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Research Article

Rising temperatures may increase fungal epizootics in northern populations of the invasive spongy moth in North America

Clare A. Rodenberg[1], Ann E. Hajek[2], Hannah Nadel[3], Artur Stefanski[4,5], Peter B. Reich[4,6,7], Kyle J. Haynes[1,8]

1 Department of Environmental Sciences, University of Virginia, Charlottesville, VA, USA
2 Department of Entomology, Cornell University, Ithaca, NY, USA
3 Forest Pest Methods Laboratory, USDA APHIS PPQ S&T, Buzzards Bay, MA, USA
4 Department of Forest Resources, University of Minnesota, St. Paul, MN, USA
5 College of Natural Resources, University of Wisconsin Stevens Point, Stevens Point, WI, USA
6 Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia
7 Institute for Global Change Biology and School for Environment and Sustainability, University of Michigan, Ann Arbor, MI, USA
8 Blandy Experimental Farm, University of Virginia, Boyce, VA, USA

Corresponding author: Clare A. Rodenberg (cr4rd@virginia.edu)

Abstract

Insect pest species are generally expected to become more destructive with climate change because of factors such as weakened host tree defences during droughts and increased voltinism under rising temperatures; however, responses will vary by species due to a variety of factors, including altered interactions with their natural enemies. Entomopathogens are a substantial source of mortality in insects, but the likelihood of epizootics can depend strongly on climatic conditions. Previous research indicates that rates of infection of the spongy moth (*Lymantria dispar*) by its host-specific fungal pathogen, *Entomophaga maimaiga*, increase with environmental moisture and decrease as temperatures rise. High temperatures may have direct and indirect (due to the associated drying) effects on the fungus, but the interactive effects between temperature and moisture level on larval infection are unclear. Here, we test the hypothesis that warmer, drier conditions will decrease rates of infection of spongy moth larvae by *E. maimaiga*. We evaluated the effects of precipitation and temperature on larval mortality caused by *E. maimaiga* with a manipulative field experiment, conducted in one of the northernmost and coldest parts of the spongy moth's non-native range in North America. We caged laboratory-reared spongy moth larvae in experimentally warmed open-air forest plots, exposing the larvae to soil inoculated with *E. maimaiga* resting spores during two consecutive trials. Caged larvae were exposed to three temperature treatments — ambient, 1.7 °C above ambient and 3.4 °C above ambient — and either supplemental precipitation (+173 mm per trial) or ambient precipitation. Opposite to our hypothesis, there was no significant effect of supplemental precipitation, nor an interaction between precipitation and temperature. There was, however, a significant positive effect of increasing temperature on the number of larvae infected. On average, in each respective trial, larval infection increased by 44% and 50% under the elevated temperature treatments compared to ambient temperature. Experimental warming may have increased infections because ambient temperatures at the field site were suboptimal for fungal germination. The results from this experiment suggest that, in colder portions of the spongy moth's invasive range, increasing temperatures due to climate change may enhance the ability of *E. maimaiga* to help control populations of the spongy moth.

Key words: Biological control, climate change, epizootiology, invasive species, spongy moth

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Introduction

Climate change is generally expected to amplify the overall impacts of forest insect pest species worldwide due to factors including increased voltinism and survival under higher temperatures, drought-induced weakening of tree defences and changes in foliage quality for defoliators (Jactel et al. 2019), although responses will vary amongst species (Pureswaran et al. 2018; Lehmann et al. 2020; Halsch et al. 2021) in part due to interactions with natural enemies (Frank 2021). Entomopathogens (i.e. bacteria-, fungi-, viruses- and nematodes-attacking insects) are extremely susceptible to changes in the environment, particularly temperature and moisture (e.g. precipitation, soil moisture, relative humidity) (Dara et al. 2019). These pathogens are major mortality agents of insects (Roy et al. 2009) and climate conditions that inhibit the transmission of pathogens may increase the probability that insect pest populations grow to outbreak levels (Skendžić et al. 2021). Most studies on the potential effects of climate change on host-pathogen systems have focused solely on the impacts of rising temperatures (Altizer et al. 2013; MacDonald et al. 2023). There are few manipulative field studies that test the independent and interactive effects of multiple abiotic variables on host-pathogen relationships. An exception is a manipulative microcosm study by van Doan et al. (2021) who found that elevated CO_2, increased temperature and decreased precipitation predicted by the extreme climate change scenario Representative Concentration Pathway 8.5 (RCP 8.5) did not directly compromise different natural enemies of herbivores. Their results suggest the potential for short term adaptations of natural enemies of herbivores to climatic change conditions (van Doan et al. 2021). As the effects of climate change are not limited to increasing air temperatures, we need to understand effects of other aspects of climate change, such as predicted increases in the frequency and severity of drought events (IPCC 2022), on insect pests and their pathogens (St. Leger 2021).

Increasing drought could have important implications for a host-pathogen interaction involving one of the most damaging forest pests in North America, the spongy moth (*Lymantria dispar*). Springtime drought may create favourable conditions for outbreaks of the spongy moth by inhibiting infections by the host-specific fungal pathogen *Entomophaga maimaiga* (Pasquarella et al. 2018). Results from laboratory and field observational studies have consistently demonstrated a positive effect of environmental moisture on germination of *E. maimaiga* resting spores, sporulation and infection of spongy moth larvae by both spore types—azygospores (resting spores) and conidia (Hajek et al. 1990b; Hajek and Soper 1992; Weseloh and Andreadis 1992b; Hajek and Humber 1997; Reilly et al. 2014). In addition, manipulative field experiments have shown that the addition of artificial rain increases rates of infection by both conidia and resting spores (Hajek and Roberts 1991; Weseloh and Andreadis 1992a; Smitley et al. 1995; Hajek et al. 1996), the latter needing water to germinate (Hajek and Humber 1997). High temperatures, especially in lab studies with constant exposure to ≥ 30 °C, generally have a negative effect on *E. maimaiga* germination, sporulation and infection (Hajek 1999). However, results from field studies with exposure to variable temperatures are not consistent. For example, results from two-year observational field studies showed that increasing soil temperature can have opposite effects on *E. maimaiga* infection levels (Hajek and Humber 1997) or may have a positive effect in the first year and no effect in the following year (Siegert et al. 2008). In another observational field study, Reilly et al. (2014) found that mortality of field-collected larvae due to *E. maimaiga* infections decreased with increasing temperature, but no clear relationship for laboratory-reared larvae exposed to field conditions. These past studies highlight the important role of weather for this host-pathogen relationship, but the effects of climate change remain unclear as no studies have investigated the independent and interactive effects of temperature and precipitation under field conditions. Additionally, most past field studies occurred in regions with more moderate climates compared to the coldest portions of the spongy moth's range (e.g. upper mid-western States, eastern Canada, Maine), making it difficult to extrapolate effects of weather across the *L. dispar* range.

Here, we experimentally tested the effects of temperature and precipitation on the relationship between the spongy moth and *E. maimaiga*. The spongy moth is a non-native defoliator of hardwood forests in north-eastern North America and causes approximately $3.2 billion of damage per year in the United States and Canada (Bradshaw et al. 2016). *Entomophaga maimaiga* is the dominant source of spongy moth larval mortality (Liebhold et al. 2013; Hajek et al. 2015) and can cause high rates of mortality in both high and low-density spongy moth populations (Hajek et al. 1990a; Elkinton et al. 1991). As epizootics can occur in low-density host populations, it is possible that the fungus can slow or stop the invasive spread of spongy moth in North America by causing mortality along the insect pest's expanding range front.

The goals of this study were to understand whether and how predicted climate changes, specifically rising temperatures and increasing summertime drought (IPCC 2022), may affect spongy moth mortality caused by *E. maimaiga* infection. The effects of soil and plant canopy temperature and precipitation on larval infection initiated from resting spores were evaluated in a manipulative field experiment. Warmer and drier conditions are generally associated with lower infection levels because *E. maimaiga* resting spores require high soil moisture to germinate and infect larvae (Hajek 1999). Temperatures of 15–25 °C are optimal for resting spore germination and high temperatures, especially those ≥ 30 °C, can directly reduce infection levels (Hajek and Humber 1997). However, our experiment was conducted in one of the northernmost parts of the spongy moth's range in North America (Fig. 1), where typical springtime and early summer conditions may be too cold to support germination (Siegert et al. 2009). In cold ecoregions of the spongy moth's range (e.g. Mixed Wood Shield (MWS), Fig. 2), above-average temperatures would likely increase germination, whereas above-average temperatures may have the opposite effect in the warmest ecoregions (e.g. Southern United States Plains (SUP), Fig. 2, H_1). Therefore, we predicted that infection levels would increase under experimentally elevated temperatures; in other words, alleviation of low temperature metabolic limitation would overwhelm any effects of warmer temperatures on enhanced soil water deficits, especially under the cold temperature range being tested. To better test the sensitivity of fungal infection to moisture levels, we added supplemental precipitation and predicted that adding supplemental precipitation would increase infection rates (Fig. 2, H_2). Understanding the effects of these abiotic conditions in the north-westernmost portion of its U.S. range is important because spongy moth spread is occurring more rapidly in this region than anywhere else (National Slow the Spread (STS) Program 2022). Furthermore, a climatic suitability study predicted that this region would become more suitable for the spongy moth under a 1.5 °C increase in mean daily temperature compared to historical averages (Gray 2004).

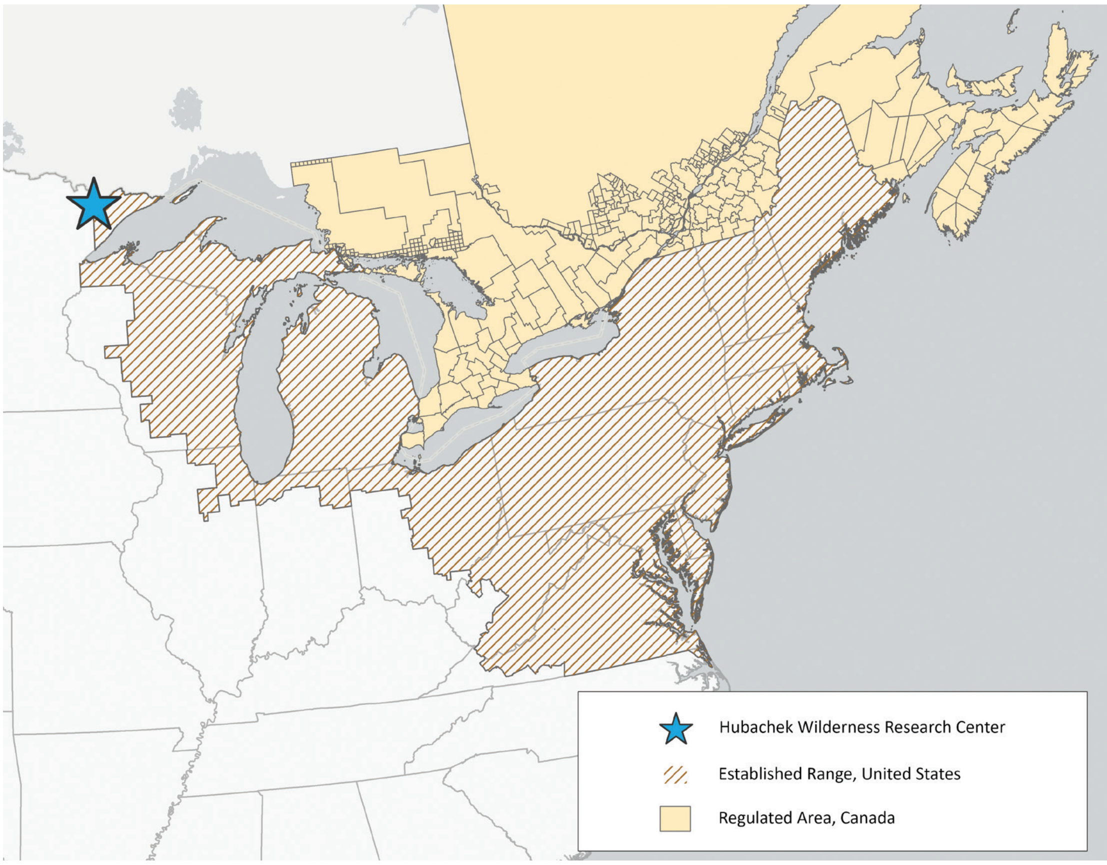

Figure 1. Location of the experimental site. The star represents the Hubachek Wilderness Research Center near Ely, Minnesota. Hatched area represents the non-native, established range of the spongy moth in the United States. Solid beige areas represent the spongy moth regulated areas in provinces and territories in Canada. In Canada, the entire Province of Quebec is regulated for the spongy moth, but the pest is only found in southern Quebec. Data for the spongy moth range in the United States were sourced from the USDA APHIS PPQ spongy moth quarantine records (National Slow the Spread (STS) Program 2022). Data for the regulated areas of Canada were sourced from the Canadian Food Inspection Agency (CFIA 2020).

Materials and methods

Study system

The infection cycle of *E. maimaiga* begins in the spring, when overwintering resting spores in the forest soil begin to germinate approximately 1–2 weeks before spongy moth larvae emerge (Hajek and Humber 1997). Resting spores are always responsible for the primary infection cycle of the season, which occurs when infectious germ conidia are released from germinating resting spores. These germ conidia can become airborne and, when they land on spongy moth larvae, they germinate, infect and then kill a larva in approximately five days (Hajek et al. 1995). Prior to larval death, *E. maimaiga* grows inside of the insect and, after host death, it grows outwards through the cuticle and produces and ejects thousands to millions of new conidia (Shimazu and Soper 1986; Hajek et al. 1993). Infections initiated by germ conidia released from resting spores (e.g. the primary infection cycle) only produce conidia, never resting spores (Hajek 1997). Conidia released from dead spongy moth larvae (cadavers) are responsible for all subsequent infection cycles during that season. When spongy moth larvae reach later instars (5^{th}–6^{th}), infected larvae begin to produce resting spores as opposed to conidia (Hajek and Shimazu 1996). The exact environmental cues

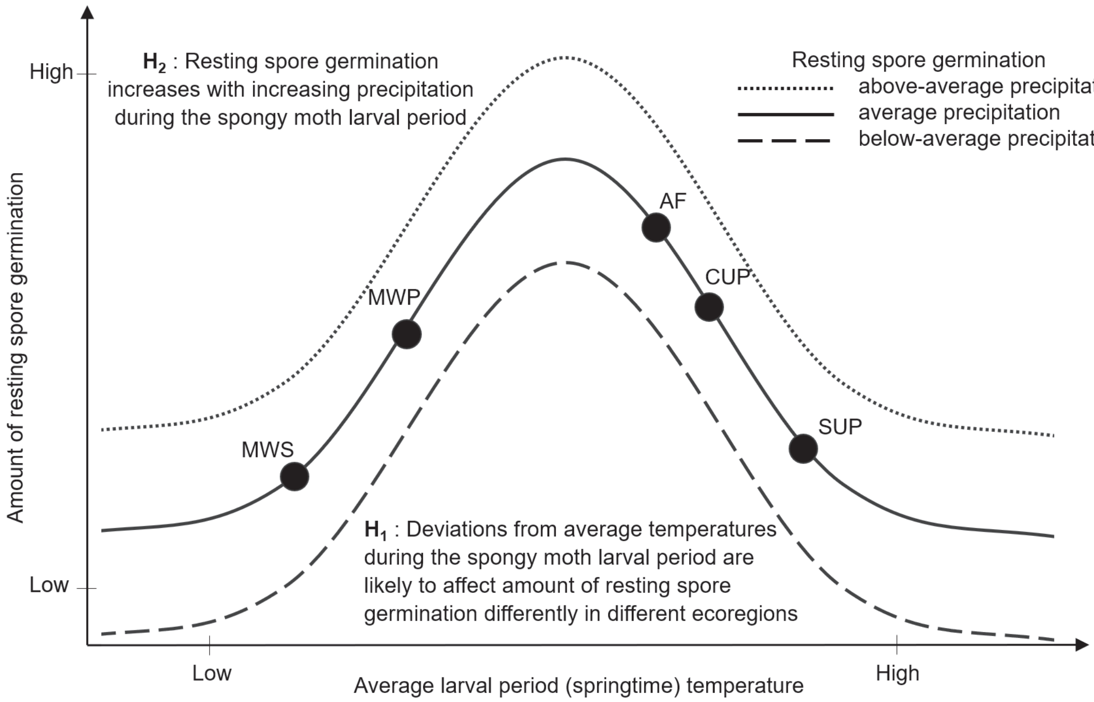

Figure 2. Hypothetical response curves of *Entomophaga maimaiga* resting spore germination. Each curve represents how resting spore germination would likely respond to different temperature and precipitation conditions during the spongy moth larval period (spring-time). The points on the average precipitation curve represent five ecoregions that are within the spongy moth's range in the North America—Mixed Wood Shield (MWS), Mixed Wood Plains (MWP), Central United States Plains (CUP), Appalachian Forest (AF) and Southern United States Plains (SUP) — based on the Environmental Protection Agency's Level II Ecoregions (Omernik 1987; Omernik and Griffith 2014). The Hubachek Wilderness Research Center is located in the MWS ecoregion.

that cause spore production to switch from conidia to resting spores are unknown, but temperature and larval instar are both contributing factors (Hajek and Humber 1997; Hajek 1999). Resting spores produced in later instar spongy moth cadavers go dormant and overwinter, adding to the reservoir of resting spores in the soil. Resting spores are typically dormant for 1–2 years prior to germination and can survive for at least 6 years in the field (Weseloh and Andreadis 1997; Hajek et al. 2004; Hajek et al. 2018).

Manipulative field experiment

The experiment was conducted in 2022 at the University of Minnesota's Hubachek Wilderness Research Center (HWRC; (47.9481, -91.7583) near Ely, Minnesota. The HWRC is in the north-westernmost portion of the spongy moth's expanding range front (Fig. 1), at an elevation of 415 m a.s.l. We used experimentally warmed forest (closed canopy) plots that were part of a long-term climate change experiment, the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) project (Rich et al. 2015). The plots were within 40- to 60-year-old stands of mixed aspen — birch — fir on coarse-textured upland soils. Tree saplings of various species that were planted for the B4WarmED project, natural understorey herbs and shrubs, as well as fallen leaves and moss, were generally found within each plot (Reich et. al 2022). The open-air plots were simultaneously warmed aboveground and belowground via ceramic heating lamps and buried resistance-type heating cables, respectively (Rich et al. 2015).

Larvae for the field experiment were reared from eggs obtained from a disease-free spongy moth colony from the United States Department of Agriculture's Forest Pest Methods Laboratory (Buzzards Bay, MA). Larvae were reared on an artificial diet (Bell et al. 1981) to the 4th instar in an environmental chamber at 25 °C, 70% relative humidity and 15 hours of light/d. We used 4th instar larvae for the experiment in alignment with established procedures (Reilly et al. 2014). To align the timing of the experiment with when wild spongy moths at the field site would be in the 4th instar, we estimated when wild larvae would reach this stage in 2022 using the spongy moth phenology model, which consists of a suite of temperature-dependent sub-models (Régnière and Sharov 1997, 1998, 1999; Gray et al. 2001). We conducted replicate trials when 5%, 50% and 95% of larvae at HWRC were predicted to have reached the 4th instar. Matching the trials with larval phenology also helped ensure the experiment aligned with *E. maimaiga*'s resting-spore germination period, which begins approximately 2 weeks before larval emergence and ends when spongy moth larvae reach later instars (e.g. 5th–6th instars) (Hajek and Humber 1997).

To ensure the presence of *E. maimaiga* in the study plots, we inoculated the plots in the summers of 2019 and 2021 with cadavers of spongy moth larvae that contained resting spores. The 2019 inoculation event was necessary because spongy moth densities were likely very low at the field station, as determined from data from the Slow the Spread (STS) Foundation (National Slow the Spread (STS) Program 2022). Due to the low spongy moth densities at HWRC, it was uncertain whether *E. maimaiga* was already present at HWRC. Originally, the experiment was planned for summer 2020, but the COVID-19 pandemic prevented the experiment from occurring 2020–2021. As some spores released in the 2019 inoculation event likely germinated (without reproducing) in the subsequent seasons, an augmentative 2021 inoculation event was necessary to ensure enough resting spores were present in 2022 when the experiment occurred. Based on our resting spore release estimates of 3.6×10^6 and 4.0×10^6 resting spores per cage site in 2019 and 2021, respectively (Hajek and Humber 1997; see Supplementary file 1: Details on soil inoculation procedure with *Entomophaga maimaiga*), the number of resting spores added to the soil at each cage site by the year 2022 was estimated to be 7.6×10^6. This estimate considers that all of the resting spores from the 2021 inoculation were present and viable in 2022.

For the inoculation events, we obtained resting spore-filled cadavers from Massachusetts and Virginia, U.S. when *E. maimaiga* epizootics were ongoing. The cadavers were found hanging upside down on the trunks of trees with prolegs extended at 90°, a visual cue that demonstrates death from infection by the fungus (Blackburn and Hajek 2018). The inoculations were carried out using procedures previously shown to be effective (A. Diss-Torrance personal correspondence; Hajek et al. 2021). In each inoculation year, first, we ground the field-collected spongy moth cadavers in a food processor and divided the ground material into 36 equal portions (0.54 grams each), one for each experimental plot. We then mixed each portion with 30 g of sterile potting soil. For each inoculation, we spread the mixture on top of the forest soil in the same two randomly selected locations in each plot. Each inoculation site was roughly 31×23 cm^2; the sites were where we would place caged spongy moth larvae during the experiment. Additional details about the inoculation procedures are provided in Supplementary file 1: Details on soil inoculation procedure with *Entomophaga maimaiga*.

The effects of temperature on the infection of spongy moth larvae by *E. maimaiga* were examined by caging larvae within 7.1 m^2 circular plots that were assigned to three different temperature treatments (ambient temperature, 1.7 °C above ambient and 3.4 °C above ambient). However, we note that the cages and the larvae experienced approximately 5–10% lower temperature increases than the overall temperature increases of 1.7 °C and 3.4 °C above ambient achieved by the system (for more details on the system performance and achieved temperatures see Rich et al. (2015)). The plots were arranged in a block design with two replicate plots of each temperature treatment in each of the three blocks (Fig. 3). Plots within a block were separated by 4–13 m and were 8 m apart on average. The blocks were spaced approximately 15–23 m from each other, with an average separation of 19 m, within the same closed canopy habitat. Temperature was manipulated in the plots annually from approximately the beginning of April until the end of November every year since 2009.

Caged larvae were exposed to temperature and precipitation treatments in three separate four-day Trials; 30 June to 04 July (trial one); 06 July to 10 July (trial two); 12 July to 16 July (trial 3). We introduced simulated rainfall to one of two cages per plot (randomly selected), creating two precipitation treatments: ambient and supplemental (Fig. 3). To determine an appropriate amount of additional, simulated precipitation, we obtained long-term daily precipitation records from two nearby NOAA weather stations (https://www.ncdc.noaa.gov/) in Ely, Minnesota, which, together, provided coverage from 2000 to 2021 (USC00212561 47.9056, -91.8283; USC00212543 47.9239, -91.8586). From these data, we calculated the long-term (2000–2021) mean and standard deviation (SD) of precipitation for the date range of the experiment, 30 June to 17 July. The total volume of water added was equal to 1 SD of the long-term mean, with this volume calculated as:

$$water\ volume\ (ml) = catchment\ area\ (cm^2) \times rainfall\ depth\ (mm) \tag{1}$$

where catchment area was the area of a single cage (31 × 23 cm^2) and rainfall depth was 1 SD of the long-term mean of 7 mm. The total volume of water added to a cage receiving supplemental precipitation was 518 ml. The rationale for choosing to supplement precipitation by this amount (1 SD of the long-term mean for the duration of the experiment) was that we wanted to substantially increase soil moisture without increasing the total amount of precipitation (ambient + supplemental) by an amount that was unusually high in recent history. We applied one-third of the total volume of water during each four-day trial, with half of the water added at dusk on the first and third days of each trial by sprinkling with a watering can. To reduce confounding effects from water contaminants (e.g. minerals, pollutant, etc.), we used deionised water.

We tested the effects of temperature and precipitation on fungal infection by deploying two cages, each containing 19 early 4^{th}-instar larvae, in each plot; for a total of 684 larvae per trial (Figs 2, 3). The cages were made by folding aluminium window screening to a dimension of 31 × 23 cm^2, with a 2-cm high interior cavity, after which the seams were stapled and taped closed (Hajek and Humber 1997; Reilly et al. 2014; Fig. 4). To ensure contact with the soil, we brushed aside the leaf litter at each cage site and then installed the cages flush with the soil. Each cage was protected from vertebrate predators and weather-related hazards (e.g. fallen limbs) by a covering box of 12-mm^2 hardware mesh and both the cage and cover were anchored to the soil with landscape staples (Reilly et al. 2014; Fig. 4). Two 0.5 g cubes of artificial wheat germ diet (Bell et al. 1981) were provided in every cage.

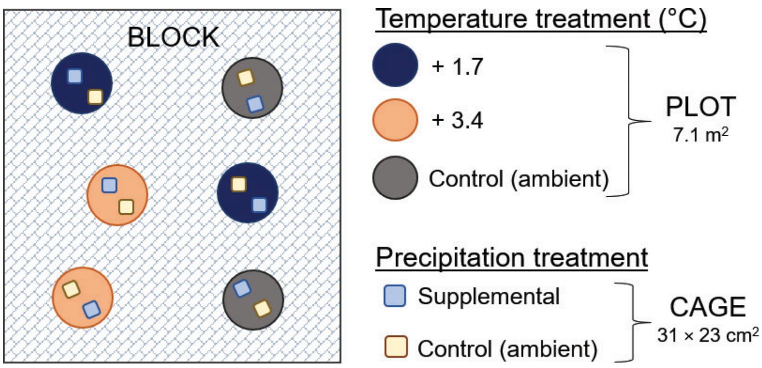

Figure 3. Diagram of one experimental block. Within each of three experimental blocks, we installed two cages of spongy moth larvae per plot (figure adapted from Rich et al. (2015)).

Figure 4. The cage design used to expose larvae to *Entomophaga maimaiga* resting spores in the soil (Reilly et al. 2014).

Following each field trial, we transferred the larvae to the lab and secured them individually in lidded 30 ml plastic cups containing a 0.20 g piece of artificial wheat germ diet (Blackburn and Hajek 2018). We maintained larvae at 18–22 °C and monitored them for 10 days or until death, whichever occurred first (Blackburn and Hajek 2018). Larvae that died within the 10-day monitoring period were placed on 1.5% water agar plates and checked daily for 3 days for conidial production (Hajek et al. 1990b). Although conidia are often visible without magnification, we noticed during the first trial's observation period that conidia were sometimes only apparent under a dissecting microscope. We did not analyse trial one results because of the possibility that we missed conidia on cadavers that we inspected without a dissecting microscope, as conidia are very short lived. We examined cadavers from trials two and three under both a dissecting and a phase contrast microscope. For phase contrast microscopy, we macerated and smeared a larva on a microscope slide and observed the specimen at 200–400× magnification (Blackburn and Hajek 2018). We counted a larva as infected by *E. maimaiga* if we saw conidia either externally or via phase contrast. It is also possible for larvae to have resting spores present instead of, or in addition to, conidia (Hajek 1999), but we did not expect this because previous research indicated that infections initiated by germ conidia from resting spores only produce conidia, not resting spores (Hajek 1997).

To understand how the weather conditions prior to and during the trials compared to average climatic conditions, we obtained long-term climate data for 2000 to 2022 from the same two NOAA weather stations used to calculate the amount of supplemental precipitation. Plot-level daily data on soil moisture for 20 June to 17 July was measured via automated, permanently installed water reflectometers (Model CS616 from Campbell Scientific), with one reflectometer per plot that collected measurements on an hourly interval for the top 30 cm soil profile.

Data analysis

We tested the hypotheses that temperature, precipitation and their interaction would affect the number of spongy moth larvae infected (and almost certainly killed) by *E. maimaiga.* We built a model with these variables and also included a fixed effect of block and random effect of plot. Including plot as a random effect allowed for the model to account for variance amongst plots, for example, potential non-independence of values within a given plot (Crawley 2013). Block was modelled as a fixed effect because there were only three levels in block and estimates of variance of random effects with so few levels are inaccurate (Arnqvist 2020). A linear mixed effects model with all of these variables was over-parameterised. Therefore, we assessed that block or plot could be dropped without adversely affecting model parsimony (fit balanced by a penalty for the number of parameters), with parsimony quantified using AIC values. Models with ΔAIC < 2 were considered to have substantial support (Burnham and Anderson 2002), where ΔAIC of model i equal to AIC_i – min(AIC) and min(AIC) is the AIC of the highest-ranking model. For both trials, we selected the model that was not over-parameterised, had substantial support (ΔAIC < 2) and the fewest number of variables dropped (See Suppl. material 2: Model selection using AIC scores). For both trials, a model that included block, but not plot was selected (Suppl. material 2). We assessed the effects of temperature, precipitation, the temperature × precipitation interaction and block on the number of infected larvae using generalised linear models (GLM). For each model, we specified a Poisson distribution and a log link. We performed all statistical analyses in R (R Core Team 2022). The models were fitted using the 'glmer' function of the 'lme4' package (Bates et al. 2015). To conduct pairwise comparisons, we performed Tukey's tests using the package 'emmeans' (Lenth 2022).

Results

Baseline climatic and abiotic conditions at the field site

Compared to the long-term mean from 2000 to 2021, NOAA weather station data (USC00212561 47.9056, -91.8283; USC00212543 47.9239, -91.8586) indicated that mean daily minimum and maximum temperatures during this experiment were near-average and slightly below-average, respectively (Table 1). Air temperature gradually increased over the course of the experiment; the average minimum and maximum daily air temperatures recorded by the NOAA weather stations during the second trial were 11.4 °C and 23.5 °C; and 12.4 °C and 24.5 °C during the third trial.

Mean daily precipitation during the experiment was 72% lower than the long-term mean (Table 1). However, prior to the experiment, mean daily precipitation in May was 38% higher than the long-term average for this month (Table 1). Soil moisture decreased during the experiment and, on the last day, average soil moisture across all plots was 0.18 Θ ($cm^3 H_2O\ cm^{-3}$soil), compared to 0.25 Θ at the start of the experiment. On average, plots under the high temperature treatment (3.4 °C above ambient) had the driest soil (Θ = 0.20 ± 0.01) and plots under the low (1.7 °C above ambient) and ambient temperature treatments had similar soil moisture, Θ = 0.24 ± 0.02 and Θ = 0.23 ± 0.02, respectively. Soil moisture in the region of the supplemental precipitation was not measured.

Effects of temperature and simulated precipitation on numbers of infected larvae

Increasing temperature had a significant, positive effect on the number of infected larvae in the second (P = 0.004) and third (P = 0.001) trials (Table 2; Fig. 5). In trial two, averaging across the precipitation treatments, the numbers of infected larvae were 24% and 65% higher in cages where temperature was elevated by 1.7 °C and 3.4 °C, respectively, compared to cages in plots at ambient temperature (Fig. 5). In trial three, the numbers of infected larvae were 56% and 44% higher under temperatures elevated by 1.7 °C and 3.4 °C, respectively, compared to ambient temperature. In both trials, very few infections occurred under ambient temperatures (Fig. 5). There were no significant effects of supplemental precipitation or interactive effects of temperature and precipitation or block on number of infected larvae in either trial (Table 2).

Table 1. Climate data for 2000 to 2022, sourced from NOAA stations USC00212561 and USC00212543 (Ely, Minnesota; https://www.ncdc.noaa.gov/). Values reported are mean daily averages ± standard deviation, during the dates of the experiment (30 June – 17 July) for the long-term (2000–2021) and in 2022. Precipitation is also reported during May because above-average rainfall in 2022 may have influenced *Entomophaga maimaiga* germination.

Year	May	30 June – 17 July		
	Precipitation (mm)	Minimum temperature (°C)	Maximum temperature (°C)	Precipitation (mm)
Long-term mean (2000 to 2021)	2.5 ± 5.4	12.7 ± 3.1	26.7 ± 4.2	0.9 ± 1.5
2022	4.0 ± 8.2	12.3 ± 3.1	24.3 ± 2.4	3.3 ± 7.1

Table 2. Results from generalised linear models on effects of temperature and precipitation on infection of spongy moth larvae by *Entomophaga maimaiga* in trials two and three.

Trial	Explanatory Variable	Degrees of Freedom	Chi-Square	*P* value
2	Block	2	2.6	0.272
	Precipitation	1	0.22	0.637
	Temperature	2	11.23	0.004
	Temperature × Precipitation	2	4.45	0.108
3	Block	2	3.67	0.16
	Precipitation	1	0.25	0.617
	Temperature	2	13.23	0.001
	Temperature × Precipitation	2	0.91	0.634

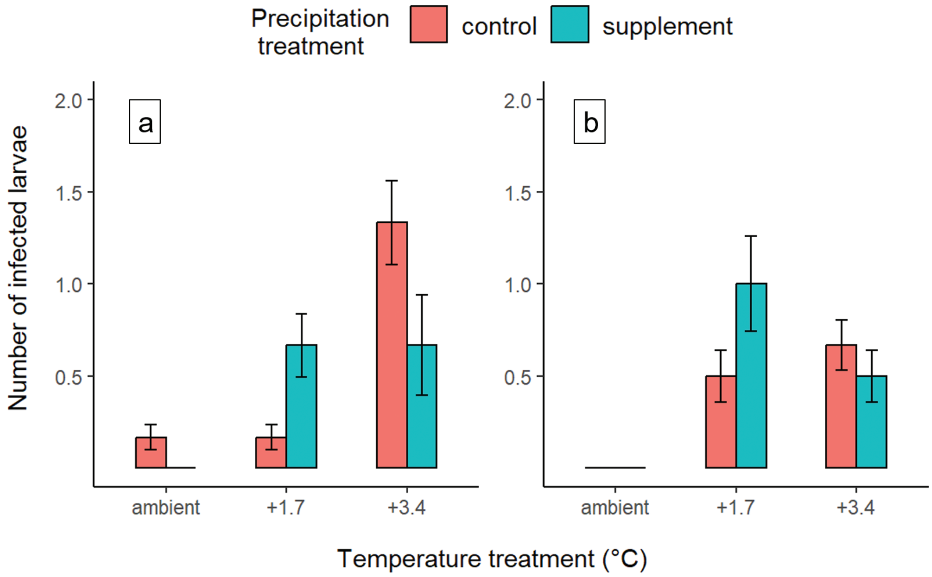

Figure 5. Results of manipulative field experiment. Effect of temperature and precipitation treatments on number of spongy moth larvae infected by *Entomophaga maimaiga* (mean ± SE) in (a) trial two and (b) trial three. The precipitation treatment consisted of adding +173 mm water to each cage over the course of a trial.

Discussion

Multiple studies have reported positive effects of environmental moisture on spongy moth larval infections by *E. maimaiga* (Hajek 1999; Reilly et al. 2014), but using a manipulative field experiment, we found that only the temperature treatments, not supplemental precipitation, affected larval infections by this pathogen. We found that larval infections increased where temperatures were elevated above ambient. This finding aligns with our prediction, based on a climate suitability study (Siegert et al. 2009), that cold temperatures may inhibit *E. maimaiga* in the north-westernmost portions of the spongy moth's invasive range. These results suggest that rising temperatures associated with climate change may increase larval mortality by *E. maimaiga* in cold portions of the spongy moth's range (e.g. Minnesota, northern Wisconsin and eastern Canada).

Contrary to our predictions, supplemental precipitation and its interaction with temperature, did not affect the number of larvae that became infected by the fungus. This finding was surprising given that multiple past studies have found positive associations between rainfall and larval infections from resting spores (Weseloh and Andreadis 1992a; Weseloh and Andreadis 1992b; Hajek et al. 1996; Reilly et al. 2014). One potential explanation for the lack of a precipitation effect in this study is that moisture requirements for resting spore germination were fulfilled before the experiment began, given that precipitation was above average during the month of May prior to the start of the experiment (Table 1). Supporting this possibility, a past study modelled the relationships between rainfall, temperature and the phenology of infection and found that the timing of rainfall, not just amount of rainfall, is a critical factor influencing infection rates (Weseloh et al. 1993). Additionally, using empirical data on *E. maimaiga* epizootics, Hajek et al. (2015) found that infection increased significantly with May rainfall, whereas the relationships between infection and precipitation in June and April were negative and not significant, respectively. Future field experiments that incorporate a reduced-moisture treatment along with an increased-moisture treatment could help resolve how infection levels will change with increasing drought severity.

Results from this study highlight the importance of considering geographic location when assessing the impacts of temperature and moisture conditions on larval infection. The general consensus of past research on the role of weather for this host-pathogen relationship is that warmer and drier conditions inhibit infections by *E. maimaiga* (Hajek 1999). Prior field studies on the effect of temperature on the spongy moth-*E. maimaiga* interaction were conducted in areas with warmer climates compared to the present study (Hajek et al. 1996; Hajek and Humber 1997; Reilly et al. 2014), which may explain why the positive effect of increasing temperature found in this experiment contrasts with that found in warmer regions. For example, mean daily air temperatures during the spongy moth's larval period are consistently lower in Ely, Minnesota (USC00212555 47.9746, -91.4495) compared to other portions of the spongy moth's non-native range — for example, Ithaca, New York (USC00304174 42.4491, -76.4491); Oakland, Maryland (USC00186620 39.4131, -79.4003); and Gassaway, West Virginia (USC00463361 38.6649, -80.7672) (Fig. 6).

While epizootics have occurred during years that were warmer and drier than average (Hajek et al. 1996), host densities must be high enough to offset the negative effects of reduced fungal germination under these abiotic conditions. A past modelling study showed that the threshold host density at which epizootics occurred changed under different abiotic environmental conditions (Kyle et al. 2020). Fungal density is also an important determinant of epizootics and, together, the interaction between host and fungal densities with weather drive epizootics (Hajek and Shapiro-Ilan 2018). While often overlooked, fungal density may be critical to understanding why epizootics can occur even when host densities are low.

Certain caveats should be considered in extrapolating our findings to the effects of climate on the host-pathogen interaction between the spongy moth and *E. maimaiga*. First, like many experimental studies on the spongy moth (Williams et al.

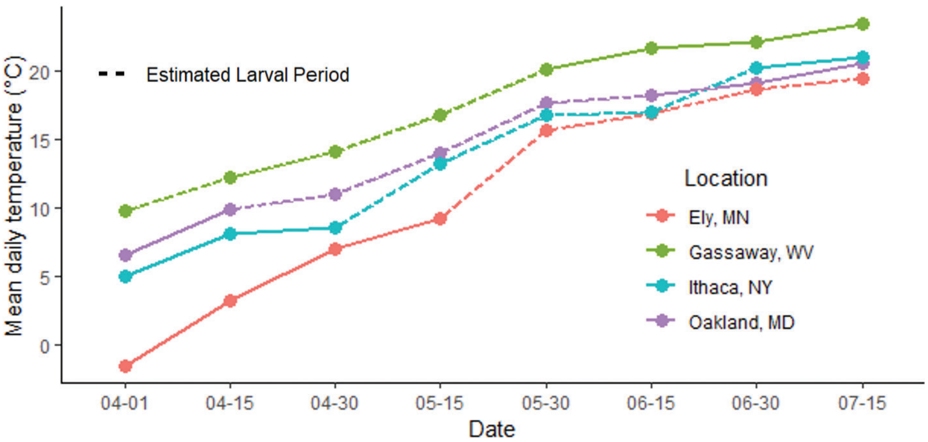

Figure 6. Mean daily temperature at different locations within the spongy moth's non-native range. Locations include: Ithaca, New York (USC00304174 42.4491, -76.4491); Oakland, Maryland (USC00186620 39.4131, -79.4003); Gassaway, West Virginia (USC00463361 38.6649, -80.7672); and Ely, Minnesota (USC00212555 47.9746, -91.4495). Temperature data are from 2000–2022 (https://ncdc.noaa.gov/). Ely, Minnesota (MN) is the weather station nearest to the experimental site. The lines are dashed during the spongy moth's larval period at each location, based on phenology model predictions (Régnière and Sharov 1997, 1998, 1999; Gray et al. 2001).

2003; Mason et al. 2014), our study was conducted using spongy moths obtained from USDA's Forest Pest Methods Laboratory and reared using artificial diet. It is not known whether this lab strain and wild spongy moths differ in their susceptibility to infection by *E. maimaiga* or if larval diet influences susceptibility to this pathogen. Therefore, wild spongy moths under the same conditions may be infected at lower or higher rates. Second, because there is evidence that temperature can alter rates of larval infection (i.e. susceptibility; Shimazu and Soper (1986)) by the fungus, we cannot rule out the possibility that changes in larval susceptibility, rather than changes in fungal germination and sporulation, influenced the observed temperature-driven changes in rates of infection.

Conducting multi-year manipulative experiments that assess the effects of annual variability in weather on this host-pathogen relationship could help quantify the range of temperature and precipitation conditions under which larval infection rates may be high enough to spark epizootics. If rising temperatures lead to increased infections of *E. maimaiga* in the colder regions of the spongy moth's range in North America, as is suggested by the results of this experiment, it is possible that rates of spongy moth range expansion in these regions could decrease in the future. However, temperatures in the north-westernmost portion of the spongy moth's range are expected to become more suitable for the pest under a 1.5 °C warming scenario (Gray 2004). Therefore, the overall effects of climate warming on spongy moth populations in these colder ecoregions is unclear. To provide information for management decisions on slowing the spread of spongy moth, particularly in the north-westernmost region of this non-native range, where rates of range expansion are fastest, it is imperative that we untangle the independent and interactive effects of temperature and precipitation on the relationship between the spongy moth and *E. maimaiga*. Doing this will help managers identify when and where climatic conditions are expected to inhibit or enhance larval mortality due to *E. maimaiga*, which in turn, would provide information for decisions on employing methods to slow the spread of the spongy moth.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: KJH, PBBR, CAR. Data curation: AS, CAR. Formal analysis: CAR. Methodology: HN, KJH, AEH, PBBR, AS, CAR. Project administration: CAR, KJH, PBBR. Supervision: KJH, AEH. Visualization: CAR. Writing - original draft: CAR. Writing - review and editing: KJH, AEH, PBBR, CAR, HN, AS.

Author ORCIDs

Clare A. Rodenberg https://orcid.org/0009-0002-1457-2950
Ann E. Hajek https://orcid.org/0000-0001-5740-4717
Artur Stefanski https://orcid.org/0000-0002-5412-1014

Data availability

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.44j0zpcp4.

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Supplementary material 1

Details on soil inoculation procedure with *Entomophaga maimaiga*

Authors: Clare A. Rodenberg, Ann E. Hajek, Hannah Nadel, Artur Stefanski, Peter B. Reich, Kyle J. Haynes

Data type: pdf

Link: https://doi.org/10.3897/neobiota.95.126311.suppl1

Supplementary material 2

Model selection using AIC scores

Authors: Clare A. Rodenberg, Ann E. Hajek, Hannah Nadel, Artur Stefanski, Peter B. Reich, Kyle J. Haynes

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