

Introduced pathogens follow the invasion front of a spreading alien host

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Summary

1. When an invasive species first colonizes an area, there is an interval before any host-specific natural enemies arrive at the new location. Population densities of newly invading species are low, and the spatial and temporal interactions between spreading invasive species and specific natural enemies that follow are poorly understood.

2. We measured infection rates of two introduced host-specific pathogens, the entomophthoralean fungus *Entomophaga maimaiga* and the baculovirus *Lymantria dispar* nucleopolyhedrovirus (LdNPV), occurring in spreading populations of an invasive forest defoliator, *L. dispar* (gypsy moth), in central Wisconsin.

3. Over 3 years, we found that host density was closely associated with the presence and prevalence of both pathogens. The fungal and viral pathogens differed in the sensitivity of their response as *E. maimaiga* was present in lower-density host population than LdNPV.

4. We examined the relationship between weather conditions and infection prevalence and found that activity of both the fungus and virus was strongly seasonally influenced by temperature and rainfall or temperature alone, respectively.

5. Purposeful releases of pathogens (median distances of study sites from release sites were 65.2 km for *E. maimaiga* and 25.6 km for LdNPV) were not associated with pathogen prevalence.

6. A generalist fly parasitoid, *Compsilura concinnata*, also killed *L. dispar* larvae collected from the study sites, and parasitism was greater when infection by pathogens was lower.

7. Our results demonstrated that although infection levels were low in newly established host populations, host-specific pathogens had already moved into host populations close behind advancing populations of an invasive host; thus, spreading hosts were released from these enemies for only a relatively short time.

Key-words: disease spread, enemy release hypothesis, *Entomophaga maimaiga*, gypsy moth, invasion ecology, nucleopolyhedrovirus, pathogen ecology

Introduction

The interactions between spatially structured host populations and the transmission dynamics of their macro- and microparasites can result in a range of spatial and temporal dynamic behaviour (Hess *et al.* 2002). In a host–parasite system, the environment of a parasite can be considered either as an individual host or as a population of hosts, both of which can be further linked to population structure across space (Hanski & Gilpin 1997). With an emphasis on environments based on populations of hosts, spatially explicit studies of host–pathogen interactions have been instrumental for understanding intricate dynamics (Dwyer, Elkinton & Hajek 1998; Hilker *et al.* 2005). Most prior studies have focused on

the long-term dynamics between host and pathogen species, particularly with established hosts (e.g. Grenfell, Bjørnstad & Kappey 2001; Getz *et al.* 2006). However, in recent years, with increasing effects of globalization resulting in increasing biological invasions, there is much interest in understanding the spread of non-native species and their macro- and microparasites that follow, whether parasites are originally introduced with their hosts or are subsequently introduced as biological control agents (Fagan *et al.* 2002; Bar-David, Lloyd-Smith & Getz 2006; Xu *et al.* 2009). In this paper, we show the extent to which host-specific infectious diseases following a spreading non-native host species are influenced by host population dynamics and climate.

The chance that disease will spread into new areas occupied by a host is dependent on numerous factors, including the nature of the pathogen, mechanisms for maintenance of

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infection, population dynamics of the host and the relative frequency of transmission within and between populations (Hess *et al.* 2002). In particular, the transmissibility level has been shown to strongly impact the speed with which a pathogen keeps up with spreading host populations (Bar-David, Lloyd-Smith & Getz 2006). The dynamics along the leading edge of a spreading invasive species can be complex because the respective colonization dynamics of hosts and parasites can differ (With 2002). For example, Allee effects (Taylor & Hastings 2005), which act upon low-density populations probably in a species-specific manner, can greatly influence establishment success of newly arriving colonies (Liebhold & Bascompte 2003), which in turn can influence the rate of spatial spread (Johnson *et al.* 2006; Tobin *et al.* 2007b).

We used spreading populations of a non-native species, *Lymantria dispar* (gypsy moth), to investigate the spatial dynamics of the micro- and macroparasites that follow. *Lymantria dispar* is native to temperate Asia, Europe and northern Africa and was introduced from Europe to North America in 1869 (Elkinton & Liebhold 1990). Beginning in the early 1900s, parasitoids and pathogens have been introduced to North America accidentally or purposefully for biological control (Hajek 2007). Gypsy moth is host to a range of pathogens and parasitoids that have been introduced to North America, including the entomophthoralean fungus *Entomophaga maimaiga* (Hajek 1999) and the baculovirus *L. dispar* nucleopolyhedrovirus (LdNPV), both highly host-specific (Barber, Kaupp & Holmes 1993; Hajek 2007), as well as hymenopteran and tachinid parasitoids, including *Comptosilura concinnata* (Meigen). *Entomophaga maimaiga* infects larvae when spores germinate and the fungus penetrates through the larval cuticle. This fungus principally disperses via airborne conidia that are actively ejected from cadavers or from environmentally persistent resting spores (Dwyer, Elkinton & Hajek 1998; Hajek 1999). *Lymantria dispar* nucleopolyhedrovirus infects larvae when eaten or during parasitoid oviposition (Dwyer & Elkinton 1995) and disperses via infected early instars or when vectored by parasitoids or predators (Reardon & Podgwaite 1976). Both pathogens cause acute infections resulting in larval death and have stages that persist in the environment. They also can coinfect larvae (Malakar *et al.* 1999) and usually coexist in established host populations in the field (A.E.H. & A.M. Liebhold, unpublished data). Parasitoids attacking *L. dispar* lay eggs in or on host eggs, larvae or pupae, or on foliage that *L. dispar* larvae eat, and all parasitoid species subsequently kill *L. dispar*.

Despite the long history of *L. dispar* in North America, populations continue to spread to the west and south as only roughly one-fourth of the habitat susceptible to *L. dispar* is currently infested (Morin *et al.* 2005). It has been assumed that as *L. dispar* invades and becomes established in new areas, both *E. maimaiga* and LdNPV will eventually follow and infest these new host populations. In addition, both pathogens have been intentionally released to facilitate faster establishment and control in newly established *L. dispar* populations (Tobin & Blackburn 2007; A. Diss-Torrance, pers. comm.). The importance of both pathogens in regulating

L. dispar outbreaks has been previously reported, as both are frequently observed to play a role in the crash of high-density populations (Elkinton & Liebhold 1990; Hajek 1999). It is not known whether these pathogens behave similarly or differently in low-density host populations at the leading edge of the *L. dispar* invasion. Empirical studies aimed at understanding how quickly these natural enemies move into expanding *L. dispar* populations are unprecedented. Specifically, we investigated which biotic and abiotic conditions are associated with the movements of pathogens following a host's invasion front. Because of the importance of understanding the dynamics of invasive hosts and the natural enemies that kill them, we examined spreading *L. dispar* populations along their leading edge to address the spatial and temporal trophic interactions between this host and its associated macro- and microparasites.

Materials and methods

HOST COLLECTION, REARING AND DIAGNOSIS OF CAUSE OF DEATH

Study sites were chosen along the western leading edge of the *L. dispar* distribution in central and southern Wisconsin over an area of c. 13 000 km² (Fig. 1). A total of 37 sites, each c. 2–3 ha, consisting of mixed forests in which oaks (*Quercus* spp.) were a primary species,

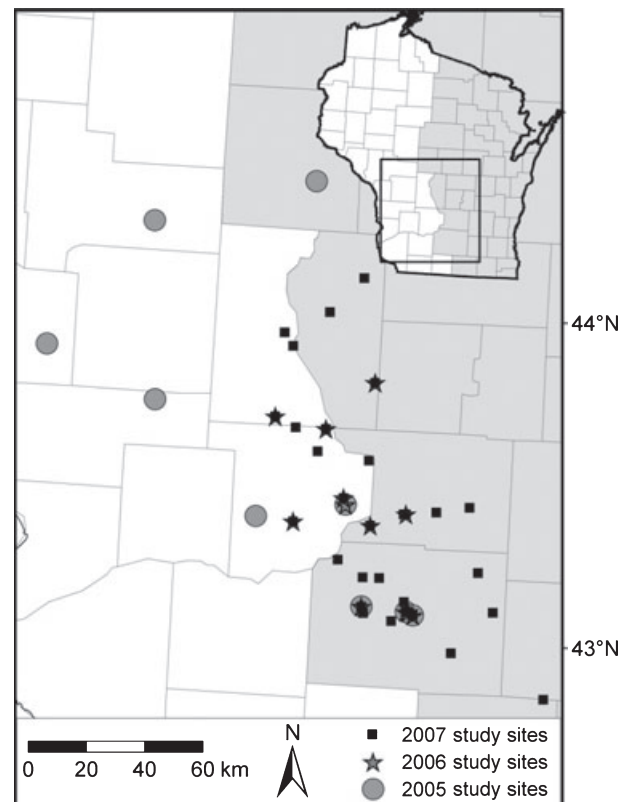


Fig. 1. Location of study sites in southern Wisconsin, 2005–2007. Shaded counties are those in which *Lymantria dispar* was considered to be established and was regulated as of 2007 (U.S. Code of Federal Regulations, Title 7, Chapter III, Section 301.45).

were sampled from 2005 to 2007 (Table S1). In 2005 and 2006, we collected three types of data to evaluate the presence and prevalence of pathogens and to evaluate the sensitivity of *E. maimaiga* detection methods: (i) cause of death of live larvae collected from the field (nine sites in 2005, 12 sites in 2006), (ii) pathogens within larval cadavers collected in the field (nine sites in 2005, 12 sites in 2006) and (iii) prevalence of fungal infection in healthy laboratory-reared larvae that were caged on the soil or in the tree canopy in the field (six sites in both years). In 2006, we added another method to detect *E. maimaiga* presence and document prevalence: (iv) exposing uninfected laboratory-reared larvae to soil samples that had been collected from the field (nine sites). Based on an analysis of the sensitivity of these methods in detecting pathogens, we only collected live larvae and cadavers from the field in 2007, which allowed us to increase the number of study sites to 31. In all years, sites were generally sampled during the period of fourth to sixth *L. dispar* instars, during June. In 2005, each site was sampled 1–3 times for *c.* 1 h each time. In 2006, sites were sampled 2–8 times for *c.* 6 h total for each site, and in 2007, sites were each sampled four times for *c.* 5 h total per site.

Each live larva collected was placed in a 29-mL clear plastic cup containing high wheat germ artificial diet (Bell *et al.* 1981), reared at room temperature ($23 \pm 1^\circ\text{C}$) and monitored daily for death for 10 days (in 2005) or up to 30 days (in 2006 and 2007). Any larvae that died were checked daily for 3 days after death to detect conidial production by *E. maimaiga*. Seven to 10 days after death, cadavers were stored at 4°C for subsequent individual dissection and microscopic observation at $200\text{--}400\times$ to diagnose the cause of death. Cadavers collected in the field were also stored individually at 4°C , and cause of death was diagnosed microscopically. Dissected cadavers were diagnosed as death owing to *E. maimaiga* if they contained characteristically shaped resting spores and death owing to *LdNPV* if, under phase contrast, shining polyhedral particles of the correct size dissolved with 1 M KOH (Lacey & Brooks 1997). Parasitoids emerging from larvae were identified based on adult flies after eclosion or, for those flies not emerging from puparia, based on morphology of the puparia (Simons, Reardon & Ticehurst 1974).

For deploying larvae in cages in the field in 2005 and 2006, we chose five dominant oaks at the centre of each site. *Lymantria dispar* larvae were obtained from a laboratory colony at the USDA Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Buzzards Bay, MA. Twenty early fourth instars were placed in a cage made by folding aluminium window screening into a pocket (20.3×25.4 cm) and stapling the sides so that larvae could not escape. For soil exposures, at each dominant oak, the leaf litter was brushed away on one side at the base of a tree, and one cage was placed on top of the organic layer soil. For canopy exposures, a cage was suspended from a lower branch, *c.* 2 m above the ground. Cages remained in the field for 4 days, after which larvae within cages were individually placed in cups of artificial diet and monitored for mortality and conidial production. We deployed cages twice in succession in 2005 and four times in succession in 2006. Across all sites and years, a total of 2891 and 2976 larvae were deployed in cages on the ground and in the canopy, respectively, and recovered for analysis.

In 2006, soil was collected from nine sites for subsequent larval exposures in the laboratory. From the base of each of the five dominant oaks where cages had been deployed, a sample of the top 3 cm of soil was collected from within 10 cm of the tree base. Soil samples from trees at each site were merged and stored at 4°C for 5 months. For each site, 35 g of soil were placed into each of three clear polypropylene containers (4.5 ht $\times$ 10.5 cm diam) at 15°C . Distilled water was added so that soil was moist but below water-holding capacity. Seven, 21 and 35 days later, ten early fourth instars were added to each

soil container. Larvae remained in soil containers for 4 days, after which they were reared individually on artificial diet at 20°C and monitored daily for mortality and conidial production. A total of 4047 larvae were exposed to soil collected in the field.

SITE CHARACTERISTICS

Lymantria dispar populations at all sites were too sparse to rely on counts of egg masses, which is the most sensitive predictor of larval densities (Gray *et al.* 2008). Instead, to estimate host density, we used male moth counts from pheromone-baited traps that were deployed under the Gypsy Moth Slow-the-Spread program (Tobin & Blackburn 2007). Counts from pheromone traps are very sensitive at low densities, are specific to adult males (adult females do not fly) and are most often used to detect newly founded populations (Tobin & Blackburn 2007). Within our study areas, traps were set $0.5\text{--}2$ km apart. We used trap catch data from 1997 to 2007 to interpolate, for each year, the number of male moths over a network of 1×1 km cells using median indicator kriging (Isaaks & Srivastava 1989). We then estimated the interpolated density (male moths/trap) at each of our study sites in the prior year (indicative of the male parent population of the larvae we sampled) and year of study (indicative of the adult male population that subsequently developed from the larvae we sampled). We used estimated *L. dispar* abundance back to 1997 to provide a historical time series of *L. dispar* populations at each study site. We also used three standard thresholds for *L. dispar* abundance that are consistent with newly established, pre-outbreak populations along the leading edge (Sharov, Liebhold & Roberts 1996; Tobin & Whitmire 2005): the number of prior years at which the interpolated male *L. dispar* abundance exceeded 1, 10 and 100 moths per trap. These male moth densities were used as proxies for larval densities because at these low densities, locating any larvae in the field is often not possible (at 1 and 10 moths per trap) or difficult (at 100 moths per trap). Along the leading edge where our sites were located, newly established populations were not spatially autocorrelated as they would be in well-established populations (Fig. S1; Sharov, Liebhold & Roberts 1996; Tobin & Blackburn 2007). *Lymantria dispar* populations tend to increase in abundance following initial establishment, and thus, the use of population thresholds provided us with a method for associating prior *L. dispar* history with pathogen prevalence as *L. dispar*-colonized areas.

Both pathogens had been purposefully released in Wisconsin at varying times before our study. The Wisconsin Department of Natural Resources (DNR) had released *E. maimaiga*-killed *L. dispar* cadavers containing resting spores at various DNR-managed parks and forests (Fig. S2). Resting spores can be dormant for at least 6 years (Hajek 1999). Thus, releases of fungal resting spores were often made in advance of outbreaks as a means to mitigate future impacts, because it was not known precisely how long it would take for *E. maimaiga* populations to follow *L. dispar* populations naturally. In addition, certain areas with newly established *L. dispar* populations had been treated under the Gypsy Moth Slow-the-Spread program using Gypchek® (USDA Forest Service, Hamden, CT, USA), the commercial formulation of *LdNPV* (Fig. S2; Tobin & Blackburn 2007). In our analyses, we explored the minimum distance between the closest prior release or recovery of each pathogen (1997–2007) and each study site (2005–2007), the length in time between the year of the closest release or recovery and the year of study for each site, and the distance-by-time interaction.

We obtained daily surface maximum and minimum temperature and precipitation data for 2005–2007 from the U.S. National Climatic Data Center (2009). Data from 41 climate stations from

Adams, Columbia, Dane, Dodge, Jefferson, Juneau, Marquette, Rock, Sauk, Waushara and Wood Counties were used. For each station and year, we calculated the mean temperature, total accumulation of rain and frequency of rain for April, May and June, which corresponds phenologically with larval development of *L. dispar* larvae at our study sites (Régnière & Sharov 1998). Climate data were spatially interpolated at a 5×5 km scale using kriging (Isaaks & Srivastava 1989) over the general area of all study sites. The estimated values for temperature and rainfall were extracted from the interpolated grid for each study site and month.

STATISTICAL ANALYSES

We compared differences in the detection of *E. maimaiga* infection among the four methods used in 2005–2006. At each site and year, the number of larvae infected with *E. maimaiga* relative to the sample size and sampling method (i.e. number of live larvae collected, number of cadavers collected, number of larvae placed in cages and number of larvae exposed to soil) was analysed using logistic regression. Overdispersion in the count data was corrected using the scaled Pearson chi-squared. Significance among methods was based on the Wald chi-squared for type 3 analysis, and odds ratios and associated confidence intervals were estimated based on the Wald chi-squared.

In all subsequent analyses, we used only data from field-collected live larvae plus cadavers for pathogen and parasitoid detection and prevalence. The number of larvae infected with *E. maimaiga*, *LdNPV* or parasitized (number of events) was analysed relative to the number of larvae and cadavers collected at each site (number of cases) using logistic regression (SAS Institute 1999). We usually used different study sites in different years (Fig. 1) as we chose sites in each year based on the movement of the *L. dispar* leading edge; thus, a repeated measures approach was not used. Significance was based on the likelihood ratio chi-squared (G^2) for type 3 analysis, and when appropriate, odds ratios and associated confidence intervals were estimated based on the Wald chi-squared. We first tested for the association between the proportion of *E. maimaiga* or *LdNPV* infection and the distance and time from release locations of *E. maimaiga* or Gypchek[®] treatment blocks, respectively, to determine whether these prior pathogen releases were associated with the patterns of *L. dispar* pathogens at our sites. We then tested for the association between proportions of *E. maimaiga* or *LdNPV* infection, and larval parasitism, and the following variables: (i) the background male moth abundance in year $t - 1$ (prior to the study), (ii) the change in male moth abundance from year $t - 1$ to year t , as determined by $\log_e$ (background male moth abundance in year t /background male moth

abundance in year $t - 1$) and (iii) the number of prior years for which the interpolated male *L. dispar* abundance exceeded 1, 10 and 100 moths per trap. The association between *E. maimaiga* or *LdNPV* infection, and monthly mean temperature, total accumulation of rain, and frequency of rain was analysed using stepwise logistic regression (SAS Institute 1999). We also tested the association between the combined rates of infection by both pathogens and parasitoids at our sites and parasitism (sites that recorded $< 5\%$ of both pathogen infection and parasitism were omitted from this analysis).

Results

ENTOMOPHAGA MAIMAIGA DETECTION METHODS

We observed a significant difference among 2005–2006 sampling methods in detecting *E. maimaiga* infection ($\chi^2 = 15.4$, d.f. = 3, $P < 0.01$). Infection was most likely to be detected from field-collected cadavers and least likely to be detected from larvae caged in the field or larvae exposed to soil collected from the field, both of which did not differ significantly from each other ($\chi^2 = 0.3$, d.f. = 1, $P = 0.86$). Overall, *E. maimaiga* infection was 48.5 (95% CI = 12.9–182.7) and 201.8 (95% CI = 18.8–999) times more likely to be detected in cadavers than field-collected live larvae, and the combined group of caged larvae and larvae exposed to soil, respectively. Detection of infection in field-collected live larvae was also significantly higher than for the combined group of caged larvae and larvae exposed to soil (odds ratio = 3.9, 95% CI = 2.9–5.1). Based on these results, we focused our efforts on only collecting cadavers and live larvae from the field in 2007.

INFECTION AND PARASITISM RATES

We collected a total of 4214 and 632 live larvae and larval cadavers, respectively, across all sites over the 3 years, from which we detected no pathogens, *LdNPV* only, *E. maimaiga* only and both pathogens at 22, 0, 7 and 8 sites, respectively. We detected parasitoids at 16 of the 37 sites. The frequency and prevalence of both pathogens and parasitoids across 2005–2007 are presented in Table 1. Across years, *E. maimaiga* was consistently more abundant than other natural

Table 1. Frequency and prevalence of pathogens and parasitoids attacking *Lymantria dispar* from 2005 to 2007^a

Year	No. of sites	No. of sites <i>L. dispar</i> larvae and cadavers collected (range of no. larvae plus cadavers collected/site)	Mean number of larvae and cadavers collected per site ($\pm$ SE)	<i>Entomophaga maimaiga</i>		<i>L. dispar</i> nucleopoly- hedrovirus		Parasitoids	
				No. of sites present	% Infection (mean $\pm$ SE)	No. of sites present	% Infection (mean $\pm$ SE)	No. of sites present	% Parasitism (mean $\pm$ SE)
2005	9	4 (3–162)	22.0 $\pm$ 17.8	2	45.5 $\pm$ 8.8	2	2.9 $\pm$ 0.4	1	1.9 $\pm$ –
2006	12	12 (1–320)	111.1 $\pm$ 34.3	6	18.6 $\pm$ 8.3	5	2.8 $\pm$ 0.9	7	11.9 $\pm$ 2.6
2007	31	28 (1–348)	106.9 $\pm$ 17.0	14	16.0 $\pm$ 5.6	5	0.8 $\pm$ 0.2	11	1.6 $\pm$ 0.5

^aPercentage infection and parasitism are calculated based on sites where the pathogens or parasitoids were present.

enemies with infection ranging from 16.0% to 45.5% at sites where it was present. In contrast, *LdNPV* prevalence was much lower at 0.8–2.9%. Parasitoids reared were predominantly the generalist-introduced tachinid fly (>90%) *C. concinnata* (Table S2), and parasitism ranged from 1.6% to 11.9%.

The presence of infection at our field sites was not significantly related to the distance from prior releases of pathogens or subsequent recoveries (that were predominantly near releases) (*E. maimaiga*: $G^2 = 0.07$, d.f. = 1, $P = 0.70$; *LdNPV*: $G^2 = 0.43$, d.f. = 1, $P = 0.53$). We also did not observe a significant effect of the time between releases or recoveries and the year of our study (*E. maimaiga*: $G^2 = 1.76$, d.f. = 1, $P = 0.18$; *LdNPV*: $G^2 < 0.01$, d.f. = 1, $P = 0.98$) or a significant distance-by-time interaction (*E. maimaiga*: $G^2 = 0.04$, d.f. = 1, $P = 0.84$; *LdNPV*: $G^2 = 0.46$, d.f. = 1, $P = 0.50$), suggesting that these biological control releases did not account for the observed patterns of pathogen distribution (Fig. 2). Although some of our sites were within 0.5 and 6.1 km of prior releases of *E. maimaiga* and *LdNPV*, respectively, the median distances from sites where pathogens were detected to release sites were much greater (65.2 and 25.6 km, respectively).

The background male moth abundance (a proxy for larval density) in the prior year ($G^2 = 7.9$, d.f. = 1, $P < 0.01$) and in the year of study ($G^2 = 8.5$, d.f. = 1, $P < 0.01$) was a significant predictor of infection by *E. maimaiga*. However, only the background male moth abundance in the year of study was a significant predictor of infection by *LdNPV* ($G^2 = 7.7$, d.f. = 1, $P < 0.01$). Infection by *E. maimaiga*

was more likely than not to occur at male moth abundances of >81 and >143 from the prior and current year, respectively, while infection by *LdNPV* was more likely than not to occur at male moth abundances of >164 from the year of study. Thus, only for *E. maimaiga* did the predicted probability of infection at a site increase with increasing male moth abundance from the prior year. There was no association between infection by *LdNPV* and the subsequent change in male moth abundance from the prior year to the survivors from the year of study ($G^2 = 0.9$, d.f. = 1, $P = 0.33$). However, there was a significant association between infection by *E. maimaiga* and subsequent decrease in male moth abundance ($G^2 = 68.9$, d.f. = 1, $P < 0.01$). The odds ratio indicated that at sites where *E. maimaiga* infection was present, the adult male population was 2.1 (95% CI = 1.6–2.7) times more likely to decrease from the prior year (i.e. male parent population of the larvae we sampled) to the year of study (i.e. the adult male populations that subsequently survived from the larval populations that we sampled).

When considering the cumulative prior history of male *L. dispar* abundance at each site, there was a significant relationship between the number of years in which male moths exceeded 100 per trap and infection by both *E. maimaiga* ($G^2 = 11.9$, d.f. = 1, $P < 0.01$) and *LdNPV* ($G^2 = 4.0$, d.f. = 1, $P = 0.04$), while the number of years for which male moths exceeded 10 per trap was a significant predictor for only *E. maimaiga* ($G^2 = 5.7$, d.f. = 1, $P = 0.02$) (Fig. 3). The respective logistic regression models for *E. maimaiga* and *LdNPV* differed. The predicted probability of *E. maimaiga* infection is *c.* 0.8 when the site has exceeded the 100-moth threshold for only one prior year, whereas for *LdNPV*, the same probability is predicted to occur when the site has exceeded the 100-moth threshold for four prior years. A similar probability (0.8) of *E. maimaiga* infection was also predicted when a site has exceeded the 10-moth threshold for *c.* 5 years (Fig. 3). For both pathogens, exceeding the 1-moth threshold was a non-significant predictor of infection ($P > 0.4$ for both).

Quantifying the rate of spread in an invading species can be challenging because of the difficulty in obtaining the spatial and temporal data that are required to estimate invasion speed. In the case of *E. maimaiga*, which was shown to be significantly related to the cumulative prior history of *L. dispar* at two population thresholds (the 10- and 100-moths-per-trap thresholds; Fig. 3), it is possible to relate the change in infection levels based on the prior host history using *L. dispar* spread rates and rate of population transition time at the time of our study, which are feasible to estimate (Tobin & Whitmire 2005; Tobin, Liebhold & Roberts 2007a). For example, *c.* 50% of larvae from sites were infected with *E. maimaiga* when the prior *L. dispar* population exceeded 100 moths per trap for < 1 year, and 50% of larvae from sites were infected with *E. maimaiga* when the prior *L. dispar* population exceeded 10 moths per trap for *c.* 3 years (Fig. 3). At the time and place of our study, *L. dispar* was spreading at a rate of *c.* 12.2 km year⁻¹, while the average distance between the 10- and 100-moth population thresholds was *c.* 37.1 km

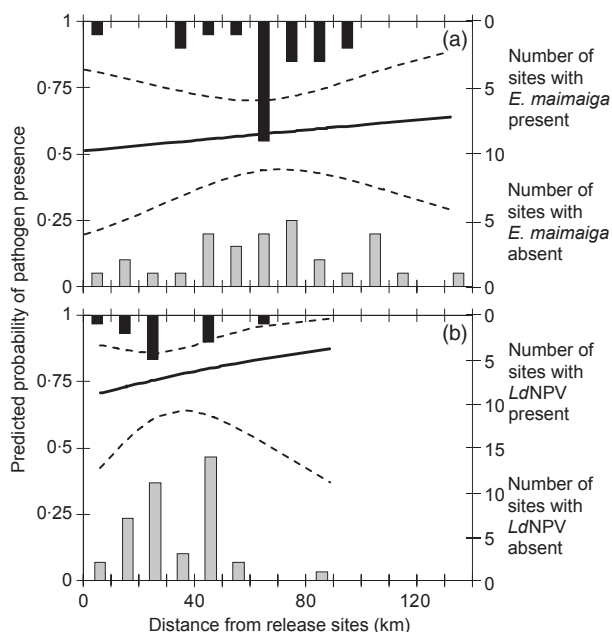


Fig. 2. Predicted model probabilities (solid black line, with 95% confidence intervals as dashed black lines) of the presence of *Entomophaga maimaiga* (a) and *Lymantria dispar* nucleopolyhedrovirus (*LdNPV*) (b) relative to the distance from known release or recovery sites (see Fig. S2). The histograms represent the number of sites with *E. maimaiga* (a) or *LdNPV* (b) present (black bars) or absent (gray bars).

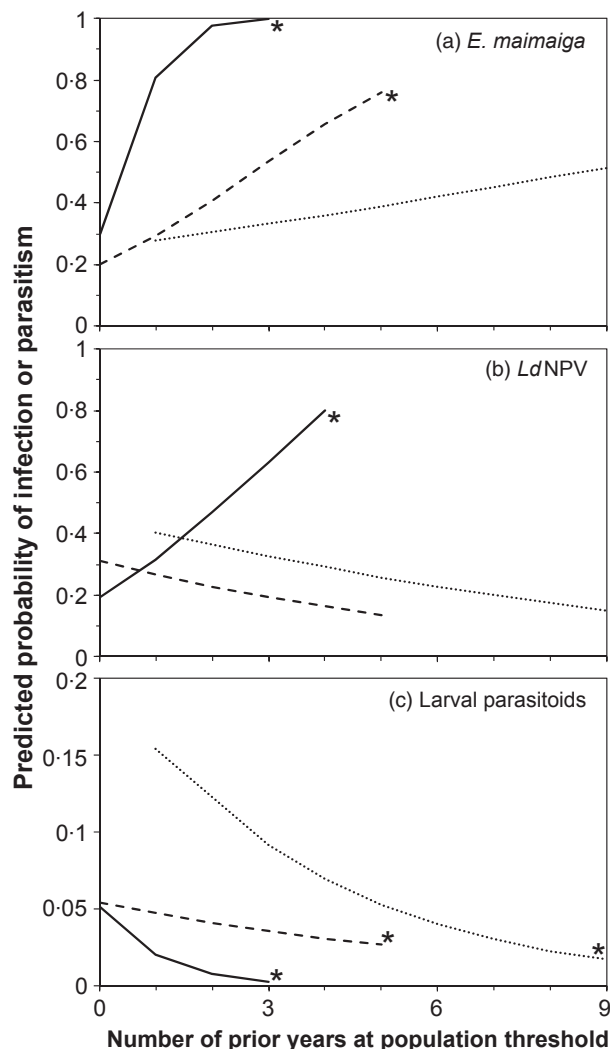


Fig. 3. Predicted probability of infection or parasitism based on prior *Lymantria dispar* population density history for *Entomophaga maimaiga* (a), *L. dispar* nucleopolyhedrovirus (*LdNPV*) (b) and larval parasitoids (c). The dotted, dashed and solid black lines represent the 1-, 10- and 100-moth population thresholds, respectively. Lines with an asterisk denote significant relationships.

(Fig. S3); thus, it took *c.* 3 years ($37.1 \text{ km}/12.2 \text{ km year}^{-1}$) for *L. dispar* populations to transition from a 10-moth to a 100-moth population threshold at our field sites. Because there was a similar 3-year lag in *E. maimaiga* infection (Fig. 3) when considering these two *L. dispar* thresholds, it is possible that *E. maimaiga* moved at the same speed as *L. dispar* at our study sites but was lagged in space.

For the 12 sites that were sampled in successive years, owing to the variability in the densities of host colonization when sampling and the overall low densities, no general trends were evident. However, in a few instances, we caught populations as infection prevalence increased, e.g. in 2006 at Rocky Arbor, we found only two larvae infected with *E. maimaiga* on the last of eight sample dates. (A total of 265 larvae were collected in 2006.) The next year, *E. maimaiga* infections began at 9.6% on 3 June and ranged from 33.7% to 66.7% on the three successive sampling dates.

Using stepwise logistic regression, three of nine climate variables were significantly associated with infection by *E. maimaiga* (Fig. 4): total April rainfall (positively associated; $G^2 = 23.9$, d.f. = 1, $P < 0.01$), May temperature (negatively associated; $G^2 = 288.2$, d.f. = 1, $P < 0.01$) and June temperature (positively associated; $G^2 = 171.6$, d.f. = 1, $P < 0.01$). For infection by *LdNPV*, only April temperatures ($G^2 = 18.4$, d.f. = 1, $P < 0.01$) and June temperatures ($G^2 = 7.3$, d.f. = 1, $P < 0.01$) were significantly positively associated with rates of infection (Fig. 4).

Rates of larval parasitism by all tachinids were not associated with male moth abundance from the prior year ($G^2 = 0.1$, d.f. = 1, $P = 0.77$), nor was there a significant association between parasitism rate and the change in male moth abundance from the prior year to the year of the study ($G^2 = 0.6$, d.f. = 1, $P = 0.42$). When considering the cumulative prior history of male *L. dispar* abundance at each site (Fig. 3), there was a significant negative relationship between the number of years for which male moths exceeded 1 ($G^2 = 18.4$, d.f. = 1, $P < 0.01$), 10 ($G^2 = 5.4$, d.f. = 1, $P = 0.02$) and 100 ($G^2 = 51.7$, d.f. = 1, $P < 0.01$) moths per trap and parasitism. Therefore, rates of larval parasitism were highest when *L. dispar* history was the shortest but declined rapidly with longer histories of *L. dispar* presence.

INTERACTIONS AMONG PATHOGENS AND PARASITIDS

Parasitism and infection proportions were frequently relatively low, as would be consistent with both recently invading hosts and natural enemies (Table 1). In our attempts to investigate the extent that different natural enemy species could successfully co-attack the same larval hosts, we did not observe any instances where both fungal and viral pathogens reproduced within the same host. We collected five *L. dispar* among the 762 infected by *E. maimaiga*, from which both fungal conidia were produced and *C. concinnata* successfully developed, and one larva, of the 26 infected by *LdNPV* in which *C. concinnata* also successfully developed.

When investigating the relationship between pathogen infection and parasitism, we observed a natural break in the data when considering sites with $< 30\%$ of pathogen infection (1369 larvae) and $\geq 30\%$ (1088 larvae) and thus considered these two groups separately in our analysis. In both groups, there was a significant difference between proportion of infection and parasitism ($G^2 = 36.7$, d.f. = 1, $P < 0.01$ in the $> 30\%$ group; $G^2 = 318.3$, d.f. = 1, $P < 0.01$ in the $\geq 30\%$ group). However, the relative differences for these two groups varied; at sites with $< 30\%$ infection, larvae were only 2.0 (95% CI = 1.6–2.5) times more likely to be infected relative to being parasitized, while at sites with $\geq 30\%$ infection, larvae were 34.1 (95% CI = 23.1–50.2) times more likely to be infected relative to being parasitized (Fig. 5).

Discussion

Many factors affect the spread of an introduced species, including resource availability, spatial heterogeneity, preda-

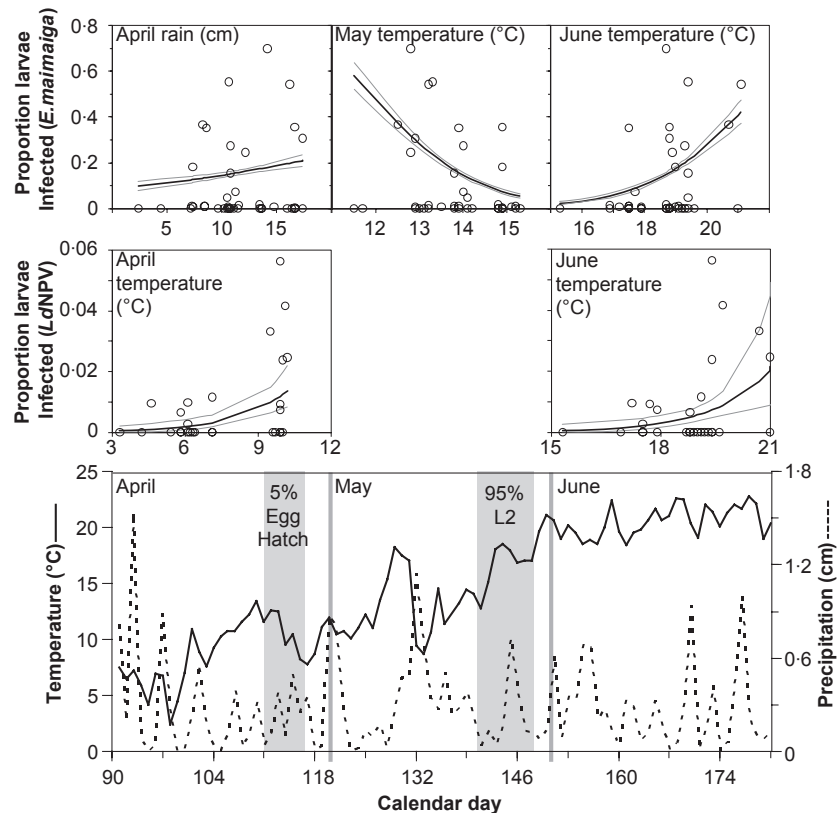


Fig. 4. The association between April rainfall, May temperature and June temperature and the proportion of *Entomophaga maimaiga*- or *Lymantria dispar* nucleopolyhedrovirus (*LdNPV*)-infected larvae per site (top two panels). Lines represent fitted logistic regression curves and 95% CI. Only significant associations are shown. The daily mean temperature and precipitation across all sites and years, April to June, are shown in the bottom panel with the predicted periods of 5% egg hatch and 95% completion to second instar (Régnière & Sharov 1998).

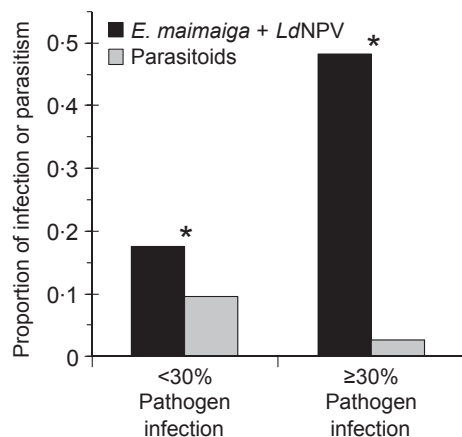


Fig. 5. Relationship between pathogen (*Entomophaga maimaiga* plus *LdNPV*) and parasitoid levels at sites with combined pathogen infection rates of <30% or ≥30%. Although there were significant differences between pathogen infection and parasitism (as denoted by asterisks) in both infection level groups, the difference was greatest when the rate of infection was ≥30%.

tion and parasitism, competition, evolutionary changes, weather conditions, long-range dispersal or transport and stochasticity (Hilker *et al.* 2005; Lockwood, Hoopes & Marchetti 2007). During this study, we principally investigated the influence of host (=resource) availability and weather on the dynamics of two introduced entomopathogens as they followed the invasion front of their host. The strongest association between both the fungal and viral pathogens occurred with host density; both pathogens were not detected in most of the very lowest-density *L. dispar* populations but

were present and increased in prevalence as *L. dispar* populations increased. In established *L. dispar* populations, *LdNPV* prevalence is dependent on host density (Woods *et al.* 1991) although contrary to our findings, and the activity of *E. maimaiga* has previously been shown to be independent of host density in at least some newly established *L. dispar* populations (Hajek *et al.* 1990; Webb *et al.* 1999). Activity of these pathogens was also related to weather conditions. Increases in rain around the time of egg hatch and cooler temperatures during early instar development were conducive to *E. maimaiga* infections, which would have been initiated predominantly by germinated resting spores. In June, when *E. maimaiga* infection would have been mainly attributable to conidia actively ejected from cadavers, prevalence was positively associated with warmer temperatures (up to the average daily maximum of 22.7 °C) (Fig. 4). Higher temperatures during egg hatch and late instar development were positively associated with *LdNPV* prevalence. Activity of both pathogens was not associated with distance from biological control releases or recoveries after releases. Perhaps this is not unexpected because for both the fungus and virus, release sites were localized, releases had occurred far from our study sites and many releases, at least for the fungus, had occurred many years before this study was conducted (Fig. S2).

Seven species of parasitoids have been introduced to North America for *L. dispar* control and have become established (Hajek 2007). The most common parasitoid found during our study of low-density *L. dispar* populations was *C. concinnata*. This generalist tachinid was probably already present in the study sites before *L. dispar* populations became

established because it was released in Wisconsin beginning in 1974 in anticipation of the presence of *L. dispar* populations (Krauth *et al.* 1977). In studies conducted in Massachusetts, when large numbers of *L. dispar* eggs were introduced to sites hosting low-density *L. dispar* populations, abundant parasitism by *C. concinnata* occurred (Liebhold & Elkinton 1989), demonstrating an impressive ability of this fly to rapidly elevated high densities of *L. dispar* hosts.

We investigated to what extent competition among natural enemies could be occurring in these newly colonizing populations where densities were often low. Although reproduction by both pathogens in coinfecting *L. dispar* larvae has been reported previously, this occurrence requires initial infection by *LdNPV* with a lag period before *E. maimaiga* infects because *E. maimaiga* kills larvae much more quickly than *LdNPV* at typical spring temperatures (Malakar *et al.* 1999). While we did not find reproduction by both pathogens in any individual host larvae, it is possible that because *E. maimaiga* infections were more prevalent than *LdNPV*, coinfections could have occurred but the fungus killed the coinfecting larvae before *LdNPV* reproduction would be detected by microscopy.

In contrast to the prevalence of fungal and viral infection, levels of parasitism were greatest at sites with shorter histories of *L. dispar* and least at sites with longer histories of *L. dispar*. This relationship between the host-specific invading pathogens and already-established generalist parasitoids could suggest that competition is occurring. Unfortunately, competition between these pathogens and *C. concinnata* has not been investigated in the laboratory, although field studies of virus/*C. concinnata* interactions have documented successful viral reproduction in large percentages of parasitized larvae (White & Webb 1994). In established *L. dispar* populations, *C. concinnata* did not demonstrate a between-generation numerical response to *L. dispar* populations (Ferguson *et al.* 1994); thus, populations of this generalist parasitoid could be limited not by competition but by a lack of response to host populations increasing from year to year, over which time pathogens would increase.

It was previously unknown how long it would take these pathogens to arrive at host populations that were invading new sites. We found that along the expanding *L. dispar* population front, the fungal pathogen *E. maimaiga* was more likely to be present in lower-density host populations and when prior *L. dispar* history was shorter compared with *LdNPV* (Fig. 3), suggesting that the fungal pathogen arrives in these newly established *L. dispar* populations faster than *LdNPV*. *Entomophaga maimaiga* primarily disperses by ejecting asexual spores from cadavers, and the spores become airborne (Hajek, Olsen & Elkinton 1999). Models of *E. maimaiga* dispersal have suggested different mechanisms for short- vs. long-range dispersal (Dwyer, Elkinton & Hajek 1998); short-range dispersal occurs within forest canopies, but longer-range dispersal is hypothesized to occur when spores escape above the canopy and are blown longer distances while surviving under humid conditions (Weseloh 2003). Ballooning by infected first instars can explain *LdNPV*

dispersal up to 120 m (Dwyer & Elkinton 1995). Longer-distance dispersal of *LdNPV* could occur when it is vectored by birds and mammals eating infected larvae or by parasitoids during oviposition (Reardon & Podgwaite 1976; Lautenschlager & Podgwaite 1979; Dwyer, Elkinton & Hajek 1998; Reilly 2009). In particular, the parasitoid *C. compsilura* can disperse longer distances fairly rapidly and has thus been hypothesized as providing an important mechanism for longer-distance *LdNPV* dispersal (Dwyer & Elkinton 1995). One laboratory study of the potential for vertical transmission of *LdNPV* suggested that it was possible that transmission of virus from female to eggs could occur at low levels (Myers, Malakar & Cory 2000; but see Kukan 1999) although the principal mode of transmission assumed for *LdNPV* is horizontal owing to environmental contamination with environmentally persistent viral occlusion bodies (Cory & Myers 2003). Adult *L. dispar* females are flightless, and flying males have never been shown to transmit the virus to progeny, so it is improbable that adult females would spread *LdNPV* on their own. However, the inadvertent movement of *L. dispar* egg masses by humans is known to be an important means of long-range dispersal (Liebhold & Tobin 2006; Hajek & Tobin 2009), so it is possible that *LdNPV* could be moved by this means. Our results demonstrate the need for further studies in order to better understand transmission and spread dynamics of these pathogens.

Newly established low-density *L. dispar* populations that form along the expanding population front could be subject to an Allee effect owing to mate-finding failures in sparse populations (Tobin *et al.* 2009), and Allee effects can affect the rate of spread in invading *L. dispar* (Johnson *et al.* 2006; Tobin *et al.* 2007b). Predation in low-density *L. dispar* populations may also be a cause of an Allee effect, especially in the presence of a pathogen (Bjørnstad, Robinet & Liebhold 2010). Thus, the absence of natural enemies (e.g. the enemy release hypothesis) along a leading edge could be a benefit for invasive species that are expanding their range (Keane & Crawley 2002; but see Colautti *et al.* 2004). The lack of natural enemies is also considered to contribute to the development of damaging insect outbreaks (Torchin *et al.* 2003). However, we have shown that in the case of spreading *L. dispar*, populations do not escape from host-specific pathogens for many years before pathogens catch up with hosts. Evidence of the ability of natural enemies to overtake and potentially decimate established host populations is very limited. Hilker *et al.* (2005) demonstrated in theoretical models that virulent pathogens introduced to spreading host populations could slow down or reverse invasion fronts. In a natural setting, Elkinton, Parry & Boettner (2006) provided strong evidence that the parasitoid *C. concinnata*, over many years, was a primary cause of the collapse and range retraction of the invasive brown-tail moth, *Euproctis chrysorrhoea*. Over the 3 years of this study, *L. dispar* populations were more likely to decrease from the prior year in association with *E. maimaiga* infection; however, the same association was not observed for *LdNPV*, which occurred at much lower prevalence than *E. maimaiga* throughout our study.

(Table 1). Future work that addresses changes in the *L. dispar* invasion front over more robust spatial and temporal scales could reveal that pathogens tracking host populations slow down the speed of *L. dispar* invasion.

The accidental transport of species continues to increase in frequency, whether inter- or intracontinental (Work *et al.* 2005; McCullough *et al.* 2006). Although not all non-native species that arrive in new habitats successfully establish (Simberloff & Gibbons 2004) or are considered pests (Mack *et al.* 2000), some that become established pests cause considerable environmental and economic harm (Pimentel, Zuniga & Morrison 2005). Among non-native invasive insect species, biological control is still considered as a management option, despite historical blunders (Strong & Pemberton 2000), because appropriate steps are now undertaken to ensure that non-target risks are minimal. However, empirical observations on the spread dynamics of introduced natural enemies as they in turn track the spread of their hosts are rare (Fagan *et al.* 2002). Indeed, information on the spread dynamics of individual non-native species across a landscape is limited, often because we lack the tools to monitor newly arriving, low-density populations that can be critical in driving rates of spread (Liebhold & Tobin 2008). Our field study is thus unique in that we jointly addressed the changes in abundance of interacting hosts, specific pathogens and a generalist parasitoid along the leading edge of an invasion.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Spatial autocorrelation in male *Lymantria dispar* trap catch data from 2005, 2006, and 2007, using data from the area in Wisconsin where gypsy moth was considered to be established and along the leading edge of gypsy moth spread where the majority of our sites were located.

Fig. S2. Historical records of *Entomophaga maimaiga* releases and known recoveries in Wisconsin, locations of Gypchek® (*Lymantria dispar* nucleopolyhedrovirus) treatment blocks deployed under the Gypsy Moth Slow-the-Spread program, and 2005–2007 study sites.

Fig. S3. Population thresholds used to estimate *Lymantria dispar* spread rate and the rate at which populations transition from a 10- to a 100-moth threshold.

Table S1. Study sites sampled in southern and central Wisconsin, 2005–2007.

Table S2. Tachinid parasitoid species richness and frequency of occurrence in 182 parasitized *Lymantria dispar* larvae collected from the leading edge of *L. dispar* spread.

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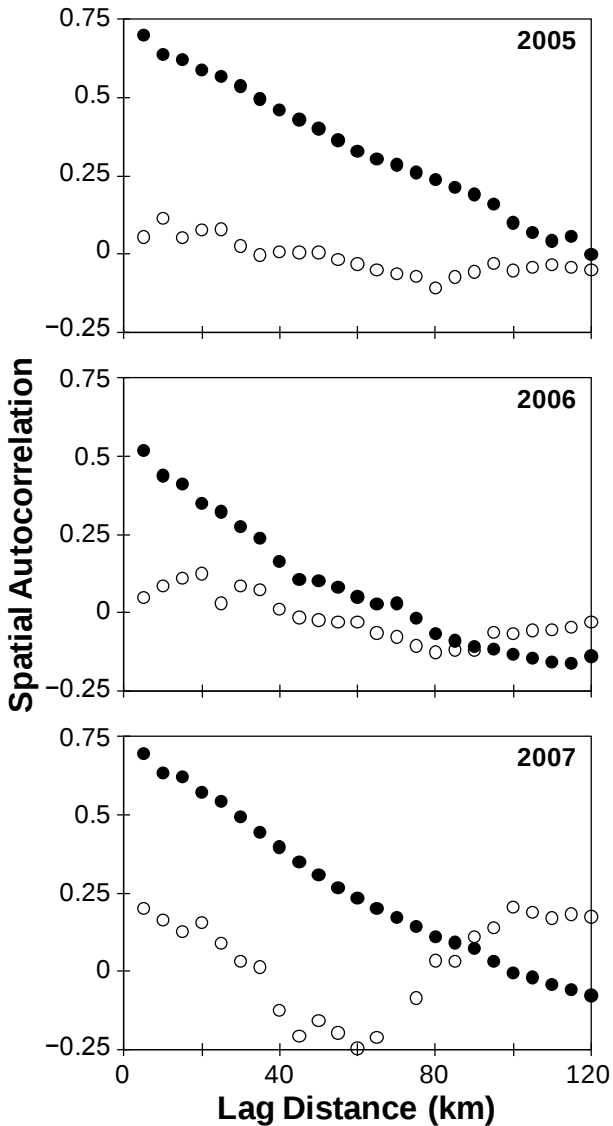


Fig. S1. Spatial autocorrelation in gypsy moth trap catch data from 2006, 2006, and 2007, using data from the entire trapping area that includes the gypsy moth established area in Wisconsin (closed circles), and when using a subset of the trapping area that reflects the locations of our sites (open circles), which were generally ahead of the established area. Note the strong linear spatial trend when including the established area, which was previously described by Sharov *et al.* (1996), but the lack of spatial autocorrelation in the area from where we sampled. The weak spatial trend in 2007 at our sites reflects the inclusion of a number of sites closer to the established area in an attempt to maximize our collection efforts. Traps that comprise this dataset

are set 500 m to 2 km apart in areas ahead of the established area, and 3 to 8 km apart within the established area (Tobin & Blackburn 2007). Trap data locations were georeferenced, and the number of moths trapped was transformed using $\log_{10}(z + 1)$ prior to analysis. We used 1 – correlogram values in GSLIB (Deutsch & Journel 1998) to estimate spatial autocorrelation.

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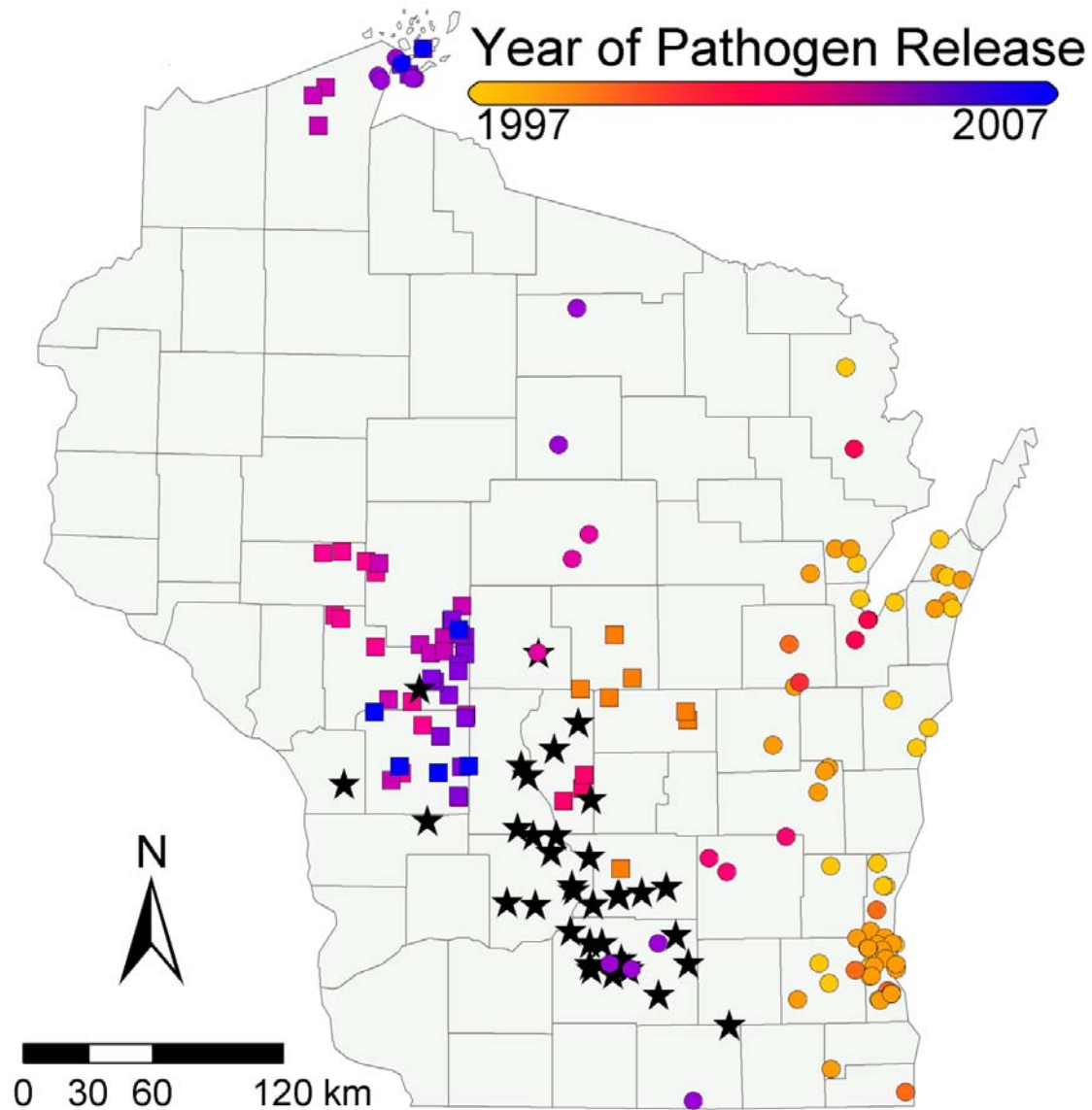


Fig. S2. Historical records of *E. maimaiga* releases and known recoveries in Wisconsin (circles), locations of Gypchek® (*LdNPV*) treatment blocks deployed under the Gypsy Moth Slow-the-Spread program (squares) and 2005-2007 study sites (stars).

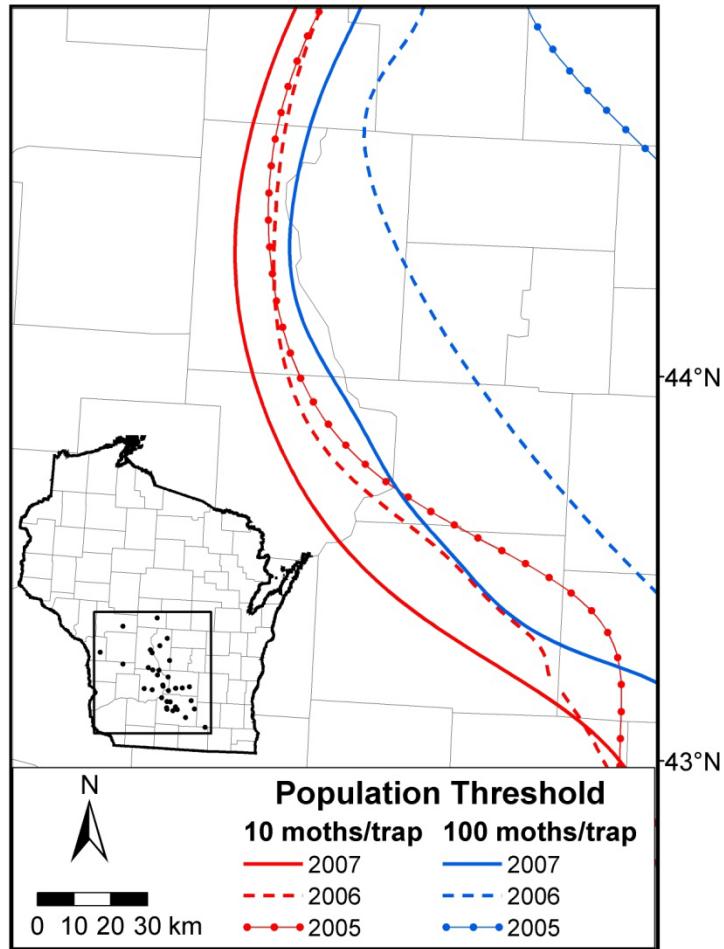


Fig. S3. Methods to estimate the rate of spread, and the rate of population transitioning, in *L. dispar* have been previously described (Sharov *et al.* 1995; Sharov, Liebhold, & Roberts 1996; Sharov, Liebhold, & Roberts 1997; Tobin & Whitmire 2005; Tobin & Blackburn 2007; Tobin, Liebhold, & Roberts 2007). Male moth trap catch data from a grid of pheromone-baited traps, placed from 0.5 to 8 km apart, are used to spatially interpolate a smooth surface using median indicator kriging (Isaaks and Srivastava 1989). Various male moth population boundaries (e.g., the 10-moth threshold and the 100-moth threshold) are estimated from the interpolated moth abundance surface using an optimization technique (Sharov *et al.* 1995). The displacement between like boundaries in consecutive years (i.e., the 10-moth threshold in year $t-1$ and year t , the 100-moth threshold in year $t-1$ and year t) can be used to estimate the rate of *L. dispar* spread

(cf. Tobin, Liebhold, & Roberts 2007), while the distance between boundaries in the same year (i.e., the distance between the 10-moth threshold and the 100-moth threshold in year t) can be used to estimate the interboundary distance (cf. Tobin & Blackburn 2007).

In the area around our field sites, as noted by the dots in the insert map, the mean rate of *L. dispar* spread was 12.2 km yr^{-1} from 2005-2007, while the mean interboundary distance between the 10-moth and 100-moth threshold was 37.1 km. Thus, when using a spread rate of 12.2 km yr^{-1} and an interboundary distance of 37.1 km, during this time, *L. dispar* populations transitioned from the 10-moth threshold to a 100-moth threshold in approximately 3 years ($37.1 \text{ km} \div 12.2 \text{ km yr}^{-1}$).

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Table S1. Study sites in southern and central Wisconsin, 2005-2007

Site	County	Year	Lat	Long
Bass Hollow County Recreation Area*	Juneau	2006-07	43.67	-90.03
Black Earth Creek State Fishery Area	Dane	2007	43.08	-89.61
Black River State Forest*	Jackson	2005	44.25	-90.6
Buckhorn State Park	Juneau	2007	43.93	-90.01
Castle Rock County Park	Juneau	2007	43.89	-89.97
Colburn Wildlife Area	Adams	2007	44.11	-89.68
Coulee Experimental State Forest*	La Crosse	2005	43.85	-91.03
Cross Plains (Route 14, nr Stagecoach Rd.)	Dane	2005-07	43.1	-89.62
Deansville State Wildlife Area	Dane	2007	43.22	-89.13
Dell Creek State Wildlife Area	Juneau/Sauk	2007	43.64	-89.94
Devil's Lake State Park, Northern site*	Sauk	2006-07	43.43	-89.72
Devil's Lake State Park, Southern site*	Sauk	2005-06	43.41	-89.71
Frautschi Point (UW Natural Area, Madison)	Dane	2005-07	43.09	-89.43
Gibraltar Rock*	Columbia	2006-07	43.35	-89.6
Goose Lake State Wildlife Area	Dane	2007	43.1	-89.06
Governor Nelson State Park	Dane	2007	43.12	-89.44
Grassy Lake State Wildlife Area (Doyleston)	Columbia	2007	43.42	-89.18
Indian Lake County Park	Dane	2007	43.19	-89.62
Lake Kegonsa State Park	Dane	2007	42.97	-89.23
Lima Marsh State Wildlife Area	Rock	2007	42.84	-88.83
Lower Wisconsin State Riverway	Dane	2007	43.24	-89.73
Mirror Lake State Park	Sauk	2007	43.57	-89.84
Mud Lake State Wildlife Area (Poynette)	Columbia	2007	43.4	-89.32
Muir Woods (UW Natural Area, Madison)*	Dane	2005-07	43.08	-89.4

Natural Bridge State Park*	Sauk	2006-07	43.35	-89.93
Picnic Point Peninsula (UW Natural Area, Madison)	Dane	2005-2007	43.09	-89.42
Pine Island State Wildlife Area	Sauk	2007	43.55	-89.62
Roche-A-Cri State Park	Adams	2007	44	-89.82
Rocky Arbor State Park*	Juneau/Sauk	2006-07	43.64	-89.81
Rowan Creek State Fishery Area	Columbia	2006-07	43.39	-89.45
Upper Neenah Creek State Fishery Area	Adams	2006-07	43.79	-89.61
Waunakee State Wildlife Area	Dane	2007	43.19	-89.55
White Mound County Park*	Sauk	2005	43.36	-90.09
Wildcat Mountain State Park*	Vernon	2005	43.7	-90.55
Woodridge Trace	Wood	2005	44.4	-89.91
6122 Mineral Point Rd., Madison	Dane	2007	43.06	-89.49
Interstate 90/94	Dane	2007	43.39	-89.46

* Intensive sites where soil exposures were conducted in 2005-2006.

Table S2. Tachinid parasitoid species richness and frequency of occurrence in 182 parasitized *Lymantria dispar* larvae collected from the leading edge of *L. dispar* spread.

Tachinid species	Occurrence among infected larvae (%)
<i>Compsilura concinnata</i> (Meigen)	90.7
Tribe Exoristini (possibly <i>Parasetigena silvestris</i> (Robineau-Desvoidy)) [*]	4.4
Unidentifiable	4.9

^{*} Tachinid larvae emerged from gypsy moth larvae and created puparia but did not eclose to adults so definite identification to species is not possible.