible hyphomycete mycelium associated with gypsy moth cadavers were processed to isolate and identify the hyphomycetes. For each sample, under sterile conditions small sections of mycelium were placed on three 50-mm petri dishes containing half-strength Sabouraud dextrose agar plus 5% yeast extract (SDAY) (Hajek et al., 1993). Petri dishes were maintained at 20 C in the dark and checked weekly for fungal growth. Fungal isolates belonging to genera that are known to be insect pathogens were identified to species. Isolates of each potentially pathogenic species were tested for pathogenicity to gypsy moth larvae following bioassay methods described by Hajek et al. (1993). Only fungal species infecting at least 1 of 10 larvae challenged were considered to be pathogenic. Infection was defined as mortality plus subsequent outgrowth by the fungus that had been inoculated. Representative isolates of each species of entomopathogen isolated were deposited in the USDA, Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, New York.

For data analysis, the presence of a fungal pathogen was evaluated separately if the pathogen was isolated from cadavers of larvae collected alive versus field-collected cadavers. We did this because we do not know whether those pathogens isolated from field-collected cadavers were the cause of host death; these fungi have the potential to grow saprophytically and could have colonized cadavers after hosts died from some other cause, e.g., predators, parasitoids, etc.

Infection by each hyphomycete species was quantified using methods of Gould et al. (1990, see Appendix therein) to estimate marginal probabilities of mortality due to simultaneous mortality agents. Methods described by Hajek et al. (1990) were used to estimate season-long infection. For those hyphomycetes isolated from cadavers within which a more obligate parasitoid or pathogen had also developed, e.g., gypsy moth nuclear polyhedrosis virus or tachnid parasitoids, the hyphomycete was not considered to be the cause of death. For specimens in which fungal species cooccurred, the cooccurring fungal species were considered to be equally responsible for host death.

Results.—Low levels of infection due to hyphomycete fungi occurred in the majority of plots (TABLE I). *P. farinosus* was by far the most abundant hyphomycete pathogen isolated from insects dying after collection. *P. farinosus* was fairly ubiquitous, being isolated from living larvae that died and/or cadavers at 31 of 35 plots (88.6%) in 1991 and 8 of 10 plots (80.0%) in 1992. However, *P. farinosus* infections were never abundant, with average infection of 4.9% in 1991 and
12.2% in 1992. The maximum infection level for *P. farinosus* was 25.5% at one site in 1992.

To evaluate cooccurrence of *E. maiimaiga* and *P. farinosus* in gypsy moth populations, levels of infection were evaluated at 21 plots in 1991 and 7 plots in 1992. *E. maiimaiga* infection levels were low at most plots during 1991 (22.2 ± 5.5%; mean ± SE) although this disease caused epizootics at many sites during 1992 (74.2 ± 12.7%). No statistically significant association was found between arc sine-transformed percentages of infection by *E. maiimaiga* and *P. farinosus* for either 1991 or 1992 (p > 0.05). *P. farinosus* and *E. maiimaiga* were never found in the same cadaver.

To evaluate potential associations between infection levels and weather, weather variables were estimated for the isolated study sites by interpolation using latitude, longitude, and elevation of study sites (analyses were conducted by ZedX, Boalsburg, PA). This technique has previously been successfully used to predict plant disease occurrence (Royer et al., 1989) and gypsy moth phenology (Russo et al., 1993). Prevalence of *E. maiimaiga* infections was positively associated with May rainfall and negatively associated with May and June temperature during 1991 with positive associations between May rainfall and May and June temperature during 1992 (Hajek et al., 1996b). Infection by *P. farinosus* was not associated with May or June rainfall abundance or frequency or May or June temperature (p > 0.05).

*B. bassiana* was the only other entomopathogen isolated from gypsy moth larvae that had been collected alive. *B. bassiana* was found in few plots, percent infection was extremely low, and *B. bassiana* was only isolated from larvae collected alive and dying after collection in 1991 (Table 1). During 1991, *B. bassiana* was isolated from a total of three live-collected specimens and in two of these larvae, *B. bassiana* coinfectected with *P. farinosus*.

*Verticillium lecanii* (Zimmerman) Viégas and *Fusarium polysphialidicum* Marasas, Nelson, Tousson & Van Wyk were both isolated only from field-collected cadavers. While isolates of both of these species killed gypsy moth larvae during laboratory bioassays, we do not know whether these pathogens killed the individual larvae from which they were isolated or colonized the cadavers after death from another cause. *F. polysphialidicum* has previously been isolated from gypsy moth larvae that were collected live and subsequently died (Hajek et al., 1993); based on this information, we assume that this pathogen causes infection in the field but at very low levels. *V. lecanii* has never before been reported in association with gypsy moth larvae in North America, even though this species is widely recognized to be a pathogen of a diverse range of insects.

**Discussion.**—Before *E. maiimaiga* was documented in North America in 1989, a survey of fungal pathogens associated with gypsy moth in one county in Pennsylvania identified *P. farinosus* and *B. bassiana* as the most common pathogens (Machrowicz and Yendol, 1973). Our study covering four different states, once again demonstrated that these two species were the most common hyphomycetes associated with gypsy moth larvae, albeit at low levels. However, epizootics caused by hyphomycetes have never been reported from North American gypsy moth populations. The present study frequently documented lower levels of infection by *P. farinosus* and *B. bassiana* than those reported by Machrowicz and Yendol (1973). However, results from this previous study were based on field-collected cadavers, and therefore could easily overestimate the actual levels of infection of live larvae in the field.

By far the most abundant hyphomycete infecting gypsy moth larvae was *P. farinosus*. However, season-long levels of infection never exceeded 25.5%, which is well below the prevalence of *E. maiimaiga* or nuclear polyhedrosis virus during typical epizootics in gypsy moth populations. Studies with *Choristoneura funebrana* Clemens (Lepidoptera: Tortricidae; spruce budworm) have shown that *P. farinosus* is abundant in forest litter, where it can saprophytically increase in abundance (Horney and Widdon 1991a, b). Therefore, the abundance of this pathogen may not be strongly dependent on the presence of insect hosts, as is more characteristic of entomopathogenic fungi that more frequently cause epizootics. In agreement with findings in North America, hyphomycete infections were consistently low in low density gypsy moth populations native to Bulgaria (Mirchev, 1986) and in outbreak populations native to the Slovak Republic (Novotny and Zubrik, 1995). Interestingly, *Paecilomyces canadensis* (Vuill.) Brown & Smith (a possible synonym of *P. farinosus* according to Brown and Smith, 1957) was found causing mixed infections with *E. maiimaiga* (reported as *Entomophthora auticae*) in 20% of cadavers during an epizootic in gypsy moth in Japan (Aoki 1974). During the present study *P. farinosus* and *E. maiimaiga* were never found in the same cadaver and, in fact, the prevalence of *E. maiimaiga* was neither positively nor negatively associated with the abundance of *P. farinosus*. The meager evidence available to date does not indicate that *E. maiimaiga* is displacing *P. farinosus* but to specifically address this subject, further studies with this focus are necessary.

One previous laboratory study testing infectivity of
fungi against gypsy moth larvae found that *P. farinosus* could not infect gypsy moth larvae (Wasti and Hartmann, 1982). While we do not know the origin of the strain that was used in this study, our crude laboratory bioassays using isolates from gypsy moth larvae readily demonstrated infection. Harney and Widden (1991b) demonstrated that strains of *P. farinosus* isolated from *C. fumiferana* grew better on gelatin while saprophytic strains isolated from litter grew better on cellulose. Differences between our findings of ready infectivity and findings by Wasti and Hartmann (1982) of lack of infectivity would further support the observation that strains of *P. farinosus* may have markedly different pathogenic capacities.

The other fungal species isolated from gypsy moth during our study were exceedingly uncommon and it is questionable whether they actually had killed the specimens from which they were isolated. *V. lecani* was isolated from a cadaver from which a tachinid parasite also emerged; this fungal species has not previously been reported in association with gypsy moth. *F. polyphialidicum* is considered to be an opportunistic pathogen (Hajek et al., 1993). Although *A. flavus* was previously recorded from gypsy moth cadavers and moribund larvae (Podgwaite, 1981), we did not find this species during our study.

While *E. maimaiga* is clearly the most important fungal pathogen of gypsy moth in North America, this species has only been investigated in North America since 1989 and, at present, methods for mass production have not been developed. In general, the methodology for mass production of hyphomycetes is much simpler and further developed than that for mass production of species of Entomophthorales. To our knowledge, the only fungus that has been applied as a mycoinsecticide against gypsy moth is *B. bassiana*. Applications in southwestern Slovakia in 1985 and 1986 (Novotny, 1988) and Maryland in 1989 (D. M. Kolodny-Hirsch, pers. comm.) were not successful in controlling gypsy moth populations. From the present study as well as previous surveys (Majchrowicz and Yendol, 1973; Podgwaite, 1981), it appears that *B. bassiana* does not frequently infect gypsy moth larvae under natural conditions in the field. Laboratory bioassays with *B. bassiana* against gypsy moth larvae demonstrated LT$_{50}$ of 11 d for first instars and 20 d for second and third instars at 23 C (Markova and Samsinakova, 1990). Based on these results, Markova and Samsinakova (1990) suggested that *B. bassiana* would not be a promising candidate for development for gypsy moth control; this statement is in agreement with the aforementioned results from field applications as well as our data on the natural occurrence of *B. bassiana* infections. We suggest that, based on its greater natural abundance, *P. farinosus* would be a much better choice than *B. bassiana* if hyphomycetes are further considered for development as mycoinsecticides.

We thank J. L. Perry for excellent assistance with fungal isolation and bioassays, D. Pilarska for Bulgarian translations, and F. Murrin and D. Smiley for helpful reviews of this manuscript. We also thank the many people who assisted with collection of the samples from which fungi were isolated. This research was supported by USDA, Forest Service, Cooperative Agreement No. 42-607.

**LITERATURE CITED**


Hajek et al.: Hypomycetes from gypsy moth larvae


