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# Introduction of the entomopathogenic fungus *Entomophaga maimaiga* Hum., Shim. & Sop. (Zygomycetes: Entomophthorales) to a *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) population in Bulgaria

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## Abstract

**The entomopathogenic fungus *Entomophaga maimaiga* was introduced into a *L. dispar* population situated in South Bulgaria for the first time in Europe.**

**It was found that of all larvae collected in the five experimental plots, 6.3 % contained conidia and resting spores of *E. maimaiga*, 14.2 % contained parasitoids and 2.3 % were infected with nucleopolyhedrovirus (NPV). The presence of the fungus in cadavers collected from each experimental plots and on five of the six collection dates was observed.**

## 1 Introduction

The fungus *Entomophaga maimaiga* Humber, Shimazu & Soper is an entomopathogen of the gypsy moth, *Lymantria dispar* (L.) that is native to Japanese *L. dispar* populations (KOYAMA, 1954). It was introduced into North America nearly 90 years ago (SPEARE and COLLEY, 1912); however it was not recovered from gypsy moth populations until 1989 when it caused a pandemic in the Northeastern United States (ANDREADIS and WESELOH, 1990; HAJEK et al., 1990). *L. dispar* larvae are infected by conidia that are produced by germinating resting spores that overwinter in the forest litter. *E.maimaiga* is effective in both high- and low- density gypsy moth populations. The effects of the fungus on non-target insects was studied by HAJEK et al. (1996) who found only two *E.maimaiga*-infected larvae among 1,511 non-target larvae collected from moderate-density *L. dispar* populations, whereas 41–97.5 % of *L. dispar* larvae were infected by E. *maimaiga*. The non-targets represented 52 species belonging to 7 families. In low-density *L. dispar* populations the authors found that none of 279 non-target larvae representing 34 species in 8 families were infected.

HAJEK et al. (1995) also conducted a laboratory study that determined that 6 of 20 species of non-target Lepidoptera larvae became infected when they were dipped in suspensions of *E. maimaiga* spores. Therefore, she concluded that host specificity data collected in laboratory bioassays are not necessarily a good predictor of the ecological host specificity of an entomopathogen. This suggests that this fungus is quite host specific and can be an effective control agent of *L. dispar* populations not only in Japan and the United States but also in Europe. *E. maimaiga* into a European *L. dispar* population in Bulgaria to improve natural biological control of *L. dispar*, and to evaluate the impact and spread of the entomopathogen.

The objective of this study was to introduce the fungus. For this purpose, we chose a medium density *L. dispar* population located in Central Bulgaria. We selected an oak-dominated forest site for the introduction of *E. maimaiga* located in the region of the Plovdiv Forest Protection Station, Karlovo Forestry (140 km southeast of Sofia). The control site was approximately 7 km from the release site.

## 2 Materials and methods

The population density of *L. dispar* in the experimental and control sites was determined at the beginning of February 1999. At the approximate center of each site a circular plot was created with a radius of 5,64 m and that contained at least 6 overstory trees. Within this plot all new egg masses on trees, on other vegetation, and on the ground were counted. Four additional circular plots each 50 m from the central plot to the north, south, east and west were sampled the same way. Using this method, we determined that the average density of gypsy moth egg masses was 660 egg masses/ha in the experimental release plot and 440 egg masses/ha in the control plot.

*L. dispar* cadavers containing *E. maimaiga* resting spores were obtained from A. E. HAJEK (Cornell University) via the U. S. Department of Agriculture, European Biological Control Laboratory, in Montpellier, France. The cadavers were placed in the release plot on April 10, 1999, prior to hatch of *L. dispar* eggs. The dried cadavers were crushed and placed within 10 cm of the base of 5 overstory trees in the release sites; large leaves and stones were removed from around the base of the trees prior to placement of the cadavers. After the cadavers were in place, 4 l of water were sprinkled around the base of each tree to improve conditions for the germination of resting spores. The area at the base of the release trees was watered weekly thereafter until gypsy moth larvae were 4th instars.

Burlap bands were placed on 15 overstory trees (including the release tree) nearest to the center of the release plot. The Burlap bands encircled the tree trunk and were attached at breast height (1,4 m above ground). Vertical cuts were made in both layers of each burlap band to facilitate collection of larvae.

After observating the presence of late third and early fourth star larvae, collections of larvae beneath the burlap bands were initiated in the experimental site between May 15 and June 25,

*Table 1.* Survival and mortality of gypsy moth larvae collected from the release site.

| Collection Date | Total number of larvae collected | Survived (number) | Number of larvae killed | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *E. maimaiga* | | NPV* | | Parasitoids | | Others | |
| | | | N | % | N | % | N | % | N | % |
| 15.05. | 10 | 3 | 1 | 10 | 0 | 0 | 2 | 20 | 4 | 40 |
| 27.05. | 37 | 20 | 0 | 0 | 1 | 2.7 | 6 | 16.2 | 10 | 27 |
| 03.06. | 29 | 12 | 3 | 10.3 | 2 | 6.8 | 2 | 6.8 | 10 | 34.4 |
| 09.06. | 19 | 7 | 1 | 5.2 | 0 | 0 | 1 | 5.2 | 10 | 52.6 |
| 19.06. | 16 | 10 | 1 | 6.2 | 0 | 0 | 4 | 25 | 2 | 12.5 |
| 25.06. | 15 | 10 | 2 | 13.3 | 0 | 0 | 3 | 20 | 0 | 0 |
| Total: | 126 | 62 | 8 | 6.3 | 3 | 2.3 | 18 | 14.2 | 36 | 28.5 |

* NPV – nucleopolyhedrosis virus

1999 (table 1). Only two collections (total 31 larvae) were made in the control sites due to their inaccessibility. The larvae were individually placed in diet cups.

The larvae were reared individually in the lab at 20–25 °C on oak foliage and observed daily. When larvae died, the day of death was recorded and the cadaver was placed at 20 °C in a humid chamber (we used sterile petri dishes with humid filter paper) for 3–4 days. Subsequently, all cadavers were kept at room temperature for a week to form resting spores, at which time they were stored at 4 °C. Each cadaver was individually dissected and observed under light microscope for the presence of conidia or resting spores.

## 3 Results and discussion

The survival and cause of mortality of all gypsy moth larvae collected under burlap bands from the release site is presented in table 1. Of all larvae collected in the experimental plots, 6.3 % contained conidia and resting spores of *E. maimaiga*, 14.2 % contained parasitoids and 2.3 % were infected with nucleopolyhedrovirus (NPV). We were not able to identify the cause of the mortality for 28.5 % of all collected larvae. *E. maimaiga* was found in cadavers collected from each of the five release trees and on five of the six collection dates. *E. maimaiga* was not found in larvae collected from the control plot. The results obtained indicate that our attempt to introduce *E. maimaiga* in the Karlovo Forestry District in Bulgaria was successful. This is the first reported introduction of this entomopathogen in Europe.

Our results are similar to those of BAUER et al. (1994) who also obtained low levels of infection (5.5 %) in the year that *E. maimaiga* was released in Michigan. However, the level of infection increased substantially in the year after release.

It will be necessary to continue monitoring this gypsy moth population in subsequent years in order to assess the establishment and prevalence of *E. maimaiga* among the larval population. However one of our concerns is that the gypsy moth population is at very low density which is not conducive to maintaining a titer of the fungus in the larval population.

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## References

ANDREADIS, T.; WESELOH, R., 1990: Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. Proc. Natl. Acad. Sci. USA, 87, 2461–2465.

BAUER, L. S.; SMITLEY, D. R.; HAJEK, A. E.; SAPIO, F. J; HUMBER R. A., 1994: Introduction and establishment of *Entomophaga maimaiga* in Michigan. Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Forum, 1994, p. 6.

HAJEK, A. E.; HUMBER, R. A.; ELKINTON, J.; WALSH, S; SILVER, J., 1990: Allozyme and restriction fragment length polymorphism analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. Proc. Natl. Acad. Sci. USA , 87, 6979–6982.

HAJEK, A. E.; BUTLER, E. L.; WHEELER, M. M., 1995: Laboratory bioassays testing the host range of the gypsy moth fungal pathogen *Entomophaga maimaiga*. Biol. Control, 5, 530–544.

HAJEK, A. E.; BUTLER, E. L.; WALSH, S; SILVER, J. C; HAIN, F. P.; HASTINGS, F. L.; ODELL, T. M.; SMITLEY, D. R., 1996: Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus the laboratory. Environ. Entomol. 25, 709–721.

KOYAMA, R. A., 1954: Two epizootic diseases of the gypsy moth. Shirin-Boeki, 27, 296–298. (In Japanese).

SPEARE, A. T.; COLLEY, R. H., 1912. The artificial use of the brown-tail fungus in Massachusetts, with practical suggestions for private experiment, and a brief note on a fungous disease of the gypsy moth caterpillar. Wright and Porter, Boston.

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