Introduction and Establishment of *Entomophaga maimaiga*, a Fungal Pathogen of Gypsy Moth (Lepidoptera: Lymantriidae) in Michigan

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ABSTRACT In 1991, late instars of gypsy moth, *Lymantria dispar* (L.), were sampled and diagnosed for infections of the pathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper and for gypsy moth nuclear polyhedrosis virus (NPV) at 50 sites in Michigan. Approximately 1,500 larvae were collected and reared from these sites, and no infections of *E. maimaiga* were detected. From 1991 to 1993, we tested the efficacy of 2 inoculative-release methods for *E. maimaiga* in replicated plots at 3 research sites by relocating soil containing *E. maimaiga* resting spores from Massachusetts to Michigan or by releasing inoculated larvae onto boles of trees. In the 2nd yr after introduction, *E. maimaiga* became established (9–40% infection) where both inoculation methods were used, and a low level of infection was detected in control plots (0.5–2.4%). In the 3rd yr, epizootics of *E. maimaiga* occurred at all 3 research sites, with the incidence of infection ranging from 20 to 99% in both treated and control plots. Infection levels were correlated with precipitation and relative humidity ≥90% for 2 wk preceding larval sampling. In 1993, egg mass densities at the 3 *E. maimaiga* study sites averaged 3- to 10-fold lower than in adjacent oak forest. We found that it is easy to introduce *E. maimaiga* to new locations even in the midst of an epizootic of gypsy moth NPV and that *E. maimaiga* reduces gypsy moth populations to levels lower than that caused by NPV alone.

KEY WORDS *Lymantria dispar*, Entomophthorales, *Entomophaga maimaiga*, nuclear polyhedrosis virus, biological control

Entomophaga maimaiga HUMBER, Shimazu & Soper is a virulent fungal pathogen of gypsy moth, *Lymantria dispar* (L.). In Japan, this relatively host-specific pathogen is important in the natural regulation of gypsy moths, causing extensive epizootics (Koyama 1954, Takamura and Sato 1973, Sato and Takamura 1975). The basic biology and history of this fungus in North America is summarized in recent reviews (Reardon and Hajek 1993, Hajek et al. 1995). In an early effort to suppress expanding gypsy moth infestations in North America, gypsy moth cadavers with *E. maimaiga* were collected in Japan and used to infect gypsy moth larvae that were released at several locations near Boston in 1910–1911 (Speare and Colley 1912). No infected larvae were found in 1910 or 1911 at the release sites, and establishment of *E. maimaiga* was presumed a failure (Soper et al. 1988, Hajek et al. 1990b). Despite several surveys conducted in the past 25 yr for gypsy moth pathogens in general (Campbell and Podgwaite 1971, Doane 1976, Podgwaite 1981), and 1 survey for fungal pathogens of gypsy moth (Majchrowicz and Yendol 1973), *E. maimaiga* was not found until 1989.

It is interesting that in 1989 *E. maimaiga* was discovered causing widespread gypsy moth mortality throughout Massachusetts, Connecticut, southern New Hampshire and Vermont, eastern Pennsylvania and New York, and northern New Jersey (Andreadis and Weseloh 1990, Hajek et al. 1990b). A survey of 12 eastern states in 1990 documented the spread of *E. maimaiga* into central Pennsylvania and New York, northern New Hampshire and Vermont, and southern Maine (Elkinton et al. 1991). Researchers and gypsy moth program leaders became interested in facilitating the spread of *E. maimaiga* to the leading edge of the contiguous gypsy moth infestation in eastern North America. After obtaining the proper permits, soil containing *E. maimaiga* resting spores was relocated to 34 sites in Virginia, West Virginia, Maryland, and Pennsylvania in 1991 (Hajek and Elkinton 1992). The apparent success of these introductions exceeded all expectations, with establishment of *E. maimaiga* in 28 of the 34 release sites during 1991. Like most fungal pathogens, *E. maimaiga* needs moist weather conditions to sporulate and infect its host (Hajek et al. 1990a, Elkinton et al. 1991, Hajek and Soper 1992; Weseloh and Andreadis

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Table 1. Incidence of *E. maimaiga* and gypsy moth NPV at 50 sites in Michigan surveyed in 1991

County	No. of locations sampled	% larvae infected with NPV[a]	% larvae infected with *E. maimaiga*
Midland	10	36.4 ± 17.4	0
Clare	10	39.6 ± 18.0	0
Gladwin	6	29.2 ± 10.1	0
Arenac	4	30.3 ± 15.4	0
Gratiot	2	45.0	0
Saginaw	1	69.0	0
Osceola	4	46.8 ± 15.3	0
Missaukee	3	39.0 ± 1.7	0
Lake	4	14.8 ± 6.2	0
Wexford	6	26.5 ± 17.1	0

Thirty larvae (3rd–5th instars) were sampled at each location.
[a] Mean ± SD.

1992a, b), and presumably the wet springs of 1989–1990 were responsible for the rapid rate of spread of *E. maimaiga* throughout the northeastern states (Elkinton et al. 1991). Although the unexpected appearance and subsequent rapid spread of *E. maimaiga* in North American gypsy moth populations is not fully understood (Reardon and Hajek 1993), it is considered a welcome addition to the list of biological control agents active against gypsy moth.

Gypsy moth is well established in the lower peninsula of Michigan, defoliating ≈283,000 ha of forests in 1993. Because gypsy moth populations in Michigan are not contiguous with those of the eastern states, surveys performed for *E. maimaiga* in 1989 and 1990 did not include Michigan. To determine if *E. maimaiga* was present, we initiated a survey of Michigan gypsy moth populations in 1991. Based on reports of epizootics in eastern states, we believed that introduction of *E. maimaiga* into Michigan would help mitigate defoliation caused by gypsy moth in the future. Therefore, in 1991 and 1992 we conducted a study to compare 2 field introduction methods for *E. maimaiga*: relocating soil containing *E. maimaiga* resting spores from Massachusetts to Michigan, or releasing inoculated larvae onto the boles of trees. We also dispensed soil containing resting spores around the base of single trees at 20 additional sites in 1991. We report the results of surveys for *E. maimaiga* from 1991 to 1993, *E. maimaiga* establishment in Michigan by 2 release methods, correlation of *E. maimaiga* activity with weather conditions, impact of *E. maimaiga* on gypsy moth populations, and indirect impact on defoliation at our research sites.

Materials and Methods

Survey for *E. maimaiga*. Fifty survey sites in 10 Michigan counties were selected from areas with a history of gypsy moth infestation from 1984 to 1990 (Table 1; Fig. 1). In June 1991, 30 gypsy moth larvae per site, ranging in age from 3rd to 5th instar, were collected, a total of 1,500 larvae. Larvae were maintained in individual diet cups until they died or pupated. Dead larvae were frozen for pathogen diagnoses. Because gypsy moth ca-

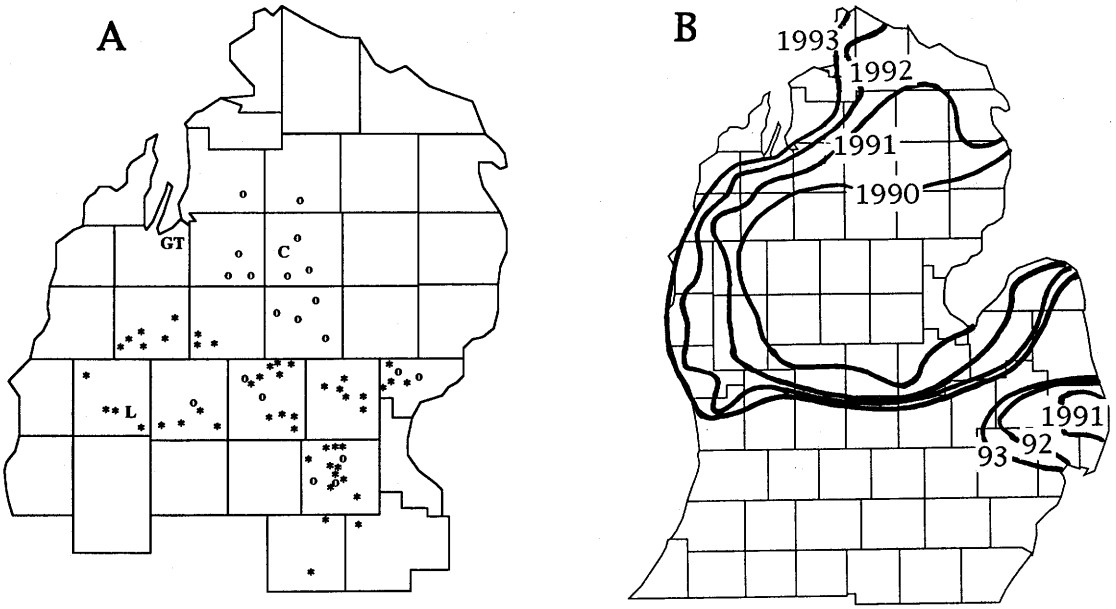

Fig. 1. (A)Location of survey sites (*) and field plots where *E. maimaiga* was introduced. Research sites where inoculation method and spread were studied are noted by an L, C, or GT for Lake, Crawford, or Grand Traverse County, respectively. Sites where *E. maimaiga* was released at the base of a single tree in 1991 are marked by an "0." (B) The leading edge of gypsy moth defoliation of Michigan forests in 1990, 1991, 1992, and 1993.

davers with *E. maimaiga* can often be distinguished from cadavers with nuclear polyhedrosis virus (NPV), we collected more samples in 1992, looking specifically for *E. maimaiga* cadavers, but collecting other cadavers as well (Hajek and Roberts 1992). In August 1992, we returned to 6 of the original survey sites and sampled larval cadavers adhering to the boles of trees. At each site, at least 30 trees were examined for cadavers, and no more than 25 cadavers were collected. Gypsy moth cadavers that displayed symptoms of *E. maimaiga* infection were favored during cadaver collection. Cadavers were placed individually in vials and frozen for pathogen diagnoses at a later date.

***Entomophaga maimaiga* Field Inoculation Methods.** Research sites were established in oak forests along the 1991 leading edge of gypsy moth infestation in Michigan's northern lower peninsula (Fig. 1). The proportions of *Quercus* spp. trees at the Crawford, Lake, and Grand Traverse sites were 79, 90, and 85%, respectively. *E. maimaiga* was introduced using 2 inoculation methods at research sites in Lake and Crawford counties in 1991. Twelve 0.04-ha plots were established at each research site, with 4 replicated plots receiving 1 of 3 treatments: (1) application of soil containing resting spores around the base of trees, (2) release of larvae inoculated with *E. maimaiga* onto the boles of trees, or (3) untreated control plots. In 1992, a 3rd research site was established in Grand Traverse County where the oak forest along the leading edge of gypsy infestation in Michigan had recently become infested with a healthy expanding population. This site was added to provide more data on how weather conditions affect establishment of *E. maimaiga* (Fig. 1). Eight new 0.04-ha plots were established there with 4 replications each of application of soil containing resting spores, or untreated control plots. The plots at each research site were spaced 53 m apart. Four northern red oaks, *Quercus rubra* L. (≥15 cm diameter at breast height [dbh]), designated the epicenter trees, were selected in each plot to receive the treatments.

Relocated Soil Containing Resting Spores. In April of 1991 and 1992, the top 10 cm of soil was scraped from around tree bases in a woodlot in Springfield, MA, after an *E. maimaiga* epizootic in 1990. Immediately after collection, the soil was poured through a coarse screen, placed in plastic bags, shipped overnight to Michigan State University, and stored outdoors in the shade for 2 wk until placement in early May. The entire batch of soil was mixed thoroughly before placement in the research plots. A subsample of soil (≈500 g) was used to estimate the number of resting spores present in the contaminated soil using the methods described by Hajek and Wheeler (1995).

On 7 May 1991 at Lake and Crawford counties, and on 12 May 1992 at Grand Traverse County, bare soil was exposed around the bases of 4 trees in each soil introduction plot by raking away the leaves within 1.0 m of trunk, and 1.5 liter (701 g) of soil containing 937 resting spores per gram was scattered around each tree. The leaves were then raked back over the soil. At both the Lake County and Crawford County research sites in 1991, $2.63 \times 10^6 \pm 5.15 \times 10^5$ (SE) spores were introduced per plot, with 4 plots at each site. In 1992, $3.32 \times 10^6 \pm 2.84 \times 10^5$ spores were released at each plot at the Grand Traverse County research site.

Release of Inoculated Larvae. Gypsy moth larvae were reared from egg masses provided by the USDA–APHIS Otis Method Development Center facility in 60-ml polystyrene cups on meridic diet (Bell et al. 1981) lacking antimicrobial agents, at 24 ± 2°C and a photoperiod of 16:8 (L:D) h. Larvae were reared so that their development coincided with the development of larvae at the field research sites. Third instars were inoculated with *E. maimaiga* by injecting 523 ± 102 protoplasts from a liquid culture (ARSEF 2779, USDA–ARS, Ithaca, NY) into a proleg with a finely drawn-out capillary tube. Larvae were maintained individually in 60-ml cups with diet for transport to the study sites. On 21 May 1991, 2–3 d after inoculation, 15 live larvae were released onto each of the 4 epicenter oak trees in each plot (60 inoculated larvae per plot). A cohort of 84 inoculated larvae was maintained in the laboratory to determine incidence of *E. maimaiga* infection. All larvae infected with *E. maimaiga* died within 14 d after they were inoculated. We determined that 37% of the injected larvae produced resting spores and 15% produced conidia, for a total infection level of 52%.

Spread. Because *E. maimaiga* was detected in control plots in 1992, an additional set of 12 plots was added in 1993 at each location to determine how far *E. maimaiga* spread from the introduction points. The new plots were located 175, 300, and 425 m away in each cardinal compass direction from the nearest introduction point (Fig. 2).

Sampling. In 1991 and 1992, live larvae were sampled twice. In 1991, larvae were collected on 6 and 17 June, when they were mostly 3rd–4th and 4th–6th instars, respectively. In 1992, larvae were collected on 11 June and 15 July, when they were mostly 3rd–4th and 4th–6th instars, respectively. No larvae were found at the Crawford County site in 1992 because of a viral epizootic that occurred in 1991. In 1993, larvae were collected once, on 29 June in Crawford and Grand Traverse counties and on 30 June in Lake County. At all 3 locations the larvae were mostly 4th–6th instars. Larvae were sampled from under burlap bands (60 cm wide) stapled around the 4 epicenter trees ≈1.5 m above the ground. If larvae were scarce, they were also collected from the trunks of other trees in the plot. At least 20, but no more than 40, larvae were removed from each plot. Larvae were placed individually into 60-ml polystyrene cups containing ≈15 ml meridic diet (Bell et al. 1981), taken to the laboratory, and reared at 24 ± 2°C and a photoperiod of 16:8 (L:D) h until death or pupation. Larvae were screened every other day for mortal-

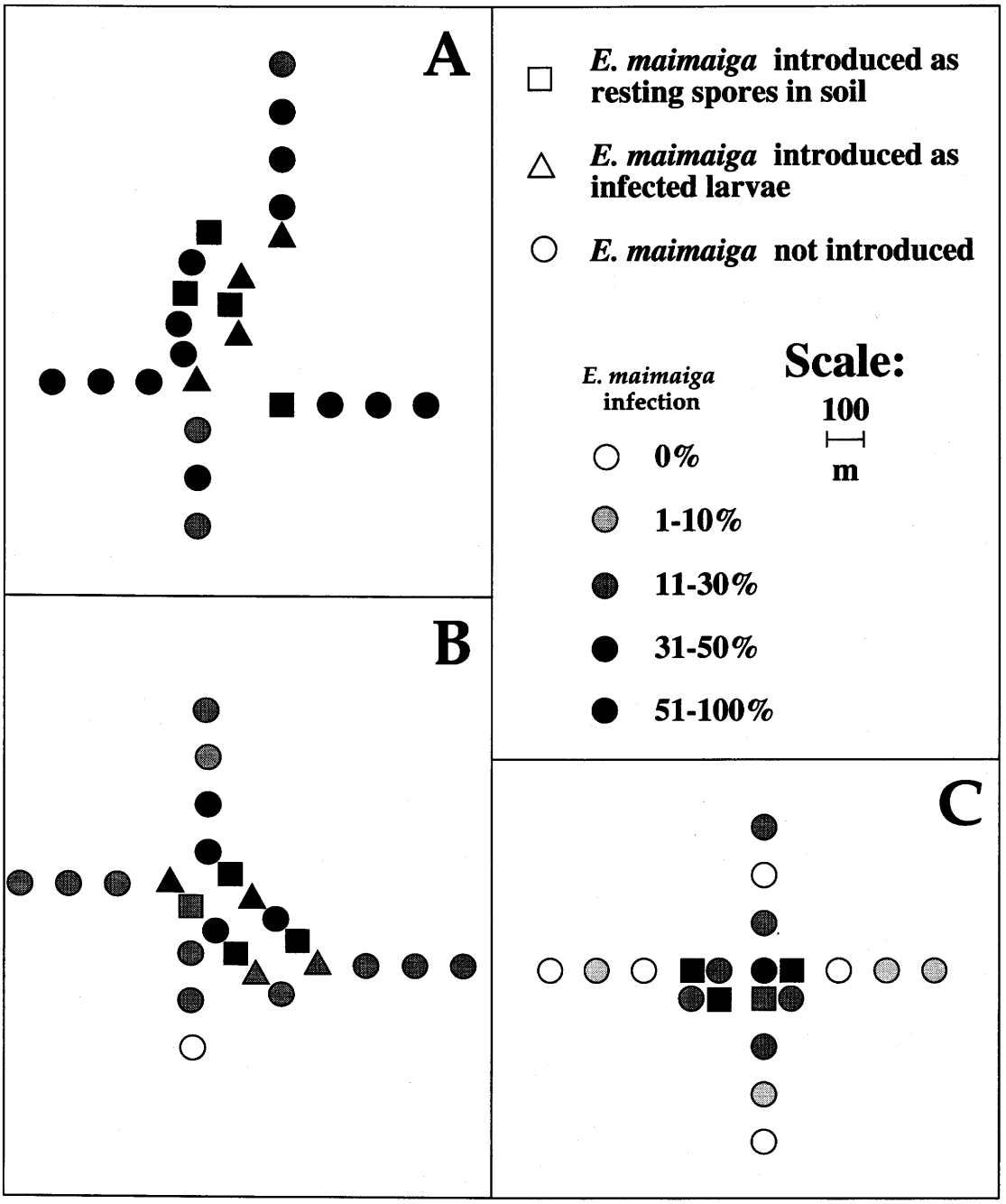

Fig. 2. Spread of *E. maimaiga* from plots where it was introduced in 1991 or 1992 (□, Δ) to nearby control plots (○) in 1993. Plot diagrams are for Lake County (A), Crawford County (B), and Grand Traverse County (C). Percentage *E. maimaiga* infection was determined for live larvae (B and C) and for cadavers (A).

ity, and cadavers were frozen for pathogen diagnoses at a later date. In 1993, larvae were sampled once at the Crawford and Grand Traverse counties sites on 29 and 30 June, respectively. In July 1993 at the Lake County site, an *E. maimaiga* epizootic near the introduction points combined with the effects of gypsy moth NPV to cause 100% mortality of larvae before our sampling. Consequently, we collected cadavers at the Lake County site in 1993. The cadavers were collected in 60-ml polystyrene cups, taken to the laboratory, and frozen for diagnosis at a later date.

To evaluate the effect of *E. maimaiga* on changes in gypsy moth population density, egg masses were

counted each year in 0.04-ha permanent plots by examining each tree in September or October and counting all egg masses visible from the ground. Egg mass density was determined each year for all field inoculation and control plots. Tree defoliation by gypsy moth was estimated each year from the 4 epicenter trees in each plot as described by Smitley and Davis (1993).

Temperature and relative humidity were recorded daily at the 3 research sites from the time of gypsy moth egg hatch until pupation with a hygrothermograph housed inside a ventilated wooden box (70 by 70 by 35 cm). Records from weather stations located within 20 km of each research site were used for daily precipitation measures.

***Entomophaga maimaiga* Introductions at Single Trees.** In early May 1991, 467.5 g of soil, containing $4.4 \times 10^5 \pm 8.6 \times 10^4$ spores, was placed around the base of a single tree at 20 separate sites in Michigan's lower peninsula (Fig. 1). On 13 and 14 July 1993, 2 yr later, we returned to the epicenter tree at each of the 20 introduction sites to sample gypsy moth cadavers, as described above for the 1992 survey sites. We searched each site for 30 min, and no more than 20 cadavers were removed. Sites where we found 5 or more cadavers were also sampled at distances of 0.8 km north and south of the epicenter tree to determine if *E. maimaiga* had spread that far. North and south were chosen as the cardinal compass directions because most sites were located on a road running north and south. Cadavers were taken to the laboratory and frozen for diagnostics at a later date.

Diagnostics of Cadavers for *E. maimaiga* Infection. *E. maimaiga* was diagnosed by staining a tissue sample from each larval cadaver with lactophenol blue (Poinar 1984) and scanning the stained slide under a compound microscope (100× and 400×) for resting spores (Andreadis and Weseloh 1990). The presence of NPV polyhedral inclusion bodies was also recorded for each larva. This study only investigated larval mortality; any larvae that pupated were counted as healthy larvae. No attempt was made to evaluate sublethal effects of the pathogens or to determine if adults emerged from pupae.

Data Analysis. The variances about percentage infection and percentage defoliation means were often significantly different by the Bartlett test for homogeneity. Because the variances were homogeneous after converting data to arcsine of the square root of percentage (x), all percentage infection and percentage defoliation data were converted before analysis (Sokal and Rohlf 1981). Means for percentage infection, percentage defoliation, and gypsy moth egg mass density from plots where *E. maimaiga* was introduced as resting spores in soil were compared with means from plots where *E. maimaiga* was introduced as infected larvae and with means from control plots by a Tukey test ($P = 0.05$) (Wilkinson 1989). The relationship between gypsy moth egg mass density and the proximity of plots to the *E. maimaiga* introduction point was determined by regression analysis (Sokal and Rohlf 1981). In the regression model, y = egg masses per 0.4 ha and x = distance from the nearest *E. maimaiga* introduction point. The incidence of *E. maimaiga* recovered from cadavers collected from single-tree introduction points was compared with incidence from cadavers collected 0.8 km north or south of each introduction point by paired t-tests.

The influence of precipitation and relative humidity on the proportion of larvae infected with *E. maimaiga* was determined by regression analysis. The total number of hours where the relative humidity exceeded 90% or the total precipitation in the 10-d period before larvae were collected was regressed against percentage infection. Data points for the regression were the mean percentage infected larvae in the 4 plots where *E. maimaiga* was introduced as resting spores in soil, or where *E. maimaiga* was introduced as infected larvae. Thus, 2 data points came from research plots at the Crawford County and Lake County locations, each time larvae were collected, but only 1 data point came from research plots at the Grand Traverse County location because *E. maimaiga* was only introduced as resting spores in soil at that site. Data from Crawford County in 1992 were not used because no larvae were found. Data were transformed to arcsine of the square root of percentage infected larvae before regression. Data were fit to an exponential function: $y = 10^x + C$, where y = arcsine of the square root of percentage infection and x = precipitation or relative humidity. The exponential function was used because the correlation coefficient was improved from $r^2 = 0.55$ for a linear relationship to $r^2 = 0.67$ for the exponential function.

The effect of *E. maimaiga* on defoliation of oak trees by gypsy moth in Grand Traverse County in 1993 was determined by regressing the proportion of larvae infected with *E. maimaiga* against the percentage defoliation of oak trees in each plot. The amount of error expected from visual estimates of percentage defoliation was described by Peterson and Smitley (1991).

Results

Survey for *E. maimaiga*. In 1991, none of the 1,500 larvae collected from 50 sites in Michigan (Table 1; Fig. 1) were infected with *E. maimaiga*. The levels of NPV infection ranged from 7 to 69%. Parasitoids were recovered from <1% of the gypsy moths reared. In 1992, *E. maimaiga* was not detected in cadavers collected at any of the 6 original survey sites.

***Entomophaga maimaiga* Field Inoculation Methods.** *1991. E. maimaiga* infection was not detected in the Crawford County research sites (Table 2). However, larvae infected with *E. maimaiga* were present in the Lake County plots that re-

Table 2. Proportion of gypsy moth larvae infected with NPV or *E. maimaiga* in sites where *E. maimaiga* was introduced and in nearby control plots from 1991 to 1993

Year	Location	Treatment	*n*	Egg masses per 0.4 h in April[a]	1st sample		2nd sample		% defoliation[a]
					% larvae with NPV[a]	% larvae with *E. maimaiga*[a]	% larvae with NPV[a]	% larvae with *E. maimaiga*[a]	
1991	Crawford County	Soil introduction	4	805 ± 287	42.5 ± 7.8	0 ± 0	24.1 ± 4.3	0 ± 0	97.0 ± 1.1
		Inoculated larvae	4	1,043 ± 256	38.4 ± 4.6	0 ± 0	29.1 ± 4.9	0 ± 0	98.7 ± 0.7
		Control	4	998 ± 183	43.3 ± 4.3	0 ± 0	27.9 ± 4.7	0 ± 0	98.8 ± 0.8
1991	Lake County	Soil introduction	4	293 ± 115	11.2 ± 5.2	0 ± 0a	20.8 ± 4.3	0 ± 0	39.4 ± 13.5
		Inoculated larvae	4	255 ± 67	16.5 ± 1.3	7.1 ± 2.6b	16.2 ± 3.1	1.6 ± 7.0	35.9 ± 4.7
		Control	4	233 ± 57	19.5 ± 4.4	0 ± 0a	19.1 ± 3.4	0 ± 0	40.4 ± 16.0
1992	Crawford County	Soil introduction	4	8 ± 10	—	—	—	—	1.4 ± 0.4
		Inoculated larvae	4	3 ± 5	—	—	—	—	1.6 ± 0.7
		Control	4	3 ± 5	—	—	—	—	1.4 ± 0.4
1992	Lake County	Soil introduction	4	6,493 ± 1,125	34.7 ± 4.6	0 ± 0	75.3 ± 6.3	9.3 ± 4.5	84.4 ± 7.6
		Inoculated larvae	4	6,703 ± 1,482	36.7 ± 3.1	0 ± 0	76.2 ± 7.0	11.7 ± 3.9	84.5 ± 10.1
		Control	4	8,278 ± 1,580	38.3 ± 1.8	0.5 ± 0.5	76.3 ± 2.4	2.5 ± 1.4	97.3 ± 1.5
1992	Grand Traverse County	Soil introduction	4	403 ± 118	6.4 ± 2.6	0 ± 0	48.2 ± 1.6	5.5 ± 2.4a	80.4 ± 8.0
		Control	4	398 ± 324	9.4 ± 1.8	0.4 ± 0.4	40.8 ± 9.6	0 ± 0b	77.4 ± 10.1
1993	Crawford County	Soil introduction	4	100 ± 38a	—	—	21.6 ± 15.2	42.6 ± 13.9	4.0 ± 1.0
		Inoculated larvae	4	333 ± 35b	—	—	44.5 ± 13.6	29.6 ± 12.4	3.0 ± 1.0
		Control	4	415 ± 46b	—	—	35.7 ± 16.5	35.1 ± 10.8	4.5 ± 0.9
1993	Lake County	Soil introduction	4	373 ± 117	—	—	1.3 ± 2.6[b]	99 ± 2.5[b]	7.1 ± 2.8
		Inoculated larvae	4	498 ± 166	—	—	3.8 ± 4.8[b]	96 ± 2.5[b]	6.7 ± 1.7
		Control	4	605 ± 166	—	—	6.4 ± 6.3[b]	92 ± 6.3[b]	6.6 ± 4.3
1993	Grand Traverse County	Soil introduction	4	7,188 ± 2,120	—	—	57.6 ± 10.0	39.8 ± 9.6a	36.8 ± 11.6
		Control	4	3,815 ± 1,053	—	—	64.1 ± 9.3	20.0 ± 10.1b	50.9 ± 18.1

Means followed by the same letter are not significantly different ($P = 0.05$; Tukey test).

[a] ± SD.

[b] Percentage infected cadavers. No live larvae were found.

Table 3. Gypsy moth egg masses per 0.4 ha in April of 1994 in plots where *E. maimaiga* was introduced and in plots located 53, 175, 300, and 425 m away

Location	*n*	$\bar{x} \pm$ SD no. gypsy moth egg masses at various distances from *E. maimaiga* introduction plot					Regression model[a]			
		0 m	53 m	175 m	300 m	425 m	Model	r^2	*P*	*F*
Crawford County	4	28 ± 10	43 ± 25	73 ± 52	75 ± 58	—	$y = 0.28x + 28$	0.32	<0.006	9.5
Lake County	4	5 ± 5	5 ± 10	18 ± 24	55 ± 44	28 ± 48	$y = 0.09x + 4$	0.22	<0.02	6.3
Grand Traverse County	4	45 ± 24	143 ± 134	470 ± 134	443 ± 296	532 ± 167	$y = 1.1x + 28$	0.45	<0.001	14.5

E. maimaiga was introduced in Crawford and Lake counties in 1991 and in Grand Traverse County in 1992.
[a] In the regression model, y = egg masses per 0.4 ha and x = distance (m) away from pltos where *E. maimaiga* was introduced.

ceived the inoculated larval treatment, but not in control plots or where soil with resting spores was introduced (Table 2). The 1st larval sample was taken on 6 June when larvae ranged in age from 3rd to 4th instars, and ≈7.1% of the larvae were infected with *E. maimaiga*. On 17 June, after 2 wk of dry weather, only 1.6% of the larvae collected from the same plots were infected with *E. maimaiga*.

1992. Larvae were collected when they were in a stage of development similar to those collected in 1991, but at later dates because weather conditions were cooler. In 1992, a population collapse caused by NPV eliminated larvae at the Crawford County research site. Gypsy moth larvae infected with *E. maimaiga* were found on 15 July in Lake County plots that received inoculum by both introduction methods (Table 2). There was no significant difference between the levels of infection, which were <12%. *E. maimaiga* infection was also detected in the untreated control plots, ranging from 0.5% in the 1st sample to 2.5% in the 2nd sample. At the Grand Traverse County research site, newly established in 1992, almost no infection was detected in larvae collected on 11 June, except for 1 individual in an untreated plot (0.4%) (Table 2). Higher levels of infection were detected on 15 July in the treatment plots (5.5%), and none from the untreated control plots.

Cadaver samples taken in July determined that 5% of the 20 cadavers collected were infected with *E. maimaiga* at the Lake County epicenter, with 0% *E. maimaiga* spores in the 24 cadavers found 1.6 km away from that epicenter. No cadavers infected with *E. maimaiga* were found at the Grand Traverse research site or at a distance 1.6 km from it.

1993. E. maimaiga was found at all inoculation sites. In Crawford County, levels of infection ranged from 30 to 42% and were similar in plots that received relocated soil with resting spores, inoculated larvae, or no treatment ($P > 0.05$). At the Lake County research site, >92% of the cadavers sampled were infected without significant differences among release methods ($P > 0.05$). At the Grand Traverse County site, *E. maimaiga* was also detected in larvae sampled from plots receiving relocated soil or no treatment. The treated plots, however, had a higher incidence of *E. maimaiga* infection (40%) than that measured in the untreated control plots (20%) ($P < 0.05$).

Egg mass counts and defoliation were used to indicate the impact of *E. maimaiga* on gypsy moth population density. Gypsy moth population dynamics at all 3 sites were driven primarily by gypsy moth NPV. Infection levels at all locations ranged from 11 to 48% when the gypsy moth egg mass density was below 2,500 per ha, and from 57 to 76% when the egg mass density was above 2,500 per ha (Table 2). Peak populations were characterized by heavy defoliation (51–99%) and a high incidence of gypsy moth NPV. Gypsy moth populations declined rapidly the year after heavy defoliation.

In the 2nd and 3rd yr after *E. maimaiga* was introduced, gypsy moth egg mass densities were lower in plots where *E. maimaiga* was introduced as resting spores in soil when compared with control plots (Crawford County 1993 and 1994, Lake County 1993 and 1994, Grand Traverse County 1994 [Tables 2 and 3]). Apparently, *E. maimaiga* has an impact on gypsy moth populations that goes beyond the effects of gypsy moth NPV.

In 1993, data were also collected from an additional 12 plots at each site located along transects radiating from the epicenter of each research site. Diagnoses of larvae and cadavers collected at the 3 research sites revealed that levels of *E. maimaiga* infection were higher in the epicenter plots than in plots established 175, 300, and 425 m away (Fig. 2). Egg mass density in 1994 was positively correlated with distance away from plot epicenters at all 3 research sites (Table 3). *E. maimaiga* establishment covered a greater area surrounding the Lake and Crawford County research plots when compared with the more recently established Grand Traverse County research site. Because *E. maimaiga* was introduced into rapidly building or peaking populations of gypsy moth and it required 2 yr to establish and spread throughout 0.04-ha plots, by the 3rd year, when a large proportion of larvae became infected, populations at the Lake and Crawford County locations had already declined below levels necessary to cause defoliation because of gypsy moth NPV. In contrast, at our study site in Grand Traverse County, a high level of infection was observed in the 2nd year, when the surrounding area was heavily defoliated. De-

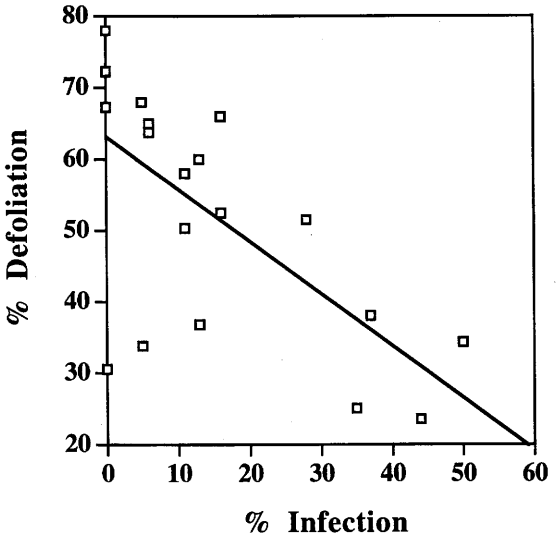

Fig. 3. Relationship between percentage defoliation and the proportion of larvae infected with *E. maimaiga* at research plots in Grand Traverse County in 1993. The equation for the line is $y = -0.73x + 63.2$ (slope SEM = 0.20, $r^2 = 0.44$, $n = 20$, $P = 0.002$).

foliation estimates from all plots in Grand Traverse County in the spring of 1993 were negatively correlated with *E. maimaiga* infection ($r^2 = 0.44$, $P < 0.01$; Fig. 3).

Daily precipitation data from weather stations within 10 km of each research site were tabulated, by year, for ≈60 d of gypsy moth larval activity. There was considerable variation between sites and from year to year, with total rainfall at our 3 research sites ranging from 17 to 18 cm in 1991, 8 to 12 cm in 1992, and 14 to 24 cm in 1993. *E. maimaiga* infection was positively correlated with the amount of precipitation recorded at the nearest weather station during the 14-d period before larval sampling ($r^2 = 0.67$, $P < 0.001$; Fig. 4A). Similarly, relative humidity at each research site was also a good predictor of percentage infection during the 10-d period before larval sampling ($r^2 = 0.66$; $P < 0.001$; Fig. 4B).

***Entomophaga maimaiga* Introductions at Single Trees.** The 20 locations where *E. maimaiga* was introduced in 1991 by the relocation of soil containing resting spores were visited again in 1993 to collect cadavers. Gypsy moth cadavers were found at 7 of the 20 release sites. *E. maimaiga* infections were confirmed in cadavers at 6 of those 7 sites, ranging from 83 to 100% cadaver infection at the epicenter (Table 4). Sites established 0.8 km north and south of the epicenter had a consistently smaller proportion of infected cadavers (0–75%) than that at the epicenter ($t = 4.95$, df = 3, $P < 0.01$ for north and $t = 4.05$, df = 4, $P < 0.01$ for south). In general, sites with higher prevalence of infection at the epicenter also had higher prevalence of infection at a distance.

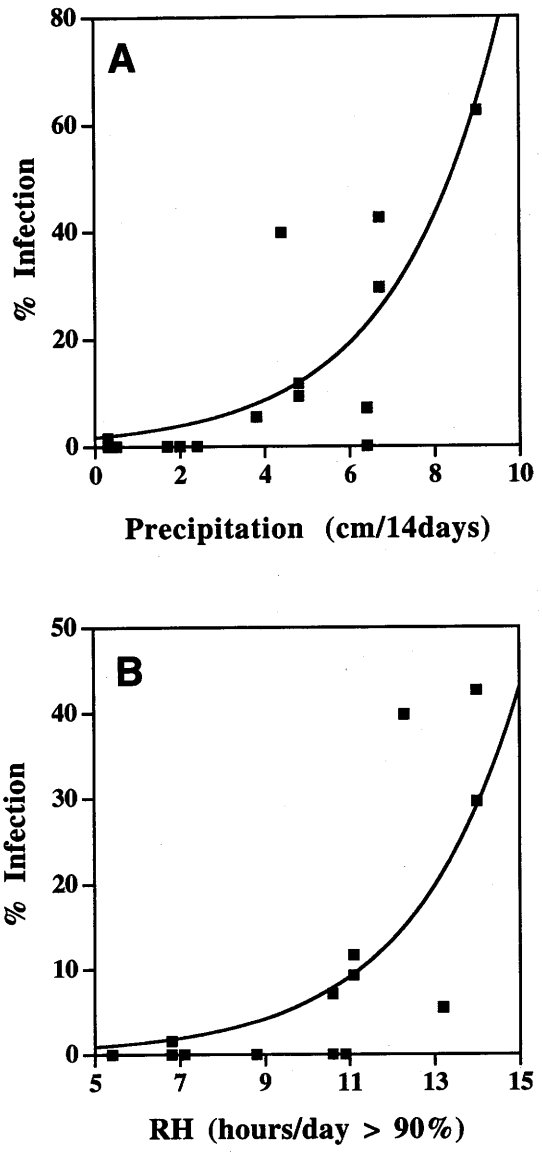

Fig. 4. Relationship between the prevalence of *E. maimaiga* infection in gypsy moth larvae and (A) precipitation during the 14-d period before larvae were collected, or (B) hours per day when the RH was >90% for the 10-d period before larvae were collected. The equations for the curves are as follows: (A) $y = 10^{17x} - 1.5$ (slope SEM = 3.5, $r^2 = 0.67$, $P = 0.0001$, $n = 18$); (B) $y = 10^{17x} - 2.7$ (slope SEM = 3.0, $r^2 = 0.66$, $P = 0.0001$, $n = 17$).

The location where no *E. maimaiga* was recovered was a river-bottom area where flooding occurs at least once every spring.

Discussion

Based on the results of our 1991 survey, we believe that *E. maimaiga* was probably not estab-

Table 4. Recovery of *E. maimaiga* 2 yr after introduction at single-tree release sites and 0.8 km away

County—Site no.	% cadavers with *E. maimaiga* resting spores and no. of cadavers collected (*n*)		
	At release point	0.8 km N	0.8 km S
Roscommon—1	92 (12)	0 (12)	8 (12)
Arenac—1	83 (12)	(0)	8 (12)
Arenac—2	100 (12)	25 (12)	42 (12)
Kalkaska—1	100 (12)	25 (12)	38 (3)
Kalkaska—2	100 (12)	75 (12)	—
Roscommon—2	100 (10)	(0)	0 (3)
Clare	0 (5)	(0)	—

lished in Michigan before our field inoculation studies. It could be argued that *E. maimaiga* was not detected because of dry weather conditions in 1991. However, the amount of rainfall reported at local weather stations in 1991 (17–18 cm) was adequate for *E. maimaiga,* and some infected larvae were recovered from our research sites at about the same time larvae were collected for the survey. In addition, control sites in our research areas had significantly lower levels of infection until 2 yr after treatments. At the single-tree release sites, a higher level of *E. maimaiga* infection was found around the epicenter tree than at a distance, suggesting that *E. maimaiga* spread ≈1 km over the 2-yr period after inoculation with contaminated soil.

Entomophaga maimaiga produces conidia abundantly at relative humidities >90% (Hajek et al. 1990a). The dependency of *E. maimaiga* on moist weather conditions for sporulation and infection of gypsy moth larvae may account for reports of a positive correlation between rainfall and the intensity of epizootics (Elkinton et al. 1991, Weseloh and Andreadis 1992a, Hajek et al. 1993). In New England, the total rainfall for May and June of 1989 and 1990, the 1st years that *E. maimaiga* epizootics were observed, was 5–15 cm above the 60-yr average. The highest levels of infection were found at sites with >20 cm of rainfall in May and June, the months when gypsy moth larvae were present (Elkinton et al. 1991). The region where our research sites are located in the northern lower peninsula of Michigan receive an average of 81 cm of precipitation per year. The precipitation totals at our test sites during the 2-mo period of larval activity ranged from 8 to 24 cm. *E. maimaiga* activity was minimal when precipitation totals were <4 cm in the 10-d period before larvae were collected, or when the number of hours per day with >90% RH averaged <8 for 2 wk before larvae were collected. Based on the results of this study, *E. maimaiga* appears capable of sustaining itself in areas receiving 10–20 cm of precipitation from 15 May to 15 July, although the most critical time for wet weather is when larvae are 4th–6th instars.

The amount of soil with resting spores needed to establish *E. maimaiga* successfully in a new area is small. At the single-tree introduction sites, we only used 467 g of soil (4.4×10^5 resting spores) around the base of a single tree at each site; yet at 5 of 7 sites where gypsy moth larvae were collected 2 yr later, *E. maimaiga* was detected 0.8 km away from the epicenter.

Introduction of *E. maimaiga* by release of inoculated larvae or by introduction of soil with resting spores are viable options. Injecting larvae is a tedious but reliable way of introducing *E. maimaiga* into test plots. Injection may be useful in situations where it is not desirable to import soil from another state. The rate of infection we observed (52%) after injection is low for a virulent pathogen like *E. maimaiga.* The most likely cause was a low inoculum dose (523 protoplasts per larva) or inconsistency in puncturing the cuticle and injecting the protoplast solution. In 1991, at the Lake County site, *E. maimaiga* infections were not detected in plots where it was introduced as resting spores although 7% infection was detected at larval release sites. Subsequently, in 1992, infection levels were equivalent in plots receiving these 2 treatments. Plots receiving these different treatments were only 53 m apart. Because of the ability of airborne *E. maimaiga* conidia to disperse or the movement of infected larvae, one could postulate that infections in the resting spore release plots were caused by airborne conidia or infected larvae coming from the larval release plots. However, barely detectable levels of infection in control plots suggest that fungal dispersal did not account for most of the infection in resting spore release plots in 1992. The low levels of infection in control plots certainly might have been caused by aerial movement of conidia from plots where *E. maimaiga* was released. The suppressive effects of *E. maimaiga* on gypsy moth were observed in the 2nd and 3rd years after *E. maimaiga* was introduced and were greatest close to the introduction point. In 1994, egg mass densities at all 3 locations were 3 to 10-fold less at the introduction sites compared with plots 300 m away. Apparently, under moist conditions *E. maimaiga* reduces the number of larvae surviving to reproduce, even when gypsy moth NPV is active or at low host densities (3–8 egg masses per 0.4 ha). Although *E. maimaiga* is believed to be an important pathogen of gypsy moth, our field experiments are the first to describe the effect of *E. maimaiga* on gypsy moth populations. Epizootics of *E. maimaiga* may also help prevent defoliation of forest trees by gypsy moth. At the Grand Traverse County research site in 1993, we found that the incidence of infection by *E. maimaiga* explained 44% of the variation in defoliation. Although our research shows that *E. maimaiga* may cause declines in gypsy moth populations beyond the precipitous decrease of dense populations caused by NPV, more research

is needed to elucidate the role of *E. maimaiga* in moderating gypsy moth population cycles and its interactions with other natural control factors.

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