

Relating Aerial Deposition of *Entomophaga maimaiga* Conidia (Zoopagomycota: Entomophthorales) to Mortality of Gypsy Moth (Lepidoptera: Erebididae) Larvae and Nearby Defoliation

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Abstract

We collected data on mortality of late-instar gypsy moth, *Lymantria dispar* (L.), from outbreak populations over 4 wk in June 2017 at 10 sites in the New England region of the United States, along with estimated rainfall at these sites. Deposition of airborne conidia of the fungal pathogen, *Entomophaga maimaiga* Humber, Shimazu & R.S. Soper, was measured at these same sites as well as at seven other locations in New England. We also quantified the geographical distribution of gypsy moth-caused defoliation in New England in 2017 and 2018 from Landsat imagery. Weekly mortality of gypsy moth larvae caused by *E. maimaiga* correlated with local deposition of conidia from the previous week, but not with rainfall. Mortality from this pathogen reached a peak during the last 2 wk of gypsy moth larval development and always exceeded that caused by *LdNPV*, the viral pathogen of gypsy moth that has long been associated with gypsy moth outbreaks, especially prior to 1989. *Cotesia melanoscela* (Ratzeburg) was by far the most abundant parasitoid recovered and caused an average of 12.6% cumulative parasitism, but varied widely among sites. Deposition of *E. maimaiga* conidia was highly correlated with percent land area defoliated by gypsy moths within distances of 1 and 2 km but was not significantly correlated with defoliation at distances greater than 2 km. This is the first study to relate deposition of airborne conidia of *E. maimaiga* to mortality of gypsy moths from that agent.

Key words: defoliator, forest insect, insect pathogen, contemporaneous mortality, marginal mortality rate

The fungal pathogen of gypsy moth, *Entomophaga maimaiga* Humber, Shimazu & R.S. Soper, was introduced inadvertently to North America and became widespread in populations in southern New England in 1989 (Hajek et al. 1990b). In the following year, it spread halfway across Pennsylvania (Elkinton et al. 1991), and by 1996, it had occupied most of the gypsy moth's invaded range in eastern North America (Hajek 1999). This fungal pathogen became the dominant source of mortality in both low- and high-density populations (Hajek et al. 2015). Spread of the pathogen is facilitated by production of airborne conidia ejected from cadavers of gypsy moth larvae killed by *E. maimaiga* (Hajek et al.

1999). Rate of *E. maimaiga* spread has been estimated in various studies (Dwyer et al. 1998, Hajek et al. 1999). In a recent study, Bittner et al. (2017) developed a trap to quantify rates of conidial aerial deposition by way of quantitative PCR detection of *E. maimaiga* DNA and showed that spore deposition was correlated with distance to nearest gypsy moth defoliation in an outbreak occurring in Pennsylvania in 2016. Spore deposition was not, however, associated with rainfall levels at spore collection sites (Bittner et al. 2017) although previous studies with *E. maimaiga* have demonstrated strong associations between moisture levels and activity of *E. maimaiga* (Hajek 1999).

In 2015, a gypsy moth outbreak began in eastern Massachusetts, Connecticut, and Rhode Island (Pasquarella et al. 2018b). The outbreak expanded in 2016 and 2017 to encompass most of the southern New England region of the United States. This was the first truly widespread outbreak of gypsy moth in this region since 1981, although smaller outbreaks occurred in 1989 and 2006 (Morin and Liebhold 2016). Prior to 1989, gypsy moth outbreaks occurred regularly in this region about every 10 yr (Haynes et al. 2009, Bjørnstad et al. 2010). Unlike the previously established *LdNPV* (Baculoviridae), a viral pathogen of gypsy moth larvae that invariably caused the collapse of high-density populations prior to 1989 (Campbell and Podgwaite 1971, Elkinton and Liebhold 1990), *E. maimaiga* causes high mortality even in low-density populations and thus often prevents initiation of outbreaks (Hajek 1999).

The purpose of this study was to investigate hypotheses regarding associations between agents causing mortality of gypsy moth larvae, during an outbreak, and abiotic and biotic conditions. We determined whether deposition of airborne conidia of *E. maimaiga* and/or rainfall would predict mortality of late-instar gypsy moths caused by *E. maimaiga* at various sites in New England. We also related total deposition of *E. maimaiga* conidia over 4 wk to defoliation at varying severity thresholds and distances from the study sites, as estimated from Landsat imagery (Pasquarella et al. 2018b). Finally, we compare weekly mortality caused by *E. maimaiga* to that caused by *LdNPV* and parasitoids.

Materials and Methods

Selection of Study Sites

With the help of collaborators at various locations in Massachusetts, Rhode Island, and Connecticut, we selected 18 sites near the edge of stands dominated by oak trees, where we deployed spore traps for weekly collections of airborne conidia of *E. maimaiga* beginning at the end of May 2017 (Fig. 1, Supp Table S1 [online only]). At 10 of those sites with high-density gypsy moth populations, we made simultaneous weekly collections of 50 late instars beginning the first week of June 2017 and continuing until larvae pupated during the last week of June.

Quantifying *E. maimaiga* Spore Deposition

Design and deployment of wet-cup modified Tauber traps was described in Bittner et al. (2017). Traps were deployed beginning on 23 May 2017 and collection buffer was retrieved and traps reset with new buffer cups weekly, with the last collection occurring on 27 June 2017. When rainfall exceeded the capacity of the deli cup, all liquid in the trap base was also collected along with the main sample. Sample bottles were refrigerated or frozen and sent to Cornell University for processing. Conidia were filtered from the trap liquids, as described in Bittner et al. (2017) using the Swinnex system, except that deli cups were not rinsed and sample volumes were not measured. Vials of prepared conidial samples were stored frozen in buffer until they were mailed to ArqGenetics (Bastrop, TX) for custom DNA extraction and quantitative PCR. Total DNA was extracted from samples using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA; cat no. 69106) according to the manufacturer's instructions with a 50- μ l final elution volume. An assay for the detection of *E. maimaiga* DNA (Castrillo et al. 2007) was utilized at a working concentration of 600 nM forward and reverse amplification primers and 200 nM probe in a reaction volume of 10 μ l.

Quantitative PCR was performed on the Bio-Rad CFX384 Real-Time System (Bio-Rad, Hercules, CA). For *E. maimaiga* samples,

each reaction well contained 5 μ l of TaqMan Universal Master Mix II (Applied Biosystems, Cat#4440038), 2 μ l of template, and 0.5 μ l of the *E. maimaiga* detection assay in a reaction volume of 10 μ l. Cycling conditions were as follows: 95°C for 10 min for polymerase activation, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Samples of pure *E. maimaiga* DNA, T5 and T9, were prepared from conidia as described in Bittner et al. (2017) and quantified on a Nanodrop ND1000 (Thermo Scientific). Both standards were used to construct standard curves for the purpose of quantifying *E. maimaiga* in unknown samples. The T5 standard curve consisted of an 8-point, 10-fold serial dilution, ranging from 10 ng to 1 fg. The T9 standard curve was identical except for quantity, which ranged from 20 ng to 2 fg.

Data analysis of the *E. maimaiga* qPCR products was performed using CFX Manager software from Bio-Rad, version 3.1. The experimental Cq (cycle quantification) was calibrated against each standard curve (T5 and T9) to determine target quantity in each reaction. The data were imported to MS Excel for further analysis. The quantifications from the two different standard curves were averaged and then log-transformed. Data on gypsy moth mortality, but not spore samples, were collected from the site in Belchertown, MA.

Rearing of Larvae and Quantifying Cause of Death

Fifty larvae were collected from foliage of oak trees each week beginning the first week of June. Larvae collected on the last week of June that survived to the following week mostly all pupated that week, so we ended our collection at that time. The larvae were reared individually in 2 oz. (20 ml) cups on artificial diet (Bell et al. 1981) in shaded outdoor rearing facilities, where they were checked 1 wk following collection. Any larval cadavers were then held for 2–3 d at room temperature to allow sporulation of *E. maimaiga* conidia. Cadavers were then examined under a compound microscope (400 power) to confirm the presence of *LdNPV* occlusion bodies, *E. maimaiga* conidia, or *E. maimaiga* azyospores (resting spores). Emerged parasitoids were also noted. After each week, live larvae were discarded and analysis proceeded with new larvae collected that week. From the last week of collection, live larvae were retained to determine the fraction that successfully molted to the pupal stage, or to recover any parasitoids, such as *Parasetigena silvestris* (Robineau-Desvoidy) (Diptera: Tachinidae), that may emerge from the pupal stage.

Weekly mortality from each cause (*E. maimaiga*, *LdNPV*, parasitoids, and unknown mortality) was calculated as marginal rate of mortality from each cause of death (Royama 1981, Elkinton et al. 1992). These mortality rates represent an estimate of the proportion of larvae each week that would have died from a particular cause had there been no other competing contemporaneous causes of death. The products of the corresponding marginal rates of survival ($1.0 - \text{the marginal rates of mortality}$) for all causes of death equals the total proportion surviving during the week. This procedure entails determining which agent is usually the observed cause of death, thereby obscuring coinfections when more than one agent infects the same individual larva. Where that information is unknown, the appropriate procedure is to use the proportional hazards calculation (Elkinton et al. 1992). In our case, however, we know from previous research that *E. maimaiga* typically out-competes both *LdNPV* and parasitoids when both infect the same host larvae because *E. maimaiga* kills larvae more quickly than these other agents (Malakar et al. 1999, Hajek and van Nouhuys 2016). As a result, we used a technique originally advocated by Varley et al. (1973), wherein mortality from contemporaneous mortality factors is treated as if they act sequentially. Hence we call this method the sequential method.

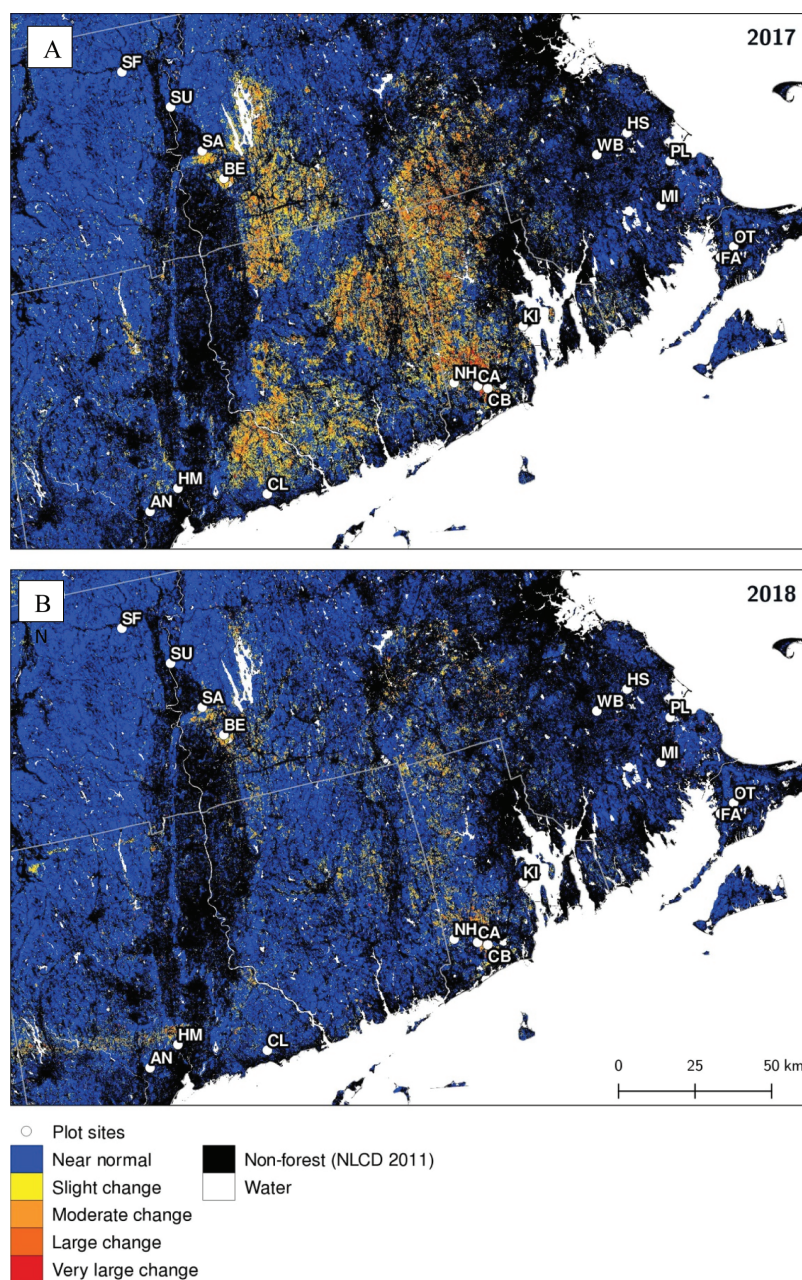


Fig. 1. Defoliation of varying severity by gypsy moth in New England in (A) 2017 (from Pasquarella et al. 2018a) and (B) 2018 with gypsy moth study sites (Table 1 and Supp Table S1 [online only]).

This procedure is what we would use for a predator and a parasitoid (Elkinton et al. 1992) and has been widely used (e.g., Broadley et al. 2019, Murphy et al. 2018). Like a predator, *E. maimaiga* almost always ‘wins’ and obscures coinfections with *LdNPV* and parasitoids. That is usually, but not always true. The actual winner no doubt depends on the timing of infection (Malakar et al. 1999). Thus, the marginal rate of attack for *E. maimaiga* (m_i) is the same as the observed death rate or proportion dying from *E. maimaiga* after 1 wk in the weekly collection: $m_i = (f)/(L - u)$, where L is the number of larvae collected at each site and week, f is the number of cadavers containing *E. maimaiga* conidia or resting spores at the end of the week, including half of those that also contained *LdNPV*, and u is the number of cadavers that died from unknown causes. For *LdNPV*, the marginal rate of mortality m_v is calculated from the larvae that remain alive after mortality from *E. maimaiga* has been

extracted: $m_v = v/(L - u - f)$, where v is the number of cadavers that contain *LdNPV*, but not *E. maimaiga*. Here, v also includes half of the larvae with coinfections of *LdNPV* and *E. maimaiga*. We treated larval mortality with no observed cause (unknown mortality) as resulting from suboptimal rearing conditions and unrelated to actual mortality in the field. Consequently, we discarded those larvae from our calculations (i.e., they were not included in the denominator of our estimates of the marginal rates of mortality).

Cumulative mortality from each agent was calculated as $1.0 -$ the cumulative marginal survival from each agent. Cumulative survival was the product of the weekly marginal survivals ($1.0 -$ the weekly marginal rate of mortality) from each agent multiplied across the 4 wk. We and our colleagues have used these calculations to calculate mortality to gypsy moth larvae from various causes in several previous studies (Gould et al. 1990, Hajek 1997, Liebhold et al. 2013). For

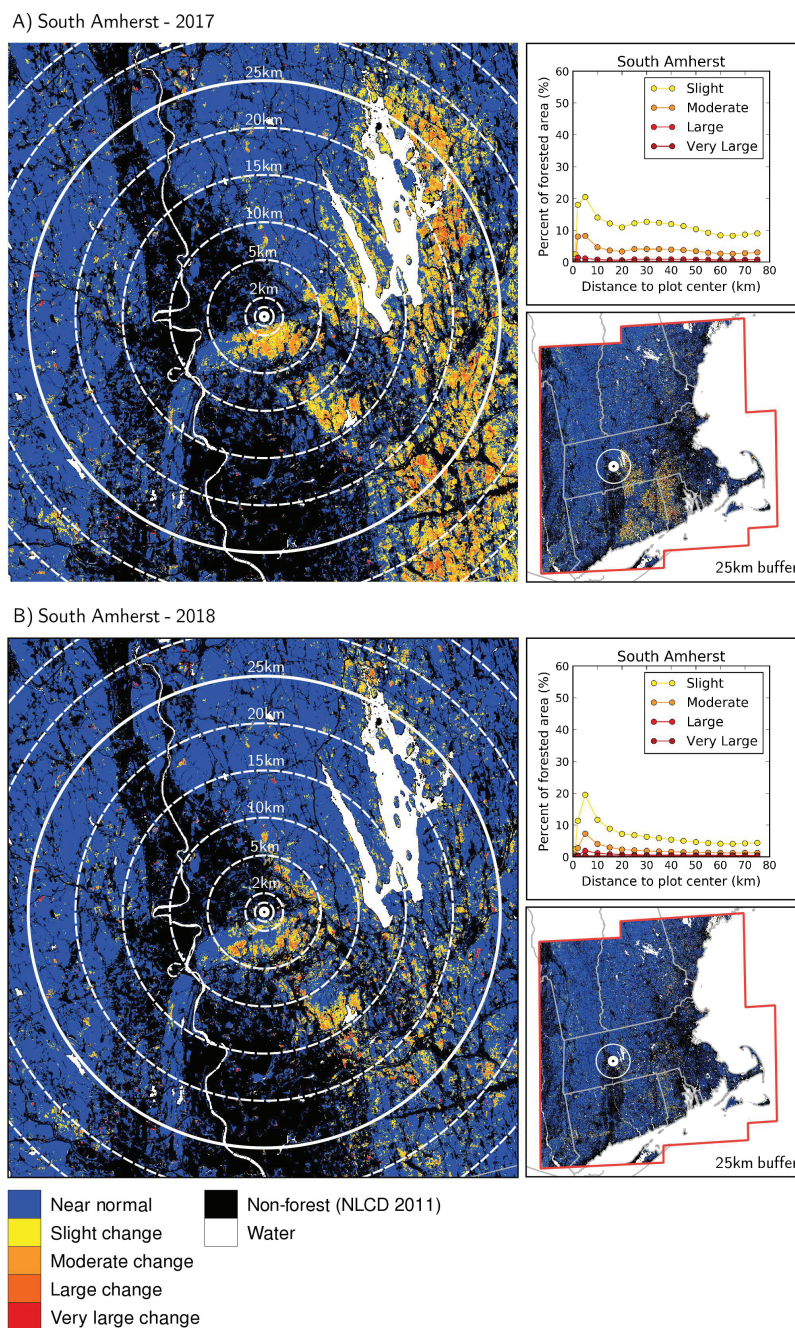


Fig. 2. Concentric circles encompassing defoliation surrounding the sample site in South Amherst for (A) 2017 and (B) 2018 along with associated plots of total areas of defoliation of differing severity as a function of distance from plot center and regional map showing plot locations.

purposes of comparison, we also present cumulative mortality that we would calculate, had we used the proportions that we observed to die from each agent each week, instead of marginal mortality rates, to calculate our weekly survival from each agent. This will enable readers to discern the differences in magnitude or impact that one gets by calculating marginal rates, instead of observed proportions dying.

Estimating Defoliation at Various Distances from Study Sites

Time series of Landsat satellite observations were used to estimate changes in forest condition associated with gypsy moth defoliation following methods previously presented (Pasquarella et al. 2017,

Pasquarella et al. 2018a). Surface reflectance measurements for each Landsat pixel were transformed to Tasseled Cap Greenness (TCG), a metric that specifically characterizes the presence and vigor of green vegetation (Crist 1985, Cohen and Goward 2004). Harmonic regression models were then fit to time series of TCG observations for the period 2004–2014 to establish a baseline model of forest ‘greenness’ for each 30 m × 30 m Landsat pixel. The baseline period was selected to represent a period of time during which minimal defoliation occurred in the study area, and the harmonic modeling approach provides a robust estimate of phenology at the pixel scale that can be used to predict TCG for any day of the year based on the historic record (Pasquarella et al. 2017).

Baseline harmonic models were used to predict TCG values for the date of each new Landsat acquisition during a ‘monitoring period’ from May 15 to September 15. During the monitoring period, the difference between observed and predicted TCG is divided by the baseline model RMSE to account for general noise in the TCG signal. This standardized forest ‘condition score’ is calculated for each clear acquisition during the monitoring period, then all condition scores are averaged to produce an estimate of change in forest condition for that year’s monitoring period. Although we focus on results from 2017 to 2018, Landsat-based monitoring has been conducted each year since 2015.

Forest condition scores for 2017 and 2018 were used to determine the percentage of defoliated forest within a range of distances from each mortality monitoring field site. Continuous condition scores were binned using severity thresholds. Severity scores ranged from 0: near normal, -1: slight change, -2: moderate change, -3: large change, to -4: very large change (Fig. 1).

A series of buffer zones was created around each mortality monitoring site at distances of 1, 2, and 5 km, then from 5 to 75 km at 5-km intervals I (Fig. 2). The total number of pixels at each severity threshold (-1, -2, -3, and -4) was counted and divided by the total number of forested pixels in each cumulative buffer zone to determine the percentage of defoliated forest at each distance from the plot center. Percentages of defoliated forest for all buffer zones were calculated for 2017 and 2018 and used to analyze the spatial and temporal relationships between detected defoliation and larval mortality. The cumulative percentage of defoliated forest within each buffer zone at each severity threshold (Supp Figs. S1 and S2 [online only]) was compared with gypsy moth mortality from *E. maimaiga*.

Estimates of Rainfall and Relative Humidity

We estimated daily rainfall for the months of May and June 2017 at each of the 10 sites where we collected mortality data using data downloaded from the PRISM Climate Group website (<http://prism.oregonstate.edu>). Daily hours of relative humidity > 90% were obtained from the nearest weather stations to each site reported on the website (<http://uspest.org>). Estimates of rainfall in May and June for 2016 and 2017 for Massachusetts as a whole, and the 30-yr averages were obtained from the PRISM Climate Group website.

Statistical Analyses

We used logistic regression via generalized linear models with a logit link and quasi-binomial distribution (PROC GLIMMIX in SAS 9.4; SAS Institute Inc. 2016) and the glm function in R (R Core Team 2013) to determine the relationship between the proportion of larvae dying each week from *E. maimaiga* (1 - the weekly marginal survivorship) to the estimated nanograms of *E. maimaiga* DNA deposited and to centimeters of rainfall at each site during the interval of time between 1 and 2 wk prior to the date larvae were collected. Our rationale was that larvae dying during a given week were probably infected the week before, given that it takes approximately 1 wk for larvae infected by *E. maimaiga* to die from that infection (Hajek 1999). We used the quasi-binomial distribution because of overdispersion or extra-binomial sources of variation in the mortality data, a nearly universal feature of mortality estimated from different samples in ecological data. In these analyses, week was included as a main fixed effect in the statistical model. We calculated goodness-of-fit for these models with McFadden’s (1974) pseudo- R^2 .

We used paired comparisons (PROC MEANS, SAS 9.4; SAS Institute Inc. 2016) to compare weekly marginal mortality rates from *E. maimaiga* to *LdNPV* across the 10 collection sites over the 4 wk of our study. We calculated Pearson’s correlation coefficient (r) to relate cumulative spore deposition over the 4 wk at each of the 17 sites to moderate or severe defoliation within concentric circles at 1 km and 5–75 km at 5-km intervals from each site (PROC CORR, SAS 9.4; SAS Institute Inc. 2016). Population change is typically defined as $R_t = (\text{density in year } [t + 1]) / (\text{density in year } [t])$ (Berryman 1999). Here we used the ratio of moderate or severe defoliation within 5 km of each site in 2018 to defoliation area in 2017 as our measure of gypsy moth population change between the 2 yr. The ratio was log-transformed to linearize it and regressed against logit-transformed total mortality ($p = \ln(p/(1 - p))$) where p is the cumulative proportion dying from all causes via PROC GLM, SAS 9.4; SAS Institute Inc. 2016) to test whether these two measures were correlated.

Results

Weekly mortality of larvae from *E. maimaiga* and *LdNPV* reached a peak during the last 2 wk of June, just before pupation (Fig. 3). Cumulative mortality from all agents over 4 wk (Table 1) exceeded 90% at 7 of the 10 sites. At 7 of 10 sites, mortality caused by *E. maimaiga* exceeded that caused by *LdNPV* (Fig. 3, Table 1), and this difference was significant across all weeks ($t = 3.51$, $n = 40$, $P < 0.001$). A total of 12 individuals of *Cotesia melanoscela* (Ratzeburg) (Hymenoptera: Braconidae), two *Compsilura concinnata* (Meigen) (Diptera: Tachinidae), three *Phobocampe disparis* (Viereck) (Hymenoptera: Ichneumonidae), and one unidentified tachinid larva were reared from the 2,008 gypsy moth larvae we collected and reared from the 10 sites in this study. Nevertheless, all but one of the 12 *C. melanoscela* emerged from larvae collected during the last week and they emerged from the few larvae that had survived both from *E. maimaiga* and *LdNPV*, so the marginal rates of cumulative mortality they caused were nontrivial averaging 12.6% ($\pm 6.7\%$; Table 1), although they varied greatly from one site to another.

Local gypsy moth population levels around sites in western and southeastern Massachusetts experienced relatively little change expressed as the ratio of 2018 defoliation/2017 defoliation within 5 km (Table 1). Modest declines of 15–30% were recorded on Cape Cod, and

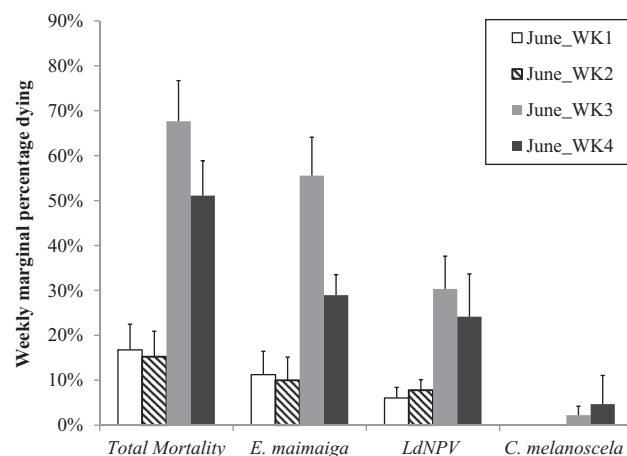


Fig. 3. Mean ($\pm$ SE) weekly mortality (marginal percentage dying) of late-instar gypsy moths caused by *Entomophaga maimaiga*, *LdNPV*, and *Cotesia melanoscela* averaged across 10 sites in New England.

Table 1. Cumulative mortality from *Entomophaga maimaiga*, *LdNPV*, *Cotesia melanoscela*, and total mortality at each of 10 sites based on the weekly estimates of marginal probabilities of dying and observed proportions dying from each cause.

Fig. 2 symbol	Site	Region	2018/2017 Defoliation	Total mortality	Cumulative percentage dying					
					<i>E. maimaiga</i>		<i>LdNPV</i>		<i>C. melanoscela</i>	
					Observed %.	Marginal %.	Observed %.	Marginal %.	Observed %.	Marginal %.
BE	Belchertown	W. Mass	1.02	94.6%	89.5%	89.5%	24.4%	45.5%	2.1%	5.6%
SA	Amherst	W. Mass	0.88	37.7%	31.2%	31.2%	6.2%	7.3%	1.7%	2.3%
WB	W. Bridgewater	SE Mass	1.07	87.5%	59.8%	59.8%	61.3%	68.9%	0.0%	0.0%
HS	Hanson	SE Mass	1.10	99.6%	80.0%	80.0%	65.0%	98.1%	0.0%	0.0%
OT	Otis AF base	Cape Cod	0.74	99.2%	97.7%	97.7%	20.7%	62.2%	6.3%	10.3%
FA	Falmouth	Cape Cod	0.85	93.3%	83.2%	83.2%	15.7%	42.4%	13.1%	31.4%
MI	Middleboro	SE Mass	1.32	73.9%	53.2%	53.2%	32.8%	40.0%	4.1%	6.9%
PL	Plymouth	SE Mass	1.08	95.0%	62.9%	62.9%	68.0%	86.2%	2.0%	2.9%
CA	Charleston	Rhode	0.45	94.1%	91.1%	91.1%	11.7%	34.1%	0.0%	0.0%
CB	RI A	Island								
	Charleston	Rhode	0.49	100.0%	100.0%	100.0%	13.0%	51.2%	7.4%	66.7%
	RI B	Island								
	Mean		0.90	87.5%	74.9%	74.9%	31.9%	53.6%	3.7%	12.6%
	SE		0.09	6.0%	7.0%	7.0%	7.6%	8.3%	1.3%	6.7%

Cumulative proportion dying equals $1.0 - \text{the cumulative survivorship}$, which equals the product of the cumulative marginal weekly survivorships, or $1.0 - \text{the weekly proportions dying of each cause of death}$. Column labeled 2018/2017 defoliation refers to the ratio of area receiving moderate and severe defoliation between the 2 yr 2018 and 2017 (2018 defoliation/2017 defoliation) within 5 km of each site.

larger declines (50%) occurred at the two sites in Rhode Island (Table 1) corresponding to the overall lower levels of defoliation in those regions evident in 2018 compared with 2017 (Fig. 1). Logit-transformed total mortality (Table 1), however, was not significantly related to the observed decline or increase in defoliation ($\log_{10} [2018/2017]$) at 1, 5, or 10 km from each site ($F = 0.66$, $df = 1$, $P = 0.44$; $F = 2.30$, $df = 1$, $P = 0.17$; $F = 2.03$, $df = 1$, $P = 0.19$, respectively).

Mortality from *E. maimaiga* was positively related to conidial spore deposition the previous week (Fig. 4A) across all weeks ($t = 2.37$, $df = 31$, $P = 0.024$, $\text{pseudo-}R^2 = 0.57$). Mortality from *E. maimaiga* was not significantly related to rainfall the previous week (Fig. 4B; $t = -1.13$, $df = 35$, $P = 0.266$, $\text{pseudo-}R^2 = 0.53$). Nor was it related to rainfall over the previous 10 d ($t = -1.17$, $df = 35$, $P = 0.25$), nor to hours of relative humidity > 90% over the previous week ($t = -0.21$, $df = 35$, $P = 0.83$). Defoliation within 1 and 2 km from each site was highly correlated with total spore deposition over 4 wk (Table 2), marginally correlated ($P < 0.1$) at distances of 5–35 km, and not significantly correlated with defoliation at greater distances, although the estimated correlation coefficients (r) were >0 over all distances (Table 2). Because each concentric defoliation circle was comprised to a large extent of defoliation in the smaller circles within them, these estimates of defoliation were highly correlated with one another.

Discussion

Peak larval mortality occurred in the second half of June, probably as a result of the successive waves of mortality resulting from multiple generations of both pathogens that propagate over the larval stages (Woods et al. 1991, Hajek 1999). Our results indicate that mortality of gypsy moth larvae caused by *E. maimaiga* was correlated with deposition of *E. maimaiga* conidia (Fig. 4A), but not with rainfall (Fig. 4B) nor with relative humidity. The lack of correlation with rainfall or relative humidity was surprising given that various previous studies have shown that high levels of rain or humidity are positively correlated with infection by *E. maimaiga* (Weseloh and Andreadis 1992, Reilly et al. 2014) and laboratory studies show that

production or germination of conidia require either free-standing water or high levels of humidity (Hajek et al. 1990a). Estimates of rainfall in May and June 2017 for Massachusetts as a whole (PRISM Climate Group) was 26.1 ± 4.5 cm SD, which exceeded the 30-yr average of 20.9 ± 1.7 cm. These values contrast with the much lower rainfall (12.4 ± 3.3) observed in 2016, which may account, at least in part, for the recent gypsy moth outbreak. We hypothesize that perhaps too much rain can serve to wash the airborne conidia out of the air or off foliage or larval integuments.

We recorded mortality exceeding 90% at 7 of 10 sites, yet defoliation between 2017 and 2018 remained largely unchanged within 5 km of these sites (Table 1), although it declined statewide in Massachusetts and in Rhode Island (Fig. 1). A limitation of our measure of population change based on defoliation within 5 km is that there was very little defoliation in either year at 8 of the 17 sites (Supp Figs. S1 and S2 [online only]). Fecundity of gypsy moth can exceed 600 eggs per female in low-density populations (Campbell and Sloan 1978), although these numbers typically decline to around 100 eggs per female in outbreak populations (Campbell and Sloan 1978) such as of those we report here. In either case, this implies that mortality over all life stages must exceed 98% to result in population declines of gypsy moth. Here we recorded mortality only during the last 4 wk of the larval stage. Thus, we cannot account for mortality during the rest of the gypsy moth life stages. Previous research suggests that mortality from the two pathogens *E. maimaiga* and *LdNPV* at the end of the larval stage dominates total mortality in outbreak populations of gypsy moth (Campbell and Podgwaite 1971, Hajek et al. 2015).

The lowest mortality from *E. maimaiga* occurred at the site in Amherst, MA (Table 1). This was the only site where the oak tree from which larvae were collected occurred on a lawn devoid of leaf litter immediately beneath the tree. Hajek (2001) showed that a significant source of infection of larvae by *E. maimaiga* is by way of germinating azygospores that late instars contact when they seek daytime resting locations in the leaf litter beneath the tree. Such larval behavior, however, occurs much less in outbreak populations (Lance et al. 1987), such as most of those we studied here (Supp Fig.

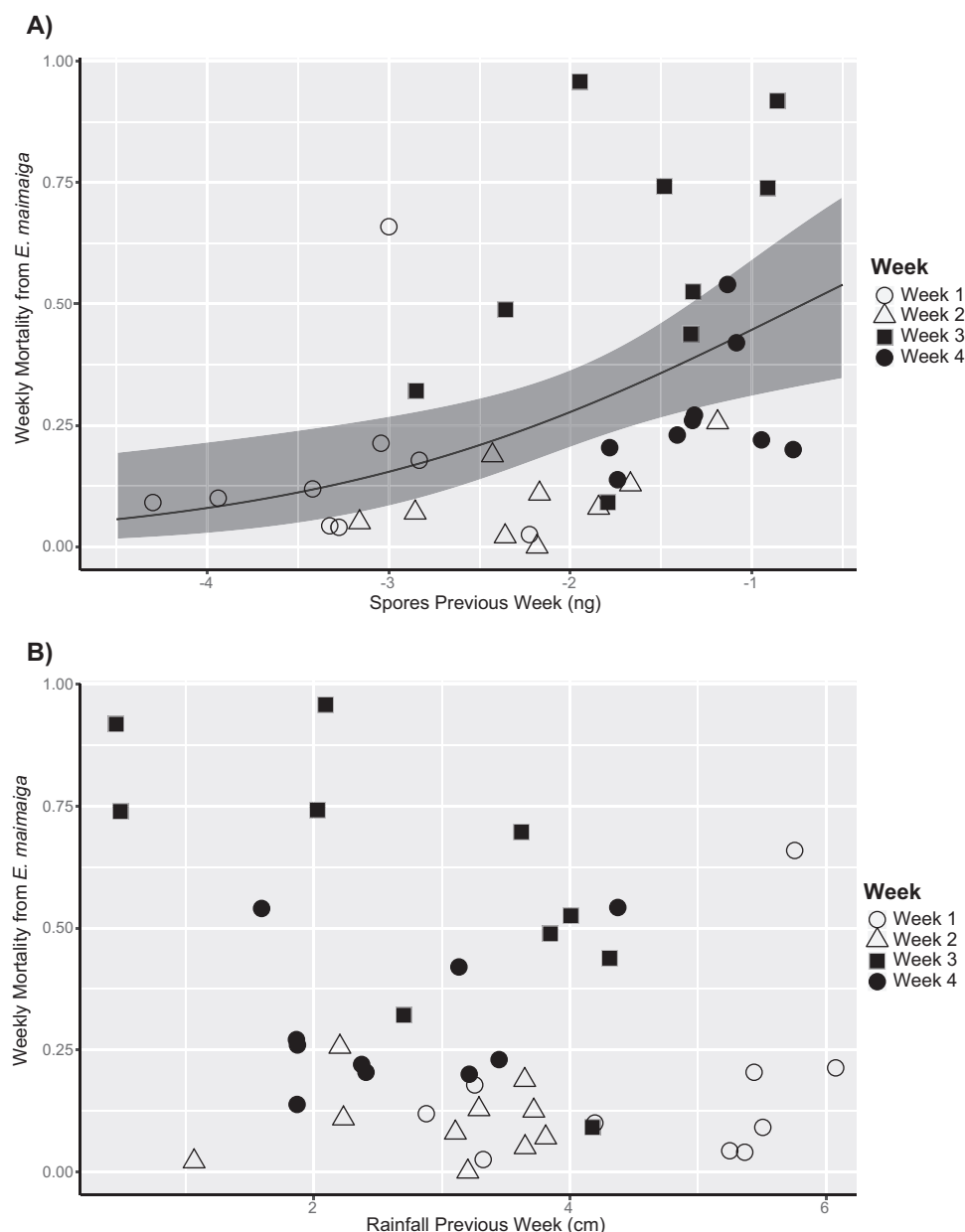


Fig. 4. (A) Logistic regression of weekly mortality (marginal probability of dying) of late-instar gypsy moths from *Entomophaga maimaiga* plotted vs. deposition of ng *E. maimaiga* DNA extracted from conidia collected the prior week vs. (B) rainfall (cm) during the prior week at each of 10 sites in New England.

S1 [online only]), because larvae remain in the canopy and continue feeding during daytime hours.

Our results suggest that airborne *E. maimaiga* conidia infecting outbreak gypsy moth populations mainly emanated from populations within 1 or 2 km, provided that some high densities existed within this distance. This was true of all but 5 of the 18 sites from which we sampled conidia (Supp Fig. S1 [online only]). Our study used Landsat-based estimates of defoliation, in contrast to the traditional estimates used in Bittner et al. (2017) based on aerial surveys, which yield defoliation maps that are much coarser in grain and do not detect smaller pockets of defoliation on a landscape. Thus, although Bittner et al. (2017) may have underestimated actual nearby defoliation, many of the sites they studied were 10 km or more from the nearest defoliation and deposition correlated with defoliation up to 20 km away. Their findings are thus not inconsistent with the current results. It is clear from data collected when *E. maimaiga*

first invaded North America that conidia sometimes spread over distances exceeding 50 km (Hajek et al. 1990a, Elkinton et al. 1991) and readily follow the advancing spread of gypsy moth in mid-western states (Hajek and Tobin 2011).

The close correlation between conidial deposition rates and mortality rates in local populations (Fig. 4a) indicates that fungal propagule pressure plays a dominant role in determining *E. maimaiga* infection rates and subsequent gypsy moth mortality. Rates of conidial deposition on larvae and subsequent infections are apparently affected by production of conidia in surrounding gypsy moth populations (Supp Fig. S1 [online only]). Mobile natural enemies are known to potentially synchronize spatially disjunct host populations (Ims and Andreassen 2000). Gypsy moth populations in North America are well known to exhibit spatial synchrony up to distances of ca. 500 km but the emergence of *E. maimaiga* has not been clearly shown to cause an increase in synchrony (Allstadt et al. 2015).

Table 2. Correlation between total amount (ng) of *E. maimaiga* conidial DNA aerially deposited over 4 wk in June 2017 and moderate to severe defoliation within circles of various radii (distance in km) around each of 17 sites

Distance, km	Correlation coefficient	P-value
1	0.79	0.0002
2	0.77	0.0003
5	0.45	0.071
10	0.42	0.093
15	0.45	0.066
20	0.47	0.054
25	0.5	0.04
30	0.48	0.05
35	0.46	0.06
40	0.41	0.11
45	0.35	0.17
50	0.31	0.23
55	0.29	0.24
60	0.31	0.22
65	0.33	0.18
70	0.35	0.16
75	0.37	0.14

The low levels of parasitism we recorded here are in contrast to those reported in earlier studies (Reardon 1976, Williams et al. 1992). Hajek and van Nouhuys (2016) reported that coinfections of gypsy moth larva with *E. maimaiga* and some parasitoid species result in suppression of parasitoid emergence due to the quicker incubation time of *E. maimaiga* within the host. Possibly the dominant role of *E. maimaiga* in both low- and high-density populations of gypsy moth (Hajek et al. 2015) has reduced the population densities of some parasitoid species that either specialize or depend on gypsy moth. The very low proportion of total larvae dying (12 out of 2,008 larvae reared or <1%) we recorded in this study from *C. melanoscela*, in contrast to the higher marginal attack rates (mean of 12%, Table 1), are almost surely caused by the fact that most of the larvae attacked by his parasitoid were killed by either *E. maimaiga* or *LdNPV* prior to parasitoid emergence. The cumulative mortality estimates for *LdNPV* and *C. melanoscela* based on the observed proportion dying was lower than the corresponding marginal probabilities of dying (Table 1). That was expected because the latter measure attempts to estimate mortalities from each of these agents that would have occurred had not most of the larvae coinfecting by the other agents had already died from *E. maimaiga* in the case of *LdNPV* or from both pathogens in the case of *C. melanoscela*. Our method assumes that *E. maimaiga* is usually the observed cause of death in the case of such coinfections, so the two estimates are equal for that agent (Table 1).

The smaller number of adult parasitoids produced as a result of prior death on coinfections is nevertheless important because it would surely influence attack rates on subsequent generations, as measured by the marginal attack rate. A similar consideration would reduce *LdNPV* inoculum available to infect gypsy moth larvae because many coinfecting larvae would die first from *E. maimaiga*. An additional consideration is that here we report what is surely the second generation of *C. melanoscela* in the gypsy moth larval stage. An earlier generation attacks and emerges from early-instar gypsy moths in May before we initiated our study in June (Weseloh 1975).

Possibly, the dominant role of *E. maimaiga* in both low- and high-density populations of gypsy moth (Hajek et al. 2015) has reduced the population densities of some parasitoid species that either specialize or depend on gypsy moth. A recent study by Baranowski et al. (2019) has shown that impact of the generalist species *C.*

concinata on giant silk moths was much lower in 2017 and 2018 at a site in central Massachusetts than it was in 1998 when this phenomenon was first studied (Boettner et al. 2000), not long after *E. maimaiga* was first introduced; this pattern may result from lower rates of parasitoid emergence from gypsy moth since the arrival of *E. maimaiga*.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Fig. S1. Defoliation versus distance from the 17 study sites in 2017.

Fig. S2. Defoliation versus distance from the 17 study sites in 2018.

Table S1. Study site locations and GPS coordinates.

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