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Functional Ecology

RESEARCH ARTICLE

Prior heat waves improve survival of field but not domesticated populations of tobacco hornworm exposed to repeated bacterial infections

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Abstract

1. In insects and other invertebrates, prior pathogen exposures can improve immune responses and survival to subsequent infections through immune priming. Alternatively, stress and metabolic costs of multiple infections can impair host immunity and survival. The effects of high-temperature extremes on host–pathogen interactions are not well understood despite the increasing occurrence of heat waves caused by climate change.
2. The response of insects to heat waves and pathogens depends on recent evolutionary history with selective pressures. Domestication of insect pests has occurred in lab colonies of model species, reducing selective pressures for immune and heat stress responses. Lab strains are often used in immunological or heat stress experiments to represent wild field strains, but the efficacy of this approach is seldom evaluated.
3. Using the tobacco hornworm (*Manduca sexta*), we tested the impact of a heat wave during initial pathogen exposure on survival of a secondary infection with *Bacillus thuringiensis* bacteria. We used a domesticated lab population and a naturally occurring field population of *M. sexta* to evaluate the impacts of recent domestication on immune and thermal responses.
4. A heat wave during initial infection significantly increased survival of the secondary *B. thuringiensis* infection in the field, but not the lab population of *M. sexta*.
5. In the field population, survival of the repeated infection was temperature dependent: exposure to an initial infection event reduced survival of the secondary infection at the control temperature regime, consistent with a stress effect. However, a heat wave during the initial infection event increased survival of the secondary infection, consistent with immune priming effects.
6. The results of this study demonstrate that (a) insect response to thermal stress and pathogens can depend on recent domestication and (b) responses of hosts to repeat pathogen exposures can be temperature-dependent, suggesting that

cross-talk between the heat stress and immune memory pathways may have important consequences for host–pathogen outcomes under heat wave events.

KEYWORDS
Bacillus thuringiensis, climate change, domestication, heat waves, host–pathogen interactions, immune priming, Lepidoptera, relaxed selection

1 | INTRODUCTION

Determining how host–pathogen interactions are impacted by climate change and other anthropogenic changes is an important challenge for ecologists. Climate change is increasing the occurrence, intensity and duration of extreme temperature events, such as heat waves (HWs) (Parmesan, 2006; Stillman, 2019). The mechanisms that govern host–pathogen or host–parasite interactions, such as immunity and virulence, are temperature dependent, and ecological outcomes of these interactions can shift under novel thermal regimes (Blanford et al., 2003; Condé et al., 2021; Hector et al., 2023; Malinski et al., 2024; Poulin et al., 2024; Rohr & Cohen, 2020; Thomas & Blanford, 2003).

In nature, hosts often encounter multiple pathogens repeatedly over the course of their lives (Halliday et al., 2020). Prior infections can shape future host immune responses via immune memory mechanisms (Boots et al., 2013; Leon & Hawley, 2017), for example, adaptive immunity in vertebrates. In insects and other invertebrates, the phenomenon of immune priming is a type of immune memory, where initial infection and clearing of a pathogen can increase survival to subsequent infection within and across generations (Contreras-Garduño et al., 2016; Sheehan et al., 2020; Sułek et al., 2021; Tetreau et al., 2019). While the impact of temperature and climate change on vertebrate adaptive immunity has received some attention in recent years (Caroprese et al., 2017; Dittmar et al., 2014; Jolles et al., 2015; Moriyama & Ichinohe, 2019; Scharsack & Franke, 2022), much less is known about the effects on immune priming in invertebrates.

While the molecular and physiological mechanisms underlying immune priming are not fully understood (Coustau et al., 2016; Milutinović & Kurtz, 2016), recent work showed that primed insects display increased circulating haemocytes (Browne et al., 2015), upregulated pattern recognition proteins (PRPs) and antimicrobial peptides (AMPs) (Bergin et al., 2006; Roesel et al., 2020) and epigenetic changes (Gegner et al., 2019; Mukherjee et al., 2012; Mukherjee & Vilcinskas, 2014; Vilcinskas, 2016). Studies also suggest prior exposure to pathogen-derived material (e.g. heat-killed bacteria, microbial cell wall components), or nonpathogenic stress events (e.g. mild wounding, short periods of high temperature exposure) can confer resistance to typically lethal subsequent infections (Bergin et al., 2006; Browne et al., 2015; Mowlds et al., 2010; Ratcliffe, 1985). Such stressors can enhance survival against infection via overlapping pathways between stress responses and immune responses (e.g. molecular chaperones, reactive oxygen species metabolizing enzymes, lipid transports proteins) (Adamo, 2017; Hector et al., 2022; Sinclair et al., 2013; Wojda, 2017), but stress responses can also drain metabolic resources, which can impair immunity (Adamo, 2017; Clark & Fucito, 1998; Hector et al., 2023). Understanding how prior exposures to pathogens interact with heat stress responses to determine the ecological outcomes of subsequent host–pathogen interactions is critical for predicting invertebrate disease dynamics in the face of rapid anthropogenic change.

Much of our understanding for how organisms respond to pathogens, temperature stress and their interaction comes from studies in laboratory strains or populations, but recent evolutionary history with different selective pressures can shape these responses (Abolins et al., 2017; González-Tokman et al., 2020; Hirakawa & Salinas, 2020; Krebs et al., 2001). It is therefore necessary to explicitly test how responses of model lab populations may diverge from those of the wild field populations they often represent (Krebs et al., 2001). Adaptation of species or populations to human-made environments can result in domestication, mediated by selective pressures from either deliberate or inadvertent anthropogenic actions and environments (Lecocq, 2019; Yu et al., 2011; Zeder, 2015). This has important implications for both thermal- and immune-related responses. Adaptation to relaxed thermal selective pressures in human-modified habitats, such as indoor or otherwise temperature-buffered spaces, can reduce thermal tolerance rapidly (e.g. within 60–120 generations, Hirakawa & Salinas, 2020; Huey et al., 1991). Similarly, relaxed selective pressures for pathogen and parasite defences in domesticated settings with lower parasite prevalence or diversity can result in weaker immune responses in lab populations (Abolins et al., 2017). Genetic drift can also alter immunological responses, especially for small populations (Grueber et al., 2013) such as lab colonies. Direct comparisons of domesticated and field populations are needed to evaluate the validity of using lab strains as models for host–pathogen interactions.

Here, we explore how the combination of recent domestication, high temperature stress and prior pathogen exposure impacts the outcome of host–pathogen interactions in a repeat exposure event. We investigated the effects of an ecologically relevant HW during an initial infection event on survival of a secondary infection in the tobacco hornworm (*Manduca sexta* (Lepidoptera: Sphingidae)), an agricultural insect pest and model system for insect physiology, thermal biology and immunology (Gershman et al., 2021; Kingsolver & Nagle, 2007; Roesel et al., 2020). To test whether and how recent domestication alters responses to prior pathogen exposure and temperature stress, this study included

a naturally occurring field population of *M. sexta*, and a domesticated lab population which has been isolated in a laboratory setting for >260 generations (Diamond & Kingsolver, 2011). Previous studies demonstrate that these populations differ in their heat tolerances and immune responses, with reduced thermal tolerance and immunocompetence in the domesticated lab population (Alston et al., 2020; Diamond & Kingsolver, 2011; Kingsolver & Nagle, 2007). To test the impact of a simulated HW on immune priming capacity in both populations, we used the highly virulent bacteria *Bacillus thuringiensis*. Prior work demonstrates *B. thuringiensis* elicits an immune priming response in several insect species (Khan et al., 2017, 2019; Medina Gomez et al., 2018), including Lepidoptera (Hernández-Martínez et al., 2010; Taszłow et al., 2017; Wu et al., 2014).

The experiments described here test (1) the impact of a stressful HW on within-generation survival of a secondary infection; and (2) the role of recent domestication in driving responses to pathogens, heat stress and their combination. We test several specific predictions about these effects. High temperatures can be therapeutic for infected hosts (e.g. via behavioural fever, Hasday & Singh, 2000). Cross-talk between immune and heat stress response pathways could bolster immune priming mechanisms for *M. sexta* initially exposed to a pathogen during a HW, thereby increasing survival of a secondary pathogen infection. Conversely, these multiple stressors may result in a compounding stress effect, resulting in decreased survival of the secondary infection. If evolutionary history with pathogens is an important mediator of immune priming, prior pathogen exposure should disproportionately increase survival of a secondary infection in the field population relative to the domesticated population. If recent domestication is an important mediator of the cross-talk between heat stress and immune responses, we expect the lab population will differ from the field population in their response to the interacting stressors.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and bacterial stocks

Field population *M. sexta* were collected as eggs or first-instar larvae from tobacco leaves at Central Crops Research Station (Clayton, NC, USA). Eggs were placed on artificial wheat germ-based diet supplemented with tobacco powder as a feeding stimulus (adapted from Kingsolver & Woods, 1998) and kept at 25°C constant under 14 L:10D photoperiod in climate controlled chambers (Percival Scientific 36VL) until hatching. Lab population *M. sexta* were collected simultaneously as eggs from the University of North Carolina-Chapel Hill laboratory colony, which has been kept under lab conditions at a constant 25°C and 14 L:10D photoperiod for at least 40 years (>260 generations). All larvae were provided diet ad libitum and kept at 14L:10D photoperiod under a daily fluctuating regime of 25°C ± 10, with 2 h at the highest and lowest temperatures and constant ramping in between. Ethical approval was not required for working with *M. sexta* caterpillars, but all animals were treated as humanely as possible.

Bacillus thuringiensis bacteria (subsp. *kurstaki*) (Dulmage, 1973) was cultured in LB liquid media at 37°C overnight. Dosages for infection treatments are described below. Note that *B. thuringiensis* is typically ingested by *M. sexta* orally in agricultural settings, where crystalline toxins perforate the gut tissue (Reinoso-Pozo et al., 2016; Schesser & Bulla, 1978). Following other recent work, the present study design inoculated hosts via injection to avoid cross-contamination with gut microbiota (Li et al., 2020; Wu & Yi, 2018), which are diet- and environment-dependent and can induce different immune priming reactions than *B. thuringiensis* alone (Hernández-Martínez et al., 2010).

2.2 | Experimental design

The study was fully factorial and consisted of three treatment types (initial infection, temperature regime during initial infection and secondary infection), two levels per treatment (sham control and infection) and two *M. sexta* populations (lab and field) (Figure 1). Initial infection treatment levels were injection with a low dose of *B. thuringiensis* (Bt), or the same volume injection with sterile PBS (sham control) to control for wounding effects. A low dose was chosen to invoke an immune priming response (Khan et al., 2016; Mowlds et al., 2010), rather than killing hosts which could result in survivor bias. Injection with PBS induces a low-level immune response (Mowlds et al., 2008); therefore, the Bt versus sham control treatments should differ only in the *B. thuringiensis* pathogen-specific effects.

Treatment levels for temperature regime during initial infection were 3 days of HW at 32°C ± 10, or no heat wave (noHW) at 25°C ± 10. The HW temperature and duration were chosen because daily maximum operative temperatures often exceed 40°C and sometimes reach 42°C on tobacco hostplants during HWs in North Carolina (Moore et al., 2023), and because HWs reaching highs of 42°C for 3 days were previously demonstrated to elicit stress responses in both field and lab *M. sexta* (Kingsolver et al., 2021). Secondary infection treatment levels were high dose of *B. thuringiensis* (Bt) or sterile PBS (sham control). Starting sample sizes at initial infection were between 29 and 50 larvae per group (Table S1).

2.3 | Initial infection and HW treatments

In the morning (between 6 and 8 AM), newly moulted fourth-instar larvae were moved from the rearing thermal regime of 25°C ± 10 and placed in either the HW treatment of 32°C ± 10 or noHW treatment of 25°C ± 10 for the 3-day initial infection temperature regime (Figure 1). Because development rate is temperature dependent (Kingsolver & Huey, 2008), the timing of treatments was based on developmental time (instar) rather than absolute time. Note that lab

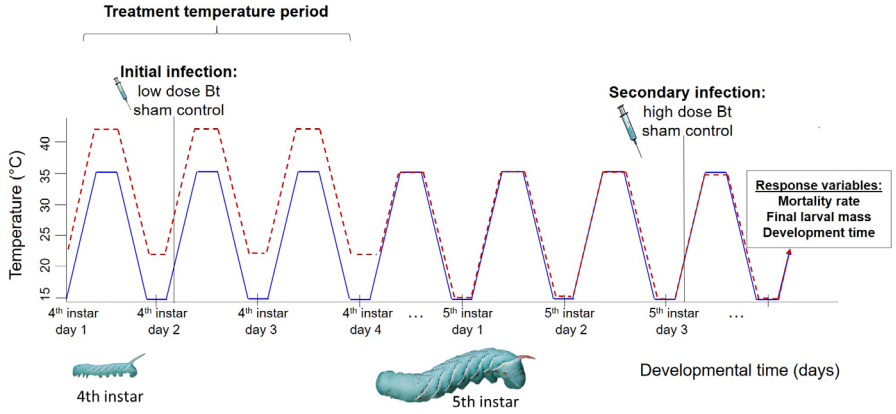

FIGURE 1 Diagram of experimental design. The *y*-axis depicts temperature (°C). The *x*-axis depicts developmental stage of lab and field population *Manduca sexta*; ellipses indicate continued thermal regimes until the next developmental stage. Daily thermal regimes are depicted as line type and colour (solid blue, 25°C ± 10 (no heat wave); dashed red, 32°C ± 10 (heat wave)). The 3-day initial infection temperature regime began on the day of moult to fourth instar. On the morning of the second day of fourth instar, larvae received the initial infection (low dose of *Bacillus thuringiensis*, or sham control). On the morning 4 days after the moult to fourth instar, the initial infection temperature treatment ended and all larvae were placed at 25°C ± 10 until 3 days after the moult to fifth instar was observed. Larvae then received the secondary infection (high dose of *B. thuringiensis*, or sham control) and were thereafter observed for death or wandering (survival).

population *M. sexta* grew faster (10 ± 1.8 days for field, 8.5 ± 0.7 days for lab) and larger (148.1 ± 94 mg for field, 258 ± 134.6 for lab) than field population *M. sexta* between hatching and fourth instar, consistent with prior research of population differences in these strains (Kingsolver & Nagle, 2007).

In the morning of the second day of the experiment (during the temperature treatments, Figure 1), *M. sexta* were injected with the initial infection treatments: either sterile PBS (sham control) or a low dose of *B. thuringiensis* diluted in sterile PBS. Larval cuticle was surface sterilized with 70% ethanol at the base of the last larval proleg and injections were administered at a volume of 10 μL consistent with prior work (Roesel et al., 2020) using a 25-gauge 100 μL syringe (Hamilton 700 series). Larvae were injected at a nearly parallel angle to the body wall to avoid puncturing the gut. Dosages for the initial infection were chosen as 1/20th of dosage at which 50% of larvae die (LD_{50}) in first day fourth-instar larvae. The lethal dose curve was determined using lab population *M. sexta* at 25°C constant prior to the start of the present experiment (Figure S2A). Because field population *M. sexta* are smaller than lab population *M. sexta*, the initial infection dosage for the field population was adjusted to keep the dose:larval mass ratio consistent between populations (0.872 CFU/1 mg). Mean mass at fourth instar was 258 mg and 148.1 mg yielding initial infection dosages of 225 CFU and 130 CFU for the lab and field populations, respectively.

Following initial infection, larvae were returned to the temperature treatments. On the morning of the fourth day of the experiment (after three full days in the treatment temperature regime, Figure 1), all larvae were returned to 25°C ± 10 thermal regime. Larvae were checked daily for moult to fifth instar or death. On the morning of the third day of fifth instar, larvae received the secondary infection treatment (Figure 1). Mean development time from fourth to fifth instar was similar between populations (4.1 ± 1.1 days for field, and 3.8 ± 1.1 days for lab), and the HW increased development time by 0.4 days during this time frame in both populations. Mortality across the duration of the experiment was evaluated; some hosts died before the secondary infection treatment (Table S1), but relative final mortality rates across treatment groups were consistent whether mortality was measured from the experiment start or from the secondary infection event (Figure S3).

To confirm that the initial bacterial infection was cleared prior to the secondary infection, 4–5 additional larvae were run in parallel to each treatment group and population and were tested for the presence of *B. thuringiensis* bacteria in the haemolymph daily after initial infections. Larvae were surface-sterilized on the dorsal horn, and the dorsal horn tip was cut to allow for bleeding of 200 μL of haemolymph onto LB agar plates, which were cultured overnight at 37°C. The presence of bacterial colonies was evaluated the next day. Larvae subsampled for haemolymph testing were excluded from the remainder of the experiment on account of wounding of the dorsal horn. Bacterial colonies were not present in the blood of any sham control larvae, but were present in 80%–100% of low-dose *B. thuringiensis*-infected larvae on the day after the initial infection, and 0%–20% on the first day of fifth instar, and no larvae the day before the secondary infection, indicating the infection was likely cleared by most larvae at the time of secondary infection (Table S2).

2.4 | Secondary infection treatments

On the morning of the third day of fifth instar, hosts received the secondary infection treatments: either sterile PBS (sham control) or a high dose of *B. thuringiensis* diluted in sterile PBS (Figure 1).

Secondary infections were administered at a volume of 25 μL to account for larger *M. sexta* body size in the fifth instar. Larval cuticle was locally surface-sterilized and injections were performed using the same methodology and equipment as described in the initial infection (above). Secondary infection dosage was the LD_{50} (1.5e4 CFU) for day 3 fifth-instar lab *M. sexta* at 25°C (Figure S2B). In preliminary trials using a secondary infection dosage which kept dosage:larval mass consistent between lab and field populations (as in the initial infections), nearly 100% of field population *M. sexta* survived, so the same secondary infection dosage of 1.5e4 CFU was used for lab and field populations regardless of body size. Following secondary infection injections, hosts were monitored daily for death or wandering (a characteristic pre-pupation behaviour) (Figure 1). Preliminary trials indicated all hosts surviving to wandering successfully pupated and eclosed as adults, so *M. sexta* exited the experiment following advancement to wandering.

2.5 | Statistical analyses

2.5.1 | Effect of population and secondary infection on host mortality

To test whether the secondary infection increased mortality relative to the sham control and to evaluate differences between populations, a generalized linear model (GLM) approach was used via the '*glm*' function in the *dplyr* package (v 1.1.3) in R (v 4.3.1). Models used a binomial distribution and logit link; included a binary response variable of death or survival to wandering; and fixed variables of initial infection, initial infection temperature regime, secondary infection, and population. Analyses of deviance (ANODEVs) were used to compare the model fit including all variables and their interaction terms with the model fit including all variables in isolate. Including interactive terms did not significantly improve the model fit (ANODEV, $p = 0.12$), so the simpler model is reported (Table 1). Because final sample sizes differed between the populations and secondary infection types (Figure 2; Table S1), separate GLM analyses for each population were performed to evaluate interactive treatment effects (below).

2.5.2 | Effect of *B. thuringiensis* secondary infection on host populations

We evaluated the effects of prior infection and HW exposure on survival of a second *B. thuringiensis* infection separately for each population using a GLM approach as described above. Models included a binary response variable of mortality or survival to wandering, and fixed response variables of initial infection, initial infection temperature regime and the interaction between initial infection and initial infection temperature regime. Models included only the *B. thuringiensis* secondary infection treatment groups. ANODEVs were run on all model fits (Table 2).

TABLE 1 Results from analyses of deviance of the generalized linear model analysis of mortality following secondary infection in lab and field *Manduca sexta* populations. The model included fixed effects of population (lab, field), initial infection (low-dose *Bacillus thuringiensis*, sham control), initial infection temperature regime (heat wave, no heat wave) and secondary infection (high-dose *B. thuringiensis*, sham control).

Variable	df	Deviance	Pr(>χ)
Initial infection	1	1.985	0.1588
Temperature	1	2.082	0.1490
Secondary infection	1	77.200	<2.2e-16*
Population	1	16.903	3.934e-05*

Note: Significant *p*-values are indicated with an asterisk (*) symbol.

2.5.3 | Host growth and development

Sublethal effects on host growth and development were analysed for both populations in combination and separately due to confounding differences in sample sizes of treatment groups between populations (Figure 2; Table S1). For larvae that survived to wandering, development time from the secondary infection to the date of wandering was evaluated via GLM analysis using a Gaussian distribution. To assess population differences, the model included fixed effects of population, initial infection, temperature regime and secondary infection. To evaluate populations separately, models for each population were run with predictor variables of initial infection, temperature regime, secondary infection and the interaction between initial infection and temperature regime. Because sample sizes differed between secondary infection types (Bt, sham), interaction terms with secondary infection were not included (Table 3). The effect of temperature and infection treatments on final larval mass of *M. sexta* populations was evaluated using models with the response variable of final larval mass at wandering (Table S3). ANODEVs were run on all model fits. All data have been deposited in Dryad Digital Repository (Malinski et al., 2025).

3 | RESULTS

3.1 | Effect of population and secondary infection on host mortality

The *B. thuringiensis* secondary infection significantly increased mortality rate relative to the sham secondary infection group, as expected (ANODEV, $p < 0.05$, Table 1) (Figure 2). Population was a significant predictor of mortality following the *B. thuringiensis* secondary infection, with higher average mortality rates observed in the lab relative to the field population across all initial infection and temperature treatments (ANODEV, $p < 0.05$, Table 1) (Figure 2). Lab *M. sexta* also exhibited faster mean time to death (Figure S1), collectively suggesting the lab population was more sensitive to the high-dose *B. thuringiensis* infection than the field population.

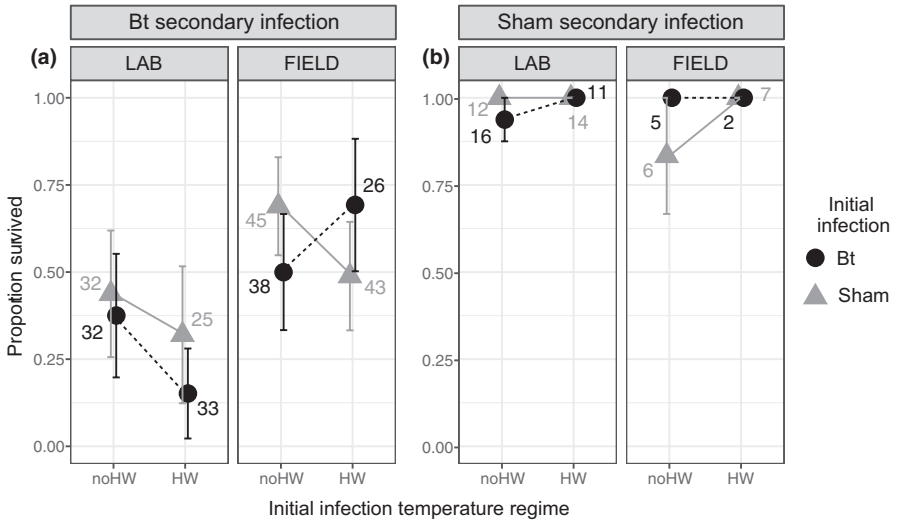

FIGURE 2 Proportions and confidence intervals of *Manduca sexta* surviving to wandering following the secondary infection treatment. (a) High-dose *Bacillus thuringiensis* secondary infection; (b) Sham control secondary infection. Within each panel, lab population *M. sexta* are shown in the left facet, field population right facet. Black circles, low-dose *B. thuringiensis* initial infection; grey triangles, sham control initial infection. Treatment temperature regime during the initial infection is shown on the *x*-axis (noHW, no heat wave; HW, heat wave). Sample sizes entering into the secondary infection are indicated by numbers adjacent to points.

TABLE 2 Results from analyses of deviances of the generalized linear model analyses of mortality following the *Bacillus thuringiensis* secondary infection in *Manduca sexta* populations separately (top, lab; bottom, field). Models included fixed effects of initial infection (low-dose *B. thuringiensis*, sham), initial infection temperature treatment (HW, noHW), and the interaction between initial infection and temperature treatments. Models included only the *B. thuringiensis* secondary infection.

Population	Variable	df	Deviance	Pr(>χ)
Lab	Initial infection	1	2.162	0.141
	Temperature	1	4.370	0.036*
	Initial infection: Temperature	1	0.747	0.388
Field	Initial infection	1	0.025	0.874
	Temperature	1	0.228	0.633
	Initial infection: Temperature	1	5.832	0.015*

Note: Significant *p*-values are indicated with an asterisk (*) symbol.

TABLE 3 Results from analyses of deviances of generalized linear models for development time (from secondary infection to wandering) for survivors. (A) Model including combined *Manduca sexta* populations; (B) models for lab and field populations separately.

Population	Variable	df	Deviance	Pr(>χ)
(A)				
Lab and field	Population	1	90.11	0.008*
	Initial infection	1	9.898	0.384
	Temperature	1	86.67	0.010*
	Secondary infection	1	0.667	0.821
(B)				
Lab	Initial infection	1	6.798	0.418
	Temperature	1	94.99	0.002*
	Secondary infection	1	4.595	0.505
	Initial infection: Temperature	1	1.331	0.720
Field	Initial infection	1	1.076	0.794
	Temperature	1	11.24	0.397
	Secondary infection	1	3.546	0.635
	Initial infection: Temperature	1	0.028	0.967

Note: Significant *p*-values are indicated with an asterisk (*) symbol.

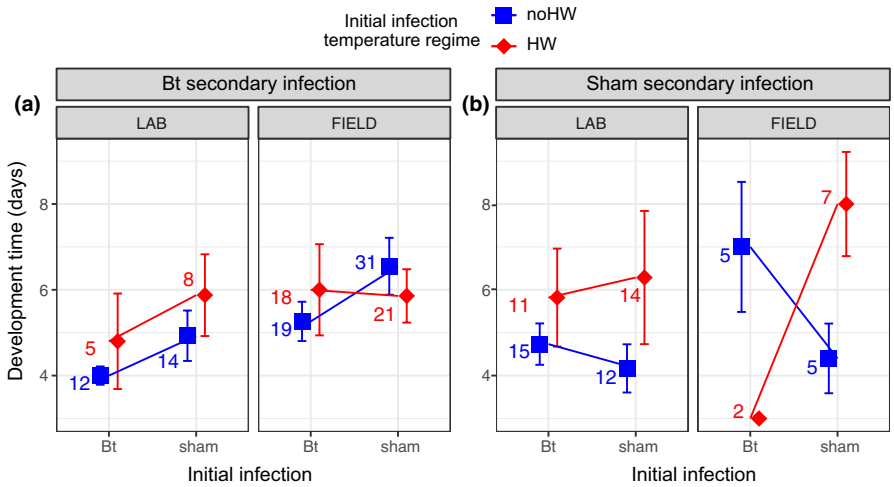

FIGURE 3 Means and standard errors for development time of surviving *Manduca sexta* from the day of the secondary infection (third day of fifth instar) to wandering. (a) *Bacillus thuringiensis* secondary infection; (b) Sham control secondary infection. Within each panel, lab population *M. sexta* are shown in the left facet, field population right facet. Initial infection treatment is shown on the *x*-axis (Bt, *B. thuringiensis*; sham, sham control). Temperature treatment is indicated by colour and shape of points (blue squares, no heat wave; red diamonds, heat wave). Sample sizes for survivors to wandering are indicated as numbers adjacent to points.

3.2 | Effect of *B. thuringiensis* secondary infection on host populations

Separate analyses of lab and field populations were conducted to evaluate interactive effects between temperature and infection treatments. For the lab population, the HW treatment significantly increased mortality following the secondary infection (ANODEV, $p < 0.05$, Table 2; Figure 2), with no interactive effects and no independent effect of initial infection. For the field population, initial *B. thuringiensis* pathogen exposure increased or decreased mortality following a secondary infection event depending on the thermal regime during the initial exposure (Figure 2). Relative to naïve larvae, field hosts exhibited increased mortality of the secondary infection if the initial low-dose exposure took place at control temperatures, but reduced mortality if it took place during a HW. This interactive effect of initial infection and temperature regime in the field population was statistically supported (ANODEV, $p < 0.05$, Table 2). These results suggest that pathogenic exposures can be beneficial or stressful depending on environmental temperature at the time of infection, and the directionality and magnitude of these effects can be population-specific.

3.3 | Host growth and development

For survivors of the secondary infection, the lab population developed significantly faster than the field population (ANODEV, $p < 0.05$, Table 3; Figure 3), consistent with prior developmental work in these populations (Kingsolver & Nagle, 2007). In the lab population, exposure to the prior HW treatment significantly slowed development time later in life (ANODEV, $p < 0.05$, Table 3; Figure 3), while development time of the field population was not impacted by any treatment (but note small sample sizes in the sham control secondary infection treatments, which limits the detection power of the statistical test). These results suggest the lab and field populations diverge in their sublethal responses to heat, with prior HW exposure resulting in prolonged deleterious developmental effects in the lab population.

For final mass at wandering, lab *M. sexta* grew significantly larger than field *M. sexta* (ANODEV, $p < 0.05$, Table S3; Figure S4), consistent with prior research in these populations (Kingsolver & Nagle, 2007). The secondary infection significantly impacted final mass at wandering in the combined analyses (Figure S4; Table S3), but we suggest this result should be interpreted with low confidence as it is likely confounded with low sample sizes in the field population sham secondary infection group (Table S1). No treatment impacted final body size in the separate population analyses (Table S3).

4 | DISCUSSION

The objectives of this study were to determine (1) how extreme high temperatures impact host–pathogen interactions during repeat exposures in insects, and (2) how recent domestication may alter these thermal and immune effects.

4.1 | Heat waves improved immune priming in the field population

We found evidence for significant interactive effects of initial infection and HW treatments in the field population: protection against the secondary infection either increased or decreased depending on the temperature at which the initial infection occurred (Figure 2; Table 2). For example, prior *B. thuringiensis* exposure exacerbated secondary infection mortality in the control temperature group, but reduced

mortality in the HW group (Figure 2). Survivor bias did not drive these patterns: While low levels of mortality occurred before the secondary infection treatment (Table S1), relative final mortality rates across treatment groups were consistent whether mortality was measured from the start of the experiment or from the secondary infection event (Figure S3). These results demonstrate that survival of secondary pathogen exposures can depend on the temperature at which the first infection event occurred, and HWs can enhance the survival of repeat infection events in wild insect populations.

Note that *B. thuringiensis* infection is typically acquired orally (Schesser & Bulla, 1978) but here was injected to avoid confounding effects with gut microbiota, consistent with other recent work (Taszłow et al., 2017; Wu & Yi, 2018). While caterpillar gut microbiomes are typically very low density (Hammer et al., 2017), rearing on artificial diet (as was done in this study for logistic purposes) can significantly alter microbiome content compared to feeding caterpillars leaf material (Hammer et al., 2017; Van Der Hoeven et al., 2008). Oral infections can produce different effects on host mortality depending on the concentration and identity of opportunistic microbes present in the gut (Li et al., 2020), and indeed prior work demonstrated that increased gut microbial load can induce immune priming protection against *B. thuringiensis* oral infection in larval Lepidoptera (Hernández-Martínez et al., 2010). Therefore, oral route of infection could alter the host's response to repeated infection, which may be further exacerbated by the interaction with heat stress. More research is needed to characterize interactions between the gut microbiome and recurring oral *B. thuringiensis* infections.

While molecular mechanisms were not evaluated in this study, cross-talk between immune and temperature stress responses may play a role in shaping the survival effects demonstrated here. Immune, thermal and stress responses can share signalling pathways: For example, heat stress alone can increase host survival to later immune challenges in insects (Browne et al., 2015; Condé et al., 2021), and mechanisms that protect against damage from temperature stress may also protect against damage resulting from infection (Sinclair et al., 2013). Consistent with this idea, heat stress and pathogenic infection can independently induce molecular chaperones such as heat shock proteins (HSPs), which can perform antiviral and antimicrobial functions across diverse invertebrate species (Altincicek et al., 2008; Cellura et al., 2007; Condé et al., 2021; Dimos et al., 2019; Gonsalves et al., 2011). Further investigation of the interactions between heat stress and immune response pathways is a promising area for future research as climate change intensifies, especially for species of public health concern or systems in which pathogens control pest populations (such as insects Flores-Mejia et al., 2017; Parker & Kingsolver, 2024; Tougeron et al., 2020; Wenda et al., 2023).

4.2 | Lab and field populations differ in immune priming and heat stress responses

Our results show that domesticated and field *M. sexta* populations differed significantly in their survival outcomes with prior pathogen and temperature exposure (Figure 2; Table 1). In the lab population, the HW increased mortality following the secondary infection in both naïve and previously exposed larvae, but in the field population, temperature had no independent effect (Figure 2; Table 2). These results demonstrate that increased survival of hosts to repeated *B. thuringiensis* infection is temperature dependent in the wild field population, but does not occur at either thermal regime in the lab population. *Bacillus thuringiensis* is an extremely important entomopathogen with high virulence against *M. sexta* and other insect herbivores (Dulmage, 1973) and elicits immune priming responses in many insects (Khan et al., 2017, 2019; Medina Gomez et al., 2018; Taszłow et al., 2017; Tate & Graham, 2015), including domesticated populations (Ferro et al., 2019; Hernández-Martínez et al., 2010). We suggest the survival effects demonstrated here may be driven by immune priming mechanisms (discussed in the section above). The lack of this response in the lab *M. sexta* population may be attributable to its long period of relaxed selection with no *B. thuringiensis* exposure (>260 generations) relative to other domesticated insects, but this requires further testing with other isolated lab populations.

Thermal performance of the domesticated lab population was lower than in the field population, consistent with prior research (Alston et al., 2020; Kingsolver & Nagle, 2007). Prior exposure to the HW significantly increased mortality of lab *M. sexta* in the secondary *B. thuringiensis* infection (Figure 2; Table 3), decreased body size (Figure S5) and resulted in prolonged developmental delays long after the acute heat stress ended (Figure 3). Furthermore, recent transcriptomic work in these two *M. sexta* populations demonstrated that the field population has a greater ability to handle stress at moderate temperatures, mediated by HSP expression (Alston et al., 2020). This reduced heat stress tolerance in the lab population at the genetic and transcriptomic levels is likely an important driver of the prolonged developmental effects and reduced survival of pathogenic exposures in hosts previously subjected to stressfully high temperatures.

Host populations also differed in their responses during infection. The lab population was more sensitive to the high dose secondary *B. thuringiensis* infection across treatment groups (Figure 2), despite their larger body size relative to the field population (Figure S5). Time to death following the secondary infection was faster in the lab population (Figure S1), collectively suggesting that the domesticated population may be less immunocompetent in both acute infection response and immune memory responses than the field population. Consistent with these results, recent transcriptomic work comparing these two populations demonstrated that field *M. sexta* displayed increased constitutive expression of AMPs in the Toll and IMD pathways, suggesting innate antimicrobial defences may be lower in the domesticated population (Alston et al., 2020). Recent work in another model insect, the red flour beetle (*Tribolium castaneum*), showed that recent selective pressures (within 14 generations) are important drivers of immune priming effects, mediated by regulation of immune genes (Ferro et al., 2019).

Collectively, the results of this study suggest that recent domestication in populations of lab model organisms may result in

divergent host–pathogen interactions in repeat exposure events compared to their wild field counterparts, and these divergent responses were exacerbated by high temperature events. Importantly, while this study shows that the domesticated and field *M. sexta* populations clearly differ in thermal, immune and developmental traits consistent with domestication effects, we note that only two populations were compared. Further comparisons are needed to confirm these survival effects result from evolutionary adaptation to domesticated environments in this and other species. Still, these results carry important implications for future work using lab models to represent wild field populations. Relying on studies in domesticated model organisms without consideration of the differences between domesticated and field populations could result in missing nuance of host–pathogen interactions under climate change, and in some cases inaccurate findings. For example, in the case of the present study, results for the field population suggest that HWs could decrease the effectiveness of biocontrol agents after repeated infections, while the opposite is true for the domesticated population. Studies using lab strains are critical to our mechanistic understanding across levels of biological organization, but we suggest the unique selective pressures acting on recent generations of model species in the lab environment should be considered when extrapolating expectations or interpretations onto natural populations.

AUTHOR CONTRIBUTIONS

Katherine H. Malinski, Christopher S. Willett and Joel G. Kingsolver conceived the ideas and designed methodology; Katherine H. Malinski collected the data, analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no competing interests.

DATA AVAILABILITY STATEMENT

All data and code used in analysis and visualization are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.pvmcvdnv9 (Malinski et al., 2025).

STATEMENT ON INCLUSION

Our study included collaborations with scientists and agriculturalists local to the region of study (North Carolina, see acknowledgements).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Cumulative daily survival proportions (*y*) of *Manduca sexta* from the day of secondary infection (*x*) until all individuals had either wandered (survived) or died.

Figure S2. Lethal dose curve for *Bacillus thuringiensis* in lab population *Manduca sexta* in the first day of fourth instar (panel A), or third day of fifth instar (panel B).

Figure S3. Proportion (*y*) of *Manduca sexta* in the *Bacillus thuringiensis* secondary infection treatment groups surviving to each experimental timepoint (*x*) from the experiment start on the first day of fourth instar (first timepoint).

Figure S4. Final larval mass of *Manduca sexta* in grams (*y*-axis) on the day of wandering for survivors of secondary infection.

Figure S5. Mass of *Manduca sexta* in grams (*y*-axis) on the day of secondary infection (third day of fifth instar).

Table S1. Sample sizes throughout the experiment.

Table S2. Results of hemolymph culturing to test for the timing of clearing of initial infection across treatment groups.

Table S3. Results from analyses of deviances of generalized linear models for final larval mass at wandering for survivors following the secondary infection.

Table S4. Model coefficients from the generalized linear model analyses of within-population mortality rates following the *Bacillus thuringiensis* secondary infection.

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