Impact of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) on Outbreak Gypsy Moth Populations (Lepidoptera: Erebidae): The Role of Weather

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ABSTRACT The fungal pathogen *Entomophaga maimaiga* Humber, Shimazu, and Soper is prevalent in gypsy moth [*Lymantria dispar* (L.)] populations throughout North America. To understand how weather-related variables influence gypsy moth–*E. maimaiga* interactions in the field, we measured fungal infection rates at 12 sites in central Pennsylvania over 3 yr, concurrently measuring rainfall, soil moisture, humidity, and temperature. Fungal mortality was assessed using both field-collected larvae and laboratory-reared larvae caged on the forest floor. We found significant positive effects of moisture-related variables (rainfall, soil moisture, and relative humidity) on mortality due to fungal infection in both data sets, and significant negative effects of temperature on the mortality of field-collected larvae. Lack of a clear temperature relationship with the mortality of caged larvae may be attributable to differential initiation of infection by resting spores and conidia or to microclimate effects. These relationships may be helpful in understanding how gypsy moth dynamics vary across space and time, and in forecasting how the gypsy moth and fungus will interact as they move into warmer or drier areas, or new weather conditions occur due to climate change.

KEY WORDS *Lymantria dispar*, epizootiology, environmental driver, outbreak insect, invasive species

Since its discovery in 1989, the fungal pathogen *Entomophaga maimaiga* Humber, Shimazu, and Soper has become ubiquitous in gypsy moth [*Lymantria dispar* (L.)] populations in North America. It is apparently native to Japan, Korea, northeastern China, and the Russian Far East (Hajek 1999), but it has now spread throughout the range of the gypsy moth in North America, closely following the spread of host populations (Hajek and Tobin 2011). *E. maimaiga* is largely specific to the gypsy moth, but it can infect a small number of other lepidopteran species. Its resting spores (azygospores) overwinter in the soil for one or more years and begin to germinate just before gypsy moth eggs hatch in the spring. Each germinating resting spore discharges a single infectious germ conidium that can become airborne. When germ conidia land on gypsy moth larvae, they germinate and infect, killing the larva after approximately 5 days (Hajek et al. 1995). *E. maimaiga* growing within cadavers of larvae killed by *E. maimaiga* produce and eject from thousands to millions of new conidia (Shimazu and Soper 1986, Hajek et al. 1993); alternatively, the fungus may instead produce resting spores within the cadaver or it may produce both spore types (Hajek and Shimazu 1996). Larvae infected by the germ conidia discharged from resting spores will only produce conidia, not resting spores (Hajek 1997). Conidia that fail to land on a larva can discharge secondary or even tertiary conidia that are infective (Hajek 1999).

Infection by *E. maimaiga* is often prevalent at both high and low host densities and appears to be largely density-independent (Hajek 1999, Liebhold et al. 2013). This is in contrast to the other primary gypsy moth pathogen, *Lymantria dispar* nucleopolyhedrovirus, which is strongly density-dependent and only infects significant numbers of larvae during outbreaks (Liebhold et al. 2013). The activity of *E. maimaiga* in the field is thought to be highly dependent on suitable weather conditions for successful transmission (Hajek 1999).

There have been a number of studies that have examined the response of *E. maimaiga* to changes in moisture or temperature. In the laboratory, sporulation from cadavers and germination of conidia both required high humidity or free water (Hajek et al. 1990). The number of conidia produced and the duration of discharge were also positively related to humidity (Hajek and Soper 1992). The germination of resting spores could only be initiated in the presence of free water in the laboratory (Hajek and Humber 1997), and in the field, resting spore germination has

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Table 1. Description of sites used in the study for both field-collected and caged larvae

Site	Location	Latitude	Longitude	Field data (# collection dates)			Cage data (# collection dates)	
				2007	2008	2009	2007	2008
RRA	Rothrock State Forest, Centre, Co., PA	40.75857	77.75588	3	6	8	5	3
RRB	Rothrock State Forest, Huntington Co., PA	40.72746	77.81071	3	6	8	5	3
RRC	Rothrock State Forest, Huntington Co., PA	40.7275	77.84769		6	8	2	3
RRD	Rothrock State Forest, Centre, Co., PA	40.75157	77.75738			8		
33A	State Game Land 33, Centre, Co., PA	40.78364	78.20803	4	4	8	4	2
33B	State Game Land 33, Centre, Co., PA	40.79913	78.21283	4	7	9	4	3
33C	State Game Land 33, Centre, Co., PA	40.81238	78.16042	5	6	4	5	4
33D	State Game Land 33, Centre, Co., PA	40.79427	78.20967		3	7		2
33E	State Game Land 33, Centre, Co., PA	40.79731	78.21504			8		
176A	State Game Land 176, Centre, Co., PA	40.79874	77.9464	5			5	
176B	State Game Land 176, Centre, Co., PA	40.75552	77.98293	5			5	
176C	State Game Land 176, Centre, Co., PA	40.77784	77.00875	5			5	

Numbers in the five right-hand columns indicate the number of times a given site was sampled in a given year.

been positively associated with soil moisture (Hajek and Humber 1997) and rainfall (Weseloh and Andreadis 1992; Weseloh 1999, 2002). Smitley et al. (1995) were able to correlate infection levels in field populations with precipitation and relative humidity. Hajek et al. (1999) found positive correlations between leaf wetness and the flux of airborne conidia, but they failed to detect a correlation between conidial flux and larval mortality. Sieger et al. (2008) found positive correlations between mortality from *E. maimaiga* infections and precipitation over 2 yr, but less consistent relationships with humidity and soil moisture.

Temperature has been suggested as affecting all stages of the fungal infection cycle, particularly if temperatures become too high, but the relationship is not straightforward. Prolonged temperatures near or above 30°C have been shown to hinder or stop conidial sporulation and germination, but these processes may occur faster at moderate temperatures than at cold temperatures (Hajek et al. 1990). The proportion of cadavers that produce resting spores versus conidia is also affected by temperature, shifting to more resting spores as temperature increases (Hajek and Shimazu 1996). In one study (Hajek and Humber 1997), there was some evidence that overall field infection rates followed a nonlinear relationship with temperature. Similarly, Sieger et al. (2008) found that infection was greater when temperatures were between 15° and 25° in one of 2 yr. Because of the important connections between the *E. maimaiga* infection cycles and weather-related variables, it seems likely that moisture or temperature changes over space and time could be responsible for the heterogeneous levels of fungal infection that are typically observed among gypsy moth populations.

Despite the availability of this information indicating an association between meteorological conditions and *E. maimaiga* infection, we still have an incomplete understanding of how weather conditions determine the impact of this pathogen on gypsy moth population dynamics. The extent to which *E. maimaiga* suppresses gypsy moth populations below outbreak levels is characteristically variable both among sites and among years and therefore difficult to predict. As such, there remains a need to clarify the linkages among weather, *E. maimaiga,* and gypsy moth dynamics. In this study, we measured fungal infection rates over 3 yr concurrently with meteorological variables at a number of sites in central Pennsylvania, associating meteorological conditions with *E. maimaiga* infection rates to better understand gypsy moth–fungus dynamics.

Methods

Quantifying Fungal Infection Rates Over the Season. To quantify mortality rates of *L. dispar* larvae due to *E. maimaiga,* studies were carried out at 12 forest sites in central Pennsylvania in 2007, 2008, and 2009 (Table 1). All sites were ≈7 ha in size and *Quercus*-dominated. Soils were primarily sandy loams and did not differ greatly across sites (Supp Table 1 [online only]). Nine, seven, and nine sites were sampled in 2007, 2008, and 2009, respectively (Table 1). At each site, infection was measured in two ways: 1) wild larvae were collected from trees at each site and then reared in the laboratory to quantify naturally occurring infection levels, and 2) larvae from a laboratory colony were placed in cages in the field for defined periods and then brought back to the laboratory to measure infection. To prevent transfer of *E. maimaiga* resting spores by humans among sites, personnel wore disposable gloves and rubber boots that were sanitized in 0.06% sodium hypochlorite between sites. To estimate background population levels of gypsy moths at each site, egg mass counts were performed following the protocol of Liebhold et al. (1994). At each site, all egg masses were counted within a 0.01-ha area surrounding each of six red oak (*Quercus rubra*) trees that were ≈60 m apart. Counts were conducted before larval eclosion, when current egg masses could be distinguished from old ones that had hatched the previous year.

Field-Collected Larvae. Collections of up to 100 larvae were made every 4 d from early to late June in 2007 and 2008, resulting in ≈6 samples/site/yr. In 2009, collections were made every 7–14 d from mid-May to early July, resulting in ≈8 samples from each site.

Larvae were mostly collected from leaves and the bark of trees within the study area, but not from trees under which cages had been placed (see below in Bioassays With Caged Larvae section). After collection, gypsy moth larvae were placed individually into 29-ml cups containing artificial diet (Bell et al. 1981). Cups were maintained at 18–22°C, and larvae were checked daily for 10 d to check for conidial production from cadavers. Dead larvae were macerated, suspended in distilled water, and a smear was then examined using phase contrast at 200–400× for the presence of *E. maimaiga* resting spores or other pathogens. The presence of either conidia or resting spores was used to diagnose fungal infection.

Bioassays With Caged Larvae. Fungal mortality was measured in laboratory-reared larvae that were deployed in bioassays designed to predominantly measure impacts of germinating resting spores. Cages of 20 fourth-instar larvae were placed on the ground at the bases of six trees (total of 120 larvae) at each site that were ≈60 m apart. Larvae were obtained from a disease-free laboratory colony maintained by the United States Department of Agriculture (USDA) APHIS at Buzzards Bay, MA. Cages were made from 31- × 46-cm^2 aluminum window screening, which was folded in half and stapled closed on the three remaining sides to make a 31- × 23-cm^2 cage. The top surface of each cage was pinched to create a 2-cm-high interior space to contain the larvae. Cages were placed directly under trees, next to tree trunks, and in randomly selected cardinal directions. The leaf litter was removed immediately before placing the cage on the ground, but the cages were placed on top of any moss that was present. The edges of the cages were anchored to the forest floor, and each cage was covered with a wire mesh (12-mm^2 mesh) box (28 by 31 by 12 cm) to exclude vertebrate predators. After 4 d in the field, cages were returned to the laboratory and replaced with an identical cage of 20 fresh larvae. In 2007, gypsy moth egg hatch was first recorded on 3 May in Centre County. Six sets of cages were deployed from early through late June and picked up 30 d, 34 d, 38 d, 47 d, 51 d, and 55 d after the first egg mass hatch. In 2008, gypsy moth egg hatch was first recorded on 28 April; five sets of cages were deployed and picked up 39 d, 43 d, 47 d, 51 d, and 57 d after the first egg mass hatch. On the return of cages to the laboratory, living larvae were removed and placed in individual 29-ml cups containing artificial diet and were maintained at 18–22°C. Larvae in cups were checked daily for 10 d for death and production of *E. maimaiga* conidia from cadavers. Larvae from cages collected in late June (collected after 50 d in 2007 and after 45 d in 2008) that died within the 10-d period were dissected to check for *E. maimaiga* resting spores. Resting spores are not produced early in the larval period.

Calculation of Mortality Rates. Sampled larvae were simultaneously exposed to multiple mortality agents including *E. maimaiga,* and therefore, marginal mortality rates were calculated using the proportional hazards method of Elkinton et al. (1992, eqn 9). Estimated this way, the marginal mortality rate due to *E. maimaiga* (i.e., in the absence of other contemporaneous mortality agents), m_F, is a function of its observed death rate due to the fungus, d_F, and the observed combined death rate from all causes, d:

$$m_F = 1 - (1 - d)^{\frac{d_F}{d}} \quad [1]$$

Measuring Weather-Related Variables. The following weather variables were measured using HOBO weather sensors (Micro Station H21–002, Onset Computer Corporation, Cape Cod, MA) and logged at 10-min intervals throughout the study period: rainfall, relative humidity, air temperature, soil temperature, and soil moisture. Air temperature and humidity were measured at 1 m above ground, and soil temperature and moisture were measured at 3 cm below ground. The sensor was placed at the center of the group of six trees used for cages at each of the 12 sites (Table 1). Relative humidity, air temperature, and soil temperature measurements were available in all sites and years, except RRC in 2007, when these sensors malfunctioned. Rainfall measurements from tipping-bucket sensors were taken in 2008 and 2009, but were unavailable in 2007. When direct values were unavailable or missing from 2008/2009 data, they were replaced by precipitation readings from the nearby Community Cooperative Rain Hail and Snow Network (CoCoRaHS) station Ramblewood3.7WNW (station PA-CN-7). If values from this station were missing, they were replaced by an averaged reading from two other nearby stations, namely, CoCoRaHS station Boalsburg1.0NNW (PA-CN-8) and NOAA COOP (Cooperative Observer Program) station Philipsburg2S (USC00366921). Before replacement, weather station values were multiplied by a constant so that the mean rainfall value for the weather station would match the mean rainfall value for the site over the study period. On average, precipitation readings from the tipping buckets were ≈10% less than those from the nearby weather stations, presumably due to protection by the forest canopy or local climate differences.

Direct soil moisture measurements were available for all sites and years, except RRC and 176A in 2007, but we determined that soil moisture values provided by individual sensors were unreliable at a number of sites, likely due to variation in sensor depth or poor soil contact. Because these measurements were not representative of variation in soil moisture among sites, we instead fitted a hydrological model to estimate soil moisture from our rainfall data. Many different models of water flux in forest soils are available in the literature (Rajaram and Georgakakos 1989, Chang 2002). Generally, these models consider the dynamics of soil water content, precipitation, evapotranspiration, runoff, and groundwater loss. For simplicity, models assume zero groundwater loss, and that evapotranspiration and runoff are constant fractions lost daily: e and r, respectively. Thus, the soil water content, W, at time t is determined by the fraction of existing soil water W_{t-1} that does not evaporate plus the fraction of added precipitation P_t that does not run off:

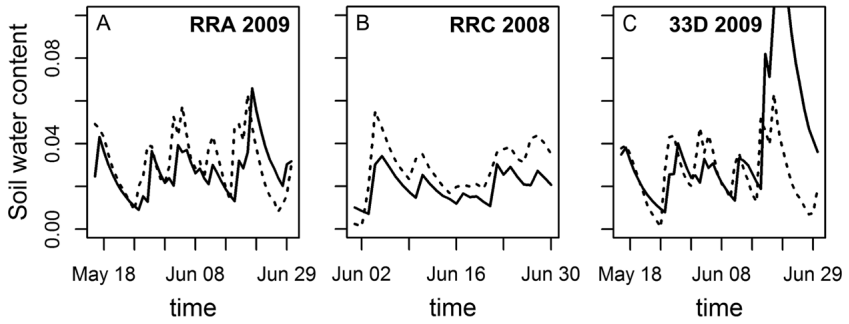

Fig. 1. Comparison of modeled soil water content estimates W_t (solid line) with direct soil moisture time series (dotted line) for three different sites. The model was parameterized based on the site RRA in 2009. The initial condition W_0 for each site was set to zero, and the model required about 2 wk of prior precipitation data (not plotted) before W_t converged with direct moisture readings.

$$W_t = (1 - c)W_{t-1} + (1 - r)p_t \qquad [2]$$

We used simple least squares to estimate values of e and r for our study area based on direct soil moisture and rainfall data from a representative site (RRA in 2009) where we felt the soil moisture time series was reliable. For this site, we found $e = 0.159$ and $r = 0.998$. When the parameterized model was applied to the other sites, the resulting time series of modeled soil moisture values matched the general pattern of the direct soil moisture measurements reasonably well (Fig. 1). For all further analyses, we used the modeled W_t values for soil water content rather than the direct measurements.

Quantification of Resting Spores. Resting spore densities in the soil were estimated at all sites from samples collected before the larval season in 2007 and 2009. Soil samples were taken from the bases of six trees at each site. Each sample was mixed thoroughly and three 5-g subsamples were taken. Resting spores in the soil subsamples were counted using the modified Percoll method described by Hajek et al. (2012). True resting spore densities were estimated from the resulting counts using the regression equation provided by Hajek et al. (2012). The effect of resting spore density on season-long mortality was analyzed using linear regression with the lm() function in R (R Development Core Team 2012).

Statistical Models: The Effect of Weather on Fungal Mortality. The effect of weather-related variables on larval mortality was modeled using generalized linear mixed models with the continuous weather variables as fixed effects. We treated the effects of site and year as random and estimated changes in the background mortality level across the season as a fixed effect of collection day. Because within-season dynamics tend to be poorly represented by a linear relationship, day was treated as a categorical factor. Gypsy moth population density was not built into the model directly but instead was allowed to contribute to the random effect of site or year. Despite the wide variation in population density that occurs in an outbreak, a previous analysis including some of these same data (Liebhold et al. 2013) has shown infection by *E. maimaiga* to be density-independent.

Model specification consisted of two steps:

1) The need for specification of a temporal correlation structure was evaluated using a mixed-effects general linear model on the proportion of larvae infected by fungus. In this model, a single observation is a proportion of larvae dying of fungal infection at a given site on a given day. To prevent small samples from biasing the results, we dropped all samples in which fewer than 14 larvae were collected (22/165 field samples). Models with and without first-order autoregressive correlation structure (across time within a site) were compared using corrected Akaike Information Criterion (AICc; Burnham and Anderson 2002) in R (R Development Core Team 2012) using the lme() function of the nlme package (Pinheiro et al. 2012).
2) To test the effects of weather-related variables on larval mortality, a mixed-effects generalized linear model (multiple logistic regression) was used to evaluate the number of larvae infected by fungus. This model treats the outcome of each larva as an observation of either "success: 1" if it became infected or "failure: 0" if it did not. The analysis was performed in R (R Development Core Team 2012) using the glmer() function of the lme4 package (Bates et al. 2012) with a binomial distribution and a logit link. Collinearity of weather variables was assessed using variance inflation factors calculated in R with the corvif() function in the AED package (Zuur 2010) or by looking at pairwise correlations. Model selection was based on AICc, and model averaging was performed using the model.avg() function of the MuMIn package (Barton 2012).

The following weather-related variables were considered: precipitation (mm; sum over the previous 10 d including the day of collection), relative humidity (%; daily mean over the previous 10 d), air temperature (C; daily mean over previous 10 d), soil temperature (C; daily mean over previous 7 d), and soil water content (cm^3/cm^3; daily mean over previous 7 d; Supp Table 2 [online only]). The number of days over which each variable was averaged for use in our anal-

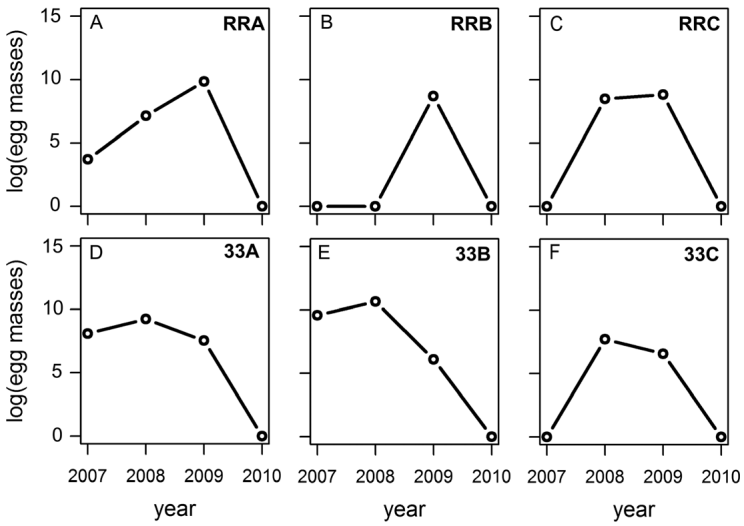

Fig. 2. Estimated number of gypsy moth egg masses per hectare at sites sampled in all years (Table 1). Estimates were calculated as the mean of the six counts at each site, extrapolated to 1 ha and natural log+1 transformed.

ysis was based on a logical assessment of when it could reasonably be impacting the chance of a test larva becoming infected. This process is not understood in great detail, but we outline our logic based on literature values in the following paragraphs. For a larva to become infected, an airborne conidium must be produced and discharged, and then land on the larval cuticle, germinate, and infect. For each weather variable considered, this process can be affected through one or more potential mechanisms.

Relative humidity, precipitation, and air temperature are known to affect the ability of the fungus to sporulate within cadavers and produce conidia. Sporulation requires certain conditions of cadaver moisture content and temperature. Cadavers can sporulate 1–2 d after larval death if conditions are favorable and can then continue to discharge conidia for 1–6 d, with a mean of ≈2 d (Hajek and Soper 1992). However, if conditions deteriorate, conidial discharge may be suspended and then restart up to a few days later. During this period, proper hydration via humidity or precipitation and suitable temperatures increase the number of conidia that can be discharged. In addition, increasing ambient temperature appears to be associated with a phenotypically plastic change from conidial production to the production of resting spores in later-instar larvae (Hajek and Shimazu 1996). This would be significant because resting spores cannot infect larvae in the same season that they are produced, and so a switch to production of resting spores would be followed by a decrease in infection rates during the present season.

Conidial germination (after landing on the larva) is thought to require the presence of free water, and is probably facilitated by precipitation and high relative humidity (possibly through condensation or microclimate effects). Conidial germination also appears to proceed faster as air temperature increases, but is checked if temperatures are too high (Hajek et al. 1990).

Putting this information together, *E. maimaiga* takes an average of about 5 d (Hajek et al. 1995) to kill a larva after infection, so infected larvae in our study could have become infected on the day of collection or on any of the previous 4 (or more) days. Adding these 5 d to ≈2 d for conidial production and 3 d for discharge

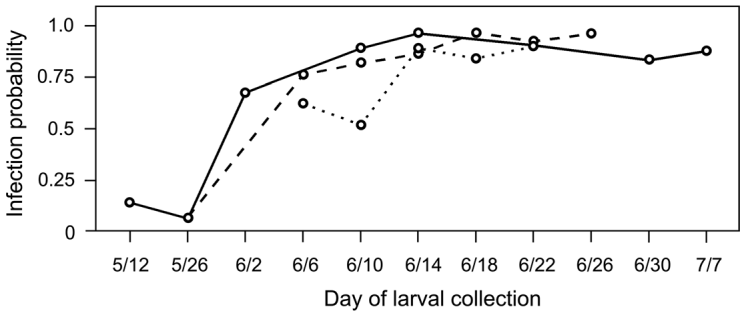

Fig. 3. Model-estimated probability of fungal infection among field-collected larvae by collection day, independent of weather-related effects. Values were calculated using model estimates for each categorical day at the mean of all continuous weather variables. Solid black line is estimates for 2009, dashed line is for 2008, and dotted line is for 2007.

Table 2. Selection for models of the association between weather variables and mortality of field-collected larvae caused by *E. maimaiga*

Model rank	Intercept	Soil moist	Soil temperature	Relative humidity	Precipitation	AICc	Delta	Weight
1	−4.84	37.5	−0.263	0.043	X	407.1	0.00	0.62
2	−4.64	31.2	−0.265	0.042	0.066	409.2	2.11	0.22
3	−1.62	38.9	−0.307	X	X	410.9	3.78	0.09
4	−1.46	31.6	−0.309	X	0.077	412.6	5.51	0.04
								0.97

The top four candidate models from AICc-based model averaging are presented. Model estimates are listed for each variable included in a given model, or are denoted with an "X" if the model does not include the variable. Day (categorical) was included in all models.

and dispersal, we arrive at a conservative figure of roughly 10 d over which such effects might have occurred in association with field-collected larvae before they were collected. Because effects on conidial germination occur essentially in real time, we could expect them to occur until larvae were collected. Smitley et al. (1995) also summed over a 10-d lag for precipitation in their study.

Soil moisture, precipitation, and soil temperature may affect the germination and conidial discharge of resting spores in the soil. Results from larvae placed in soil cages in the field by Weseloh and Andreadis (1992) suggest that resting spores probably germinate 1–2 d after a moisture trigger (assuming they have passed their dormancy requirement), although in the laboratory, Hajek and Humber (1997) found that most resting spores took 4–8 d to germinate. Weseloh (1999) found that rainfall was most strongly correlated with presumed resting spore germination over the previous 5 d, which he interpreted as indicating that rainfall was acting indirectly through soil moisture, and that generally, wet soil is a critical requirement for resting spore germination. Indeed, Hajek and Humber (1997) found a significant positive correlation between directly measured soil moisture and presumed resting spore infection in 1 yr of their study, and a significant effect of soil temperature in the other year. Based on these findings, we concluded that a 10-d average for precipitation would be ideal to encompass any association between precipitation and resting spore germination, and a 7-d average (5 d of potential infection + 2 d to germinate and discharge conidia) would ideally capture the effect of direct soil moisture and soil temperature on larval infection through the resting spore infection route.

Results

Based on egg mass counts, gypsy moth populations were high in 2007, peaked at outbreak levels in 2008 or 2009, and had crashed to virtually zero in 2010 (Fig. 2). Overall, *E. maimaiga* infections occurred in 2,742 of 7,691 larvae collected in the field and in 2,806 of 5,906 larvae placed in cages in the field. Infection levels varied across sites and over time, but were generally highest (over 50%) in mid or late June samples (Fig. 3). Counts of resting spores in the soil also varied across sites (Supp Table 3 [online only]), but showed no relationship with mortality rates among either field-collected ($P = 0.64$) or caged larvae ($P = 0.12$). Analysis of the effects of weather variables on fungal mortality proceeds as follows: 1) ruling out the need for correlation structure in the models, 2) analysis of the associations between weather variables and *E. maimaiga* infections in field-collected larvae via multiple logistic regression, and 3) analysis of *E. maimaiga* infections in the caged larvae using the same methods.

Analysis of Autoregressive Correlation Structure. For comparison, models of the proportion of larvae infected were performed with and without the estimation of first-order autoregressive correlation structure. In neither of our data sets (field-collected larvae and caged larvae) did AICc indicate enough support to warrant the addition of a correlation structure to the model ($\Delta = +1.62$ [field] and -1.35 [caged]). Thus, for simplicity in all further analyses, we instead used mixed-effects logistic regression models with no added correlation structure.

Fungal Infection of Field-Collected Larvae. We found relatively high variance inflation factors (VIF > 4) and pairwise correlations for air temperature and soil temperature in the full model, indicating excessive

Table 3. Model-averaged coefficients for the model of mortality due to *E. maimaiga* infection among field-collected larvae

	Estimate	SE	z	*P* value	Relative Importance	Shrinkage-corrected Estimate
Intercept	−4.349	1.967	2.21	0.027	1.00	−4.349
Soil moisture	35.970	9.656	3.73	<0.001	1.00	35.970
Soil temperature	−0.269	0.078	3.47	0.001	1.00	−0.269
Relative humidity	0.042	0.017	2.56	0.011	0.86	0.037
Precipitation	0.068	0.071	0.95	0.341	0.26	0.018

The effect of day (not listed) was included and highly significant in all models ($P < 0.001$). Shrinkage-corrected estimates include zeros in the average when a parameter does not appear in contributing models.

Table 4. List of best models with only a single weather variable, ranked by AICc, for the field-collected larvae

Model rank	Intercept	Soil moisture	Precipitation	Rh	Soil temperature	Air temperature	AICc	Delta	Weight
1	−4.906	45.78					425	0	0.94
2	0.3218				−0.3611		430.6	5.61	0.057
3	−3.616		0.2318				437	12.06	0.002
4	−7.611			0.06545			439.3	14.36	0.001
5	−0.4146					−0.2116	442.6	17.63	0

Model estimates are listed for each variable included in a given model. Day (categorical) and random effects were included in all models.

collinearity that may obscure statistical associations. (Supp Tables 4 and 5 [online only]). After removal of air temperature, the reduced set of variables exhibited acceptable VIF values (<4) for all remaining variables. Model selection based on AICc identified four candidate models within six ΔAICc points, with a combined Akaike weight of 0.97 (Table 2). The best model (soil moisture, soil temperature, and relative humidity) was 2.11 AICc points (Akaike weight, 0.62) better than the next best model. This model could potentially be used alone, but we elected to perform model averaging among the top four models due to the fairly small differences in AICc among them and to increase precision (Burnham and Anderson 2002). Soil moisture (positive relationship with fungal mortality, $P < 0.001$) and soil temperature (negative relationship, $P = 0.001$) were highly significant in the averaged model (Table 3) and had relative importance values of 1.0. Relative humidity was also significantly positive ($P = 0.011$) with a relative importance of 0.86. Two of these models also included precipitation (relative importance = 0.26), but it was not significant. However, precipitation could not be ruled out as unimportant by AICc. Although the explanatory power of the overall model is not affected, care must be taken when interpreting the significance of individual predictors that are correlated. This issue was partly solved by the removal of air temperature, but some collinearity still existed between soil moisture and precipitation. This means that (unsurprisingly) some of the variation that is explained by soil moisture could also be explained by precipitation. Indeed, if soil moisture is removed from the model, the AIC increases, but precipitation now becomes highly significant ($P < 0.001$). The best alternative models with only a single weather variable are listed in Table 4. This is useful for examining the sign of estimates in the absence of the other variables, showing that all moisture-related variables tend to be positively associated with mortality, whereas temperature variables tend to be negatively associated. The best two and three variables are listed in the supplementary information (Supp Table 6 [online only]). In all models, collection day (as a categorical predictor) was highly significant ($P < 0.001$), with mortality due to fungal infections increasing rapidly around the beginning of June and then slowly leveling off (Fig. 3).

Calculating from the estimates of the logistic regression, an increase in average soil moisture by 0.01 cm^3/cm^3 would increase the odds of infection by ≈43%, whereas an increase in average soil temperature by 1°C would yield an ≈24% decrease in the odds of infection. Although superficially this appears to be an extreme effect, when interpreting these results, it should be remembered that an increase in the multiday averages of moisture or temperature is much more substantial than a similar increase over a shorter interval.

Fungal Infection of Caged Larvae. Consistent with the analysis of field-collected larvae, after removal of air temperature from the analysis, collinearity was found to be within acceptable levels based on variance inflation factors (<4 for all variables). Thus, we did not remove any further variables, although the high correlation between soil moisture and precipitation (Supp Table 7 [online only]) was noted. Model selection based on AICc identified three candidate models within 4 ΔAICc points, with a combined Akaike weight of 0.97 (Table 5). The best model (precipitation and relative humidity) was 3.2 AICc points (Akaike weight, 0.69) better than the next best model. This model could potentially be used alone, but we elected to perform model averaging on the top five models due to the relatively small differences in AICc. Precipitation (positive relationship with fungal mortality, $P < 0.001$) and relative humidity (positive relationship, $P < 0.001$) were highly significant in the averaged model (Table 6) and had relative importance values of 1.0. Soil temperature and soil moisture

Table 5. Model selection table for mortality of caged larvae on soil

Model rank	Intercept	Precipitation	Relative humidity	Soil temperature	Soil moisture	AICc	Delta	Weight
1	−14.4	0.617	0.164	X	X	379.4	0	0.69
2	−15.0	0.601	0.166	X	14.6	382.6	3.20	0.14
3	−15.4	0.611	0.169	0.042	X	382.6	3.21	0.14
								0.97

The top three candidate models were used in AICc-based model averaging. Model estimates are listed for each variable included in a given model, or denoted with an "X" if the model does not include the variable. Day (categorical) was included in all models.

Table 6. Model-averaged coefficients for the model of mortality of caged larvae due to *E. maimaiga*

	Estimate	SE	z	*P* value	Relative Importance	Shrinkage-corrected Estimate
Intercept	−14.590	3.130	4.662	<0.001	1.00	−14.590
Precipitation	0.614	0.118	5.202	<0.001	1.00	0.614
Relative humidity	0.165	0.033	5.026	<0.001	1.00	0.165
Soil temperature	0.042	0.166	0.251	0.802	0.14	0.006
Soil moisture	14.629	53.155	0.275	0.783	0.14	2.106

Day effects (not listed) were included and highly significant in all models ($P < 0.001$). Shrinkage-corrected estimates include zeros in the average when a parameter does not appear in contributing models.

were included in one of the three candidate models, but were not significant ($P = 0.802$ and $P = 0.780$) and had relative importance values of 0.14. However, because of collinearity (as was true in the field-collection data), some of the variation that was explained by precipitation could also be explained (although less well) by soil moisture, such that if precipitation is removed from the model, the AIC increases, but soil moisture becomes highly significant ($P < 0.001$).

The best alternative models with only a single weather variable are listed in Table 7. As in the field data, all moisture-related variables tend to be positively associated with mortality due to fungal infection, whereas temperature variables tend to be negatively associated. The best two- and three-variable models are listed in the supplementary information (Supp Table 8 [online only]).

Discussion

In both field-collected larvae and caged larvae, there was a very strong positive relationship between moisture-related variables (precipitation or soil moisture and relative humidity) and the probability of fungal infection. This result is consistent with previous laboratory and field studies (Hajek et al. 1990, Hajek and Soper 1992, Weseloh and Andreadis 1992, Smitley et al. 1995, Weseloh 1999). Soil moisture was more significant in the analysis of the field data, while precipitation was more significant in the cage data.

The effect of soil temperature was significant for the field-collected larvae and inversely related to infection rate, but temperature was not significant in the analysis of the caged larvae. If infection by airborne conidia is impacted negatively by ambient temperature, this might explain why a negative soil temperature relationship was clear in the field-collected larvae but not in the cage larvae, as caged larvae are likely to be infected by resting spores as well as conidia.

Such a relationship between conidial infections and temperature would be consistent with previous research showing that the flux of airborne conidia was negatively associated with temperature (Hajek et al. 1999) and possibly with various findings that conidial production and germination (Hajek et al. 1990) or survival (Roberts and Campbell 1977) could be harmed when temperatures approach 30°C. In our study, temperatures rarely became this high for long, so this mechanism seems less likely. Another possibility is that we could have detected the association between increasing temperatures and the switch from conidial production to the production of overwintering resting spores (Hajek and Shimazu 1996), which would result in fewer infections because resting spores do not infect in the season in which they are produced. If infection levels due to resting spores are positively associated with temperature, we might expect to see such a relationship most strongly in our caged larvae (in contact with the soil), which might cancel out other negative effects and explain the lack of temperature significance in that data set. In the literature, the response of resting spore germination to temperature is not well explored, but Hajek and Humber (1997) found that the infection of larvae in soil cages increased with increasing temperature up to ≈11°C, but then began falling. Thus, their analysis fit a second-order polynomial to the relationship. In our models, the addition of a second-order polynomial for soil temperature did not improve the model fit (ΔAICc = +0.57 for field data, +1.20 for cage data, Supp Table 9 [online only]). Another possibility is that temperature effects were less pronounced for larvae in soil cages due to the buffering of extreme temperatures at ground level (Supp Table 2 [online only]). We found no relationship between resting spore density before the 2007 and 2009 seasons and season-long mortality due to *E. maimaiga.* This suggests that the success of conidial infections over the season is probably more

Table 7. List of best models with only a single weather variable, ranked by AICc, for the cage data

Model rank	Intercept	Soil moisture	Precipitation	Relative humidity	Soil temperature	Air temperature	AICc	Delta	Weight
1	−1.817		0.7282				404.8	0	0.891
2	−14.33			0.1995			409	4.2	0.109
3	5.688				−0.2721		450.9	46.1	0
4	−0.710	65.42					453	48.25	0
5	1.649					−0.00648	455.2	50.42	0

Model estimates are listed for each variable included in a given model. Day (categorical) and random effects were included in all models.

influential than the initial resting spore load, and therefore, weather variables would be very important for successful infection.

In summary, meteorological variables including both temperature- and moisture-related factors are clearly very important to interactions between *L. dispar* and *E. maimaiga.* Because the host–pathogen dynamics of this system appear to be density-independent (Liebhold et al. 2013), environmental factors may be the key to understanding why gypsy moth dynamics differ among different areas and through time. Our results suggest that the effect of *E. maimaiga* on gypsy moth populations could be quite different as the moth expands into warmer and drier areas of the south and west. This is consistent with predictions that the fungus could be less effective at controlling gypsy moths in warmer and drier areas (Siegert et al. 2009). Because the fungus is so dependent on moisture and temperature at multiple points in its infection cycle, it seems important to consider what implications climate change might have for this system, and further research to more completely specify the response of the fungus to temperature changes is needed.

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ONLINE SUPPLEMENTARY INFORMATION

Table S1. Soil texture analysis of selected sites used in the study, as determined by the Cornell Nutrient Analysis Laboratory. Each set of percentages is based on an average of 7 soil samples from the site.

Site	% Sand	% Silt	% Clay
176A	51.8	34.1	14.2
176B	58.8	35.6	5.6
176C	68.3	26.6	5.1
33A	45.0	41.7	13.3
33B	53.2	34.2	12.6
33C	44.2	40.7	15.1
RRA	39.1	48.2	12.7
RRB	29.1	58.1	12.8
RRC	42.4	41.2	16.4

Table S2. Weather variables analyzed in this study. For each dataset, the mean, min and max are calculated. These statistics apply to the 7 or 10 day means used in the analysis, not to values of individual days.

			Field data:			**Cage data:**		
	Units	**Lag**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Soil moist (W)	cm^3/cm^3	Daily mean over previous 7 days	0.031	0.012	0.096	0.030	0.012	0.047
Precip	mm	Daily mean over previous 10 days	2.82	0.83	11.14	3.12	1.04	5.85
Rh	%	Daily mean over previous 10 days	78.2	54.4	92.0	78.5	66.8	89.5
Soil temp	°C	Daily mean over previous 7 days	15.0	8.8	18.1	15.6	13.3	17.7
Air temp	°C	Daily mean over previous 10 days	17.4	7.4	24.1	19.0	15.6	23.3

Table S3. Mean (± SE) resting spore densities in 2007 and 2009.

	Resting spores/g dry soil	
Site	2007	2009
33A	341.1 (72.2)	243.8 (153.9)
33B	186.8 (113.1)	0 (0)
33C	169.6 (105.5)	80.2 (54.3)
RRA	0 (0)	39.2 (18.9)
RRB	87 (22.9)	87.7 (44.2)
RRC	78.9 (23.8)	23.9 (23.9)

Table S4. Correlation matrix of weather variables used in full model of field-collected larvae.

	Air temp	Soil temp	Precip	Rh	Soil moist
Soil temp	0.85				
Precip	-0.34	-0.25			
Rh	0.26	0.42	0.20		
Soil moist	-0.38	-0.16	0.81	0.29	
Day	0.28	0.51	0.09	0.54	0.20

Table S5. Variance inflation factors (VIF) associated with weather variables used in full model of field-collected larvae, before and after the removal of air temperature from the model.

	VIF (before removal of air temp)	VIF (after removal of air temp)
Air temp	5.0	--
Soil temp	5.5	1.7
Precip	3.1	3.0
Rh	1.6	1.6
Soil moist	3.7	3.1
Day	1.8	1.7

Table S6. Alternative models for field-collected larvae with two and three weather variables. Models are ranked by AICc.

BEST TWO VARIABLE MODELS

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Air temp	AICc	Delta	Weight
1	-1.617	38.88			-0.3074		410.9	0	0.895
2	-8.535	42.58		0.0562			415.8	4.94	0.076
3	-0.2202		0.2076		-0.3392		418.2	7.31	0.023
4	-2.67	40.92				-0.1537	421.2	10.34	0.005

BEST THREE VARIABLE MODELS

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Air temp	AICc	Delta	Weight
1	-4.844	37.47		0.04255	-0.2626		407.1	0	0.761
2	-1.617	38.88			-0.3074		410.9	3.78	0.115
3	-1.455	31.57	0.07721		-0.3092		412.6	5.51	0.048
4	-1.615	38.87			-0.3071	-0.00037	413.8	6.7	0.027
5	-3.477		0.1951	0.04241	-0.2933		414.5	7.39	0.019
6	-6.451	39.58		0.04885		-0.1108	415.5	8.39	0.011
7	-8.535	42.58		0.0562			415.8	8.72	0.01
8	-8.386	37.1	0.05869	0.05556			418	10.96	0.003
9	-0.2202		0.2076		-0.3392		418.2	11.09	0.003

Table S7. Correlation matrix of weather variables used in full model of caged larvae.

	Air temp	Soil temp	Precip	Rh	Soil moist
Soil temp	0.64				
Precip	-0.37	-0.23			
Rh	-0.51	-0.33	0.55		
Soil moist	-0.43	-0.08	0.76	0.52	
Day	0.13	0.35	-0.53	-0.30	-0.28

Table S8. Alternative models for caged larvae with two and three weather variables. Models are ranked by AICc.

BEST TWO VARIABLE MODELS

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Air temp	AICc	Delta	Weight
1	-14.36		0.6167	0.1641			379.4	0	1
2	3.943		0.767		-0.3881		399.7	20.32	0

BEST THREE VARIABLE MODELS

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Air temp	AICc	Delta	Weight
1	-20.1		0.5615	0.1986		0.1749	379.6	0	0.692
2	-14.97	14.63	0.6005	0.1664			382.6	3.02	0.153
3	-15.38		0.6113	0.1693	0.04161		382.6	3.03	0.152
4	-34.84	177.8		0.2871		0.4016	390.9	11.29	0.002

Table S9. Models with and without soil temperature as a second order polynomial, ranked by AICc, for the field-collected larvae and caged larvae. Model estimates are listed for each variable included in a given model. Day (categorical) and random effects were included in all models.

FIELD DATA

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Soil temp^2	AICc	Delta
1	-4.64	31.2	0.066	0.0416	-0.265	X	409.18	0
2	0.324	32.1	0.0427	0.0515	-1.087	0.028	409.75	**0.57**

CAGE DATA

Model rank	(Intercept)	Soil moist	Precip	Rh	Soil temp	Soil temp^2	AICc	Delta
1	-17.3	25.7	0.578	0.178	0.077	X	385.8	0
2	9.44	22.7	0.531	0.193	-3.46	0.113	387.0	**1.20**