Distribution and Apparent Spread of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in Gypsy Moth (Lepidoptera: Lymantriidae) Populations in North America

J. S. ELKINTON, A. E. HAJEK, G. H. BOETTNER, AND E. SIMONS


**ABSTRACT** *Entomophaga maimaiga* Humber, Shimazu & Soper was recovered from gypsy moth, *Lymantria dispar* (L.), populations from Massachusetts, Connecticut, New Hampshire, Vermont, eastern New York, northeastern Pennsylvania, and New Jersey in 1989 and 1990. No *E. maimaiga* was recovered from cadavers of gypsy moth larvae collected in western New York, western Pennsylvania, West Virginia, or Virginia in either year despite high levels of rainfall in these regions in May of both years. In 1990, *E. maimaiga* was recovered in many areas that bordered the 1989 distribution (Maine, central Pennsylvania, central New York, Delaware, and northeastern Maryland) where it had not been recovered in 1989, possibly due to spread of the disease. In 1989, mortality of gypsy moth larvae from sites in Massachusetts was correlated with rainfall, but not with population density.

**KEY WORDS** Insecta, *Lymantria dispar*, *Entomophaga maimaiga*, epizootiology

In 1989, an epizootic of *Entomophaga maimaiga* Humber, Shimazu & Soper decimated gypsy moth, *Lymantria dispar* (L.), populations throughout New England (Andreadis & Weseloh 1990, Hajek et al. 1990a). This agent is an important source of mortality in gypsy moth in Japan, but was not previously recovered in North America. Previous research has shown that *E. maimaiga* conidia germinate only under very wet conditions (Hajek et al. 1990b), a trait that is characteristic of the Entomophthorales (McCoy et al. 1988). In this study, we compare the distribution of *E. maimaiga* infections in gypsy moth populations between 1989 and 1990, and relate this to the distribution of rainfall in May and June of both years. We present evidence from Massachusetts that relates mortality caused by *E. maimaiga* to gypsy moth population density and rainfall.

**Methods**

**Distribution of *E. maimaiga***. In 1989 and 1990, cadavers of larval gypsy moths (instars 4–6) were collected by cooperators in various states who monitor gypsy moth populations in their regions. The cadavers were shipped to the University of Massachusetts or the Boyce Thompson Institute for identification of *E. maimaiga*. Each cadaver was examined under a compound microscope for the presence of conidia or resting spores of *E. maimaiga*.

Rainfall data were obtained from monthly precipitation records published by the National Oceanic and Atmospheric Administration (NOAA Climatological Data). Monthly rainfall at each weather station for May and June 1989 and 1990 was tabulated and averages were calculated for each county in the northeastern United States. These values were plotted using the ATLAS*GRAPHICS mapping program (STSC, Rockville, Md.). For counties that lacked NOAA weather stations (~17% of the total) we plotted the average value for all contiguous counties. Deviations from the 60-yr (1931–1990) average monthly rainfall were tabulated for each weather station and averaged for each state.

**Rainfall and Density Correlation**. In Massachusetts, cadavers were counted and collected at 28 permanent sites maintained by the Department of Environmental Management since 1984 for the purpose of surveying yearly trends in gypsy moth density. Sites were located in forest stands that were deemed susceptible to gypsy moth outbreaks (Houston & Valentine 1977). At each site, burlap bands were wrapped around the boles of 20 adjacent overstory trees at a height of ~1.5 m. At each site in July 1989, gypsy moth larvae, larval cadavers, and pupae were counted under the bands and on the bole of the tree below the bands. Such counts are sensitive indicators of gypsy moth population density, particularly when densities are low (Weseloh 1987, Wallner et al. 1989). Mortality was computed by dividing the number of dead larvae found under the 20 burlaps by the total number of larvae and pupae (live and dead) under all 20 burlaps. Only dead larvae were included in mor-
Table 1. Deviation (in cm) of mean total rainfall recorded for May and June 1989 and 1990 from the 60-yr average (1931-1990) at NOAA weather stations within each northeastern state

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Results and Discussion

E. maimaiga Distribution. In 1989, we recovered E. maimaiga from virtually all samples that we examined from the New England states of Massachusetts, Connecticut, New Hampshire, and southern Vermont, as well as northeastern Pennsylvania, and New Jersey (Fig. 1A) (Hajek et al. 1990a). This region represents the area infested with gypsy moth before 1980. We recovered no E. maimaiga from hundreds of larvae examined from western Pennsylvania, Maryland, or Virginia despite the fact that heavy rainfall occurred throughout this region (Fig. 1A, 2A and B; Table 1). The only samples from New England in which we detected no E. maimaiga were collected in northern Vermont and Maine in 1989. Rainfall in northern New England in May and June 1989 was generally lower than that in Connecticut and western Massachusetts (Fig. 2A and B). Thus it seems likely that previous studies suggested that few gypsy moths die from E. maimaiga in the pupal stage (unpublished data). To assure sufficiently accurate measurements of mortality, we arbitrarily restricted our analyses to sites with at least 20 gypsy moths under the 20 bands (18 sites total).

The proportion of larvae that were dead at each site was transformed to arcsin (square root) and regressed against gypsy moth density (log$_{10}$ of total number of live and dead larvae and pupae) and against rainfall for May and June of that year at the nearest NOAA weather station. We applied simple regressions of mortality versus density and rainfall separately, and a stepwise multiple regression against both variables with a model entry criterion of $P = 0.15$ using PROC REG of the SAS statistical package (SAS Institute 1985).

Fig. 1. Distribution of sites across the northeastern United States where E. maimaiga was recovered (●) and was not recovered (○) from cadavers of gypsy moth larvae. Figure 1A is reproduced from Hajek et al. (1990a). Boundary lines (---) define approximate maximum geographical extent of gypsy moth defoliation for the indicated years (Hajek et al. 1990a).
that the near-record levels of rainfall that occurred in May and June of 1989 helped promote the occurrence of *E. maimaiga* epizootics. However, regional differences in rainfall do not appear to account for the absence of *E. maimaiga* among gypsy moths in the southern and western parts of its North American distribution. The geographical distribution of *E. maimaiga* suggests that it was introduced into New England, possibly from the intentional releases that occurred near Boston in 1910 and 1911 (Speare & Colley 1912, Hajek et al. 1990a).

In 1990 we recovered *E. maimaiga* again from the same regions where we had detected it the previous year (Fig. 1B). Rainfall was again much higher than average throughout the region in May 1990 (Fig. 2C, Table 1), but June was generally much drier (Fig. 2D) and rainfall was well below average except in Maine, Vermont, and New Hampshire (Table 1). Nevertheless, the vast majority of mortality that we observed from *E. maimaiga* (97% of 2,186 cadavers collected at sites in western Massachusetts) appeared after 25 June 1990.
The apparent expansion of the range of *E. maimaiga* may be caused by spread of the disease to uninfected populations or by the increase in frequency of the disease that was present at very low incidence during 1989 at locations where we observed it in 1990, but not in 1989. Little is known about the long distance spread of fungal entomopathogens. There are several possible mechanisms of spread. Resting spores of *E. maimaiga* were found in gypsy moth egg masses in Japan (Aoki et al. 1976) and in North America (unpublished data). First instars that are either infected or that carry resting spores externally on their bodies could spread the fungus when they disperse. Furthermore, conidia are actively ejected from cadavers under high relative humidities. Studies of alfalfa fields have documented the presence of conidia from five groups of Entomophthorales in the air (Wilding 1970). Thus, conidia could be transported by the wind. Lastly, predators or scavengers feeding on diseased insects or the resulting cadavers are known to act as vectors of fungal entomopathogens (Guggnani & Okafor 1980).

**E. maimaiga—Rainfall and Density Associations.** There was a significant positive correlation between rainfall and the proportion of dead larvae among samples collected under burlap bands and on the tree boles below the bands in Massachusetts ($F = 15.16$, df = 1, $R^2 = 0.487$, $P = 0.001$) (Fig. 3A). Similar results occurred when we restricted our analysis to gypsy moths beneath the burlap bands and excluded those on the boles below the bands ($F = 5.713$, df = 1, $R^2 = 0.26$, $P = 0.029$). We observed higher levels of mortality at sites in western Massachusetts, where rainfall was much higher than in eastern Massachusetts (Fig. 2A and B). Of course, some other factor distributed east to west across Massachusetts may have caused the positive correlation. However, because *E. maimaiga* conidia germinate only under wet conditions (Hajek et al. 1990b), it seems probable that the east–west variation in rainfall was the cause of the trend in mortality.

The proportion of dead larvae was unrelated to density of gypsy moth as measured by the log$_{10}$ counts of total larvae and pupae (live and dead) under and below the burlap bands ($F = 0.005$, df = 1, $R^2 = 0.003$, $P = 0.837$) (Fig. 3B). With stepwise multiple regression, rainfall, but not density, was selected as a significant correlate of proportion dying, which resulted in a model identical to the simple regression indicated above. We did not dissect all these larvae to confirm the presence of *E. maimaiga*, but we did dissect a subsample from each site. Fifty-one of 59 larvae dissected were killed by *E. maimaiga*. None of the larvae had died from nuclear polyhedrosis virus (NPV), an agent that produces larval cadavers that are similar in appearance to those killed by *E. maimaiga*, and which is a major source of mortality in high density gypsy moth populations. Thus, we feel confident that the mortality recorded at these sites was caused primarily by *E. maimaiga*. The finding of no re-
relationship between mortality from *E. maimaiga* and gypsy moth population density is supported by data collected from four intensively sampled populations reported in Hajek et al. (1990a). Mortalities from *E. maimaiga* of >50% were recorded in these populations that were at least ten-fold lower in density, based on numbers of larvae per burlap band, than most of the populations presented in Figure 3B. The fact that *E. maimaiga* caused high mortality even at low population density suggests that this agent may have the potential to help maintain gypsy moth populations in a low density, endemic phase, as opposed to merely causing the collapse of high density populations, as is typical of other entomopathogens such as NPV (Doane 1970).

Acknowledgment


References Cited


Received for publication 26 April 1991; accepted 12 August 1991.