

**Evaluating the effects of temperature variability on a lepidopteran agricultural pest: from
life history traits to host-pathogen interactions**

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ABSTRACT

Temperature variability associated with climate change is expected to impact insect performance and alter the impacts of pest species and the efficacy of biocontrol strategies. However, our understanding of insect thermal biology is mostly based on measurements of insect performance under constant temperatures, which may not reflect the fluctuating temperatures insects experience over their lifespans in their natural environments. Moreover, thermal performance curves vary for different organismal traits and life stages; thus, understanding the impacts of temperature fluctuations requires measures of multiple traits across the life cycle. Here, I explored the impacts of temperature variability on the life history and demography of *Spodoptera frugiperda*, a widespread insect pest, and its susceptibility to a pathogen commonly used in biocontrol. Using a full factorial experiment with a wide range of temperature treatments, I systematically investigated the effects of temperature mean and fluctuation magnitude on fitness-related traits in each life stage. I then used these data to parametrize stage-structured matrix population models to understand the overall effect of temperature means and fluctuations on expected population growth rates. I found interactive effects of mean temperature and the magnitude of daily fluctuations on survival to maturity, development rates, pupal mass, reproductive output, numbers of viable offspring and population growth rates. Larger fluctuations significantly reduced larval and pupal survival but not adult survival relative to constant temperatures. Effects of variability on individual traits and population growth rates were generally larger and more negative at the highest mean temperature. Changes in expected population growth with increasing variability are primarily driven by differences in reproductive parameters and egg hatch rates. Lastly, I investigated the effects of temperature fluctuations at intermediate and elevated mean temperatures on outcomes in *S. frugiperda* larvae exposed to sublethal doses of *Bacillus thuringiensis* subsp. *kurstaki*. Mean temperature largely predicted survivorship following infection. However, temperature fluctuations modified delays in larval development in those surviving *Bt* infection, including at the intermediate mean temperature. Together, these results demonstrate the potential consequences of temperature fluctuations for multiple organismal traits and in the context of biotic stressors, which may become more salient under climate warming.

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CHAPTER 1

INTRODUCTION

Insects in their natural environments are exposed to highly variable temperatures on a daily basis and their body temperatures often closely track these changes in ambient temperatures (Harrison et al., 2012). The potential consequences of short-term temperature variability for insect physiology and ecology have been increasingly recognized by biologists over the last few decades (Colinet et al., 2015; Stoks et al., 2017). However, insect thermal performance is still often studied using insects held in constant temperatures, with estimates from these studies informing models of insect distribution and spread (Rebaudo and Rabhi, 2018; Buckley et al., 2018). Even among laboratory experiments that do incorporate fluctuations in thermal treatments, the systematic effects of temperature variability on these responses are usually not the focus of investigation – studies often include a single fluctuation treatment or examine the effects of variability at a single intermediate temperature (e.g. Prasad et al., 2022).

Assuming that we can infer how insects will fare in an environment by measuring their performance in a constant environment with the same average temperature has several shortcomings. Most relationships between temperature and organismal performance (defined here as “any measure of an organism’s capacity to function”; Angilletta, 2009) are nonlinear and several are asymmetric due to metabolic and physiological constraints (for instance, enzyme denaturation thresholds). A typical thermal performance curve (TPC) is shown in **Fig. 1-1**. With increasing temperature, performance typically accelerates from a lower performance threshold until an inflection point, decelerates from the inflection point to a performance maximum, and declines steeply thereafter (Angilletta, 2006). Temperature fluctuations are therefore generally expected to increase performance over time in the initial “concave up” portion of the curve, decrease overall performance in the “concave down” portion of the curve (for instance, around the temperature at which performance is maximized) and have minimal impacts on performance in any linear regions of the curve. Ruel and Ayres (1999) and Denny (2019), among others, encouraged biologists studying temperature-performance relationships using constant temperatures to account for the effects of variability by integrating the thermal performance curve across the sequence of temperatures actually experienced by organisms. Incorporating fine-scale temperature variability in this manner can considerably improve predictions of how

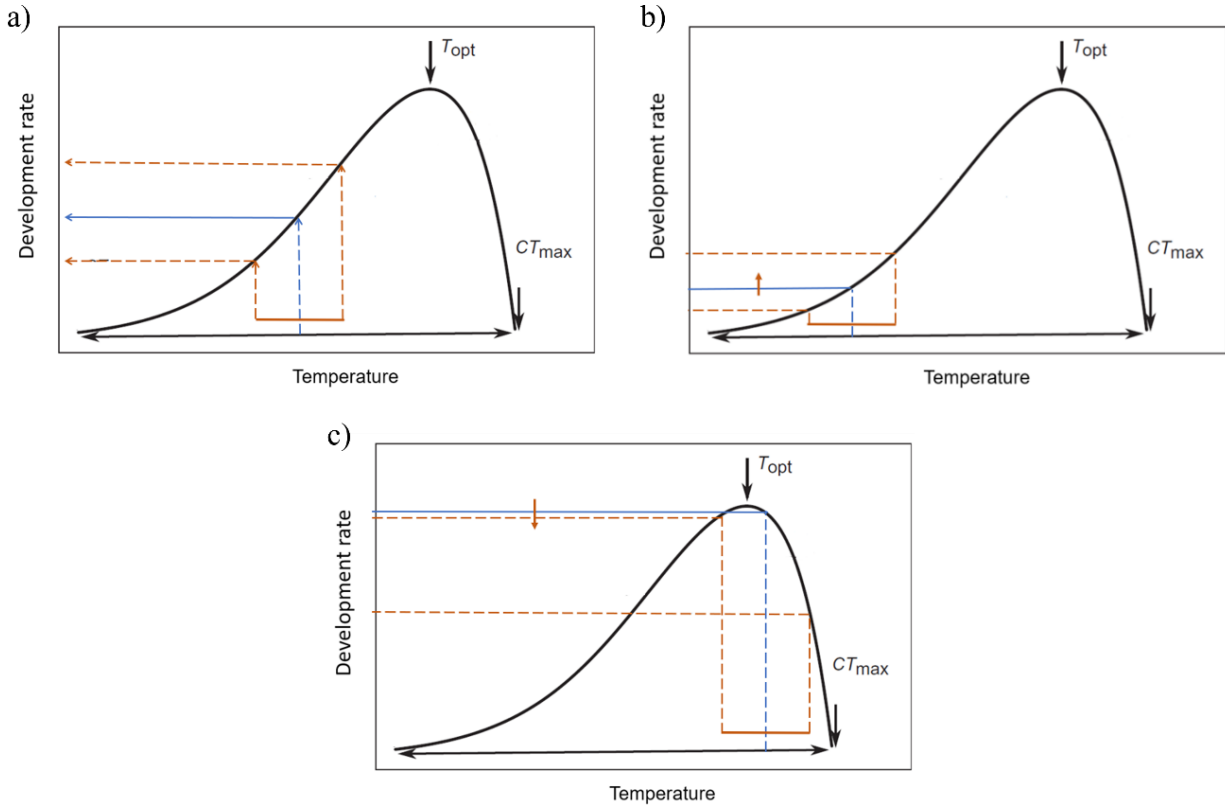


Figure 1-1. Hypothetical thermal performance curve showing the temperature-dependence of development rate. The blue line shows development rate at the mean temperature $f(\bar{x})$, while the brown lines indicate the development rate at diurnal maxima and minima. Key features include the critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}), which represent the upper and lower limits of thermal performance, and the thermal optimum (T_{opt}), at which performance is maximized. (a) In the linear portion of the curve, if variability is symmetric around the mean, the development rate at the mean temperature approximates the average development rate – any increases or decreases in development rates over the cycle are counterbalanced. (b) Variation in temperature over the concave-up portion of the curve generally increases the time-averaged development rate over $f(\bar{x})$ – the increase in development rate when conditions are favorable exceeds any decrease in the rate when conditions are unfavorable. (c) Variation in temperature over the concave-down portion of the curve generally decreases the time-averaged development rate over $f(\bar{x})$. Figures adapted from Sinclair et al., 2016.

organisms respond to temperature change (Sheldon and Dillon, 2016).

However, predicting the effects of variability by averaging thermal performance curves overlooks the impacts of temperature variability on the physiology of organisms and the energetic costs they sustain over time. These time-dependent physiological responses to temperature variability can cause nonlinear averaging predictions to diverge from actual insect performance in variable environments (Kingsolver et al., 2015). Fluctuations that expose insects to stressful temperatures could trigger hardening and heat shock responses in the short-term, exert higher energetic demands on the organism and result in the accumulation of physiological damage over time (González-Tokman et al., 2020). Conversely, depending on the range over which temperatures fluctuate, such environments may facilitate recovery during more benign phases of the fluctuation (Vajedsamiei et al., 2021) or metabolic acclimation to extreme temperatures (Williams et al., 2012; Bozinovic et al., 2013). Even more moderate fluctuations could alter organismal activity patterns and the timing of critical functions (for instance, diel narrowing; Kefford et al., 2022).

Therefore, resolving the biological effects of short-term temperature variability may require more empirical observations from a wider range of insect systems and contexts. This dissertation contributes to this effort by systematically studying the biology of a poikilothermic noctuid moth, *Spodoptera frugiperda*, under a range of temperature regimes that vary in both fluctuation magnitude and average temperature. Through controlled experiments, I assess how these components of the thermal environment interact to affect a suite of fitness-related traits and track these effects across the life cycle (**Chapter 2**). In particular, I compare the effects of variability at mean temperatures assumed to be optimal for growth and development of this species with effects at temperatures considered suboptimal and temperatures nearing upper performance thresholds, and evaluate hypotheses about differences in thermal impacts across traits and life stages.

Chapter 3 links individual-level outcomes to population-level fitness, and asks: what do thermal impacts on different life-history traits ultimately mean for rates of population increase in *S. frugiperda*? I use data from the above experiment to parametrize stage-structured matrix projection models, integrating temperature impacts over the life cycle. By further analyzing the

demographic processes that drive differences among treatments, this project helps resolve the ambiguity over how temperature fluctuations may affect insect populations.

Insects in their natural environments face a multitude of biotic stressors, yet most investigations into the consequences of thermal variability isolate organisms from this context (Stoks et al., 2017). In **Chapter 4**, I address this gap by examining how increased warming and variability influence the outcome of an important interaction between *S. frugiperda* and a pathogen used to control its populations in agricultural settings.

Spodoptera frugiperda, commonly known as fall armyworm, is a tropical species native to the neotropics that does not undergo diapause (Sparks, 1979). A major crop pest in its larval stage, it has rapidly invaded parts of Africa, Asia and Oceania over the past decade (Kenis et al., 2023). Additionally, the dispersal ability of *S. frugiperda* adults allows them to seasonally migrate into subtropical and temperate regions where this species cannot overwinter (Tessnow et al., 2023). Tropical species are expected to be especially susceptible to the effects of projected climate warming, since they already occupy niches close to their physiological optimum (Deutsch et al., 2008; Diamond et al., 2012). Temperature variability, including the frequency of heat waves and diurnal variability, is projected to increase in many portions of the year-round and seasonal range for this species (Englehart and Douglas, 2005; Bathiany et al., 2018; Duffy et al., 2022). Understanding the consequences of warming and variability for *S. frugiperda* biology in the short-term, as I seek to do here, and over longer periods of time is increasingly relevant as these trends continue.

CHAPTER 2
TEMPERATURE FLUCTUATIONS INTERACT WITH MEANS TO IMPACT LIFE
HISTORY TRAITS IN *SPODOPTERA FRUGIPERDA*

A version of this chapter is under review at the *Journal of Thermal Biology* and was coauthored by Anchal Padukone and Kimberly S. Sheldon. Anchal Padukone and Kimberly S. Sheldon conceived and designed the study. Anchal Padukone collected and analyzed data. Anchal Padukone and Kimberly S. Sheldon interpreted the data. Anchal Padukone led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Temperature variability associated with climate change is expected to impact insect performance and alter the ecological and economic impacts of both beneficial and pest species. However, our current understanding of how temperature impacts insect performance is not based on the fluctuating temperatures insects experience in their natural environments or the impact of these fluctuations over the lifespan. Yet, thermal performance curves vary for different organismal traits and life stages, and, thus, understanding the impacts of temperature fluctuations requires measures of multiple traits across the full life cycle. Using fall armyworm *Spodoptera frugiperda*, a global insect pest, we designed a full factorial experiment with a wide range of temperature treatments to study the effects of temperature mean and fluctuations on survival, development and/or reproduction in each life stage. Our results show interactive effects of mean temperature and the magnitude of daily fluctuations on survival to maturity, development time, and pupal mass as well as on the number of eggs laid by females and subsequent egg hatching. However, the impacts of temperature fluctuation varied across life stages: at the warmest mean temperature, larger fluctuations significantly reduced larval and pupal survival but not adult survival relative to constant temperatures. Our results demonstrate the importance of explicitly considering the biological effects of fluctuating temperatures across traits and life stages since these effects may become more salient under climate warming. Understanding how temperature fluctuations impact the entire life cycle of insects can support more accurate forecasts of insect populations under climate change.

Introduction

Temperature fluctuations associated with climate change are expected to have a major impact on insect performance. Insects are exposed to substantial short-term temperature fluctuations in their natural environments: the range of average daily air temperature exceeds 10°C in many parts of the world (Abatzoglou et al., 2018) and can be even greater between microhabitats (Kingsolver et al., 2011; Leith et al., 2024). Since the operating temperatures of many insects closely track ambient fluctuations, this variability impacts rates of critical biological processes (Colinet et al., 2015; Sheldon et al., 2020). In recent decades, research has increasingly recognized the impacts of fine-scale temperature variability on organismal traits, such as metabolism, growth, and development (Colinet et al., 2015; Sheldon et al., 2020). Understanding the consequences of temperature fluctuations may be especially important in a warming world in which insects are increasingly likely to experience temperatures above their thermal optimum (Buckley and Huey, 2016; Pigot et al., 2023). Indeed, compared to shifts in mean temperatures, many researchers expect temperature variability to have an equal or greater impact on insect populations (García-Carreras and Reuman, 2013; Vasseur et al., 2014).

Despite a recognized need to understand the impacts of temperature fluctuations on organisms (Clusella-Trullas et al., 2011; Paaijmans et al., 2013; Vasseur et al., 2014; Sheldon and Dillon, 2016), most studies of insect thermal performance still use constant temperature treatments that do not represent natural environments. By holding insects in the laboratory at the mean temperature of an environment, biologists assume that insects' performance at that temperature represents their overall performance in that environment. This is not always a good approximation because temperature-performance relationships tend to be nonlinear and are often asymmetrical (Angilletta, 2006; Sinclair et al., 2016). With increasing temperature, performance typically accelerates from a lower performance threshold until an inflection point, decelerates from the inflection point to a performance maximum, and declines steeply thereafter (Angilletta, 2006). Therefore, when temperatures fluctuate around a mean temperature, an organism's average performance over time may not equal its performance at the constant mean temperature (i.e., Jensen's inequality; Ruel and Ayres, 1999; Denny, 2017; Massey and Hutchings, 2021). Even if the average environmental temperature is considered near optimal for a species, diurnal extremes can have a disproportionate influence on organismal responses (Lawson et al., 2015).

Exposure to thermal fluctuations alters organisms' physiology and energetic costs over time. In thermally variable environments, metabolic rates increase and insects expend more energy to maintain homeostasis, negatively impacting fitness traits such as growth and survival (Estay et al., 2011; Folguera et al., 2011; Kingsolver and Woods, 2016; Fleming et al., 2021). However, through acclimation, insects can improve performance under fluctuating temperatures (Kingsolver et al., 2015; Kremer et al., 2018; Massey et al., 2022). For example, insects can reduce their thermal sensitivity of metabolism, reducing the energetic impacts of the warmer temperatures associated with thermal variability (Williams et al., 2012; Bozinovic et al., 2013). Temperature variability may also present opportunities for organisms to recover at regular intervals, such as recovering from mid-day heat during cooler periods at night (Vajedsamiei et al., 2021). These mechanisms may explain why realized performance of organisms under fluctuations often differs from predictions made from observations of constant temperatures, even after accounting for the nonlinear effects of thermal performance curves (Niehaus et al., 2012; Vangansbeke et al., 2015; Kingsolver et al., 2015; Marshall et al., 2021). Incorporating fluctuating temperatures into more empirical investigations is thus crucial for accurately predicting how taxa will fare under more variable climates.

The impact of temperature fluctuations is expected to vary across organismal traits and life stages (Schulte et al., 2011; Marshall et al., 2021). Thermal performance curves vary among traits and life stages, leading to differential impacts of temperature variation. For example, when examining organismal traits in woodlice (*Porcellio laevis*), Folguera et al., 2011 found that moderate fluctuations reduced survival but increased maximum size and growth rates, relative to static temperatures. Similarly, some life stages may be inherently more sensitive to thermal stress than others due to differences across ontogeny in morphology, physiology, behavior and microenvironment (Kingsolver et al., 2011; Woods, 2013). For instance, when exposed to heat stress, survival rates were lower for larvae of the leaf beetle *Ophraella communa* relative to eggs, pupae, or adults (Chen et al., 2018). Plastic responses to fluctuating temperatures also vary across life stages: in *Onthophagus taurus* beetles, metabolic sensitivity to fluctuations is higher in adults relative to pupae (Carter and Sheldon, 2020). Moreover, acclimation to fluctuating temperatures in earlier life stages can have lasting impacts on organismal traits (Carter and Sheldon, 2020; Shinner et al., 2020). Yet relatively few studies examine the consequences of

temperature fluctuations for multiple traits or life stages, limiting our understanding of the impacts of temperature variability on insects.

Our goal was to fill these gaps by examining the impact of shifts in temperature mean and fluctuation on organismal performance across traits and life stages. We used the noctuid moth and major agricultural pest *Spodoptera frugiperda* since its short generation time enabled us to track the effects of temperature mean and fluctuations across the life cycle. In recent years, this highly destructive pest species has successfully established and spread beyond its native range. Previous studies examining the effects of temperature on *S. frugiperda* life-history did so by using a series of constant temperatures (Ali et al., 1990; Du Plessis et al., 2020; Chen et al., 2022; Lee et al., 2022; Díaz-Álvarez et al., 2023; Adan et al., 2024) or by incorporating a single fluctuating treatment (Barfield and Ashley, 1987; Prasad et al., 2022). Given the widespread distribution of this pest, understanding the consequences of temperature mean and variability for its development, fecundity, and survival is critical for improving predictions of its abundance and seasonal distribution under projected climate change (Bathiany et al., 2018; Duffy et al., 2022).

Thermal effects depend on how temperatures experienced by individuals map onto trait- and stage-specific thermal performance curves. Thus, we employed nine experimental treatments; our full factorial design consisted of three mean temperatures and three amplitudes of diurnal fluctuation. We monitored individuals in temperature treatments across their lifespans, measuring traits related to survival, development and/or reproduction in each life stage. We hypothesized that the effect of diurnal temperature variation on organismal traits would depend on mean temperature, with more pronounced impacts under warmer and more variable treatments relative to more intermediate conditions. We also hypothesized that increased temperature fluctuations would have more noticeable effects on development time and reproduction than on survival since we expect these traits to be more sensitive to temperature changes.

Methods

Study system

Spodoptera frugiperda (J. E. Smith, 1797) is a holometabolous species with four major life stages. Female adults usually lay egg masses on the underside of host plant leaves (Pitre et al., 1983). Larvae go through 6 instars in favorable conditions (Kenis et al., 2023). After hatching, early instar larvae feed superficially on leaf tissue from one side, often in groups on the same plant and more actively at night, and commonly disperse between plants via ballooning (Sokame et al., 2020). By the third instar, larvae begin to perforate leaves and cause more extensive defoliation. Late-instar larvae often burrow into the growing points of host plants (leaf whorls, buds, corn ears), feeding on kernels and inhibiting plant growth. Mature larvae drop to the ground and pupate 2–8 cm in the soil within loose pupal burrows or on the surface under plant debris (Capinera, 2017). Upon eclosing, adult female moths may disperse over 500 km before ovipositing; this enables seasonal migrations into regions where this neotropical species cannot overwinter, since it does not undergo diapause (Nagoshi and Meagher, 2008; Tessnow et al., 2023).

Temperature experiments

To investigate the effects of fluctuating temperatures across traits and life stages, we reared *S. frugiperda* from eggs in incubators and monitored them through larval, pupal, and adult stages. Our nine temperature treatments were combinations of three mean temperatures (22°C, 26°C, 30°C) and three amplitudes of diurnal temperature variation ($\pm 0^\circ\text{C}$, $\pm 3^\circ\text{C}$, $\pm 6^\circ\text{C}$, hereafter “constant”, “low fluctuation”, and “high fluctuation”, respectively). In the fluctuation treatments, temperatures fluctuated sinusoidally between specified maximum and minimum temperatures to simulate natural cycles. These temperature regimes are representative of daily fluctuations in regions where *S. frugiperda* can complete development year-round or during the growing season (Early et al., 2018). These include regimes estimated to be optimal for growth and development ($26 \pm 0^\circ\text{C}$, $26 \pm 3^\circ\text{C}$, $30 \pm 0^\circ\text{C}$), regimes approaching the lower performance threshold ($22 \pm 3^\circ\text{C}$, $22 \pm 6^\circ\text{C}$), and regimes near the upper performance threshold ($30 \pm 3^\circ\text{C}$, $30 \pm 6^\circ\text{C}$) as determined by previous constant-temperature studies (Ali et al., 1990; Du Plessis et al., 2020;

Ashok et al., 2021; Huang et al., 2021; Malekera et al., 2022; Chen et al., 2022). Photoperiod in the incubators was 14D:10N to simulate day length during the summer growing season.

Eggs of *S. frugiperda* were sourced from a mass-reared stock colony (Benzon Research Inc.) held at 26°C–29°C that had been established using a strain collected from the USDA-ARS Station in Stoneville, Mississippi (USA). Over 1000 eggs were collected from the colony within 8 hours of oviposition and transferred to the University of Tennessee by the following day. Upon receipt, we separated eggs into groups of ~50, and each group was randomly assigned to a temperature treatment (Haahr, 2025). We placed eggs in incubators in sealed Ziploc bags and checked daily until larvae hatched. Given the large number of individuals we tracked, we were unable to run all nine temperature treatments at the same time. Thus, we completed the nine treatments using three different cohorts of eggs. To verify that the use of different egg cohorts did not impact our conclusions, we ran five of the nine treatments as part of a pilot study, all of which were repeated in the main study. We compared three variables (survival to pupation, time to pupation, and pupal mass) between the repeated sets of treatments and found the use of three cohorts did not alter our conclusions about the effects of fluctuating temperatures (Appendix B).

Larval and pupal survival and development

From each treatment, we randomly selected 90–120 neonates that hatched on the same day to monitor. We assigned each larva a unique identification and reared them individually in 44 mL plastic containers on ad libitum artificial diet (Frontier Agricultural Sciences Inc.). For each larva, we recorded daily survival, and larval instar duration and time to pupation in days. We recorded mass and sex of each surviving pupa one day following pupation. We monitored pupae individually in 44 mL plastic cups, which we lined with paper towels to facilitate adult emergence and wing expansion, and recorded survival and days to adult eclosion.

Adult survival and fecundity

To monitor adult survival and fecundity, we paired male and female adults that emerged from the same temperature treatment on the same day since delays in mating can affect overall fecundity in this species (Wang et al., 2021). On the day of eclosion, we transferred breeding pairs to 266 mL plastic containers and returned them to their temperature treatments. We lined

and covered breeding containers with paper towels, which served as a substrate for oviposition, and placed cotton wool saturated with 10% sucrose solution in the containers for adult sustenance. We formed the maximum number of breeding pairs possible every day until 15–20 pairs were achieved for each treatment. For two treatments ($30 \pm 3^{\circ}\text{C}$ and $30 \pm 6^{\circ}\text{C}$), we had between 9 and 11 breeding pairs. We monitored adult survival daily. Every two days, we removed eggs laid by the female on the oviposition substrate and transferred breeding pairs to fresh containers. We placed the collected eggs in Ziploc bags labelled with the collection date and female ID. We returned the collected eggs to the same treatment incubators for a seven-day observation period before we removed the bag and immediately placed it into a freezer.

For a random subset of adult females in each treatment, we counted the number of unhatched eggs and hatched larvae in each Ziploc bag under a stereomicroscope. We kept a separate tally of any eggs left behind in the container that we were unable to collect (i.e., if eggs were laid directly on the plastic container). For each female, we summed the total number of unhatched eggs and hatched larvae from each 2-day collection period together with any uncollected eggs from that period to obtain total egg production for that period. We calculated hatch rates for each period as the percentage of eggs collected on substrate during that interval that hatched into larvae. We extrapolated the total number of hatched larvae for each period by multiplying the total egg production for that period by the hatch rate. To ensure that we allowed sufficient time for mating, we excluded from this subset any females that did not overlap with their paired males for at least two nights and that had also not oviposited by the first day following eclosion. Previous studies and our observations indicate that initial mating generally occurs between the first and second day after emergence in warmer temperatures (26°C – 30°C). Oviposition follows soon after in mated females, whereas unmated females frequently have prolonged pre-oviposition periods (Montezano et al., 2019). While initial mating and oviposition may occur 1–3 days later at cooler temperatures (**Supplemental Fig. 2-7**), all females in this subset that were held in mean 22°C temperatures overlapped with their paired males for at least 7 nights.

Data analysis

Our goal was to examine the effects of temperature variation across traits and life stages for *S. frugiperda*. Prior to analyses, we excluded individuals that did not meet certain criteria. Specifically, for analyses of larval development times and pupal masses, we removed individuals with major prepupal and pupal abnormalities that inhibited further development (**Supplemental Table 2-1**). For analyses of pupal stage duration and time to eclosion, we removed individuals that did not eclose successfully (**Supplemental Table 2-1**). For analysis of survival to pupation or adulthood, we counted only those that pupated normally (without major abnormalities) and those that eclosed successfully as having survived to pupation and eclosion, respectively. Final sample sizes for all analyses are in **Supplemental Table 2-2**.

To analyze the effects of mean temperature and temperature fluctuation on the various life-history traits (below), we constructed generalized linear models (GLMs) predicting each response variable as a function of mean temperature, temperature fluctuation, and their interaction. We specified mean temperature and temperature fluctuation as factor variables in our models because each combination of mean and variance corresponded to an experimental group and because we expect the relationship between temperature and performance to be nonlinear. When possible, we also included the effect of sex to account for potential differences between males and females, and interactions between sex and temperature if sex differences were observed to vary by temperature (Appendix A). We evaluated models using the residual diagnostics tools from the DHARMA R package (Hartig, 2022) and used likelihood ratio tests to compare models including the mean by fluctuation interaction to models that either excluded the interaction or the effect of temperature fluctuation altogether. We further investigated the independent and interactive effects of temperature mean and fluctuation by conducting post-hoc pairwise comparisons of mean responses. We estimated and compared these marginal means using the emmeans R package (Lenth, 2024), and applied the "mvt" adjustment (based on the multivariate t distribution) to correct p-values for each family of comparisons. We conducted all analyses in R version 4.4.1 (R Core Team, 2024).

Survival from hatch to pupation and adult eclosion

We analyzed survival from hatch to pupation and adult eclosion using logistic regression models with binomial error distributions and logit link functions. We could only identify sex at pupation, so we could not include sex as a predictor of survival since some individuals died in earlier stages.

Time to pupation and eclosion, and pupal stage duration

We analyzed total time in days from hatch to pupation, hatch to eclosion, and the duration of the pupal stage using Conway-Maxwell Poisson GLMs (glmmTMB package with `family = compois(link = "log")`; Brooks et al., 2017) since time was measured in discrete units and data were underdispersed. We found no discernible differences in time to pupation between female and male pupae within treatments (**Supplemental Fig. 2-8**), so we did not include sex as a predictor in that model. In our models of time to eclosion and pupal stage duration, we included sex and the interaction between mean temperature and sex as predictors since pupal sex ratios were not even across treatments and sex appears to influence development time following pupation.

Pupal mass

For pupal mass, we first removed individuals with pupal abnormalities and with missing sex identification. Using a Gaussian error distribution and an identity link function, we initially modelled pupal mass as a function of temperature mean, fluctuation, sex, and all two-way and three-way interactions. Although prior work on this species suggests that male pupae are generally larger than females (Malekera et al., 2022), we observed similar mass distributions for males and females in some treatments (**Supplemental Fig. 2-9**). However, we removed the interactions between sex and temperature variables from the model because they were not supported.

Adult lifespan

For post-maturity lifespans, we used the negative binomial distribution with variance quadratically related to the mean to account for overdispersion (`glm.nb` function from the MASS

package in R; Venables and Ripley, 2002). Because females generally outlived males and differences between sexes widened with increasing mean temperature, we included sex and the interaction between mean temperature and sex in our model.

Egg production and hatching

Using data from the subset of adult females monitored for reproductive output, we analyzed lifetime egg production and larvae hatched. For lifetime egg production, we used a negative binomial distribution with variance linearly related to the mean (using the glmmTMB package and specifying family = nbinom1(link = “log”); Brooks et al., 2017) to account for overdispersion in the count data.

For lifetime larvae hatched, we removed data from three females because we did not monitor enough intervals to confidently determine hatch rates (**Supplemental Table 2-2**). We used glmmTMB to fit a hurdle model, specifying a truncated negative binomial error distribution (family = truncated_nbinom1) and log link for the hatch count component and a binomial error distribution and logit link for the zero component. In this way, we accounted for zero-inflation in the hatch counts resulting from several females not producing any eggs that hatched. We modelled the probability that a female produced zero larvae as a function of mean temperature, since this proportion appeared to primarily vary by mean and since our sample size limited model complexity. Then we modelled the non-zero outputs as a function of mean temperature, temperature fluctuation, and their interaction.

Results

Survival to pupation

Overall larval survival ranged from 91% in the lowest mean, constant fluctuation treatment ($22 \pm 0^\circ\text{C}$) to only 55% in the warmest, most variable treatment ($30 \pm 6^\circ\text{C}$). The best model of larval survival included the independent effects of mean temperature and temperature fluctuation, but not their interaction (**Supplemental Tables 2-3, 2-4**). Increasing mean temperature significantly decreased larval survival, averaging across fluctuation conditions: for instance, the probability of surviving to pupation in treatments with a mean of 30°C was about 16% and 24% lower than in treatments with a mean of 26°C and 22°C , respectively (adjusted $P \leq$

0.008; **Supplemental Table 2-5**). Large fluctuations decreased larval survival relative to constant temperatures on average (**Supplemental Table 2-4**), although this effect was small relative to the effect of mean temperature and less visible at a mean of 26°C (**Fig. 2-1a**).

Time to pupation

Among individuals that successfully pupated, the larval stage lasted between 9 and 34 days (**Fig. 2-2a**). The best model of time to pupation included mean temperature, temperature fluctuation, and their interaction (**Supplemental Table 2-6**). Increasing mean temperature decreased expected time to pupation regardless of the magnitude of temperature fluctuation (**Fig. 2-2a**; **Supplemental Table 2-7**). Overall, the effect of temperature fluctuation was noticeable at the coolest (22°C) and warmest (30°C) mean temperatures, but smaller than the effect of mean temperature. Large fluctuations around 30°C significantly delayed expected time to pupation relative to low fluctuations by 11% (1.2 days, adjusted $P = 0.0001$) or no fluctuations by 15% (1.7 days, adjusted $P < 0.0001$) (**Supplemental Tables 2-7, 2-8**). This delay began early in development: at a mean of 30°C, 28% of larvae in high fluctuations took more than 3 days to reach the third instar compared to less than 11% of larvae in low fluctuations or constant treatments (**Supplemental Fig. 2-10**). For a mean of 22°C, expected time to pupation was also delayed at low fluctuations relative to constant and high fluctuations by approximately 5% (1 day, adjusted $P = 0.022$ and 0.041 respectively) (**Supplemental Tables 2-7, 2-8**). In contrast, temperature fluctuation did not significantly alter the time to pupation among treatments with a mean of 26°C (adjusted $P > 0.32$).

Pupal mass

Pupal mass varied from 67.4 to 302.6 mg (**Fig. 2-2b**). The best model of pupal mass included mean temperature, temperature fluctuation, sex, and the interaction between temperature mean and fluctuation (**Supplemental Tables 2-9, 2-10**). We did not find significant differences between males and females ($t = 0.34$, $p = 0.74$). Relative to constant temperatures, we observed lower pupal mass under both fluctuating temperature treatments at the coolest mean temperature (22°C) (adjusted $P \leq 0.0001$; **Supplemental Table 2-11**). At the warmest mean

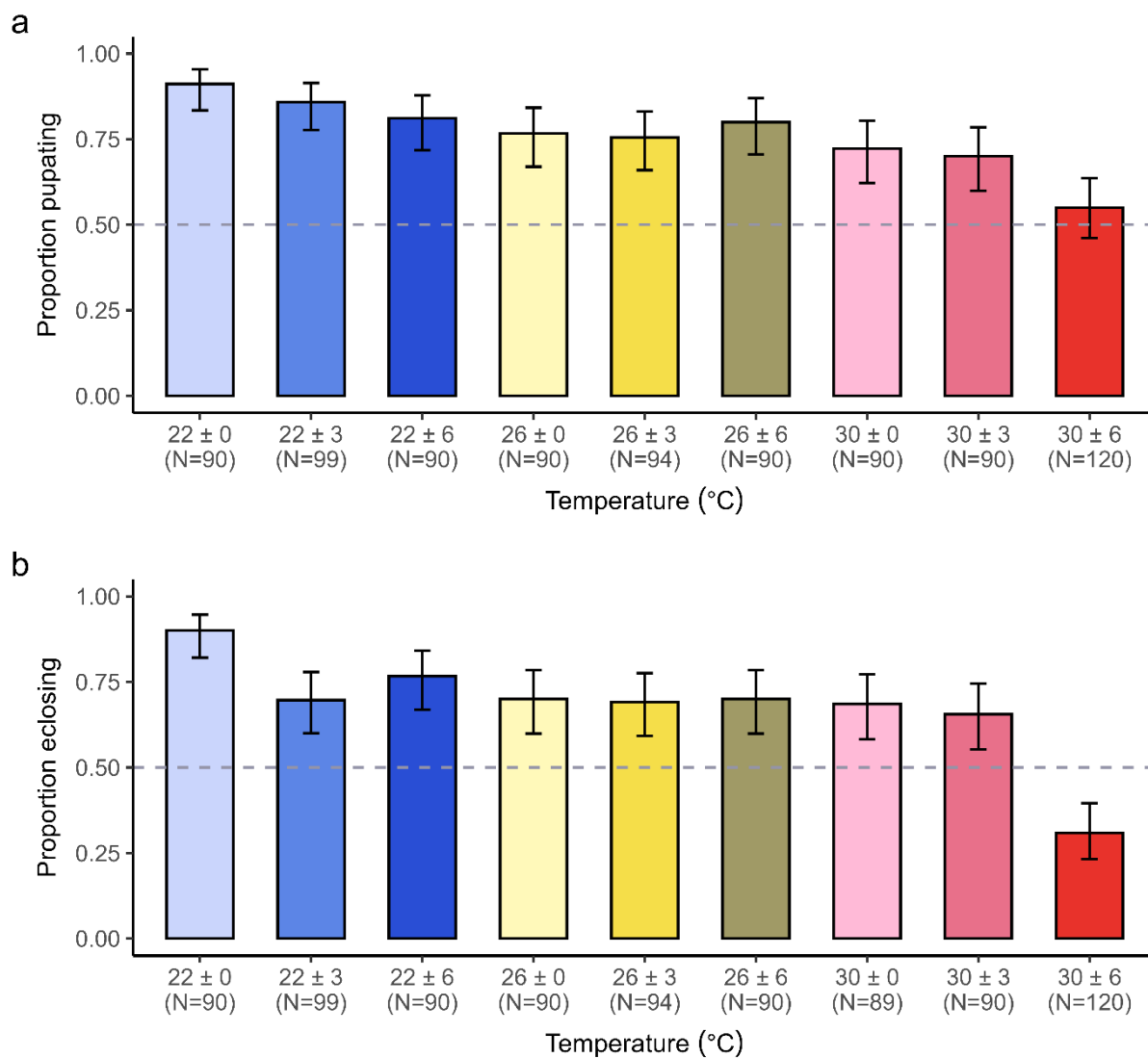


Figure 2-1. Survival of *S. frugiperda* across temperature treatments. a) Proportion of larvae in each treatment that survived to pupation (excluding individuals with major prepupal or pupal abnormalities). b) Proportion of larvae in each treatment that survived to eclosion (excluding individuals that did not eclose completely).

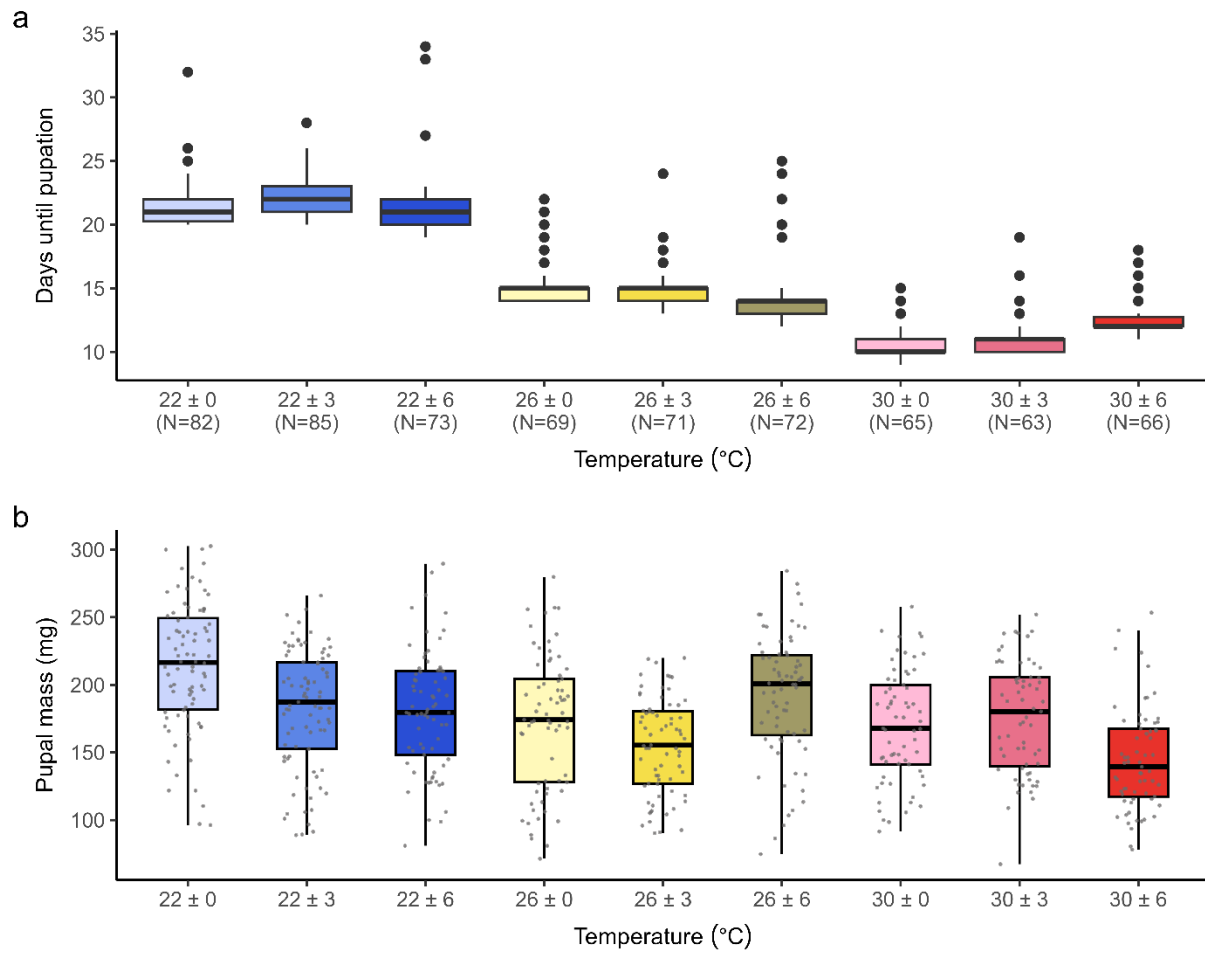


Figure 2-2. Pupal timing and mass across temperature treatments. a) Number of days between hatching and pupation for surviving pupae in each treatment. b) Distributions of pupal mass within each treatment (excluding those with pupal abnormalities). Jittered points indicate individual weights. Boxes and dark lines represent inter-quartile ranges and medians, respectively, for each treatment. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range.

temperature (30°C), the mean pupal mass in the high fluctuation treatment was significantly lower than in either the low fluctuation or constant treatments (adjusted $P \leq 0.020$). Pupal mass was lowest in the warmest, most variable ($30 \pm 6^\circ\text{C}$) treatment.

Pupal stage duration

Among all individuals that successfully eclosed, time spent in pupation varied from 6 to 16 days (**Fig. 2-3**). Our final model of pupal stage duration included the effects of temperature mean, temperature fluctuation, and their interaction, and the effects of sex and its interaction with mean temperature (**Supplemental Tables 2-12, 2-13**). Pupal stage duration decreased with increasing mean temperature (**Fig. 2-3**). Among treatments with the lowest mean temperature (22°C), pupae in constant conditions spent nearly a full day longer in pupation on average than pupae in high and low fluctuations (adjusted $P < 0.0001$) (**Supplemental Table 2-14**). In contrast, pupal stage duration did not vary significantly across fluctuation treatments at the intermediate (26°C) or warmest (30°C) mean temperatures. Male pupae spent longer in pupation than females.

Survival to eclosion

Survivorship in the pupal stage was higher than in the larval stage, with more than 81% of pupae surviving to eclosion for most treatments (**Supplemental Fig. 2-11**). However, survivorship in the pupal stage was substantially lower – 56% – in the warmest and most variable treatment ($30 \pm 6^\circ\text{C}$). Consequently, the differences in survival among fluctuation conditions observed from hatch to pupation became more salient when examining differences in survival from hatch to adult eclosion (**Fig. 2-1b**). Our final model of survival to eclosion included mean temperature, temperature fluctuation, and their interaction (**Supplemental Tables 2-15, 2-16**). Fluctuation magnitude affected survival to eclosion at the coolest (22°C) and warmest (30°C) mean temperatures, but not at the intermediate mean temperature (26°C). At a mean of 22°C, survival to eclosion was 29% higher under constant temperatures than under low fluctuations (adjusted $P = 0.015$; **Supplemental Table 2-17**). At a mean of 30°C, high fluctuations reduced the probability of survival to eclosion by over 50% compared to constant and low fluctuation treatments (adjusted $P < 0.0001$).

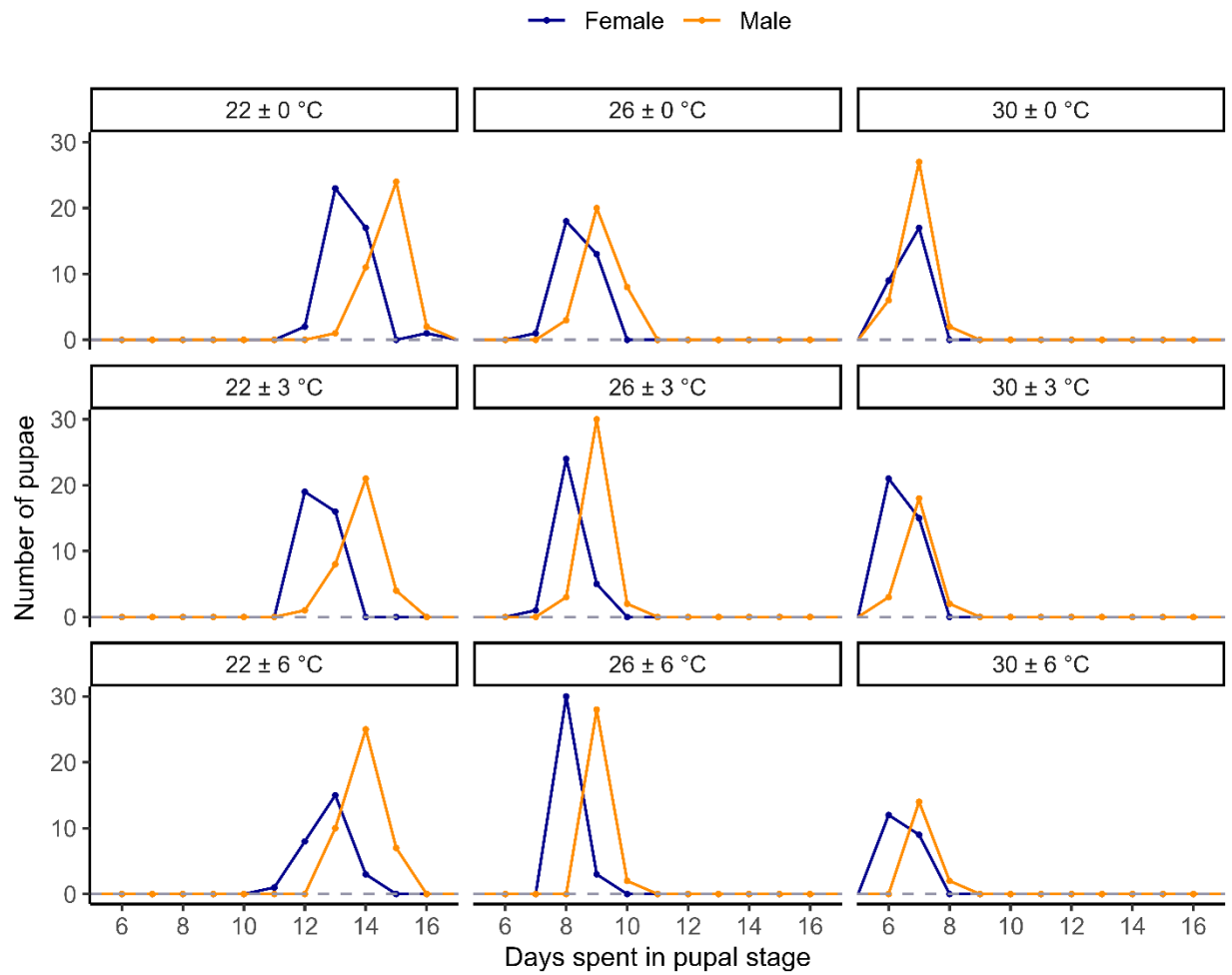


Figure 2-3. Duration of the pupal stage for individuals within each temperature treatment. Points along the lines indicate the number of pupae (blue = female; orange = male) in each treatment that spend the corresponding number of days in pupation.

Development time to eclosion

Time from hatch to eclosion varied from 16 to 47 days. The best model of development time to eclosion included mean temperature, temperature fluctuation, and their interaction as well as sex and the interaction between mean temperature and sex (**Supplemental Tables 2-18, 2-19**). In the treatments with the coolest mean temperature (22°C), females eclosed sooner than males, but these differences largely disappeared at mean temperatures of 26 and 30°C (**Fig. 2-4**). Increasing mean temperature decreased expected time to eclosion in both sexes and fluctuation conditions (**Supplemental Table 2-19**). The effect of fluctuation magnitude on time to eclosion was noticeable at our warmest mean temperature but was smaller than the effect of mean temperature. Large fluctuations ($\pm 6^\circ\text{C}$) at the warmest mean temperature (30°C) significantly increased time to eclosion compared to constant and low fluctuation treatments (adjusted $P < 0.003$; **Supplemental Table 2-20**).

Adult lifespan

Post-maturity lifespan varied from 4–33 days in females and 2–38 days in males (**Fig. 2-5**). The model that best explained adult lifespan included the effects of mean temperature, sex, and the interaction between mean temperature and sex (**Supplemental Tables 2-21, 2-22**). For both males and females, average adult lifespan decreased significantly with each increase in mean temperature (from 22°C to 26°C, and from 26°C to 30°C; **Supplemental Table 2-23**). We found significant differences in expected lifespan between sexes only in the warmest mean temperature (30°C) treatments, in which females lived 65% longer on average than males (adjusted $P < 0.0001$).

Egg production and hatching

Females laid eggs in all treatment conditions, with the greatest lifetime output of 2645 eggs produced by a female across 15 days in the $26 \pm 0^\circ\text{C}$ treatment (**Fig. 2-6a**). Lifetime egg production was best predicted by mean temperature, temperature fluctuation, and their interaction (**Supplemental Tables 2-24, 2-25**). On average, females in treatments with the warmest mean temperature (30°C) laid fewer eggs over their lifetimes relative to other treatments, though most of these comparisons were not statistically significant given large

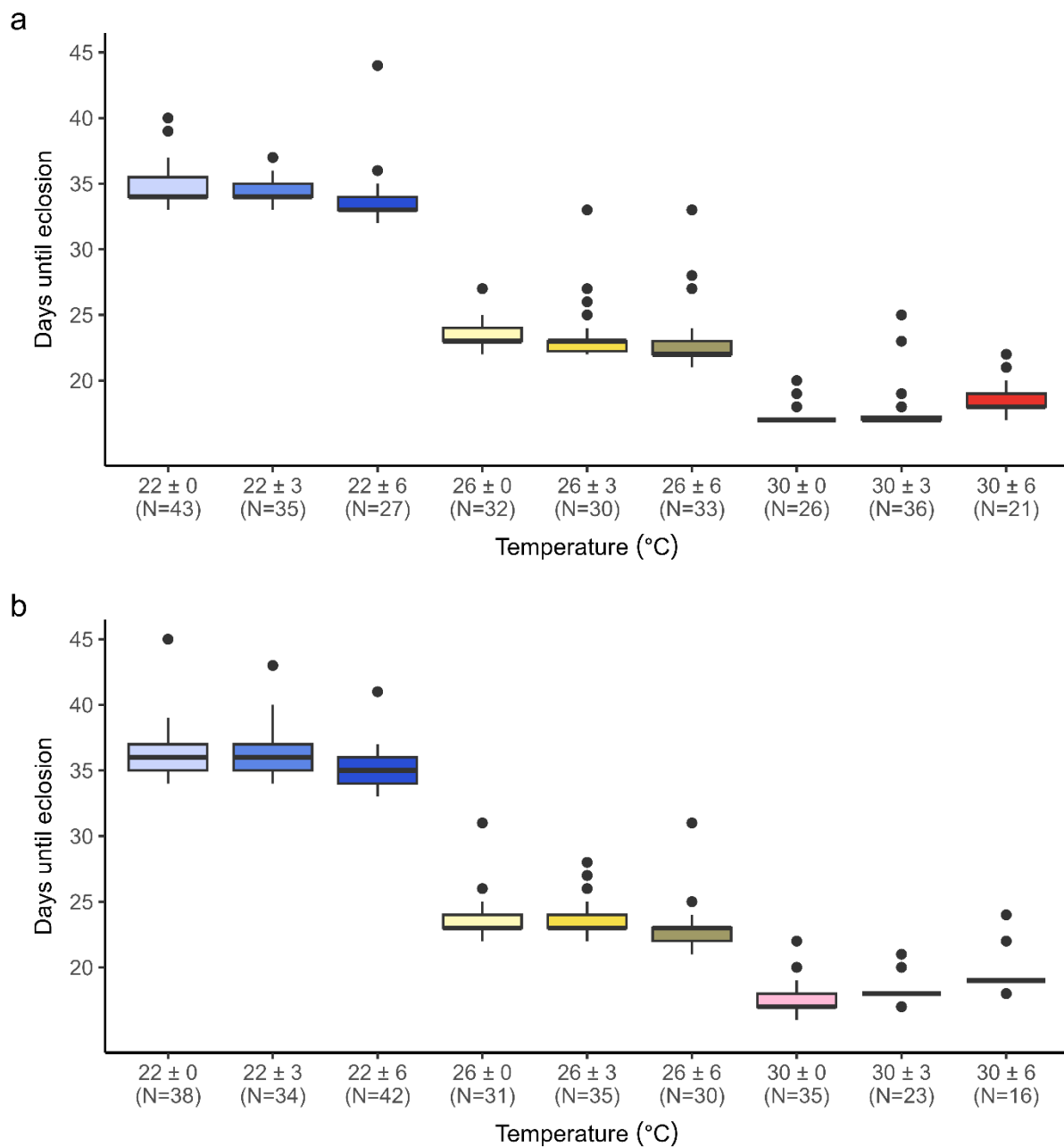


Figure 2-4. Number of days between hatching and adult emergence for a) surviving female adults and b) surviving male adults within each treatment. Boxes and dark lines represent inter-quartile ranges and medians respectively for each treatment. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range.

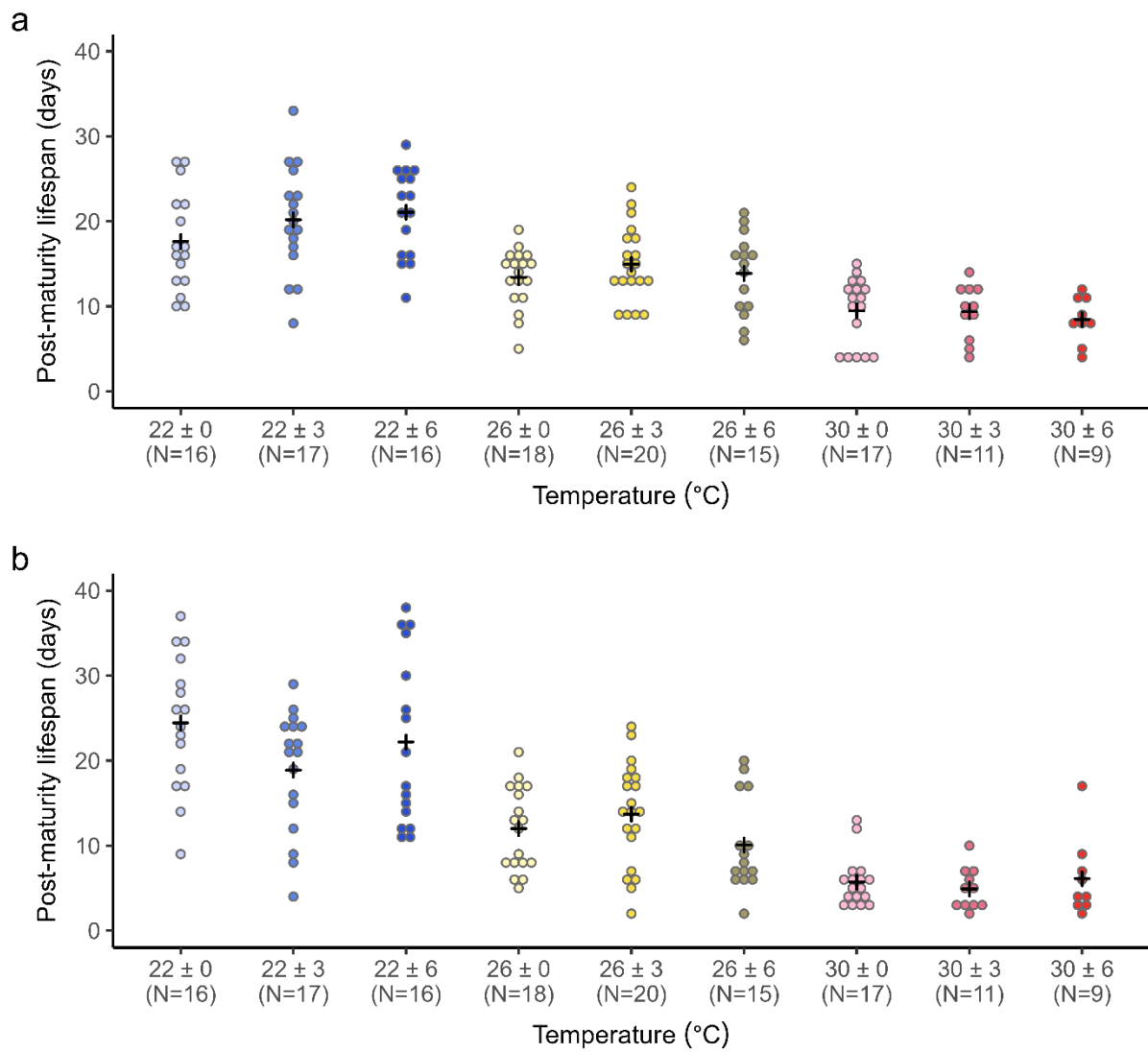


Figure 2-5. Lifespan after adult eclosion across temperature treatments. Dot-plots showing lifespans following eclosion of a) females and b) males in each treatment. Black crosses indicate mean lifespan for each treatment.

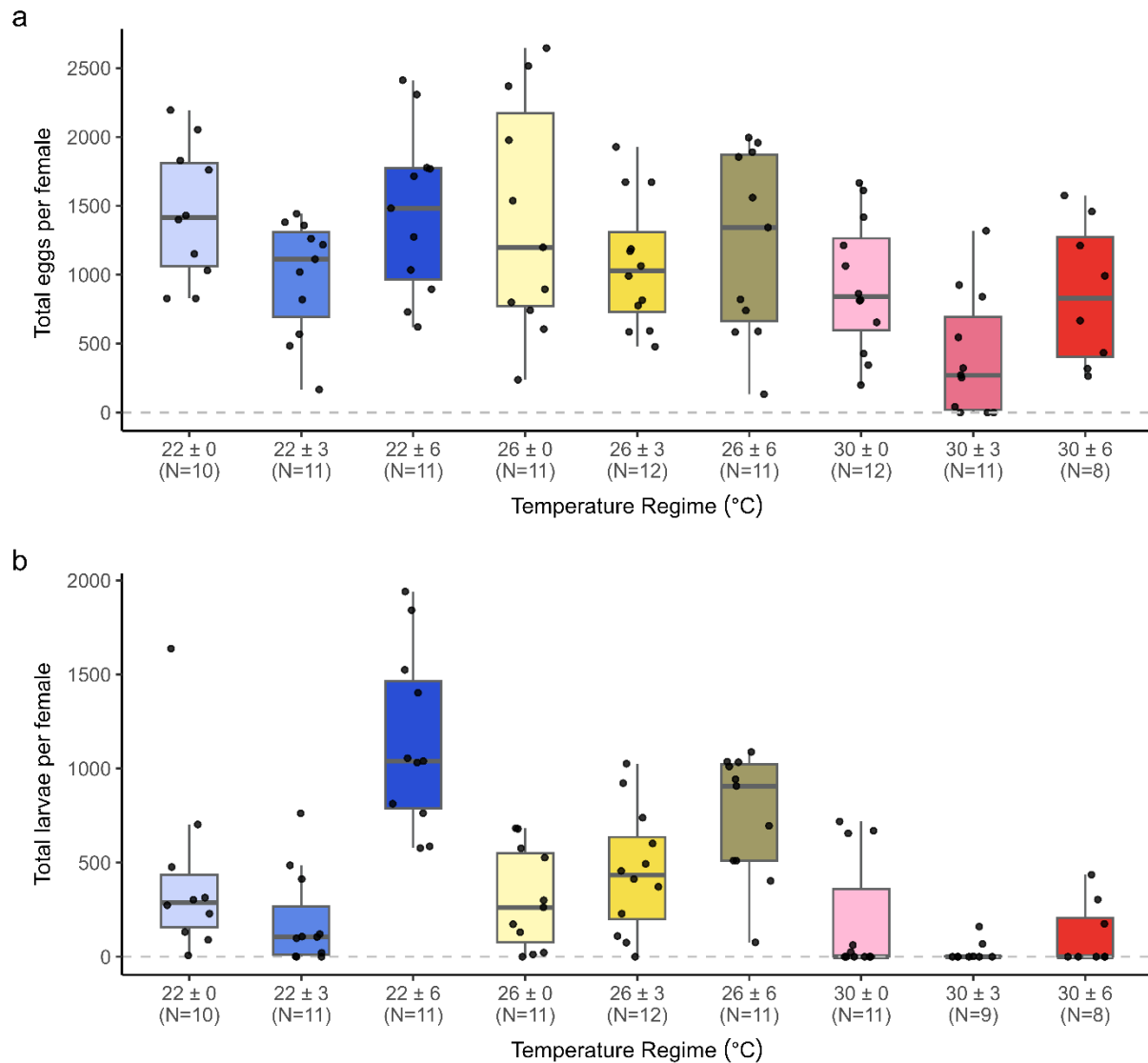


Figure 2-6. Egg production and hatching across temperature treatments. a) Lifetime total eggs laid by females in each treatment. b) Lifetime total larvae produced by females in each treatment. Boxes and dark lines represent inter-quartile ranges and medians respectively for each treatment. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range. Jittered points show data from each female. We could not determine hatch rates and extrapolate larvae totals for three females due to missing data, therefore sample sizes in part b) are lower for two treatments.

inter-individual variability within each treatment (**Supplemental Table 2-26**). Females exposed to low fluctuations around this mean temperature ($30 \pm 3^{\circ}\text{C}$) laid fewer eggs than females in other fluctuation conditions or in cooler mean temperatures (adjusted $P \leq 0.0004$).

The number of successful hatches from eggs laid by females varied widely, from no larvae to a maximum lifetime output of 1941 larvae in the $22 \pm 6^{\circ}\text{C}$ treatment (**Fig. 2-6b**). Among females that produced larvae, total eggs hatched was best predicted by mean temperature, temperature fluctuation, and their interaction (**Supplemental Tables 2-27, 2-28**). Of females that were monitored for egg hatching, most (94%) held in the cooler (22°C) and intermediate (26°C) mean temperatures produced larvae, but only 39% of females held in the warmest mean temperature (30°C) did so. At mean temperatures of 22°C and 26°C , females exposed to high fluctuations produced more larvae on average than females held in constant temperatures (**Supplemental Table 2-29**). We did not find significant differences in eggs hatched among females held in different fluctuation regimes at mean 30°C (adjusted $P \geq 0.85$).

Discussion

Despite the importance of temperature variability for insect thermal performance, we lack an understanding of how different magnitudes of diurnal temperature fluctuation impact life history traits for many species. This knowledge is particularly important for understanding how populations of widespread agricultural pests like *S. frugiperda* will fare under climate change. Our study investigated the effects of shifts in temperature mean and variance on *S. frugiperda* life-history traits, and uniquely, we tracked these effects across the entire life cycle. We found that temperature fluctuations have considerable impacts on survival, development, and reproductive output that are not captured by constant temperature treatments, and the effects of these fluctuations vary substantially by trait and life stage.

The effects of temperature fluctuations depend on the mean temperature of the environment. For most traits, we observe more pronounced effects of fluctuations at our coolest (22°C) and warmest (30°C) mean temperatures than at our intermediate mean temperature (26°C). Recalling Jensen's inequality, biological rates should be higher under variable temperatures than predicted by mean temperatures over the accelerating portions of performance curves and lower than predicted over the decelerating portion of the performance curve. In line

with predictions, fluctuations had a large negative impact at the mean temperature known to maximize performance for some traits, such as development rate (Ali et al., 1990; Du Plessis et al., 2020; Lee et al., 2022; Malekera et al., 2022; Adan et al., 2024). However, contrary to predictions, temperature fluctuations have impacts beyond the expected nonlinear effects. Fluctuations around low to moderate temperatures can quicken development in *S. frugiperda* and other insects (Barfield and Ashley, 1987; Ragland and Kingsolver, 2008; Kingsolver et al., 2015), but in our study, low fluctuations around 22°C actually delayed larval development. Similarly, in other insects, incorporating fluctuations around low mean temperatures can boost survival relative to constant temperatures (Carrington et al., 2013; Paaajmans et al., 2013), an effect we expected at our lowest mean temperature since survival of *S. frugiperda* larvae often declines at and below 22°C (Du Plessis et al., 2020; Huang et al., 2021). Instead, survival to pupation and eclosion at 22°C was lower in both fluctuation treatments relative to the constant treatment, as was pupal mass. These impacts suggest that withstanding repeated, transient exposures to stressful temperatures may divert energy from growth and other processes towards responding to thermal stress and recovering from thermal injuries, causing performance to deviate from nonlinear averaging predictions (Kingsolver and Woods, 2016).

The impacts of transient exposures to temperatures near the lower and upper performance thresholds are asymmetrical. Because thermal performance relationships are nonlinear and left-skewed for many traits, variability at warmer temperatures is more likely to hit or transcend performance thresholds than variability at cooler temperatures (Lawson et al., 2015). Past research on *S. frugiperda* shows that constant temperatures below 19°C and above 33°C reduce survival and fecundity (Ali et al., 1990; Du Plessis et al., 2020; Huang et al., 2021; Lee et al., 2022; Malekera et al., 2022). High fluctuations ($\pm 6^\circ\text{C}$) at our coolest (22°C) and warmest (30°C) mean temperatures exposed organisms to these extreme temperatures for comparable fractions of the diurnal cycle. Yet, we found large fluctuations to have more substantial negative impacts on *S. frugiperda* survival and larval development when temperatures varied around 30°C than when they varied around 22°C. Other insect studies show greater negative impacts of thermal fluctuations at higher mean temperatures (Slein et al., 2023) and on a wider range of traits for heat stress than for cold stress (Jeffs and Leather, 2014). Though acute responses to and recovery from both heat and cold stress and injury are energetically demanding, organisms in warmer

treatments have high metabolic rates, which may raise their risk of mortality from energy depletion. Moreover, while heat acclimation with repeated exposures may improve performance, these mechanisms may involve a larger increase in metabolic costs than cold acclimation (González-Tokman et al., 2020).

Performance curves vary such that temperature fluctuations have differential impacts on organismal traits. The noticeable effects—both positive and negative— of fluctuations on most traits reveal considerable variation in the underlying thermal performance curves, with some traits appearing particularly susceptible to heat stress. For instance, our data suggest that upper thresholds for reproduction may occur at lower temperatures than upper thresholds for survival of immature stages. Thus, an important takeaway from these results is that as climates warm, fertility in insects may decline before mortality. Conversely, in regions with cooler or more moderate temperatures, higher temperature variability and diurnal maxima may stimulate more oviposition – as seen in studies on other insects incorporating fluctuations (Mironidis and Savopoulou-Soultani, 2008; Nyamukondiwa et al., 2010) – and increase fertility. Since reproductive traits tend to have narrow thermal performance breadths (Walsh et al., 2019), more research into how temperature variability affects fertility limits and the mechanisms that contribute to fertility, from gamete development and viability to mating behavior, is crucial for understanding population viability and species distributions under global change (Parratt et al., 2021; van Heerwaarden and Sgrò, 2021).

Different life stages show differences in sensitivity to temperature fluctuations. We recorded a decline in larval survivorship under warmer and more variable temperatures, but pupal survivorship was similar across most temperature treatments, echoing previous findings in *S. frugiperda* (Simmons, 1993; Díaz-Álvarez et al., 2023). Unlike larval development times, pupation durations did not increase with fluctuations around our coolest and warmest mean temperatures. Similarly, Malekera et al., 2022 showed that an increase in temperature that led to faster pupal development in *S. frugiperda* resulted in slower larval development, indicating that the thermal performance curve for development is right-shifted in pupae compared to larvae. Several mechanisms could contribute to differences in sensitivity to thermal fluctuations among insect life stages. Pupae of *S. frugiperda* are exposed to large temperature fluctuations on the soil surface or in the upper regions of the soil, whereas adults and larvae can move to more favorable

microclimates (Pincebourde and Suppo, 2016). Sessile life stages, such as pupae, may thus be under greater selection than more mobile life stages to maintain physiological performance over a wider range of temperatures (Enriquez and Colinet, 2017). Expression levels of genes that encode heat-shock proteins involved in responses to temperature fluctuations and the capacity to acclimate to higher daily maxima can vary among life stages (Yang et al., 2021; Phungula et al., 2023). To fully understand the impacts of temperature fluctuations, greater consideration of how responses vary across life stages is needed, particularly for organisms with complex life cycles.

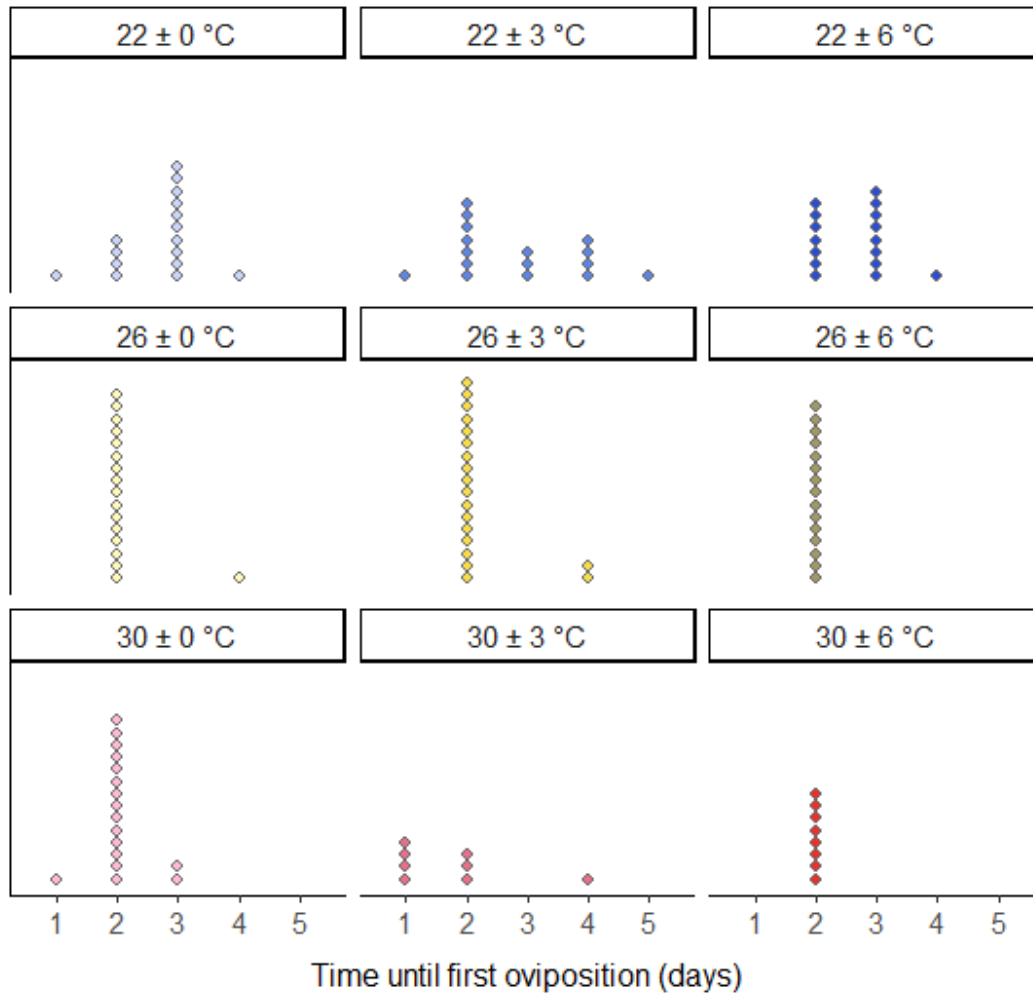
The effects of temperature fluctuations during later life stages may be a consequence of acute impacts on the later life stages as well as exposure to fluctuations in earlier life stages (Rebolledo et al., 2021). In our study, individuals experienced regular fluctuations from the time of hatch until their death. Studies in which organisms only experience these conditions within a specific life stage or only as immatures may show different outcomes. For example, chronic exposures to fluctuations can result in selective mortality or acclimation during larval development that can increase thermal tolerance and performance in later life stages (Marshall and Sinclair, 2012). However, long exposure times can exacerbate any negative impacts of fluctuations (Slein et al., 2023). For example, in our warmest and most variable treatment, damage due to incomplete recovery from thermal injury may have accumulated over time and increased rates of developmental abnormalities. The especially low reproductive output in our warmest mean treatments may also reflect organisms' altered allocation of resources between survival and fecundity due to heat stress earlier in development (Zheng et al., 2017; Awde et al., 2023). Going forward, experiments that monitor organisms over the long-term but vary the proportion of the life cycle when individuals are exposed to each treatment can help pinpoint specific acute and long-term impacts of fluctuations. This could even extend to monitoring transgenerational impacts, which have been observed for heat stress in *S. frugiperda* (Reshma et al., 2023).

Our use of a controlled laboratory setting and an *S. frugiperda* strain that has been maintained in the laboratory for generations constrains the extent to which we can extrapolate our findings to field populations. The effects of ambient temperature variability, though likely still important, may differ in magnitude for organisms in natural environments. Insects in the field or even in greenhouse settings may take advantage of microclimates on host plants that are

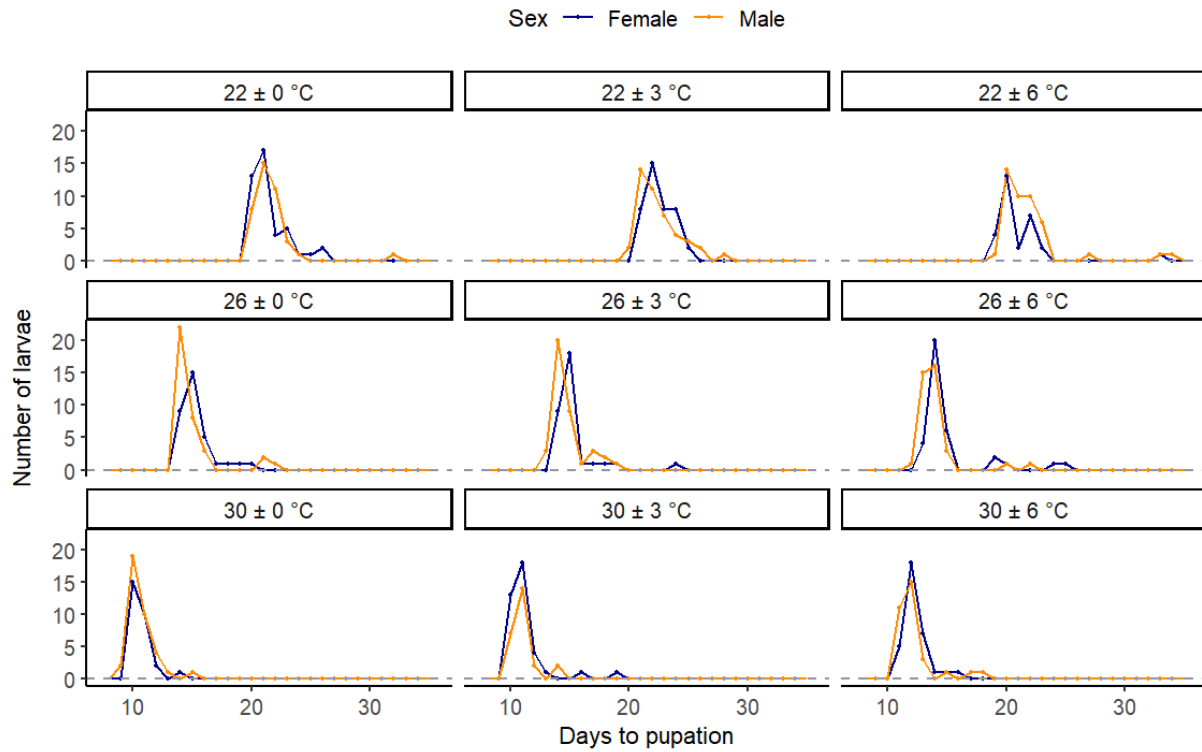
buffered from ambient fluctuations and behaviorally thermoregulate in ways that were not accessible to individuals in our study (Kingsolver et al., 2011; Leith et al., 2024). For instance, female adults may escape unfavorable temperatures by dispersing and delaying reproduction, thereby preventing early declines in lifespan and potentially securing better mating opportunities. Thermal performance may also vary among different geographic populations due to local adaptation and the nutrient composition of different food plants (Chen et al., 2022; Phungula et al., 2023).

The impacts of short-term variability may become more salient under global change as temperatures warm and insects are pushed closer to their upper threshold temperatures for more extended durations. Even where diurnal ranges in air temperatures are not projected to increase, habitat changes, such as desertification and forest canopy loss, may result in insects experiencing larger fluctuations in operative temperatures (Sheldon et al., 2018). Concurrent increases in temperature mean and variance may widen the deviation between observed organismal performance and constant-temperature predictions and increase the relevance of time-dependent effects of fluctuations. Understanding how temperature fluctuations in the upper range of insects' thermal niches impact the entire life cycle would enable us to more accurately forecast insect populations.

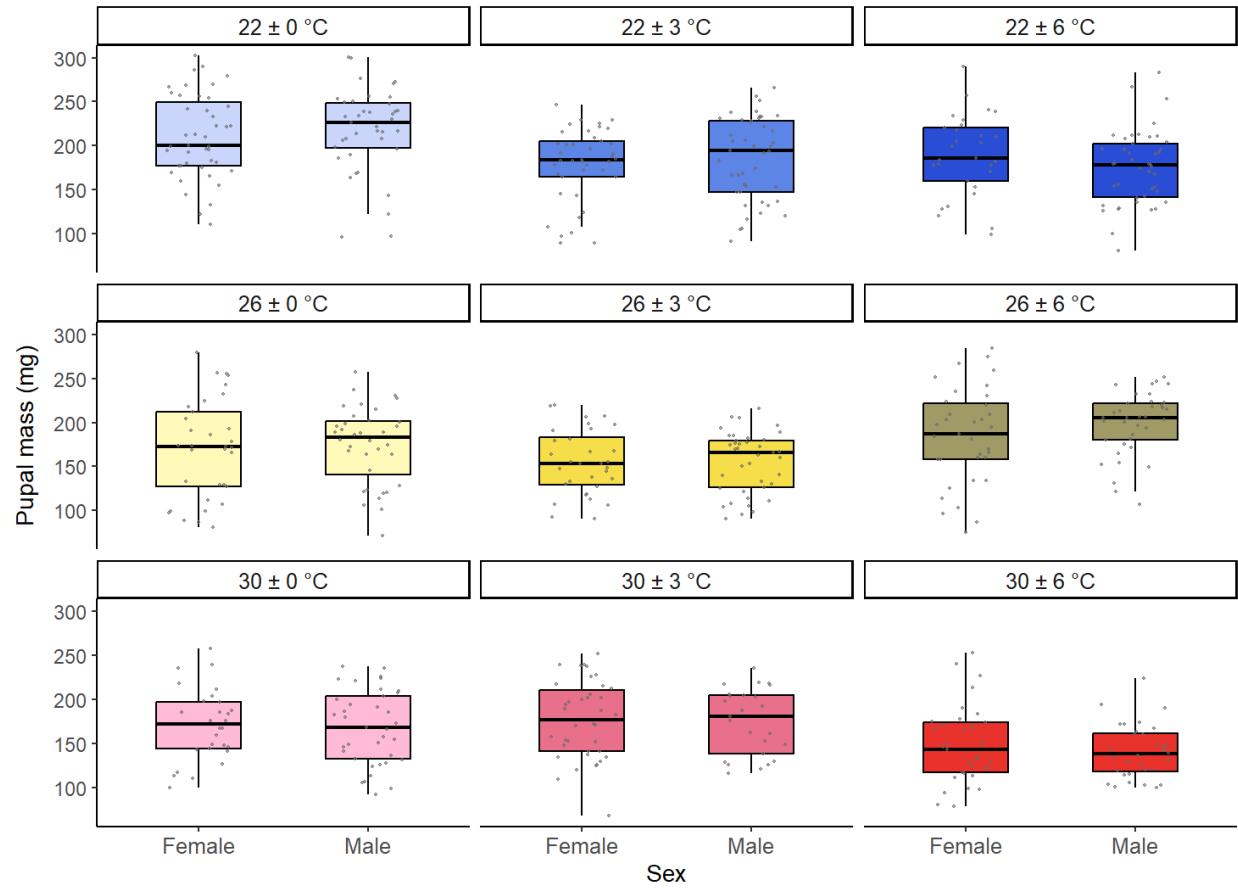
Appendix A: Supplementary material



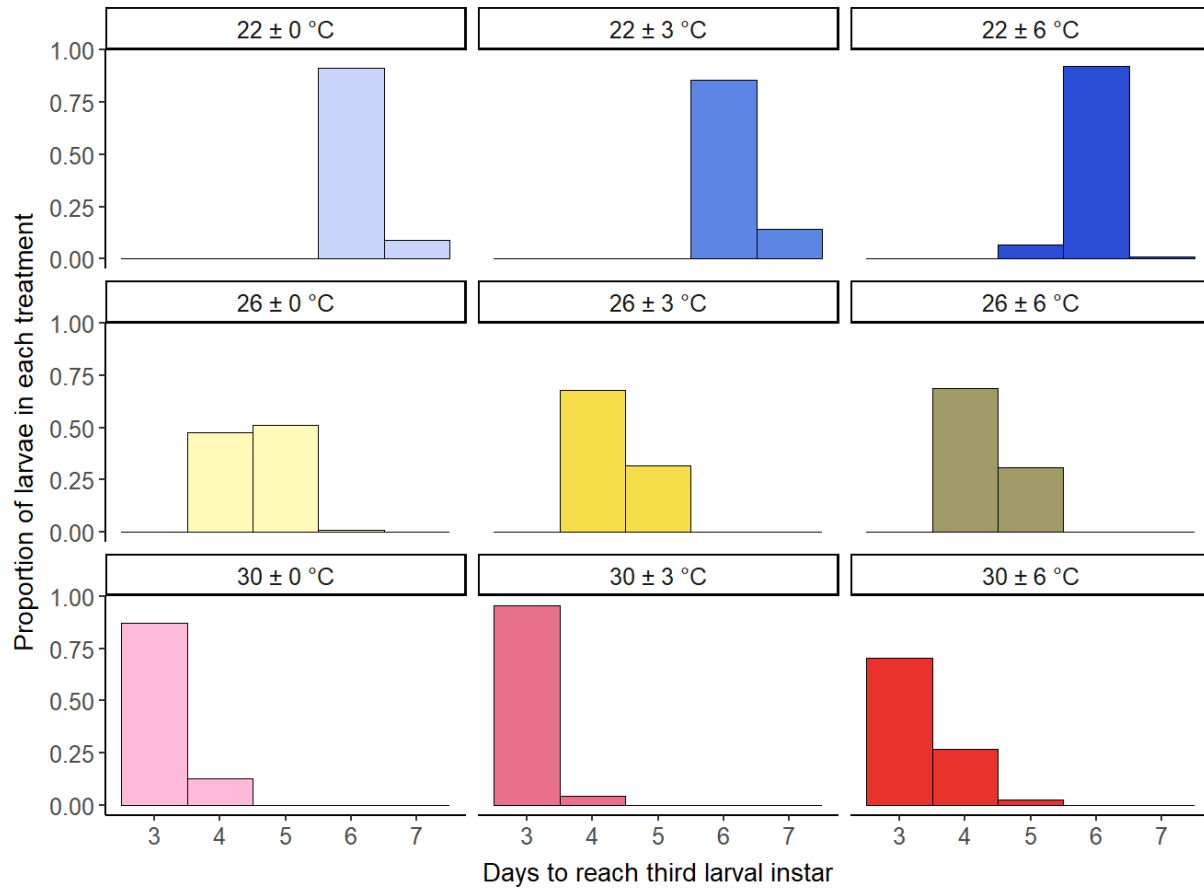
Supplemental Figure 2-7. Time (in days) between emergence and initial oviposition for each adult female observed during the breeding pair phase of the experiment as a function of temperature treatment. Each point represents the time to oviposition for an individual female.



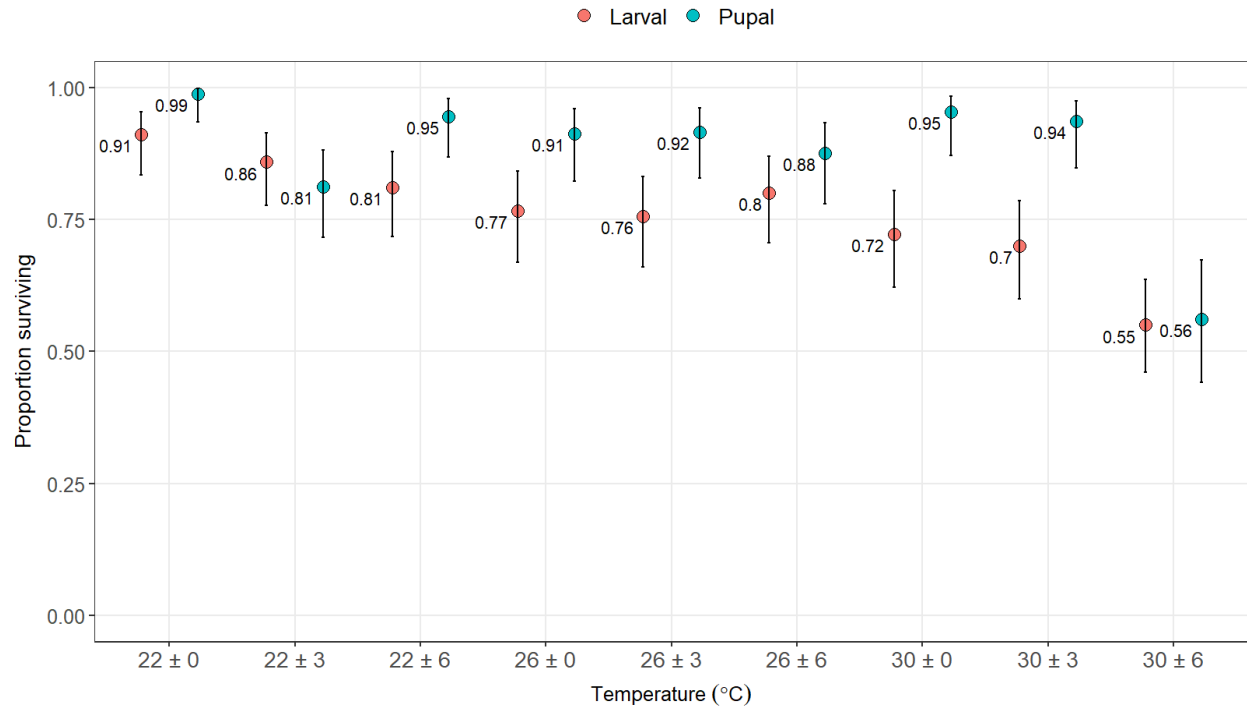
Supplemental Figure 2-8. Time (in days) between hatching and pupation for individuals within each temperature treatment. Points along the lines indicate the number of larvae (blue = female; orange = male) in each treatment that take the corresponding number of days to reach pupation.



Supplemental Figure 2-9. Mass of female and male pupae within each temperature treatment, excluding pupae with major abnormalities. Boxes and dark lines represent inter-quartile ranges and medians, respectively, for each temperature treatment and sex. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range away from the box. Jittered points indicate masses of individual pupae.



Supplemental Figure 2-10. Time (days) from hatching to reaching third larval instar in each temperature treatment. Histogram bars represent the proportion of surviving larvae in each treatment that take the corresponding number of days to reach the third larval instar.



Supplemental Figure 2-11. Survivorship of immature stages in each temperature treatment. Red points represent the proportion of all neonates in a treatment that survive the larval stage and pupate without major abnormalities. Blue points represent the proportion of all normal pupae in a treatment (i.e., without major abnormalities) that successfully eclose. Error bars indicate Wilson 95% confidence intervals for the proportions.

Supplemental Table 2-1. Prepupal and pupal abnormalities and failed eclosion.

Temperature Regime (°C)	Number of individuals that develop major prepupal and pupal abnormalities	Percentage of individuals that develop major prepupal and pupal abnormalities (%)	Number of normal pupae that fail to eclose as adults	Percentage of normal pupae that fail to eclose as adults (%)
22 ± 0	1	1.1	1	1.2
22 ± 3	6	6.1	5	6.8
22 ± 6	6	6.7	1	1.4
26 ± 0	11	12.2	2	3.1
26 ± 3	12	12.8	3	4.4
26 ± 6	4	4.4	1	1.6
30 ± 0	2	2.2	0	0.0
30 ± 3	7	7.8	1	1.7
30 ± 6	30	25.0	9	19.6

Supplemental Table 2-2. Final sample sizes at each life stage.

Temperature Regime (°C)	Individual monitoring (survival, development)			Breeding pairs		
	Neonates *	Normal pupae (without major deformities) +	Eclosed adults	Number of adults followed in pairs (post-maturity lifespan)	Females (lifetime egg output)	Females (lifetime larva output)
22 ± 0	90	82	81	32	10	10
22 ± 3	99	85	69	34	11	11
22 ± 6	90	73	69	32	11	11
26 ± 0	90	69	63	36	11	11
26 ± 3	94	71	65	40	12	12
26 ± 6	90	72	63	30	11	11
30 ± 0	90	65	61	34	12	11
30 ± 3	90	63	59	22	11	9
30 ± 6	120	66	37	18	8	8
Total	853	646	567	278	97	94

* For analyses of survival to pupation, n = 853; for analyses of survival to eclosion, n = 852 as we removed an individual that was accidentally killed during the pupal stage from this dataset

+ One pupa with missing sex information was excluded from analyses of pupal weights (n = 645).

Supplemental Table 2-3. Likelihood ratio tests for survival to pupation.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature * Temperature Fluctuation	Mean Temperature + Temperature Fluctuation	4	6.568	0.1605
Mean Temperature + Temperature Fluctuation	Mean Temperature	2	6.072	0.0480

Supplemental Table 2-4. Final regression model and coefficients table for survival to pupation.

Term	Estimate	Standard Error	z	p
(Intercept)	2.051115	0.218233	9.398747	5.52E-21
Mean 26°C	-0.59017	0.225728	-2.61452	0.008935
Mean 30°C	-1.19302	0.211556	-5.63926	1.71E-08
Fluctuation (low)	-0.18117	0.212262	-0.85353	0.393365
Fluctuation (high)	-0.48304	0.201973	-2.39159	0.016776

Number of observations = 853; Residual deviance = 902.41; Residual degrees of freedom = 848; Null deviance = 945.37; Null degrees of freedom = 852

Supplemental Table 2-5. Pairwise comparisons of odds for survival to pupation.

Contrast	Odds ratio	SE	Null	z ratio	p
Mean 26°C / Mean 22°C	0.554233	0.125106	1	-2.61452	0.047658
Mean 30°C / Mean 22°C	0.303304	0.064166	1	-5.63926	1.03E-07
Mean 30°C / Mean 26°C	0.54725	0.103548	1	-3.18604	0.008153
fluctuation (low) / constant	0.834292	0.177089	1	-0.85353	0.890229
fluctuation (high) / constant	0.616908	0.124599	1	-2.39159	0.08609
fluctuation (high) / fluctuation (low)	0.739439	0.143921	1	-1.55091	0.461663

Supplemental Table 2-6. Likelihood ratio tests for time to pupation.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature * Temperature Fluctuation	Mean Temperature + Temperature Fluctuation	4	50.61	< 0.0001
Mean Temperature * Temperature Fluctuation	Mean Temperature	6	58.59	< 0.0001

Supplemental Table 2-7. Final regression model and coefficients table for time to pupation.

Term	Estimate	Standard Error	z	p
(Intercept)	3.068053	0.010204	300.6755	0
Mean 26°C	-0.35039	0.016769	-20.8955	5.89E-97
Mean 30°C	-0.70286	0.019354	-36.3157	9.1E-289
Fluctuation (low)	0.0452	0.014144	3.195801	0.001394
Fluctuation (high)	0.001592	0.014862	0.107096	0.914713
Mean 26°C: Fluctuation (low)	-0.05388	0.023468	-2.29584	0.021685
Mean 30°C: Fluctuation (low)	-0.00245	0.027156	-0.09027	0.928077
Mean 26°C: Fluctuation (high)	-0.04224	0.023977	-1.76168	0.078123
Mean 30°C: Fluctuation (high)	0.140589	0.026856	5.23497	1.65E-07

Number of observations = 646; Deviance = 2525.8; Residual degrees of freedom = 636; Dispersion parameter = 0.180097

Supplemental Table 2-8. Pairwise comparisons of means for time to pupation.

Temperature Fluctuation	Mean Temperature	Contrast	Ratio	SE	Null	z ratio	p
constant	.	Mean 26°C / Mean 22°C	0.704415	0.011812	1	-20.8955	0
constant	.	Mean 30°C / Mean 22°C	0.495169	0.009584	1	-36.3157	0
constant	.	Mean 30°C / Mean 26°C	0.702951	0.014871	1	-16.6614	0
low	.	Mean 26°C / Mean 22°C	0.667467	0.010958	1	-24.6236	0
low	.	Mean 30°C / Mean 22°C	0.493957	0.009409	1	-37.026	0
low	.	Mean 30°C / Mean 26°C	0.740047	0.015533	1	-14.3425	0
high	.	Mean 26°C / Mean 22°C	0.675281	0.011573	1	-22.9105	0
high	.	Mean 30°C / Mean 22°C	0.569916	0.010611	1	-30.1992	0
high	.	Mean 30°C / Mean 26°C	0.843969	0.017022	1	-8.41068	3.33E-16
.	22	low / constant	1.046237	0.014798	1	3.195801	0.021635
.	22	high / constant	1.001593	0.014886	1	0.107096	1
.	22	high / low	0.957329	0.013962	1	-2.99019	0.041246
.	26	low / constant	0.99136	0.018565	1	-0.46338	0.999877
.	26	high / constant	0.960168	0.018065	1	-2.16041	0.323401
.	26	high / low	0.968536	0.018134	1	-1.70754	0.641065
.	30	low / constant	1.043676	0.024194	1	1.844077	0.540942
.	30	high / constant	1.152785	0.025786	1	6.356315	1.68E-09
.	30	high / low	1.104543	0.02462	1	4.4609	0.000123

Supplemental Table 2-9. Likelihood ratio tests for pupal mass.

Full model	Nested model	Difference in degrees of freedom (numerator d.f.)	Residual degrees of freedom (denominator d.f.)	F	p
Mean Temperature * Temperature Fluctuation * Sex	Mean Temperature * Temperature Fluctuation + Sex	8	627	0.662	0.7251
Mean Temperature * Temperature Fluctuation + Sex	Mean Temperature + Temperature Fluctuation + Sex	4	635	14.39	< 0.0001
Mean Temperature * Temperature Fluctuation + Sex	Mean Temperature + Sex	6	635	12.35	< 0.0001

Supplemental Table 2-10. Final regression model and coefficients table for pupal mass.

Term	Estimate	Standard Error	t-statistic	p
(Intercept)	213.1148	5.08029	41.94933	3.4E-185
Mean 26°C	-41.4064	7.114252	-5.8202	9.33E-09
Mean 30°C	-44.4129	7.237551	-6.13645	1.49E-09
Fluctuation (low)	-31.6509	6.740715	-4.69548	3.26E-06
Fluctuation (high)	-33.0993	7.019574	-4.71528	2.97E-06
Sex (male)	1.156133	3.450588	0.335054	0.737695
Mean 26°C: Fluctuation (low)	14.21729	9.979344	1.424672	0.154743
Mean 30°C: Fluctuation (low)	38.28544	10.25734	3.732492	0.000207
Mean 26°C: Fluctuation (high)	52.70649	10.15366	5.190888	2.82E-07
Mean 30°C: Fluctuation (high)	8.377449	10.38755	0.806489	0.420263

Residual standard error: 43.54 on 635 degrees of freedom; Multiple R-squared: 0.1651; F-statistic: 13.95 on 9 and 635 d.f., p-value: < 2.2e-16; Number of observations = 645

Supplemental Table 2-11. Pairwise comparisons of means for pupal mass.

Temperature Fluctuation	Mean Temperature	Contrast	Estimate	SE	df	t ratio	p
constant	.	Mean 26°C - Mean 22°C	-41.4064	7.114252	635	-5.8202	7.92E-08
constant	.	Mean 30°C - Mean 22°C	-44.4129	7.237551	635	-6.13645	1.69E-08
constant	.	Mean 30°C - Mean 26°C	-3.0065	7.527273	635	-0.39941	0.99996
low	.	Mean 26°C - Mean 22°C	-27.1891	7.000677	635	-3.88378	0.001844
low	.	Mean 30°C - Mean 22°C	-6.12743	7.249932	635	-0.84517	0.990973
low	.	Mean 30°C - Mean 26°C	21.06165	7.55392	635	2.788175	0.076974
high	.	Mean 26°C - Mean 22°C	11.30012	7.237854	635	1.561252	0.744151
high	.	Mean 30°C - Mean 22°C	-36.0354	7.434575	635	-4.847	2.72E-05
high	.	Mean 30°C - Mean 26°C	-47.3355	7.449422	635	-6.35426	2.44E-09
.	22	low - constant	-31.6509	6.740715	635	-4.69548	4.78E-05
.	22	high - constant	-33.0993	7.019574	635	-4.71528	4.55E-05
.	22	high - low	-1.4484	6.953584	635	-0.2083	1
.	26	low - constant	-17.4336	7.360547	635	-2.36852	0.214438
.	26	high - constant	19.6072	7.334749	635	2.673194	0.103941
.	26	high - low	37.0408	7.282781	635	5.086079	7.73E-06
.	30	low - constant	6.634562	7.720311	635	0.859365	0.989882
.	30	high - constant	-24.7218	7.641595	635	-3.23517	0.019754
.	30	high - low	-31.3564	7.704404	635	-4.06993	0.000857

Supplemental Table 2-12. Likelihood ratio tests for pupal stage duration.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Temperature Fluctuation + Sex: Mean Temperature	Mean Temperature +Temperature Fluctuation + Sex + Sex: Mean Temperature	4	29.71	< 0.0001
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Temperature Fluctuation + Sex: Mean Temperature	Mean Temperature + Sex + Sex: Mean Temperature	6	97.51	< 0.0001

Supplemental Table 2-13. Final regression model and coefficients table for pupal stage duration.

Term	Estimate	Standard Error	z statistic	p
(Intercept)	2.595194	0.005805	447.0345	0
Mean 26°C	-0.47612	0.010167	-46.8322	0
Mean 30°C	-0.72564	0.012588	-57.6468	0
Fluctuation (low)	-0.06805	0.007433	-9.15503	5.43E-20
Fluctuation (high)	-0.05403	0.007389	-7.31168	2.64E-13
Sex (male)	0.094843	0.006183	15.33986	4.14E-53
Mean 26°C: Fluctuation (low)	0.04329	0.012257	3.531803	0.000413
Mean 30°C: Fluctuation (low)	0.063811	0.01412	4.519091	6.21E-06
Mean 26°C: Fluctuation (high)	0.032272	0.012395	2.60361	0.009225
Mean 30°C: Fluctuation (high)	0.065999	0.014819	4.453626	8.44E-06
Mean 26°C: Sex (male)	0.006174	0.010071	0.613031	0.539856
Mean 30°C: Sex (male)	-0.02558	0.012415	-2.06059	0.039342

Number of observations = 567; Deviance = 861.7; Residual degrees of freedom = 554; Dispersion parameter = 0.026476

Supplemental Table 2-14. Pairwise comparisons of means for pupal stage duration.

Temperature Fluctuation	Mean Temperature	Contrast	Ratio	SE	Null	z ratio	p
constant	.	Mean 26°C / Mean 22°C	0.623109	0.005444	1	-54.1473	0
constant	.	Mean 30°C / Mean 22°C	0.477864	0.004549	1	-77.5782	0
constant	.	Mean 30°C / Mean 26°C	0.766902	0.008338	1	-24.4113	0
low	.	Mean 26°C / Mean 22°C	0.650675	0.005603	1	-49.9098	0
low	.	Mean 30°C / Mean 22°C	0.50935	0.005359	1	-64.119	0
low	.	Mean 30°C / Mean 26°C	0.782803	0.008681	1	-22.0812	0
high	.	Mean 26°C / Mean 22°C	0.643545	0.00566	1	-50.1122	0
high	.	Mean 30°C / Mean 22°C	0.510466	0.005921	1	-57.969	0
high	.	Mean 30°C / Mean 26°C	0.793209	0.009756	1	-18.8358	0
.	22	low / constant	0.934214	0.006944	1	-9.15503	0
.	22	high / constant	0.947404	0.007001	1	-7.31168	1.66E-12
.	22	high / low	1.014119	0.007918	1	1.795747	0.575877
.	26	low / constant	0.975545	0.009508	1	-2.54037	0.141278
.	26	high / constant	0.978477	0.009737	1	-2.18642	0.307653
.	26	high / low	1.003006	0.00952	1	0.31624	0.999993
.	30	low / constant	0.995771	0.011957	1	-0.35296	0.999985
.	30	high / constant	1.012042	0.012999	1	0.931906	0.983006
.	30	high / low	1.01634	0.013732	1	1.199597	0.925409

Supplemental Table 2-15. Likelihood ratio tests for survival to eclosion.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature * Temperature Fluctuation	Mean Temperature + Temperature Fluctuation	4	28.421	< 0.0001
Mean Temperature * Temperature Fluctuation	Mean Temperature	6	51.474	< 0.0001

Supplemental Table 2-16. Final regression model and coefficients table for survival to eclosion.

Term	Estimate	Standard Error	z	p
(Intercept)	2.197225	0.351335	6.253926	4E-10
Mean 26°C	-1.34993	0.419936	-3.2146	0.001306
Mean 30°C	-1.41856	0.41898	-3.38574	0.00071
Fluctuation (low)	-1.36432	0.413839	-3.29673	0.000978
Fluctuation (high)	-1.00764	0.430753	-2.33925	0.019322
Mean 26°C: Fluctuation (low)	1.324109	0.523488	2.529396	0.011426
Mean 30°C: Fluctuation (low)	1.229196	0.522089	2.354379	0.018554
Mean 26°C: Fluctuation (high)	1.00764	0.539785	1.866743	0.061937
Mean 30°C: Fluctuation (high)	-0.57895	0.526052	-1.10056	0.271089

Number of observations = 852; Residual deviance = 988.86; Residual degrees of freedom = 843; Null deviance = 1086.00; Null degrees of freedom = 851

Supplemental Table 2-17. Pairwise comparisons of odds for survival to eclosion.

Temperature Fluctuation	Mean Temperature	Contrast	Odds ratio	SE	Null	z ratio	p
constant	.	Mean 26°C / Mean 22°C	0.259259	0.108872	1	-3.2146	0.020006
constant	.	Mean 30°C / Mean 22°C	0.242063	0.10142	1	-3.38574	0.011153
constant	.	Mean 30°C / Mean 26°C	0.933673	0.30257	1	-0.21177	1
low	.	Mean 26°C / Mean 22°C	0.974513	0.304593	1	-0.0826	1
low	.	Mean 30°C / Mean 22°C	0.827489	0.257764	1	-0.60789	0.99907
low	.	Mean 30°C / Mean 26°C	0.849132	0.267274	1	-0.51957	0.999705
high	.	Mean 26°C / Mean 22°C	0.710145	0.240845	1	-1.00925	0.972103
high	.	Mean 30°C / Mean 22°C	0.135673	0.043157	1	-6.27952	3.96E-09
high	.	Mean 30°C / Mean 26°C	0.19105	0.057944	1	-5.45754	7.07E-07
.	22	low / constant	0.255556	0.105759	1	-3.29673	0.015066
.	22	high / constant	0.365079	0.157259	1	-2.33925	0.225318
.	22	high / low	1.428571	0.473669	1	1.07572	0.959264
.	26	low / constant	0.960591	0.307955	1	-0.12541	1
.	26	high / constant	1	0.3253	1	6.28E-14	1
.	26	high / low	1.041026	0.333741	1	0.125414	1
.	30	low / constant	0.873612	0.278071	1	-0.4245	0.999936
.	30	high / constant	0.204622	0.061789	1	-5.25422	4.57E-06
.	30	high / low	0.234225	0.069594	1	-4.88508	1.43E-05

Supplemental Table 2-18. Likelihood ratio tests for time to adult emergence.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Temperature Fluctuation + Sex: Mean Temperature	Mean Temperature + Temperature Fluctuation + Sex + Sex: Mean Temperature	4	35.14	< 0.0001
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Temperature Fluctuation + Sex: Mean Temperature	Mean Temperature + Sex + Sex: Mean Temperature	6	35.86	< 0.0001

Supplemental Table 2-19. Final regression model and coefficients table for time to eclosion.

Term	Estimate	Standard Error	z statistic	p
(Intercept)	3.548231	0.00727	488.0915	0
Mean 26°C	-0.38571	0.01239	-31.13	9.5E-213
Mean 30°C	-0.70339	0.014709	-47.821	0
Fluctuation (low)	-0.0019	0.009231	-0.20571	0.837019
Fluctuation (high)	-0.01897	0.009321	-2.0348	0.041871
Sex (male)	0.044508	0.0077	5.780442	7.45E-09
Mean 26°C: Fluctuation (low)	-0.00099	0.015343	-0.06422	0.948795
Mean 30°C: Fluctuation (low)	0.025861	0.017524	1.475766	0.140007
Mean 26°C: Fluctuation (high)	-0.00546	0.015522	-0.35196	0.724865
Mean 30°C: Fluctuation (high)	0.105088	0.018981	5.536535	3.09E-08
Mean 26°C: Sex (male)	-0.04273	0.012682	-3.36925	0.000754
Mean 30°C: Sex (male)	-0.02334	0.015051	-1.55104	0.120891

Number of observations = 567; Deviance = 2209.5; Residual degrees of freedom = 554; Dispersion parameter = 0.111202

Supplemental Table 2-20. Pairwise comparisons of means for time to eclosion.

Temperature Fluctuation	Mean Temperature	Contrast	Ratio	SE	Null	z ratio	p
constant	.	Mean 26°C / Mean 22°C	0.665595	0.007145	1	-37.9204	0
constant	.	Mean 30°C / Mean 22°C	0.489159	0.005944	1	-58.8484	0
constant	.	Mean 30°C / Mean 26°C	0.734921	0.009983	1	-22.6742	0
low	.	Mean 26°C / Mean 22°C	0.664939	0.007287	1	-37.2371	0
low	.	Mean 30°C / Mean 22°C	0.501975	0.006283	1	-55.0657	0
low	.	Mean 30°C / Mean 26°C	0.754918	0.01026	1	-20.6868	0
high	.	Mean 26°C / Mean 22°C	0.661968	0.007414	1	-36.8339	0
high	.	Mean 30°C / Mean 22°C	0.543362	0.007887	1	-42.023	0
high	.	Mean 30°C / Mean 26°C	0.820828	0.01275	1	-12.7105	0
.	22	low / constant	0.998103	0.009214	1	-0.20571	1
.	22	high / constant	0.981212	0.009146	1	-2.0348	0.403615
.	22	high / low	0.983077	0.0095	1	-1.76626	0.597084
.	26	low / constant	0.99712	0.012219	1	-0.23536	0.999999
.	26	high / constant	0.975866	0.012112	1	-1.9684	0.450018
.	26	high / low	0.978685	0.012077	1	-1.74602	0.612103
.	30	low / constant	1.024252	0.015257	1	1.608705	0.709825
.	30	high / constant	1.089938	0.018021	1	5.208608	2.32E-06
.	30	high / low	1.064131	0.017541	1	3.770854	0.00274

Supplemental Table 2-21. Likelihood ratio tests for post-maturity lifespan.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Temperature Fluctuation + Mean Temperature: Sex	Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Sex	4	5.71	0.2218
Mean Temperature + Temperature Fluctuation + Sex + Mean Temperature: Sex	Mean Temperature + Sex + Mean Temperature: Sex	2	0.136	0.9342

Supplemental Table 2-22. Final regression model and coefficients table for post-maturity lifespan.

Term	Estimate	Standard Error	z	p
(Intercept)	2.977194	0.050906	58.48396	0
Mean 26°C	-0.33008	0.073234	-4.50727	6.57E-06
Mean 30°C	-0.75917	0.087108	-8.71519	2.9E-18
Sex (male)	0.103592	0.071278	1.453345	0.146128
Mean 26°C: Sex (male)	-0.25797	0.10415	-2.47687	0.013254
Mean 30°C: Sex (male)	-0.60466	0.130333	-4.63936	3.49E-06

Number of observations = 278; Residual deviance = 285.66; Residual degrees of freedom = 272; Null deviance = 589.13; Null degrees of freedom = 277; Theta = 13.15 (Std. Err.: 2.24)

Supplemental Table 2-23. Pairwise comparisons of means for post-maturity lifespan.

Sex	Mean Temperature	Contrast	Ratio	SE	Null	z ratio	p
female	.	Mean 26°C / Mean 22°C	0.718864	0.052645	1	-4.50727	4.66E-05
female	.	Mean 30°C / Mean 22°C	0.468056	0.040772	1	-8.71519	0
female	.	Mean 30°C / Mean 26°C	0.651106	0.057387	1	-4.86836	2.21E-05
male	.	Mean 26°C / Mean 22°C	0.55541	0.04113	1	-7.94079	6.55E-15
male	.	Mean 30°C / Mean 22°C	0.25568	0.024788	1	-14.0677	0
male	.	Mean 30°C / Mean 26°C	0.460345	0.045814	1	-7.7951	3.09E-14
.	22	male / female	1.109148	0.079058	1	1.453345	0.616351
.	26	male / female	0.856952	0.065075	1	-2.03289	0.25174
.	30	male / female	0.605882	0.066111	1	-4.59212	4.33E-05

Supplemental Table 2-24. Likelihood ratio tests for lifetime egg output.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature * Temperature Fluctuation	Mean Temperature + Temperature Fluctuation	4	19.58	0.0006
Mean Temperature * Temperature Fluctuation	Mean Temperature	6	34.93	< 0.0001

Supplemental Table 2-25. Final regression model and coefficients table for lifetime egg output.

Term	Estimate	Standard Error	z	p
(Intercept)	7.350797	0.147911	49.69732	0
Mean 26°C	-0.15832	0.20991	-0.75421	0.450722
Mean 30°C	-0.4764	0.220198	-2.1635	0.030503
Fluctuation (low)	-0.40558	0.221975	-1.82715	0.067677
Fluctuation (high)	-0.0247	0.203941	-0.12111	0.9036
Mean 26°C: Fluctuation (low)	0.276455	0.307749	0.898312	0.369019
Mean 30°C: Fluctuation (low)	-1.31922	0.426142	-3.09573	0.001963
Mean 26°C: Fluctuation (high)	-0.10776	0.298758	-0.3607	0.718324
Mean 30°C: Fluctuation (high)	-0.05237	0.333466	-0.15704	0.875215

Number of observations = 97; Deviance = 103.6064; Residual degrees of freedom = 87; Dispersion parameter = 378.8533

Supplemental Table 2-26. Pairwise comparisons of means for lifetime egg output.

Mean Temperature	Temperature Fluctuation	Contrast	Ratio	SE	Null	z ratio	p
22	.	low / constant	0.666589	0.147966	1	-1.82715	0.548619
22	.	high / constant	0.975602	0.198966	1	-0.12111	1
22	.	high / low	1.463573	0.31977	1	1.743274	0.610692
26	.	low / constant	0.878863	0.187428	1	-0.60548	0.999073
26	.	high / constant	0.875936	0.19124	1	-0.60672	0.999061
26	.	high / low	0.99667	0.218691	1	-0.0152	1
30	.	low / constant	0.178208	0.065009	1	-4.72821	2.74E-05
30	.	high / constant	0.925828	0.244266	1	-0.2921	0.999996
30	.	high / low	5.195196	2.004284	1	4.271002	0.000374
.	constant	Mean 26°C / Mean 22°C	0.853579	0.179175	1	-0.75421	0.995653
.	constant	Mean 30°C / Mean 22°C	0.621016	0.136747	1	-2.1635	0.318353
.	constant	Mean 30°C / Mean 26°C	0.727543	0.16166	1	-1.43151	0.818842
.	low	Mean 26°C / Mean 22°C	1.125399	0.253234	1	0.525018	0.999675
.	low	Mean 30°C / Mean 22°C	0.166025	0.060787	1	-4.90429	1.09E-05
.	low	Mean 30°C / Mean 26°C	0.147525	0.053118	1	-5.3151	1.75E-06
.	high	Mean 26°C / Mean 22°C	0.766379	0.162974	1	-1.25122	0.905355
.	high	Mean 30°C / Mean 22°C	0.589332	0.147849	1	-2.10769	0.353154
.	high	Mean 30°C / Mean 26°C	0.768983	0.200502	1	-1.00748	0.971847

Supplemental Table 2-27. Likelihood ratio tests for lifetime larval output.

Full model	Nested model	Difference in degrees of freedom	χ^2	p
Mean Temperature * Temperature Fluctuation	Mean Temperature + Temperature Fluctuation	4	13.87	0.0077
Mean Temperature * Temperature Fluctuation	Mean Temperature	6	40.20	< 0.0001

Supplemental Table 2-28. Final regression model and coefficients table for lifetime larva output.

Component	Term	Estimate	Standard Error	z	p
conditional	(Intercept)	5.860653	0.24443	23.97686	4.8E-127
conditional	Mean 26°C	-0.09777	0.341847	-0.28599	0.774883
conditional	Mean 30°C	-0.01148	0.411613	-0.02789	0.977751
conditional	Fluctuation (low)	-0.52657	0.398143	-1.32255	0.185984
conditional	Fluctuation (high)	1.226453	0.277605	4.417973	9.96E-06
conditional	Mean 26°C: Fluctuation (low)	1.018066	0.507747	2.005063	0.044956
conditional	Mean 30°C: Fluctuation (low)	-1.00783	1.309168	-0.76982	0.441406
conditional	Mean 26°C: Fluctuation (high)	-0.35167	0.405693	-0.86683	0.386037
conditional	Mean 30°C: Fluctuation (high)	-1.04254	0.594564	-1.75346	0.079523
zero-inflated	(Intercept)	-2.70805	0.730297	-3.70815	0.000209
zero-inflated	Mean 26°C	-0.06455	1.031788	-0.06256	0.950119
zero-inflated	Mean 30°C	3.143371	0.826478	3.803332	0.000143

Number of observations = 94; Deviance = 1086.4; Residual degrees of freedom = 81; Dispersion parameter = 286.4996

Supplemental Table 2-29. Pairwise comparisons of expected non-zero counts for lifetime larva output.

Mean Temperature	Temperature Fluctuation	Contrast	Ratio	SE	Null	z ratio	p
22	.	low / constant	0.59063	0.235155	1	-1.32255	0.865249
22	.	high / constant	3.409116	0.946389	1	4.417973	0.000121
22	.	high / low	5.772	2.0305	1	4.983218	1.11E-05
26	.	low / constant	1.634767	0.514173	1	1.562679	0.723023
26	.	high / constant	2.398366	0.718714	1	2.919187	0.046149
26	.	high / low	1.4671	0.378575	1	1.485365	0.773966
30	.	low / constant	0.215587	0.268824	1	-1.23053	0.905956
30	.	high / constant	1.201907	0.632457	1	0.349498	0.999983
30	.	high / low	5.575043	7.063374	1	1.356235	0.8483
.	constant	Mean 26°C / Mean 22°C	0.906861	0.310008	1	-0.28599	0.999996
.	constant	Mean 30°C / Mean 22°C	0.988587	0.406915	1	-0.02789	1
.	constant	Mean 30°C / Mean 26°C	1.090119	0.454287	1	0.207057	1
.	low	Mean 26°C / Mean 22°C	2.510041	0.941752	1	2.452865	0.160237
.	low	Mean 30°C / Mean 22°C	0.360846	0.448442	1	-0.8202	0.991477
.	low	Mean 30°C / Mean 26°C	0.143761	0.174849	1	-1.59474	0.700624
.	high	Mean 26°C / Mean 22°C	0.637991	0.139494	1	-2.05552	0.3692
.	high	Mean 30°C / Mean 22°C	0.348533	0.149562	1	-2.45625	0.159573
.	high	Mean 30°C / Mean 26°C	0.546298	0.239716	1	-1.37782	0.836862

Appendix B: Comparing outcomes across cohorts

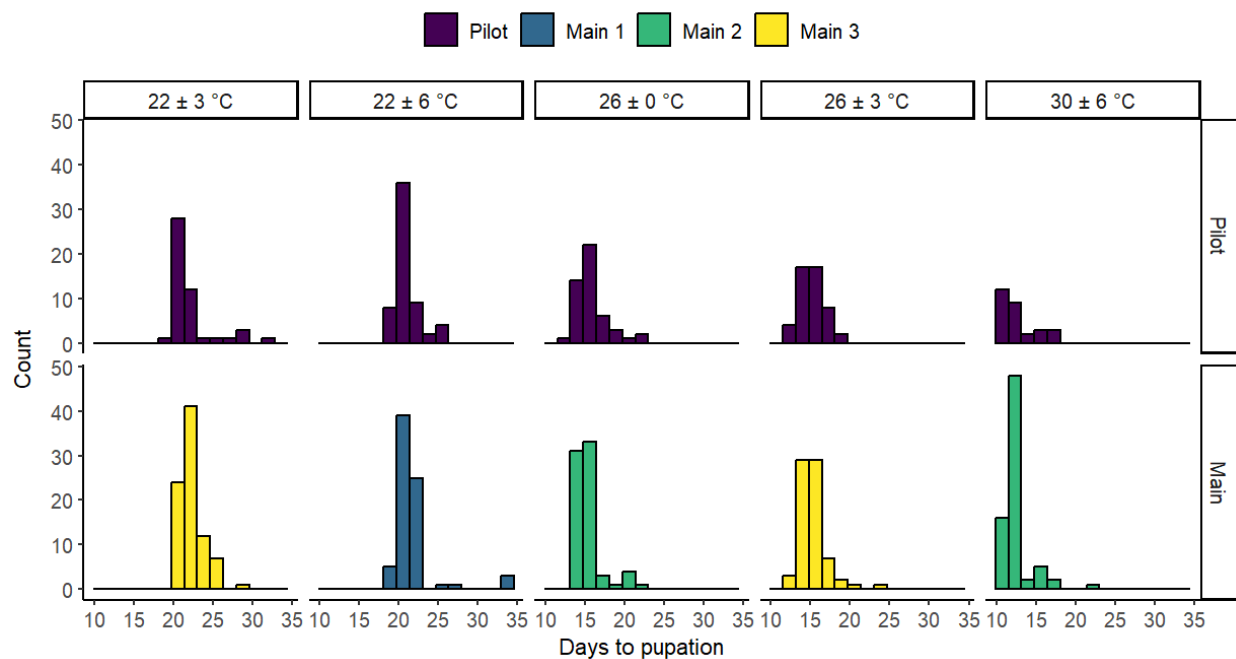
Methods for comparison of three main cohorts with pilot cohort

To verify that the use of three different egg cohorts during our main experiment did not change our conclusions, we ran five of the nine treatments as part of a pilot study. We compared three variables (survival to pupation, time to pupation and pupal mass as a fecundity proxy) between the set of treatments in each of the three main cohorts and the corresponding treatments in the pilot study. For analysis, we constructed generalized linear regression models of development time (Conway-Maxwell Poisson error distribution, log link), survivorship (binomial error distribution, logit link) and pupal mass (Gaussian error distribution, identity link) as functions of temperature regime, experimental phase (pilot versus main) and their interaction (since the main cohort used depended on the temperature regime, we expected differences between phases to vary by temperature regime). We then conducted pairwise comparisons between main and pilot cohorts for each temperature regime using the emmeans package in R (Lenth, 2024).

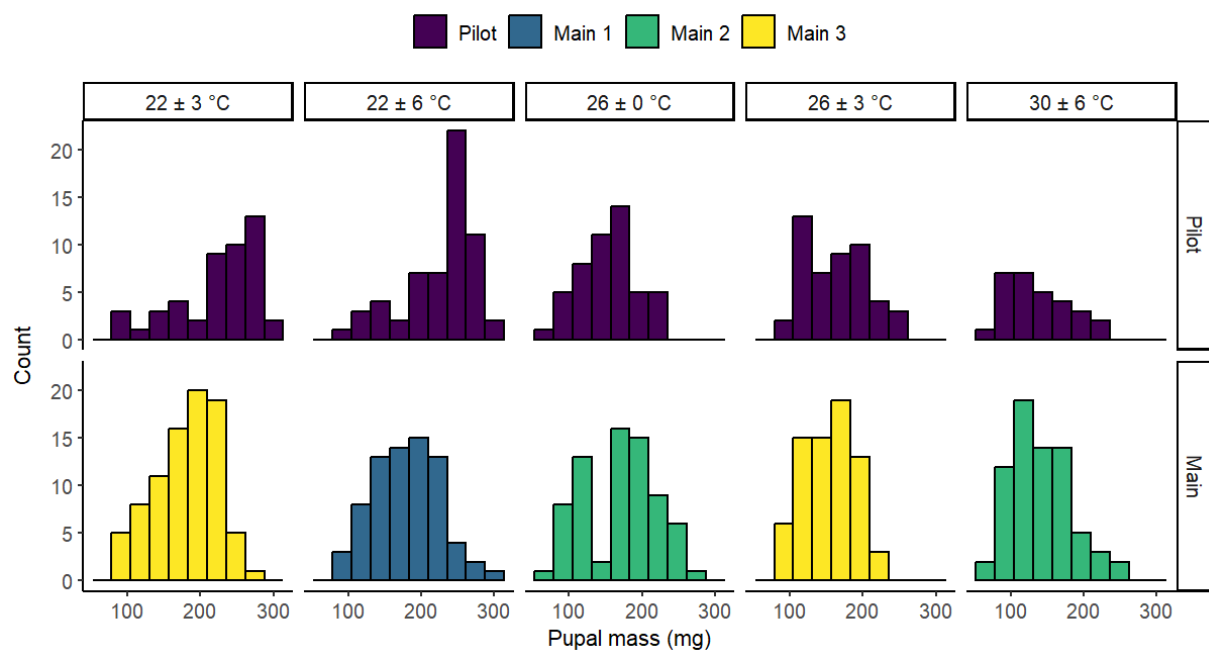
Results for comparison of three main cohorts with pilot cohort

Development time to pupation for each replicated temperature regime was very consistent between cohorts (**Supplemental Fig. 2-12**, below): pairwise comparisons of means revealed no significant difference in development time between the cohort used in the pilot study and the cohort used in the main study for each temperature treatment (Z tests, $p > 0.05$).

Pupal masses were largely comparable between the pilot study and main study for three of the temperature treatments (**Supplemental Fig. 2-13**). For two treatments ($22 \pm 3^\circ\text{C}$ and $22 \pm 6^\circ\text{C}$), pupal masses were lower for main cohorts 1 and 3 in the main experiment compared to the pilot cohort ($t = 5.09$ and 5.85 , $p < 0.0001$). However, at a warmer mean temperature ($26 \pm 3^\circ\text{C}$), we saw no difference in pupal mass between main cohort 3 and the cohort pilot, suggesting no major differences in pupae size across cohorts. Importantly, even if we assumed that main study cohorts 1 and 3 produced smaller than average pupae, or smaller pupae at cooler temperatures, and interpreted our results accordingly, we would still arrive at qualitatively similar conclusions about differences between temperature treatments. For instance, we can still conclude that pupal masses were higher at $22 \pm 0^\circ\text{C}$ compared to $22 \pm 6^\circ\text{C}$ (both run using main cohort 1).

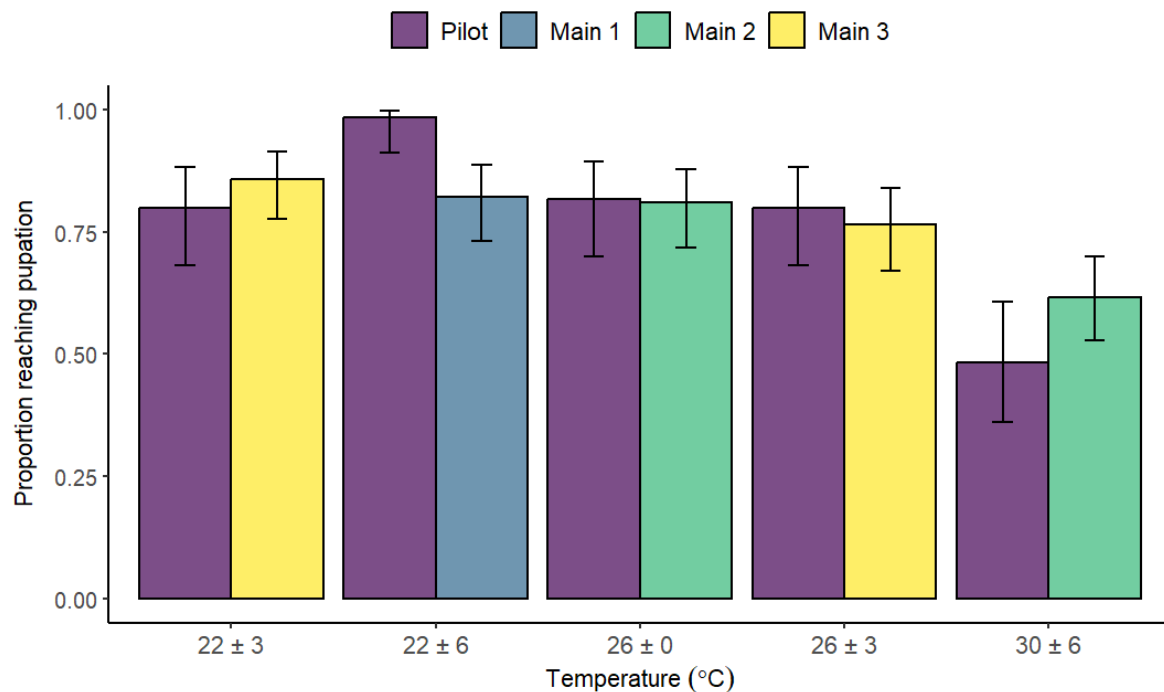


Supplemental Figure 2-12. Histograms of development times (days) between hatching and pupation of cohorts in the pilot study (top) and the main experiment (bottom) for the five temperature treatments. Different cohorts are indicated by different plot colors.



Supplemental Figure 2-13. Histograms of pupal masses of cohorts in the pilot study (top) and the main experiment (bottom) for the five temperature treatments. Different cohorts are indicated by different plot colors.

Larval survival was comparable between the pilot study and main study for four of five temperature treatments (**Supplemental Fig. 2-14**; Z tests, $p > 0.05$). For one treatment, $22 \pm 6^\circ\text{C}$, survival was lower in the main study cohort (“main cohort 1”) relative to the pilot study cohort ($Z = 2.47$, $p = 0.0149$). However, when we compared within main cohort 1, responses were as expected, with larvae at $22 \pm 6^\circ\text{C}$ significantly less likely to survive than larvae in $22 \pm 0^\circ\text{C}$. Even if we assume that main cohort 1 had lower survival on average, our qualitative results are the same. Thus, from these results, our overall conclusions for survivorship are robust to potential differences between cohorts.



Supplemental Figure 2-14. Proportion of larvae surviving to pupation for cohorts in the pilot study and the main experiment for the five temperature treatments. The color of each bar corresponds to a particular cohort (see legend).

CHAPTER 3
IMPACTS OF TEMPERATURE VARIABILITY ON POPULATION GROWTH RATES
IN *SPODOPTERA FRUGIPERDA*

Abstract

Climate change is expected to impact insect populations through increases in average temperatures as well as changes in temperature variability. However, many predictive models of insect populations are based on records of life history traits under constant temperatures, which do not reflect the fluctuations insects experience in their natural environments. Our previous work on the widespread agricultural pest *Spodoptera frugiperda* showed that chronic exposure to realistic fluctuating temperatures altered survival, development and fecundity relative to performance at a constant mean temperature. Notably, the impacts of increased fluctuation varied across life stages and biological rates. Here, we employ stage-structured matrix population models to understand how these variable impacts of temperature fluctuations on different stages and traits ultimately affect population dynamics. We parametrized models using data from *S. frugiperda* individuals that had been exposed to constant or fluctuating temperatures over their lifespans. Eigenanalyses of our models revealed that the effect of fluctuation magnitude on long-term population growth rate depended on mean environmental temperature: fluctuations around 30°C reduce projected growth, whereas fluctuations around 26°C increase projected growth. Perturbation analyses suggest that increasing temperature variability affects population dynamics primarily through changes to reproduction (egg production, hatch success and the duration of reproductive activity). Overall, our approach enables us to isolate and compare the effects of temperature variability in different thermal environments on the potential for population growth and to understand the key demographic mechanisms underlying these effects. We also suggest important extensions to these simple deterministic models to account for density-dependence, stochasticity and other factors that may influence insect mortality in the field.

Introduction

Climate change is expected to impact insect populations through increases in average temperatures as well as changes in temperature variability. Under climate warming, insect populations in the tropics are generally expected to decline as even minor temperature increases may exceed upper performance thresholds and result in demographic attritions (Deutsch et al., 2008; Diamond et al., 2012; García-Robledo and Baer, 2021), while some temperate insect populations are expected to increase (Musolin, 2007; Ju et al., 2015; Deutsch et al., 2018;

Pureswaran et al., 2019; Wagner et al., 2021). Changes in temperature variability have also been linked to altered growth rates and increased extinction risks of insect populations across latitudes, particularly for mid-latitude species during the warmer months (Kingsolver et al., 2013; Duffy et al., 2022). Moreover, climate warming may exacerbate the effects of daily temperature variation, even if the amplitude of variation does not change, since the effects of fluctuations depend on the range over which temperatures fluctuate (Lawson et al., 2015). Therefore, research into the consequences of environmental change for insect populations should consider the consequences of concurrent changes in temperature means and variance (Vasseur et al., 2014).

Though temperature variability may alter the vital rates that underpin insect demography (Colinet et al., 2015), many predictive models of insect populations are based on measurements of life history traits under constant temperatures that do not reflect the fluctuations insects experience in their natural environments (Denny, 2017; Rebaudo and Rabhi, 2018; Buckley et al., 2018). The relationships between temperature and fitness-associated traits, such as survival, development and fecundity, are nonlinear and often asymmetric (Angilletta, 2006; Damos and Savopoulou-Soultani, 2012; Sinclair et al., 2016). Consequently, the values of these traits under variable temperatures may differ considerably from their expected values under a constant environmental temperature at the same mean. The nonlinear nature of temperature performance curves is often referred to as Jensen's inequality (Denny, 2017; Ruel and Ayres, 1999). Importantly, exposure to temperature fluctuations may change the energetic costs organisms sustain relative to static temperatures, due to plastic mechanisms such as hardening (Folguera et al., 2011; Kingsolver and Woods, 2016) and acclimation (Williams et al., 2012; Bozinovic et al., 2013) as well as opportunities to accrue energetic costs or recover from thermal stress-related damage (Vajedsamiei et al., 2021; Kefford et al., 2022). These time-dependent physiological effects of fluctuations may explain why the observed performance of poikilothermic invertebrates in fluctuating environments often differs from predictions based on the nonlinear averaging of rates measured under constant temperatures (Kingsolver et al., 2015; Vangansbeke et al., 2015; Marshall et al., 2021). Indeed, previous studies report considerable variation in projected population growth rates between insects exposed to temperature fluctuations over their lifespans and insects held in static thermal environments at the same mean temperature, although

the nature of these differences depends on the species and the range of temperatures used (Bozinovic et al., 2011; Carrington et al., 2013; Zhao et al., 2015; Cheng et al., 2018).

Our overall understanding of how insects will fare under climate change may improve if we clarify the population effects of long-term fluctuations for more species in a wider range of thermal environments, particularly those including stressful high temperatures. More populations may be exposed to heat stress and temperatures in the upper range of species' thermal niches in the coming decades (Pigot et al., 2023). When organisms are regularly exposed to heat stress during diurnal fluctuations, we expect life history trait values and population dynamics to deviate even more from predictions based on constant temperature experiments (Ma et al., 2021; Slein et al., 2023). However, apart from critical thermal maxima, relatively few measurements of biological rates tend to be made beyond thermal optima in the upper ranges of thermal performance curves (Kingsolver and Buckley, 2017). Investigating organismal performance in the tails of thermal distributions and quantifying the sublethal impacts of stressful high temperatures may help resolve the effects of thermal variability on insect biodemography.

Understanding how temperature variability affects insect pests is a particularly relevant goal since the success of insect pests is often attributed to aspects of their life-histories (Wu et al., 2022), such as their fast and plastic generation times. An unresolved question for some widespread pest species is how diurnal temperature fluctuations across a range of average temperatures could affect the growth of their populations. García-Carreras and Reuman (2013) hypothesized that populations close to or in their optimal thermal environments are more likely to be affected by changes in variability than changes in mean temperatures, with pest populations likely benefitting from these changes. Others caution that the impacts of warming and variability on pests are complex and heterogeneous across species and geographies (Lehmann et al., 2020). We focus on the noctuid moth, *Spodoptera frugiperda*, which has in recent years spread beyond its native range in the Americas and successfully established in sub-Saharan Africa, Asia and Oceania (Kenis et al., 2023). The outsized economic impacts of this species and its presence across many ecoregions have made it a focus for several modelling efforts (Barfield et al., 1978; Ramirez-Cabral et al., 2017; Du Plessis et al., 2018; Garcia et al., 2019; Adan et al., 2024). However, many of these predictive models of *S. frugiperda* abundance and distribution have

been parametrized using data from constant temperature experiments (Barfield and Ashley, 1987; Clavijo A et al., 1991; Garcia et al., 2018; Adan et al., 2024).

Our previous investigation of *S. frugiperda* life history showed that exposure to realistic temperature fluctuations across the lifespan can significantly alter survival to maturity, development rates and the number of viable offspring per female relative to constant environments with the same mean temperature (see Chapter 2). Importantly, the impacts of temperature variability differed across biological rates and life stages. In the current study, we employed tools from demography to synthesize these heterogeneous effects and understand the overall consequences of variable temperatures for the population (in the absence of other limiting factors such as parasitism, predation, and competition). We used measurements of *S. frugiperda* survival, stage durations, and fecundity from our previous experiment to parametrize stage-structured matrix population models for nine temperature regimes, which were different combinations of three mean temperatures and three fluctuation amplitudes. From each matrix, we determined λ , the long-term growth rate for a stable population (i.e. a population with a relatively constant proportion of individuals in each stage). We then analyzed these models using a combination of prospective and retrospective approaches to address the following questions:

- 1) How does daily temperature variability affect long-term population growth rates at different mean temperatures?
- 2) What demographic processes drive the differences in expected population growth rates with increasing diurnal temperature range?
- 3) Within each temperature regime, which life stage transitions represent the largest potential influences on population growth rates? In other words, to which stage transitions is the long-term population growth rate most sensitive or elastic, and does this depend on temperature?

Since the effects of temperature variability on several *S. frugiperda* life-history traits depend heavily on the mean temperature (Chapter 2), we hypothesized that diurnal fluctuations would influence population growth most at the warmest mean temperature of 30°C and least at an intermediate mean temperature of 26°C. We expected large fluctuations to increase population growth rates relative to constant temperatures at a mean of 22°C, primarily by increasing fecundity and the proportion of viable eggs. Conversely, we expected fluctuations to negatively

affect population dynamics at a mean of 30°C by reducing survivorship of later larval and pupal stages, since *S. frugiperda* individuals that survive to maturity spend most of their lifespan in these stages.

Methods

Data collection

We collected all life history information required to parametrize the models during the experiment detailed in Chapter 2 (under Methods, Temperature Experiments), except for the duration of the egg stage, which we approximated from the literature (**Table 3-1**). Briefly, we reared *Spodoptera frugiperda* (J. E. Smith, 1797) from eggs under nine temperature treatments, which were combinations of three mean temperatures (22°C, 26°C, 30°C) and three amplitudes of diurnal temperature variation ($\pm 0^\circ\text{C}$, $\pm 3^\circ\text{C}$, $\pm 6^\circ\text{C}$, hereafter “constant”, “low fluctuation”, and “high fluctuation”, respectively). We simulated these temperature regimes in incubators programmed to fluctuate sinusoidally between specified maximum and minimum temperatures. We chose fluctuation treatments that were representative of daily variation in ambient temperatures in regions and during seasons that support the presence of *S. frugiperda* (Early et al., 2018). Selected temperature regimes also represented different regions of the thermal performance curves for development time for this species, from temperatures considered to be optimal to regimes that approach lower and upper performance thresholds (Ali et al., 1990; Du Plessis et al., 2020; Ashok et al., 2021; Huang et al., 2021; Malekera et al., 2022; Chen et al., 2022).

Eggs used in this experiment were sourced from a mass-reared stock colony (Benzon Research Inc.) held at 26°C–29°C that had been established using a strain collected from the USDA-ARS Station in Stoneville, Mississippi (USA). During the first phase of the experiment, we followed individuals in our temperature treatments from egg hatch to adult eclosion, tracking the duration of each developmental stage or instar in days and survival outcomes for each individual. During the second phase of the experiment, we formed breeding pairs of newly emerged adult males and females within each temperature treatment and monitored survival and time to oviposition for each female. Additionally, we monitored a subset of females within each treatment for egg laying and egg hatch rates. We collected eggs from these females every two

Table 3-1. Sources of life history information for the matrix population models.

Stage	Life history trait
Egg	• Stage duration (T_e): we did not collect egg development times due to time constraints during the experiment. Instead, we approximated egg development times for each mean temperature using a model proposed by Ali et al. (1990), which was parametrized using data from <i>S. frugiperda</i> collected near the USDA-ARS station in Mississippi. The model adequately described the linear relationship between egg development time and temperatures in the range 18°C–32°C and is given by: $Y = -0.318 + 0.025X$, where X is the temperature (°C) and Y is the development rate (days^{-1}). We assumed the same T_e for all fluctuation levels at a given mean temperature. • Daily fraction maturing from stage (γ_e) = $1/T_e$. For all life stages, γ was approximated from stage durations as discussed in Section 6.4.1 of Caswell (2001). • Stage survivorship: Proportion of eggs that hatched out of the total number of eggs laid by reproductive females in each treatment.
Larva (instars 1 and 2)	• Stage duration (T_1): Duration of the first and second instar for each individual in days. • Daily fraction maturing from stage (γ_1) = $1/T_1$, averaged across all larvae surviving to instar 3. • Stage survivorship: Proportion of all neonates that survive to the third instar.
Larva (instars 3–6, prepupae)	• Stage duration (T_L): Number of days between the transition to larval instar 3 and pupation for each individual. • Daily fraction maturing from stage (γ_L) = $1/T_L$, averaged across all surviving pupae (excluding abnormal pupae, see Chapter 2). • Stage survivorship: Proportion of all third instar larvae that survive to normal pupation.
Pupa	• Stage duration (T_p): Number of days between pupation and successful eclosion for each female. • Daily fraction maturing from stage (γ_p) = $1/T_p$, averaged across females that successfully eclose as adults. • Stage survivorship: Proportion of normal female pupae that eclose completely.
Reproductive adult	• Reproductive period duration (T_r): Number of days between the recorded eclosion date and the last collection date yielding a sample that contained hatched larvae, for each female laying eggs that successfully hatched. • Daily fraction maturing from stage (γ_r) = $1/T_r$, averaged across all females laying eggs that hatched into larvae. • Fecundity: Mean daily fecundity during the reproductive period, averaged across females. Daily fecundity was calculated as the total number of eggs laid by a female during the reproductive period divided by the length of the reproductive period. If a reproductive period duration was not calculated for an individual female, we assumed this to be the mean reproductive period duration in that temperature regime (Table 3-2).

days from eclosion, placed the eggs in sealed Ziploc bags and returned them to their treatment incubators for a seven-day period to monitor hatch success. We kept a separate tally of any eggs that we could not collect from the container (for instance, if moths laid eggs directly on the plastic surface such that we could not move them without risk of rupture). Total eggs and total hatches for each 2-day interval were calculated as described in Chapter 2 (under Methods, Temperature Experiments). To ensure that we allowed sufficient time for mating, we excluded from this subset and from our parameter estimates any females that did not overlap with their paired males for at least two nights and that had also not oviposited by the first day following eclosion.

Model setup and parameter estimation

Our previous results show that the effects of changing mean temperature or variability vary across performance traits and life stages of *S. frugiperda* (see Chapter 2). Stage-structured matrix population models (MPMs) allow us to translate these effects into consequences for population-level fitness and analyze the importance of specific demographic transitions (Caswell, 2001). These models are especially suited to studying the dynamics of holometabolous insects, since they enable us to divide the population into distinct life stages with separate stage-specific demographic rates. For each temperature regime, we constructed a 5×5 projection matrix, with each matrix dimension representing a life stage and each matrix element representing the probability of transitioning between life stages or the expected production of offspring over a single time step. The time step (projection interval) of each model was 1 day. Our simple MPMs make a few key assumptions: notably, they are deterministic (i.e. assume constant demographic rates) and density-independent.

Our models included five life stages: eggs, early-stage larvae (first and second larval instars), late-stage larvae (remaining larval instars), pupae, and reproductive adults. We separated early and late larval stages due to differences in observed mortality between these stages (Appendix B) and due to potential differences between these stages in microhabitat preferences and behavior (for instance, early-stage larvae may feed in groups whereas late-stage larvae are often solitary and cannibalistic) (Prasanna et al., 2018). In our experiment, pupae that eclosed as adults during a time step were only paired and given an opportunity to reproduce in the following

time step. Our observations and data on time until initial oviposition (**Supplemental Fig. 3-6**) suggest that individuals were able to mate during the first time step after pairing, so we did not include a separate pre-reproductive stage in the model.

For each stage in the model, we calculated or estimated the proportion of individuals in each treatment that survived the stage as well as the average rate at which individuals matured from the stage (generally, as the mean of the inverses of individual stage durations) (details in **Table 3-1**). Our MPM focused on tracking the growth rates of the female population and assumed a 1:1 female: male ratio among offspring. Thus, we used pupal and adult stage survival and development rates from females only. Until pupation, development rates were either similar between sexes or we could not obtain female-specific rates as we could only identify sex in pupae onwards (Chapter 2).

The final stage in the model corresponds to the period when adult females are assumed to be reproductively active. In general, we observed declines in the daily number of eggs laid and steep declines in the number of larvae hatching from these eggs as females aged (**Supplemental Fig. 3-7**). Moreover, eggs collected towards the end of a female's lifespan may not have been fertilized (for instance, after the death of the paired male). Therefore, we estimated the mean reproductive period in a treatment using data from females laying eggs that successfully hatched. For each such female, we estimated the reproductive period as the number of days between the date they were paired and the last collection date yielding a sample that contained hatched larvae. We then calculated daily fecundity for these females as the total number of eggs laid during the reproductive period divided by the length of the reproductive period. Further details about how we estimated mean daily fecundity and egg survivorship for other categories of females are in **Table 3-2**. We did not include a post-reproductive stage in our MPM since post-reproductive females do not contribute to population growth. Moreover, excluding this category makes the long-term behavior of the model and its insights into demographic processes independent of initial population structure, as long as that initial population includes individuals in other life stages.

Table 3-2. Source of data for variables relevant to reproduction by adult females. We obtained information on eggs laid and eggs hatched from most of the females that we followed for egg production and hatching. Some females did not produce any eggs that hatched, and a few laid no eggs altogether over their lifetime. Hatch records were incomplete for three females (although we obtained complete egg tallies for these females, we did not monitor enough eggs during the reproductive period to confidently assess hatch rates). The table below shows which data we included in calculating treatment means for each variable related to reproduction. Included signifies that data from these females were included in calculating the treatment average. Value undefined/ not available signifies that we could not include data from these females in the treatment average.

Subset of adult females	Variable		
	Potential adult reproductive period (days)	Daily egg production per female	Proportion of eggs that hatch into larvae
Complete egg count and record of successful hatches	Included	Included	Included (total eggs, total hatches)
Complete egg count, incomplete record of hatches	Value undefined/ not available For these females, mean reproductive period was assumed to be the treatment average.	Included	Value undefined/ not available
Complete non-zero egg count, no successful hatches	Value undefined/ not available For these females, mean reproductive period was assumed to be the treatment average.*	Included	Included (total eggs, total hatches = 0)
No eggs laid, no hatches	Value undefined/ not available For these females, mean reproductive period was assumed to be the treatment average.*	Included (daily eggs = 0)	Value undefined

* Our methods did not allow us to evaluate why these females did not lay eggs or produce any eggs that hatched; for instance, since we did not monitor mating behavior or dissect females to determine whether spermatophores were present in the bursa copulatrix, we could not confirm or rule out whether these females mated with their paired males or whether these eggs were fertilized.

Using the life history information in **Table 3-1**, we calculated the following vital rates for each life stage j :

- Gamma (γ_j) = the probability of graduating from stage j to stage $j + 1$ within a time step = daily fraction maturing from each stage (Caswell 2001, Section 6.4)
- Sigma (σ_j) = daily stage survival, calculated from stage survivorship as $(\text{Stage survivorship})^{\frac{1}{\bar{T}_j}}$, where $\bar{T}_j$ is the mean stage duration for a stage j
- G_j (growth) represents the probability of surviving and transitioning from stage j to stage $j + 1$ during a time step. P_j (stasis) represents the probability of surviving and remaining in the same stage over the time step. For egg to pupal stages, $G_i = \sigma_i \gamma_i$ and $P_i = \sigma_i (1 - \gamma_i)$. For the reproductive adult stage, γ_r represents the fraction of individuals leaving stage r during a time step, either to enter the post-reproductive stage or due to mortality. Therefore, $P_r = 1 - \gamma_r$
- Fecundity (F_r) = $P_r (0.5 \times \text{Daily fecundity})$. Since our model tracks female population growth, we considered only the proportion of offspring that are female in the calculation. We assumed the ratio of female: male offspring to be 0.5 in all treatments, since sex-determination is not expected to be temperature-dependent in this species. Most mating happens in the early evening with oviposition following soon after (Simmons and Marti, 1992; Montezano et al., 2019). Because we checked adults in the morning, we assumed that fecundity during a time step was conditional on the female's persistence in the reproductive stage until the end of the time step (akin to a “birth-pulse” design; Equations 6.142 and 6.143 in Caswell, 2001).

The resulting projection matrices followed the general form below. Each matrix element a_{ij} (i.e. each element in the i^{th} row and j^{th} column) corresponds to the average number of individuals in stage i contributed by an individual in stage j over one time step (i.e. 1 day).

$$\mathbf{A} = \begin{pmatrix} P_e & 0 & 0 & 0 & F_r \\ G_e & P_l & 0 & 0 & 0 \\ 0 & G_l & P_L & 0 & 0 \\ 0 & 0 & G_L & P_p & 0 \\ 0 & 0 & 0 & G_p & P_r \end{pmatrix}$$

The matrix elements correspond to transitions or “arcs” between stage classes (“nodes”) in the life cycle model of *Spodoptera frugiperda* (**Fig. 3-1**).

Note that we do not include a fecundity term in the column corresponding to the pupal stage since we assume that maturing pupae cannot reproduce during the timestep in which they eclose, allowing time for wing expansion and drying post-emergence. In our experiment, pupae were held in individual containers and only paired shortly after eclosion, so they could not access mates during that time step.

Analyzing long-term (asymptotic) population dynamics under different temperatures

Per our MPMs, the population at time $t + 1$ is given by:

$$n(t + 1) = \mathbf{A}n(t)$$

where $\mathbf{A}$ is the 5×5 stage-based projection matrix and $n(t)$ and $n(t + 1)$ are vectors containing the numbers of individuals in each life stage, at the specified time point. The dominant eigenvalue (λ) of the projection matrix $\mathbf{A}$ is the asymptotic population growth rate. That is, λ is the long-term finite rate of population increase over one time step for a population that has reached an equilibrium such that the proportion of individuals in each life stage remains constant. This long-term relative abundance of different life stages is the stable stage distribution.

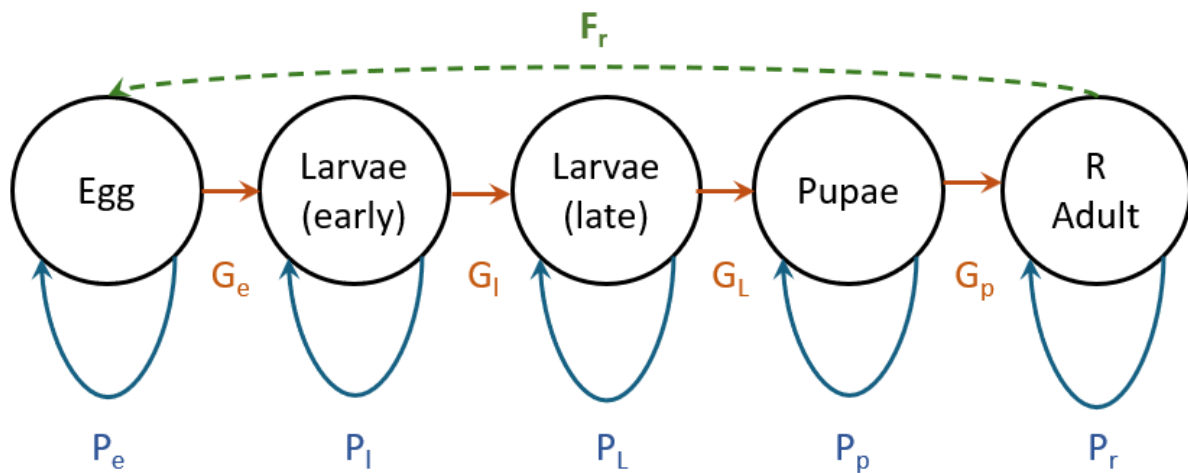


Figure 3-1. Life cycle model of *Spodoptera frugiperda*. Solid arrows indicate survival transitions whereas dashed arrows indicate fecundity transitions.

We obtained dominant eigenvalues (λ), stable stage distributions, and stagewise reproductive values for all nine projection matrices as all matrices were primitive, irreducible, and ergodic.

Identifying the demographic processes that explain observed differences or similarities in population growth rates across temperatures (Life Table Response Experiment)

Having determined how temperature variability may impact population growth rates, we then turned our focus to the mechanisms by which those impacts are achieved. Population growth may be more sensitive to changes in some biological rates than others, suggesting that small differences in life-history traits between treatments may have large effects on λ . Changes to demographic rates may also have counteracting effects on λ , such that increased temperatures and variability may alter survivorship, development rates, or fecundity but result in similar λ – for instance, if poor survivorship during the larval stage is offset by faster times to maturity. Life table response experiment (LTRE) analyses allow us to decompose the effects of temperature regime on population growth rates into contributions from different demographic rates represented in the matrix (Caswell, 1989).

We performed one-way fixed LTREs to decompose the difference in λ between fluctuation levels at each mean temperature into contributions from each stage-specific vital rate. For each mean temperature, we measured the effects of low and high fluctuations against the projection matrix for the corresponding constant temperature regime, which served as the reference matrix in each comparison. Contributions from each matrix element were calculated by multiplying the difference in each matrix element (a_{ij}) between the reference matrix and the fluctuation treatment matrix by the sensitivity of that element in the midway matrix, $(\mathbf{A}^{constant} + \mathbf{A}^{fluctuation}) / 2$ (Caswell, 2001). Therefore, contributions reflect both the difference in trait values between treatments and the sensitivity of λ to incremental changes in each trait.

Analyzing the elasticity of population growth rates to different life stages

We also conducted prospective analyses with the goal of understanding which life stages have the largest potential influences on population growth, and whether the most influential stages change with warming or increased temperature variability or the interaction of the two. That is, under each set of temperature conditions, which stage-specific vital rates, if altered,

would result in the largest change in λ ? Analyses of projection matrices for each temperature regime yielded sensitivities (the effect on the population growth rate, $\delta\lambda$, of a small absolute change in a matrix element, δa_{ij} , holding other elements constant) and elasticities (the proportional change in λ following a proportional change in a_{ij} , i.e. the proportional sensitivities) (Caswell, 2019). For a given treatment, we summed elasticities for each stage to understand the relative importance of each stage for population growth within that environment. Comparing these elasticities could be insightful for a species that is regularly targeted for control in some stages.

All demographic analyses were conducted in R version 4.4.1 (R Core Team, 2024) using the popbio (Stubben and Milligan, 2007) and popdemo (Stott et al., 2025) packages.

Results

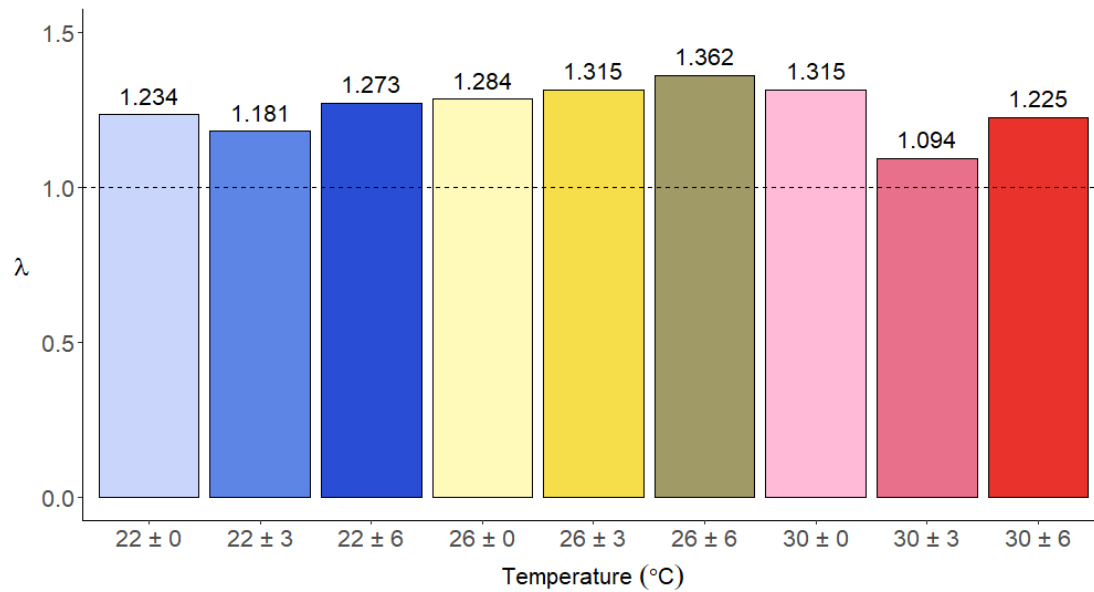
Vital rates used in the models, together with associated standard errors and sample sizes, are given in Appendix B **Supplemental Table 3-3** through **Supplemental Table 3-7**. Final projection matrices for the nine treatments are listed in Appendix C **Supplemental Table 3-8** through **Supplemental Table 3-16**.

Long-term population dynamics under different temperatures

Long-term population growth-rate (λ) ranges from 1.094 to 1.362 across treatments (**Fig. 3-2a**), with the highest rate projected for $26 \pm 6^\circ\text{C}$ and the lowest for $30 \pm 3^\circ\text{C}$. Populations that attain a stable demographic structure are expected to increase over time under all temperature conditions investigated in the study, given the assumptions of our model (**Fig. 3-2b**). The effect of fluctuation magnitude on λ varies by mean temperature. Large fluctuations around an intermediate mean temperature of 26°C increase population growth rates by around 6%. Conversely, fluctuations around the warmest mean temperature of 30°C decrease rates of population growth by close to 17% with low fluctuations and 7% with high fluctuations. Fluctuations around a mean temperature of 22°C alter population growth rates by less than 4.3%.

Despite noticeable differences in expected rates of population increase, there is little difference between most temperatures in the stable stage distribution (i.e. the long-term relative abundance of life stages). Under stable population dynamics, populations under all conditions

a)



b)

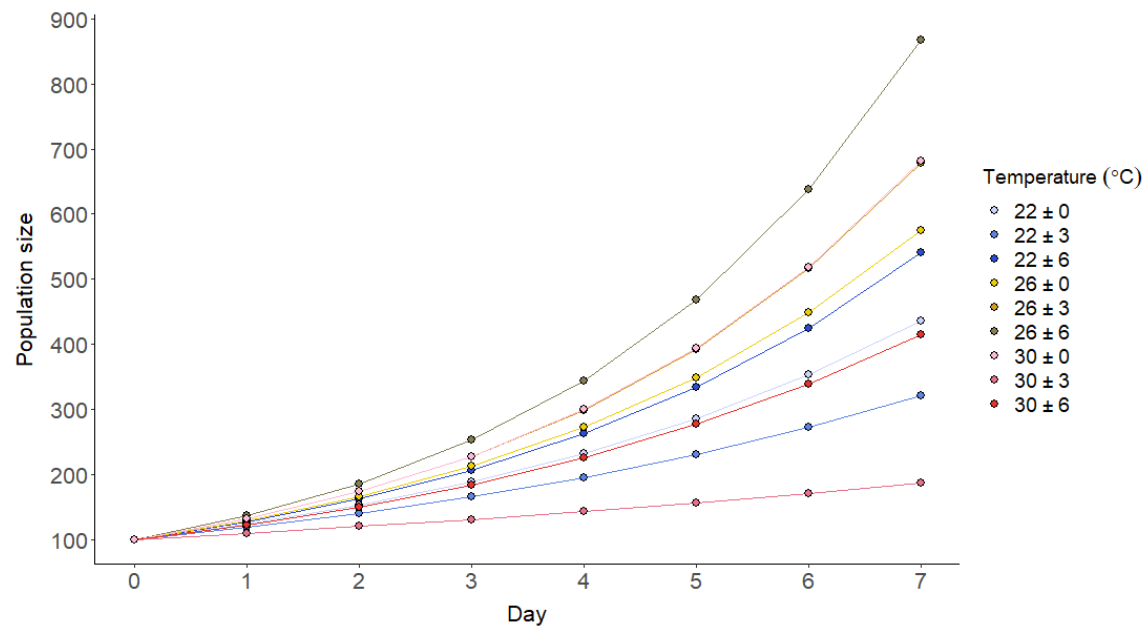


Figure 3-2. Projected long-term *S. frugiperda* population growth rates under different mean temperatures and fluctuations. a) Barplot showing changes in λ with increases in mean temperatures and diurnal fluctuation. b) Expected increases in the size of an equilibrium population (of 100 individuals on Day 0) under different temperature conditions, according to our models.

except $30 \pm 3^\circ\text{C}$ should be comprised of 51–58% eggs, 22–28% early-stage larvae, and 13–17% late-stage larvae, with pupae (2–4%) and reproductive adults (1% or less) being the least abundant life stages (**Supplemental Table 3-17**). Conversely, at $30 \pm 3^\circ\text{C}$, eggs and both larval stages should be similar in relative abundance (23–30%), with pupae and adults making up a higher proportion of stable populations under these conditions relative to other temperatures.

Retrospective and prospective analyses

Our LTRE analyses show that temperature fluctuations will alter population growth rates relative to constant mean temperatures primarily by changing fecundity, the length of the female reproductive period, and egg survivorship (**Fig. 3-3**). The increase in λ with high fluctuations at a mean temperature of 26°C is largely explained by increases in the proportion of eggs hatching (stasis and growth) and by a small increase in the persistence of adult females in the reproductively active period (stasis). Changes in projected population growth rates with fluctuations around mean 22°C also appear to be due to decreased fertility (with low fluctuations) and increased egg survivorship (with high fluctuations). A steep decline in expected fecundity and a shorter reproductive adult stage underly the reduction in projected population growth rates with a moderate increase in diurnal variability ($30 \pm 3^\circ\text{C}$) from constant 30°C .

Other life stages play a bigger role in reducing population growth rates with a larger increase in diurnal temperature variation around the warmest mean temperature (30°C). While the largest contribution to decreased λ in $30 \pm 6^\circ\text{C}$ is still from reduced fertility, lower growth of late-stage larvae, and reduced stasis of pupae contribute more to this decrease than they do to changes between other temperatures.

Contributions from a fixed LTRE reflect both differences in vital rates between treatments and the sensitivity of λ to changes in those rates. The effects of temperature variability on λ in our study appear to arise from large differences in stasis and growth in the egg and adult stage and in fecundity (**Fig. 3-4**), despite low sensitivity to changes in egg and adult stasis and fecundity across all temperature regimes.

Within each temperature condition, elasticity analyses show that the long-term population growth rate is most elastic to demographic transitions within and from the late larval and pupal stages (**Fig. 3-5**). For most treatments, the next-highest elasticities are to transitions from the

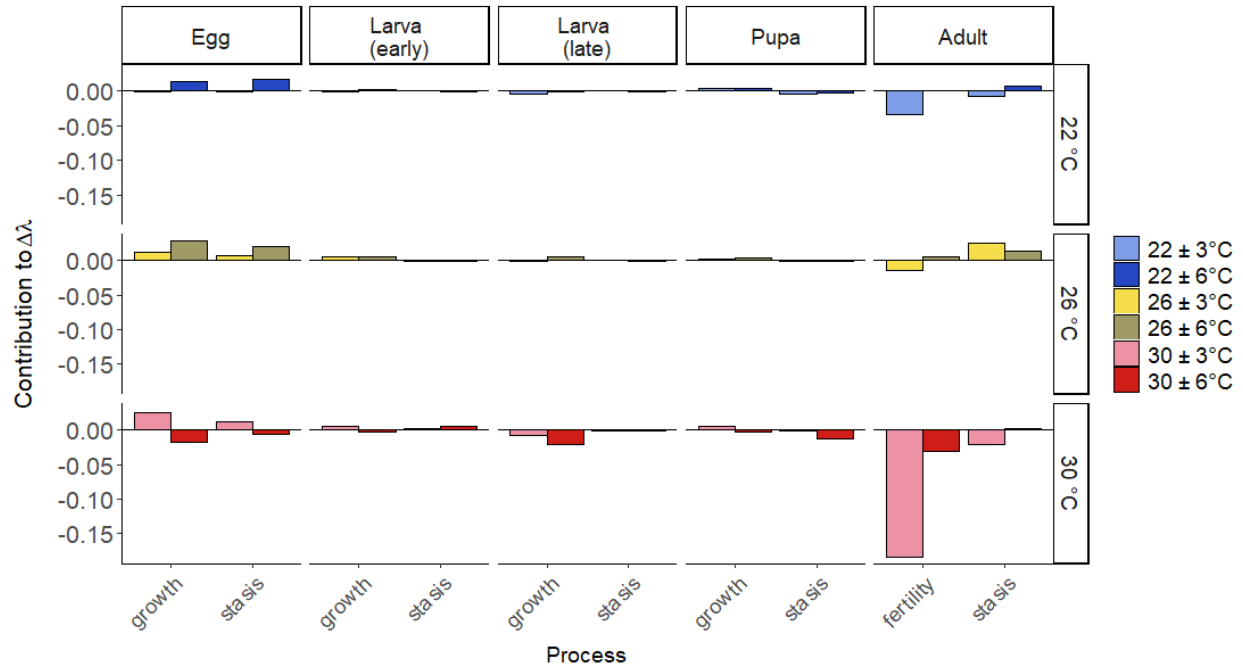


Figure 3-3. Life table response experiment (LTRE) contributions of vital rates in each life stage to changes in projected *S. frugiperda* population growth rates (λ) between constant temperature environments and fluctuating temperature environments with the same mean temperature.

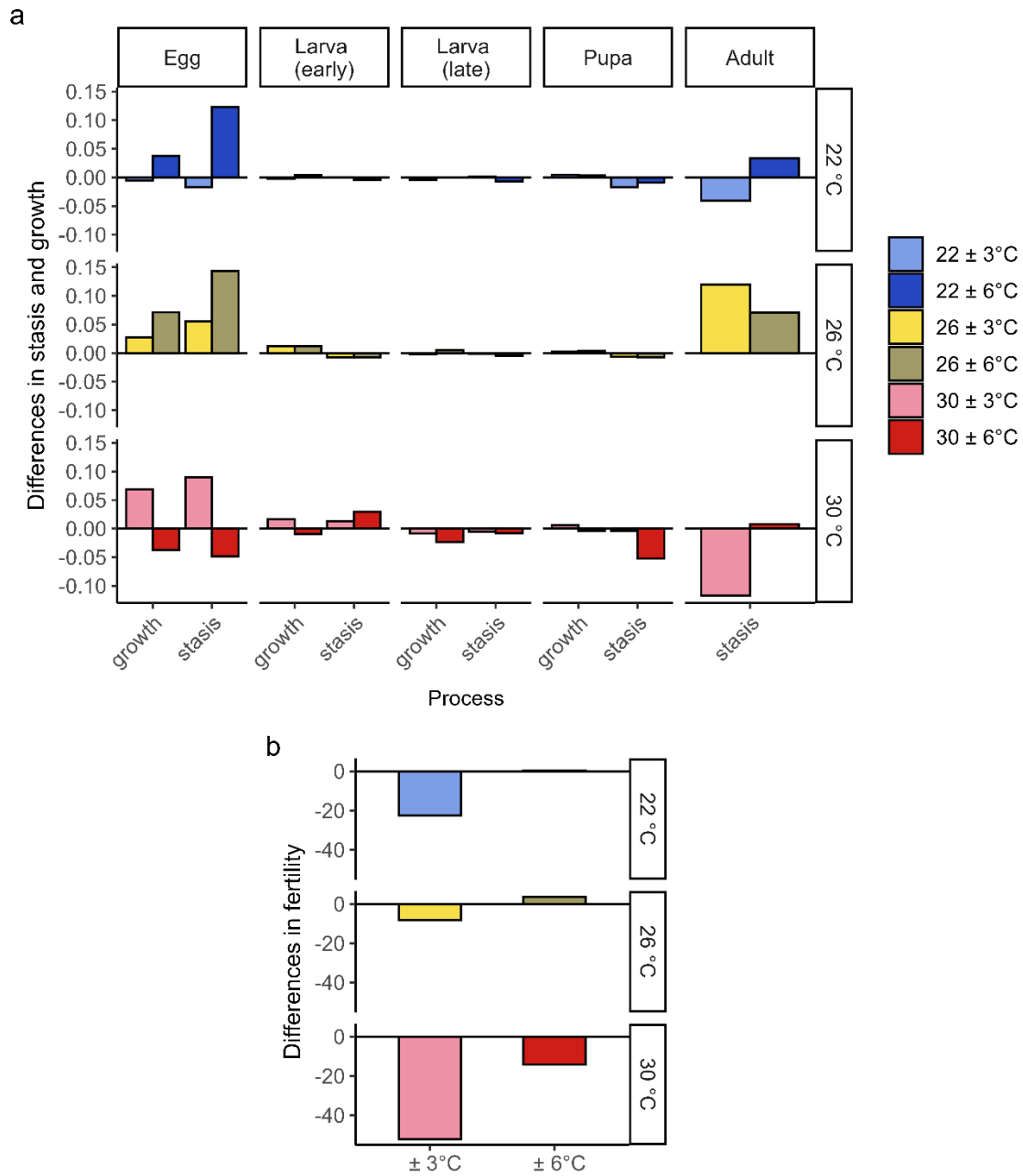


Figure 3-4. Changes in stage-specific vital rates with increasing fluctuation magnitude, relative to constant mean temperatures. Bars show differences in the projection matrix elements corresponding to a) stasis and growth and b) fertility between fluctuating temperature treatments and the corresponding constant temperature condition.

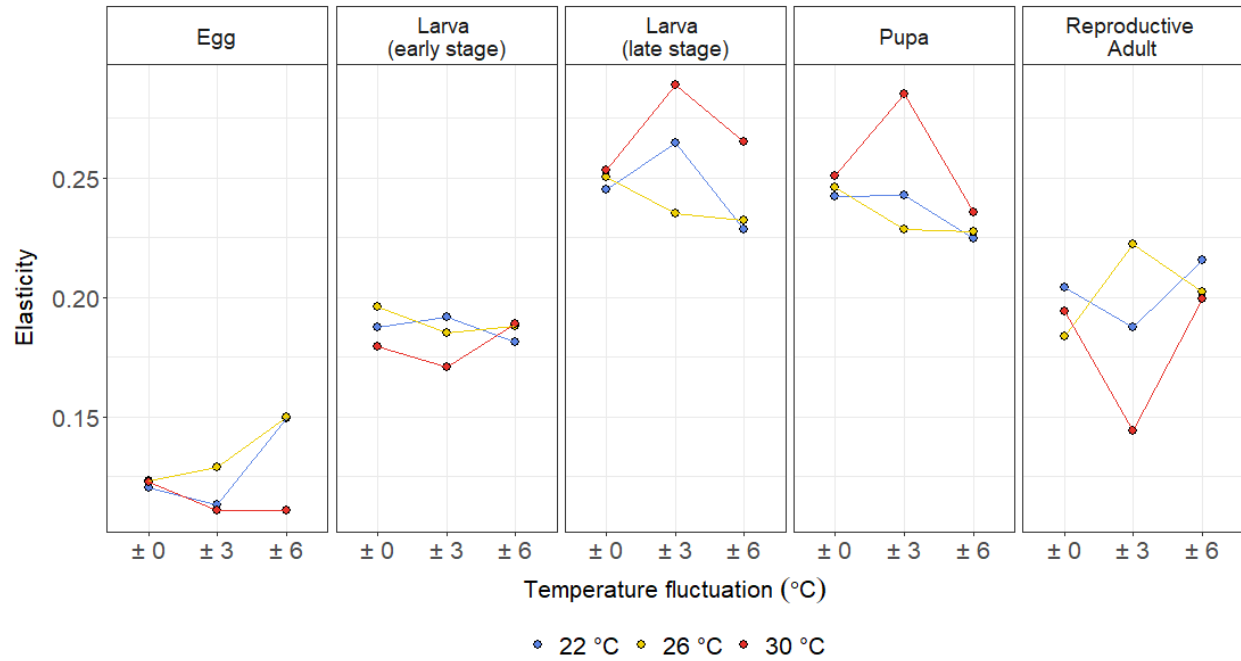


Figure 3-5. Total elasticities of λ to demographic processes in each *S. frugiperda* life stage for the nine temperature treatments. For readability, stage-summed elasticities for different fluctuation levels at the same mean temperature are connected by solid lines (blue = 22°C, yellow = 26°C, red = 30°C).

reproductive adult stage, and the lowest elasticities are associated with the earliest life stages. Reducing stasis within and growth from the late larval stage by 1% would change λ by 0.23–0.29%, whereas a similar 1% perturbation of transitions from the egg stage leads to only a 0.11–0.15% change in λ . The similarity across most temperatures in which processes have the largest potential impact on λ may be explained by the similarities in expected population structures and in the value of late-stage larvae and pupae as seeds for population growth (**Supplemental Tables 3-17 and 3-18**).

Elasticity patterns in some temperature regimes showed a few subtle deviations from the general trend. Under low fluctuations at the warmest mean temperature ($30 \pm 3^\circ\text{C}$), the total elasticity associated with reproductive adult stasis and fecundity is especially low, such that the population growth rate is twice as responsive to late-stage larval or pupal vital rates versus adult vital rates. Also, population growth is more elastic to changes in stasis and growth from the egg stage when mean temperatures are moderate and diurnal temperature variation is high ($22 \pm 6^\circ\text{C}$ and $26 \pm 6^\circ\text{C}$) than when mean temperatures are high (30°C) or when temperatures are less variable. Under variable but moderate temperatures, the proportion of eggs hatching into early-stage larvae is considerably higher than in other treatments (**Supplemental Table 3-3**), therefore individuals in the egg stage are more likely to survive to maturity and contribute to population growth.

Discussion

Our goal was to understand how daily temperature variability can affect *Spodoptera frugiperda* population growth rates at different mean temperatures characteristic of its range. To do this, we used life history data from *S. frugiperda* individuals that had been exposed to fluctuating temperatures over their lifespans to parameterize stage-structured matrix population models. By doing so, we were able to incorporate time-dependent physiological effects of temperature fluctuations (e.g., accumulated physiological damage and recovery) into our estimates of survival, development, and fecundity rates, and ultimately translate these into measures of population-level fitness. We found that whether diurnal temperature variability increases or decreases population growth rates relative to constant temperatures depends on the mean temperature of the environment, as we expected from our previous findings. Fluctuations

around 30°C are projected to reduce λ , whereas fluctuations around 26°C increase expected λ . These results align with theoretical predictions that increased temperature variability would decrease upper thermal performance thresholds, resulting in decreased population growth rates at warmer environmental mean temperatures (Estay et al., 2014).

Our results also partially agree with meta-analyses of organism-level responses to temperature fluctuations, which find more negative impacts on organisms at warmer mean temperatures and when the amplitude of fluctuation is larger (Slein et al., 2023; Stocker et al., 2024). Indeed, the effect of temperature fluctuations on population growth rates in our study is most pronounced at the warmest mean temperature of 30°C and least evident at 22°C. However, the largest reduction in population growth rate relative to constant temperatures occurs with low fluctuations ($30 \pm 3^\circ\text{C}$), and not large fluctuations ($30 \pm 6^\circ\text{C}$). It is possible that larger fluctuations around 30°C are more conducive to reproduction and survival in *S. frugiperda* adults than low fluctuations, as cooler nighttime temperatures and lower daily minima may facilitate physiological recovery from heat stress experienced during the day (Buckley et al., 2025). Moreover, “diel narrowing” may result in individuals limiting their activity to periods with more optimal temperatures for reproduction, which are generally described to be lower than optima for survival and development rates (Kefford et al., 2022). More research into the physiological consequences of repeated, intermittent thermal stress and activity patterns under fluctuating temperatures may reveal whether these mechanisms are indeed at play in *S. frugiperda* exposed to variable summer temperatures.

Across mean temperatures, changes in fecundity, the duration of the reproductive period for adult females, and egg hatch rates drive the differences we observe in population growth rates with increasing diurnal temperature range. Identifying these underlying demographic processes allows us to draw more useful conclusions. If the primary mechanism through which temperature variation reduces population growth is through effects on the adult and egg stage, then *S. frugiperda* field populations may be able to avoid these effects and maintain higher rates of growth if adults can disperse to and oviposit in cooler and less variable environments. However, with larger fluctuations around 30°C, reduced survival and development rates in the larval and pupal stages become more important in explaining declines in population growth rates alongside impacts on the adult stage. Therefore, in warmer parts of *S. frugiperda*’s current range, increases

in variability may indeed lead to reduced population growth *in situ* due to these broader impacts across life stages.

Our results support a broader argument that declines in fecundity and the proportion of viable offspring may be the most important constraints on population growth under projected climate change. Indeed, thermal fertility limits, which are generally lower than survival limits, may be even more closely related than the latter to population viability and species geographic range limits (Walsh et al., 2019; van Heerwaarden and Sgrò, 2021). Yet, among systematic investigations of how thermal variability affects biological rates, many focus on developmental rates, with fewer studies measuring impacts on reproduction (Slein et al., 2023). Therefore, focusing on how thermal variability could alter insect reproduction could improve forecasts of future population trends based primarily on lethal limits.

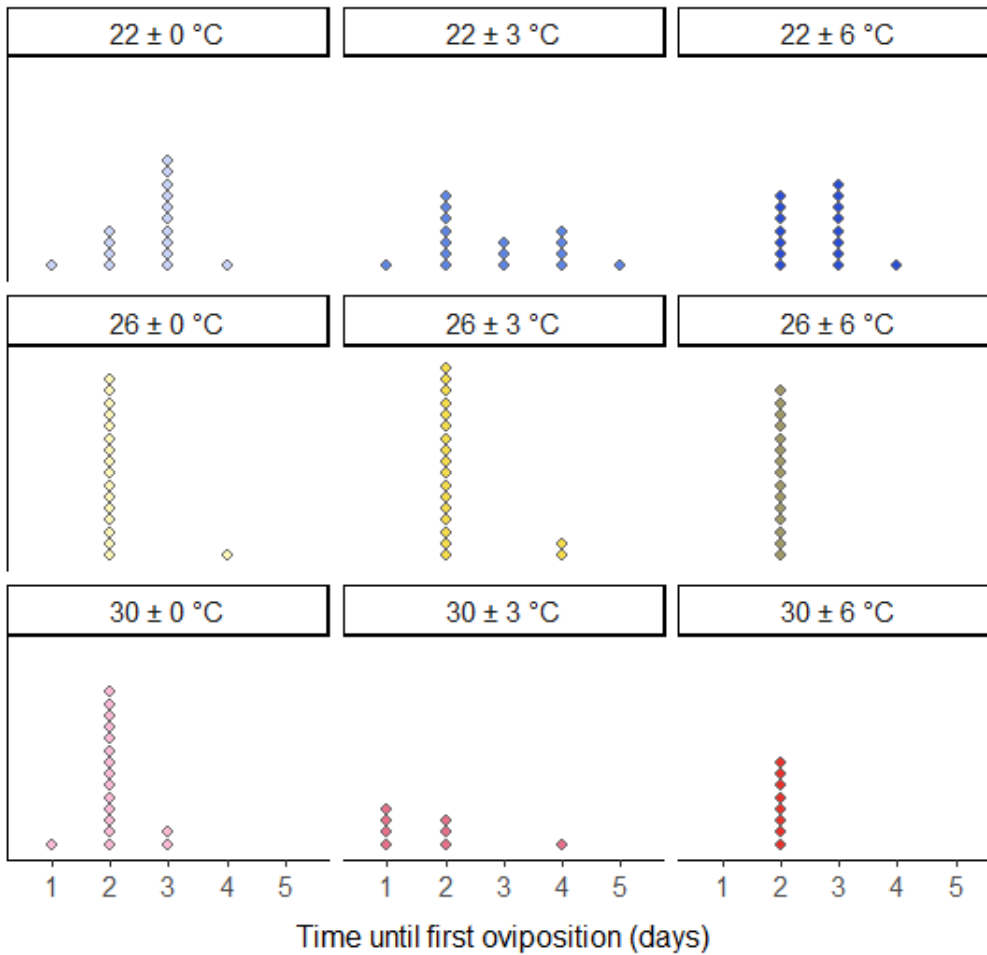
While temperature fluctuations did not impact vital rates for *S. frugiperda* larvae and pupae to the same extent as for eggs and adults, the highest stage-summed elasticities in all treatments were associated with the late larval stage and pupal stages. This suggests that further changes in the survival or development of these stages could have a larger impact on population growth rates than proportional changes to survival, development, and fecundity in other stages, regardless of environmental temperatures. Therefore, according to these results, population control strategies that manage to reduce survival or slow development in larvae and pupae may still be effective in reducing long-term population growth rates under warming and increasingly variable temperatures. The question moving forward is whether reducing those vital rates in the first place will become more challenging under projected climate change.

Our simple MPMs do not account for factors that may further limit *S. frugiperda* populations in the field. For instance, due to our experimental design, we could not incorporate density-dependence in our model. However, high population densities in the field likely reduce population growth rates, through intraspecific resource competition, larval cannibalism, and increased disease transmission. Moreover, our deterministic MPMs do not account for the random changes in vital rates that may occur across time (for instance, with anomalous climatic events such as heat waves, droughts and floods). Allowing vital rates to depend on the population size at each time step and incorporating environmental stochasticity into the models could be useful extensions (Caswell, 2001). By projecting long-term growth rates, we also assume that

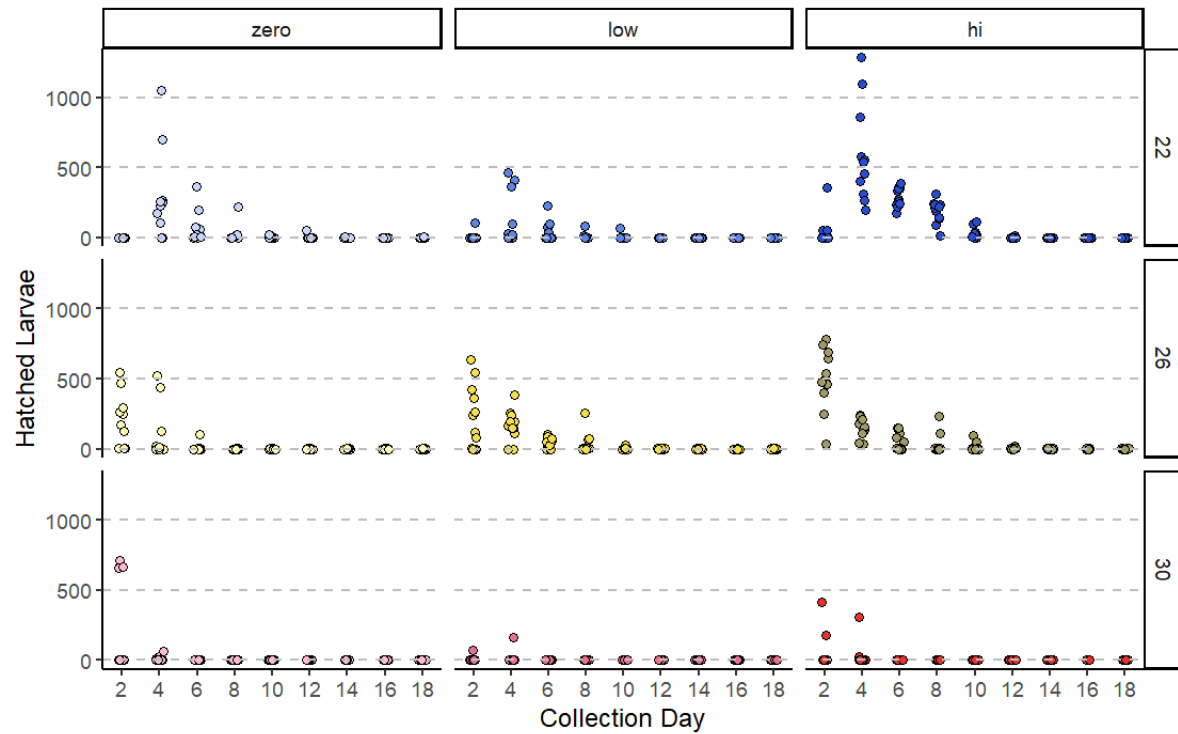
populations reach an equilibrium in which the relative abundance of different life stages stays consistent across time. However, in many locations, *S. frugiperda* populations are only present seasonally and may not reach a stable stage distribution until well into the season, unless dispersal events and other disturbances that affect life stages non-uniformly prevent them from reaching this equilibrium at all. Therefore, analyzing transient dynamics may be necessary for understanding population trends in some contexts (Ezard et al., 2010). Finally, we used data from controlled laboratory environments to parametrize these models; survivorship in the field, particularly for early-stage larvae, may be substantially lower than the rates presented here.

Nevertheless, our models are still useful for isolating the effects of temperature variability on the potential for population growth, and for understanding the mechanisms underlying these effects. The qualitative comparisons they enable among different temperature conditions provide insights into the ability of different climates to sustain large populations of *S. frugiperda*.

Appendix A: Supplementary figures



Supplemental Figure 3-6. Days between emergence and initial oviposition for adult females in different temperature treatments. Eggs were observed in breeding pair containers 1 day after pairing in four treatments and 2 days after pairing in all others. Each point represents the time to oviposition for an individual female.



Supplemental Figure 3-7. Number of eggs collected during each interval that eventually hatched into larvae. This number of successful hatches drops sharply after days 2–4 at 30°C, days 6–10 at 26°C and day 10 at 22°C. Each point represents data from one female during a single collection interval.

Appendix B: Vital rates

Supplemental Table 3-3. Vital rates for the egg stage.

Temperature Regime	γ_e	Stage survivorship
22 ± 0°C	0.232	0.378853
22 ± 3°C	0.232	0.334653
22 ± 6°C	0.232	0.834694
26 ± 0°C	0.332	0.348885
26 ± 3°C	0.332	0.486929
26 ± 6°C	0.332	0.778346
30 ± 0°C	0.432	0.308271
30 ± 3°C	0.432	0.528736
30 ± 6°C	0.432	0.216466

Supplemental Table 3-4. Vital rates for the early larval stage (instars 1 and 2). SE = standard error of the mean (γ) or standard error of the proportion (stage survivorship). n = number of individuals.

Temperature Regime	Stage duration	γ_l	SE (γ_l)	n (γ_l)	Stage survivorship	SE (surv.)	n (surv.)
22 ± 0°C	6.088889	0.16455	0.000718	90	1	0	90
22 ± 3°C	6.142857	0.163265	0.000846	98	0.989899	0.01005	99
22 ± 6°C	5.944444	0.168624	0.000927	90	1	0	90
26 ± 0°C	4.534091	0.223485	0.002742	88	0.977778	0.015538	90
26 ± 3°C	4.319149	0.234043	0.002417	94	1	0	94
26 ± 6°C	4.311111	0.234444	0.002454	90	1	0	90
30 ± 0°C	3.126582	0.322785	0.003137	79	0.877778	0.034526	90
30 ± 3°C	3.045977	0.329502	0.001882	87	0.966667	0.018922	90
30 ± 6°C	3.321429	0.30744	0.003874	112	0.933333	0.022771	120

Supplemental Table 3-5. Vital rates for the late larval stage (instars 3 to prepupa). SE = standard error of the mean (γ) or standard error of the proportion (stage survivorship). n = number of individuals.

Temperature Regime	Stage duration	γ_L	SE (γ_L)	n (γ_L)	Stage survivorship	SE (surv.)	n (surv.)
22 $\pm$ 0°C	15.42683	0.065479	0.000659	82	0.911111	0.029998	90
22 $\pm$ 3°C	16.37647	0.06153	0.000571	85	0.867347	0.034264	98
22 $\pm$ 6°C	15.63514	0.065533	0.000951	73	0.811111	0.041259	90
26 $\pm$ 0°C	10.76712	0.095877	0.001488	69	0.784091	0.043861	88
26 $\pm$ 3°C	10.875	0.094316	0.001333	71	0.755319	0.044341	94
26 $\pm$ 6°C	10.22222	0.101315	0.001817	72	0.8	0.042164	90
30 $\pm$ 0°C	7.569231	0.133981	0.001822	65	0.822785	0.042962	79
30 $\pm$ 3°C	8.125	0.126616	0.002065	63	0.724138	0.047918	87
30 $\pm$ 6°C	9.189189	0.113672	0.001669	66	0.589286	0.046486	112

Supplemental Table 3-6. Vital rates for the pupal stage. SE = standard error of the mean (γ) or standard error of the proportion (stage survivorship). n = number of individuals.

Temperature Regime	Stage duration	γ_p	SE (γ_p)	n (γ_p)	Stage survivorship	SE (surv.)	n (surv.)
22 $\pm$ 0°C	13.4186	0.074714	0.000573	43	1	0	43
22 $\pm$ 3°C	12.40541	0.080403	0.000548	35	0.853659	0.055199	41
22 $\pm$ 6°C	12.75	0.07873	0.000866	27	0.931034	0.047054	29
26 $\pm$ 0°C	8.323529	0.119916	0.00142	32	0.969697	0.02984	33
26 $\pm$ 3°C	8.129032	0.12328	0.001172	30	0.9375	0.042791	32
26 $\pm$ 6°C	8.088235	0.123737	0.000706	33	0.942857	0.039235	35
30 $\pm$ 0°C	6.653846	0.151099	0.002265	26	0.928571	0.04867	28
30 $\pm$ 3°C	6.432432	0.156746	0.001984	36	0.947368	0.036224	38
30 $\pm$ 6°C	6.535714	0.156463	0.002635	21	0.636364	0.083739	33

Supplemental Table 3-7. Vital rates for the reproductive adult stage. SE = standard error of the mean. n = number of individuals.

Temperature Regime	γ_r	SE (γ_r)	n (γ_r)	Mean daily fecundity	SE (fecundity)	n (fecundity)
22 $\pm$ 0°C	0.132222	0.011484	10	131.7	15.70721	10
22 $\pm$ 3°C	0.173148	0.020449	9	83.75379	15.26383	11
22 $\pm$ 6°C	0.098485	0.004608	11	128.203	12.89298	11
26 $\pm$ 0°C	0.2625	0.04215	10	172.2386	35.32234	11
26 $\pm$ 3°C	0.141667	0.007538	11	128.9813	18.08127	12
26 $\pm$ 6°C	0.190909	0.018985	11	165.8909	21.33223	11
30 $\pm$ 0°C	0.3	0.05	5	162.75	29.78415	12
30 $\pm$ 3°C	0.416667	0.083333	3	16.09091	6.307217	11
30 $\pm$ 6°C	0.291667	0.11024	3	120.75	22.35504	8

Appendix C: Projection matrices

Supplemental Table 3-8. Projection matrix for $22 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.736533141	0	0	0	57.696
Larva 1+2	0.222494386	0.831	0	0	0
Larva 3+	0	0.169	0.922034792	0	0
Pupa	0	0	0.064626302	0.916168309	0
R Adult	0	0	0	0.078261637	0.9015

Supplemental Table 3-9. Projection matrix for $22 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.595895999	0	0	0	34.6513
Larva 1+2	0.18001025	0.835631067	0	0	0
Larva 3+	0	0.16273341	0.930368405	0	0
Pupa	0	0	0.060967136	0.907969733	0
R Adult	0	0	0	0.079383174	0.827

Supplemental Table 3-10. Projection matrix for $22 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.613203324	0	0	0	57.288
Larva 1+2	0.185238504	0.835	0	0	0
Larva 3+	0	0.165	0.928860787	0	0
Pupa	0	0	0.065104742	0.9253	0
R Adult	0	0	0	0.0747	0.868

Supplemental Table 3-11. Projection matrix for $26 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.614583802	0	0	0	67.147
Larva 1+2	0.30545183	0.766	0	0	0
Larva 3+	0	0.234	0.879546308	0	0
Pupa	0	0	0.098814435	0.869668044	0
R Adult	0	0	0	0.123103696	0.809

Supplemental Table 3-12. Projection matrix for $26 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.526058955	0	0	0	55.341
Larva 1+2	0.261454451	0.766	0	0	0
Larva 3+	0	0.234	0.882646575	0	0
Pupa	0	0	0.091899715	0.870122719	0
R Adult	0	0	0	0.122035455	0.858

Supplemental Table 3-13. Projection matrix for $26 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.470968733	0	0	0	63.468
Larva 1+2	0.23407428	0.773193716	0	0	0
Larva 3+	0	0.221907592	0.883956553	0	0
Pupa	0	0	0.093763337	0.876784243	0
R Adult	0	0	0	0.119561488	0.738

Supplemental Table 3-14. Projection matrix for $30 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.292974968	0	0	0	42.834
Larva 1+2	0.222826032	0.678674358	0	0	0
Larva 3+	0	0.30065372	0.836409706	0	0
Pupa	0	0	0.107619308	0.787571547	0
R Adult	0	0	0	0.145570096	0.708

Supplemental Table 3-15. Projection matrix for $30 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.431400279	0	0	0	4.69315
Larva 1+2	0.328107254	0.662668913	0	0	0
Larva 3+	0	0.326389166	0.838958866	0	0
Pupa	0	0	0.122047853	0.835890712	0
R Adult	0	0	0	0.155675969	0.583

Supplemental Table 3-16. Projection matrix for $30 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.34150796	0	0	0	57.05
Larva 1+2	0.259738449	0.649435162	0	0	0
Larva 3+	0	0.309848681	0.843999475	0	0
Pupa	0	0	0.130595762	0.83964948	0
R Adult	0	0	0	0.149336951	0.7

Supplemental Table 3-17. Stable stage distributions for each temperature regime (right eigenvectors of each projection matrix).

Temperature Regime	Egg	Larva (instars 1 and 2)	Larva (instar ≥ 3)	Pupa	Adult (reproductive)
$22 \pm 6^\circ\text{C}$	0.555511	0.279907	0.13495	0.024471	0.005161
$22 \pm 3^\circ\text{C}$	0.512559	0.266969	0.173177	0.038637	0.008658
$22 \pm 0^\circ\text{C}$	0.561883	0.260996	0.141228	0.029805	0.006087
$26 \pm 6^\circ\text{C}$	0.548662	0.281309	0.136511	0.027413	0.006105
$26 \pm 3^\circ\text{C}$	0.554909	0.264398	0.143188	0.029597	0.007908
$26 \pm 0^\circ\text{C}$	0.560647	0.256888	0.14248	0.032803	0.007182
$30 \pm 6^\circ\text{C}$	0.548978	0.223712	0.172874	0.042485	0.011951
$30 \pm 3^\circ\text{C}$	0.299241	0.227891	0.292212	0.13844	0.042217
$30 \pm 0^\circ\text{C}$	0.576788	0.224944	0.147842	0.04058	0.009847

Supplemental Table 3-18. Stagewise reproductive values for each temperature regime (left eigenvectors of each projection matrix).

Temperature Regime	Egg	Larva (instars 1 and 2)	Larva (instar ≥ 3)	Pupa	Adult (reproductive)
$22 \pm 6^\circ\text{C}$	1	2.409205	6.294838	34.1432	155.4864
$22 \pm 3^\circ\text{C}$	1	3.251709	6.905834	28.41624	97.81955
$22 \pm 0^\circ\text{C}$	1	3.350197	8.097099	37.92401	156.6149
$26 \pm 6^\circ\text{C}$	1	2.4461	6.227622	30.3901	121.4781
$26 \pm 3^\circ\text{C}$	1	3.016477	7.07364	33.25803	121.1678
$26 \pm 0^\circ\text{C}$	1	3.473604	7.99663	34.12209	116.2311
$30 \pm 6^\circ\text{C}$	1	4.184896	7.611145	27.51607	82.77443
$30 \pm 3^\circ\text{C}$	1	2.017948	2.663699	5.55545	9.193175
$30 \pm 0^\circ\text{C}$	1	3.749668	8.059743	29.0951	92.69776

Appendix D: Elasticity matrices

The two transitions with the highest elasticities are shown in bold for each matrix.

Supplemental Table 3-19. Elasticity matrix for $22 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.086491342	0	0	0	0.06294674
Larva 1+2	0.06294674	0.118461394	0	0	0
Larva 3+	0	0.06294674	0.165573791	0	0
Pupa	0	0	0.06294674	0.161812248	0
R Adult	0	0	0	0.06294674	0.152927527

Supplemental Table 3-20. Elasticity matrix for $22 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.057187255	0	0	0	0.056174305
Larva 1+2	0.056174305	0.135822317	0	0	0
Larva 3+	0	0.056174305	0.208327413	0	0
Pupa	0	0	0.056174305	0.186647216	0
R Adult	0	0	0	0.056174305	0.131144275

Supplemental Table 3-21. Elasticity matrix for $22 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.059883225	0	0	0	0.060604146
Larva 1+2	0.060604146	0.126895373	0	0	0
Larva 3+	0	0.060604146	0.184610137	0	0
Pupa	0	0	0.060604146	0.181779708	0
R Adult	0	0	0	0.060604146	0.143810825

Supplemental Table 3-22. Elasticity matrix for $26 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.067626059	0	0	0	0.082214798
Larva 1+2	0.082214798	0.105709728	0	0	0
Larva 3+	0	0.082214798	0.149961051	0	0
Pupa	0	0	0.082214798	0.14530025	0
R Adult	0	0	0	0.082214798	0.120328922

Supplemental Table 3-23. Elasticity matrix for $26 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.051541645	0	0	0	0.077271591
Larva 1+2	0.077271591	0.107867293	0	0	0
Larva 3+	0	0.077271591	0.157847937	0	0
Pupa	0	0	0.077271591	0.151225009	0
R Adult	0	0	0	0.077271591	0.145160159

Supplemental Table 3-24. Elasticity matrix for $26 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.045230044	0	0	0	0.078085243
Larva 1+2	0.078085243	0.118183929	0	0	0
Larva 3+	0	0.078085243	0.172519542	0	0
Pupa	0	0	0.078085243	0.168106172	0
R Adult	0	0	0	0.078085243	0.105534099

Supplemental Table 3-25. Elasticity matrix for $30 \pm 6^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.026464524	0	0	0	0.084233361
Larva 1+2	0.084233361	0.1045475	0	0	0
Larva 3+	0	0.084233361	0.181082557	0	0
Pupa	0	0	0.084233361	0.151492855	0
R Adult	0	0	0	0.084233361	0.115245757

Supplemental Table 3-26. Elasticity matrix for $30 \pm 3^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.043810189	0	0	0	0.067238894
Larva 1+2	0.067238894	0.103420435	0	0	0
Larva 3+	0	0.067238894	0.221613842	0	0
Pupa	0	0	0.067238894	0.218173625	0
R Adult	0	0	0	0.067238894	0.07678744

Supplemental Table 3-27. Elasticity matrix for $30 \pm 0^\circ\text{C}$.

Stage at time $t+1$	Stage at time t				
	Egg	Larva 1+2	Larva 3+	Pupa	R Adult
Egg	0.031824582	0	0	0	0.090759263
Larva 1+2	0.090759263	0.088501118	0	0	0
Larva 3+	0	0.090759263	0.162482035	0	0
Pupa	0	0	0.090759263	0.160166739	0
R Adult	0	0	0	0.090759263	0.103229212

CHAPTER 4
WARMER AND MORE VARIABLE TEMPERATURES ALTER OUTCOMES FOR
SPODOPTERA FRUGIPERDA* INFECTED WITH *BACILLUS THURINGIENSIS

Abstract

Parasites and pathogens can be major regulators of insect populations in the field. Predicting how temperature warming and variability will alter these host-pathogen interactions is challenging, since outcomes depend on the thermal performance of complex biological functions in all interacting species. Moreover, the effects of temperature variability on these interactions are not well understood, since most studies investigating the effects of temperature fluctuations measure traits in organisms that are held apart from other species. Understanding the temperature-dependence of these interactions could be especially important for the biological control of insect pests with entomopathogens. Here, we investigated how ambient temperature mean and variability affect the survival and development of *Spodoptera frugiperda* larvae exposed to a fixed concentration of *Bacillus thuringiensis* var. *kurstaki*, a common biocontrol agent used against lepidopterans. We reared larvae from eggs under either moderate or elevated mean temperatures and under either constant or fluctuating temperatures, and exposed fourth instars to dietary overlays of either *B. thuringiensis* suspension or water. Survival to pupation and adult eclosion following infection with *B. thuringiensis* increased with mean temperature but was unaffected by temperature variability. Meanwhile, larval development time in infection survivors depended on both average temperature and diurnal temperature variation: temperature fluctuations shortened development times relative to constant conditions at the moderate mean temperature, but prolonged larval development relative to constant conditions at the elevated mean temperature. Overall, warming may lessen the effects of *B. thuringiensis* on this widespread agricultural pest, most noticeably by reducing mortality. By altering development rates, increasing temperature variability could also affect the growth of *S. frugiperda* populations subjected to biological control.

Introduction

Biologists increasingly recognize the importance of incorporating short-term temperature variability when studying the effects of temperature on insect performance (Vasseur et al., 2014; Colinet et al., 2015; Sheldon et al., 2020; Marshall et al., 2021; Vajedsamiei et al., 2021). However, most empirical studies and reviews that explicitly investigate the effects of temperature fluctuations focus on individual-level traits and survivorship, often using organisms

maintained in controlled settings to isolate any temperature effects (Stoks et al., 2017; Slein et al., 2023). Yet, insects in their natural environments face a multitude of biotic stressors from their interactions with other species. For herbivorous insect pests in particular, parasites and pathogens can be major regulators of populations in the field and account for a large proportion of natural insect mortality, particularly in areas that are not sprayed with pesticides (Marengo and Saunders, 1993; Meagher et al., 2016). Despite projected warming and increases in diurnal fluctuations and extremes in many parts of the world (Bathiany et al., 2018; Duffy et al., 2022), we know relatively little about how withstanding large temperature fluctuations at higher mean temperatures may change the susceptibility of insects to parasites, pathogens, and microbial biopesticides (Stoks et al., 2017; Verheyen et al., 2022).

The effects of simultaneous temperature variability and pathogen stress on insect hosts can be challenging to predict (Thomas and Blanford, 2003). The negative effects of warmer and more variable conditions on insect performance may be exacerbated in combination with other stressors: examples of these negative synergies abound in the literature and span various taxa (Dinh et al., 2016; González-Tokman et al., 2020). Direct physiological damage by pathogens or pesticides may prevent insects from effectively thermoregulating and reduce their heat tolerance (Verheyen et al., 2022). Secondly, responding to either stressor may involve the same mechanism. For instance, both immune challenge and warming (via increased development rates) may increase the demands on the melanization pathway (Murdock et al., 2012). Similarly, proteins involved in immune surveillance or detoxification can also be involved in responding to heat stress, but may be unable to serve both functions simultaneously (Adamo, 2012). These situations could result in resource allocation tradeoffs where aspects of immune function may be constrained under warmer and more variable environments – such as in *Bicyclus anynana* butterflies, which had higher fat content and body size but reduced immune responses when reared in high temperatures (Lazzaro and Little, 2009; Karl et al., 2011). At the same time, research also associates short-term thermal stress with improved resistance to immune challenge (Wojda and Taszłow, 2013). Because both heat stress and pathogenic infection can share similar signaling pathways, mounting a response to one stressor – for instance, synthesizing heat-shock proteins in responses to large temperature fluctuations (Colinet et al., 2015) – may also stimulate the immune response (Wojda, 2017). Exposure to these stressors in sequence, especially, can pre-

emptively activate common defense mechanisms and induce cross-tolerance to the subsequent stressor (Verheyen et al., 2022). Mechanisms that protect against physiological damage from temperature stress may also confer protection against pathogens: for instance, heat-shock proteins, which prevent protein denaturation and repair misfolded proteins during heat stress, may also protect host tissues from the toxic effects of reactive oxygen species produced during the immune response (Wojda, 2017). Aside from the consequences for insect hosts, hot and variable conditions also impact pathogen responses via changes in host body temperatures. Increased temperatures and daily temperature variation may directly reduce the abundance of parasites and pathogens within the organism and speed up degradation of biopesticides in the environment, or favor host performance over that of the pathogen, depending on the relative position and shape of their thermal performance curves (Thomas and Blanford, 2003; Ferguson and Sinclair, 2020).

Despite this complexity, understanding how warmer and more variable temperatures may alter insect-pathogen interactions may be especially critical for herbivorous insects that are the targets of biological control. In particular, studying the sublethal effects of pathogen infections in these contexts could be revealing, since insects are frequently exposed to low or moderate doses in the field (Guedes et al., 2017). For instance, when targeting lepidopteran larvae with microbial biopesticides, several areas that harbor larvae may receive partial or inadequate coverage (Roh et al., 2007). High rates of larval movement between plants (Pannuti et al., 2016; Faretto et al., 2017) further increase the likelihood of larvae receiving a sublethal dose as they move away from a sprayed area or into a sprayed area only after a delay. More broadly, studying responses to sublethal doses also allows us to probe the longer-term consequences of responding to concurrent thermal and pathogen stress. Immune activation and recovery from infection are associated with increases in energy expenditure (Ardia et al., 2012). Sustained exposure to high temperatures or repeated exposures to diurnal extremes could exert further energetic demands that could exacerbate the effects of sublethal infection on growth, development and survival in subsequent life stages.

In this study, we explored how temperature fluctuations around moderate and elevated mean temperatures affect the outcomes of a lepidopteran insect host-pathogen interaction. We studied the effects of a sublethal dose of *Bacillus thuringiensis subsp. kurstaki*, an

entomopathogenic bacterium commonly used in biological control, on the survival of *Spodoptera frugiperda* larvae and on the longer-term consequences for performance in those that survive larval exposure to the bacterium. The toxic effects of *Bacillus thuringiensis* (*Bt*) are primarily due to crystalline proteins produced during sporulation. When ingested by susceptible lepidopteran larvae, these protein endotoxins are proteolytically activated in the insect gut. The activated toxin can then cross the peritrophic membrane, bind to receptors on the brush border membrane of midgut epithelial cells, and eventually cause perforation of the midgut epithelial barrier. *Bt* and other gut bacteria may then be released into hemocoel, potentially killing the host via septicemia (Herrero et al., 2016; Li et al., 2020; Jurat-Fuentes et al., 2021). However, larvae may also mount responses to *Bt* that improve their chances of survival under low or moderate doses. These may include enhancing midgut epithelium repair, perhaps by upregulating specific proteins associated with cell proliferation or by forming melanized scabs to seal gaps, as well as increasing immune responses, both within the midgut (e.g. upregulating midgut antimicrobial proteins) and systemically, to limit the infection and prevent septicemia (Herrero et al., 2016; Jurat-Fuentes et al., 2021). For instance, sublethal exposure to *Bt* toxins has been shown to increase hemolymph phenoloxidase activity in several species (including *S. frugiperda*) and increase other immune functions (lysozyme-like activity and encapsulation rate) in *Galleria mellonella* (Dubovskiy et al., 2008; Cunha et al., 2013; Grizanova et al., 2014; Li et al., 2020).

The efficacy of *Bt* strains in suppressing *S. frugiperda* may depend on environmental conditions, due to environmental effects on pathogenicity (Raun et al., 1966) and/or on host susceptibility (as suggested by Lysyk and Selinger, 2012). Though studies in some insect systems have reported increases in overall mortality following exposure to *Bt* with increasing temperature (Li et al., 1995; Katbeh-Bader et al., 1999), other studies find that mortality declines when rearing temperatures are elevated (van Frankenhuyzen et al., 2008; Lysyk and Selinger, 2012), suggesting that this relationship could be specific to the particular host species and *Bt* strain.

To investigate how mean temperature and temperature fluctuations alter responses to *Bt* in *S. frugiperda*, we exposed fourth instar larvae reared under different temperature regimes ($26 \pm 0^\circ\text{C}$, $26 \pm 6^\circ\text{C}$, $30 \pm 6^\circ\text{C}$, $30 \pm 0^\circ\text{C}$) to a dietary *Bacillus thuringiensis subsp. kurstaki* dose observed to cause intermediate levels of mortality under standard rearing conditions. We then monitored stage survivorship, development rates and mass at pupation in these individuals. We

hypothesized that elevated mean temperature (30°C) and temperature fluctuations around 26°C ($26 \pm 6^\circ\text{C}$) would increase survival relative to constant 26°C following exposure to *Bt*, due to increased immune system activation and rates of repair at the higher temperature and the potential immunostimulatory effects of fluctuations (Greenspan et al., 2017; Ferguson and Sinclair, 2020). However, we expect large fluctuations around 30°C to decrease survival relative to constant 30°C, due to the inclusion of stressful high temperatures in this treatment, which may reduce immune activity and constrain energy availability. Based on previous examinations of the sublethal effects of *Bt* toxins, we expected exposure to *Bt* to delay development time and reduce pupal mass (Hardke et al., 2011; Sousa et al., 2016; Zhang et al., 2018; Botha et al., 2019). We hypothesized that the magnitude of these effects across temperatures would track patterns in mortality, with poorer outcomes for individuals at the intermediate mean temperature 26°C and with the effect of fluctuations on performance depending on mean temperature.

Methods

Study system

We sourced *Spodoptera frugiperda* eggs from a mass-reared stock colony (Benzon Research Inc.) that was established using a strain collected from the USDA-ARS Station in Stoneville, Mississippi (USA) and has been maintained at 26°C–29°C for several years in the laboratory. For each preliminary and main assay, over one-thousand eggs were collected from *Bt*-susceptible females within 8 hours of oviposition and transferred to the University of Tennessee by the following day. Upon receipt, we separated all the eggs into groups of approximately 50, and each group was randomly assigned to a treatment using a random number generator (Haahr, 2025). Eggs were placed in the assigned incubator in sealed Ziploc bags and checked daily until larvae hatched. For each assay and within each treatment, we randomly selected neonate larvae that hatched on the same day for observation and reared them individually in 37 mL plastic containers on ad libitum agar-based artificial diet (Frontier Agricultural Sciences Inc.).

Preliminary bioassays

We first conducted preliminary assays to determine a suitable moderate (sublethal) dose of *Bacillus thuringiensis subsp. kurstaki* (*Btk*) to use in the main experiment since *S. frugiperda*

populations vary in their susceptibility to different *Bt* strains and Cry toxins (Monnerat et al., 2006). We used a commercially available biopesticide formulation, Thuricide® (Certis USA, Columbia, MD), that is marketed for use on lepidopteran pests. This product contains solids, spores and toxic crystalline proteins from the SA-12 strain of *Bacillus thuringiensis subsp. kurstaki* (98.35% by weight) as the active ingredient. Formulations containing spores and protein-endotoxins are known to have toxic activity against *S. frugiperda* (Souza et al., 2019). Our goal was to find the concentration that results in approximately 50% additional mortality in exposed fourth instar larvae relative to unexposed controls at 26°C, a standard temperature for rearing *S. frugiperda* in the laboratory. Selecting such a concentration would best enable us to detect any variation in mortality across temperature and fluctuation treatments (van Frankenhuyzen et al., 2008), while having sufficient larvae survive to subsequent developmental stages to capture the effects of *Bt* exposure on longer-term outcomes. Fourth instar larvae are less sensitive to commercial *Bt* formulations than early instars and are expected to survive at higher rates in the field. Understanding the consequences for those that do survive is therefore important, especially since *S. frugiperda* larvae consume most of the total foliage they consume as larvae in the later instars and experience the largest mass gain between the fourth instar and pupation (Sparks, 1979; Capinera, 2017).

Larvae from a single cohort were reared at constant 26°C on ad libitum diet as described in the previous section (Study System) until the fourth instar. The number of individuals in each instar was monitored daily to twice daily. Less than 24 hours before larvae were expected to molt into the fourth instar, we prepared a series of 5 concentrations of *Bt* spore-protein mixture suspensions using deionized water, spanning from 0.4% volume/volume (the recommended label concentration) to 0.01% volume/volume in dilution steps by a factor of 2.5. We then overlaid artificial diet that had been prepared in 12-well plates with *Bt* suspensions. Each well (with a surface area of 3.85 cm² and containing 2.0 mL of diet) was treated with 200 µL of a *Bt* suspension and a single concentration of *Bt* suspension was used for the whole plate, with multiple plates per concentration. We dispensed an equal volume of deionized water into each well of a corresponding set of control diet plates. After filling wells in each plate, we ensured that the liquid completely covered all of the diet surface. We then allowed plates to dry, monitoring them to prevent the gel from cracking or pulling away from the well sides and

exposing untreated diet. We then transferred recently molted fourth instar larvae to the treated and control well plates, with 1 larva per well, and returned the larvae to the rearing incubator. We recorded daily survivorship and instar/ development stage for each larva in the assay at 24-hour intervals. Using data on larval survival to pupation, we calculated adjusted mortality (i.e. corrected for mortality in the control plates) for each *Bt* suspension concentration using Henderson-Tilton's formula (Henderson and Tilton, 1955). The concentration that resulted in adjusted larval stage mortality closest to 50% informed our choice of sublethal concentration in the main assay.

Temperature treatments for the main experiment

We reared and monitored *S. frugiperda* individuals from egg to adult in four temperature treatments. Treatments were combinations of one of two mean temperatures (26°C, 30°C) and one of two amplitudes of daily temperature variation ($\pm 0^\circ\text{C}$ or $\pm 6^\circ\text{C}$). For fluctuating regimes, we programmed temperatures in incubators to fluctuate sinusoidally between specified daily maxima and minima and maintain a consistent photoperiod of 14D:10N to simulate natural cycles and expected summer day length. We selected fluctuating temperature regimes that are representative of diurnal variation in regions where *S. frugiperda* can complete development year-round or during the growing season (Early et al., 2018) and constant temperature treatments with the same mean temperature for comparison. The two mean temperatures chosen for this study also represent different locations on the thermal performance curve for development in this species, such that in the absence of infection or other stressors, increased temperature variability around a mean of 26°C has negligible impacts on development, whereas increased temperature variability around 30°C has significant negative effects (see Chapter 2).

Survival and development assays

Larvae from a single cohort were reared under the four temperature regimes described in the previous section (Temperature Treatments) on ad libitum diet (as described under Study System) until the fourth instar. We monitored survival and molting to each subsequent instar regularly (daily to twice daily) until larvae reached fourth instar. Less than 24 hours before larvae were expected to molt into the fourth instar in each temperature treatment, we prepared *Bt*

suspension of 0.06% (volume/volume) concentration using deionized water. We then dispensed 515 μL volume of *Bt* suspension onto the surface of artificial diet that had been freshly prepared in 37 mL rearing containers. Each rearing cup contained 4 mL of diet (diet surface area was approximately 7.6 cm^2). Corresponding control containers included 515 μL of deionized water applied over 4 mL of diet. We maintained a similar liquid overlay volume to surface area ratio as for the pilot study (67 $\mu\text{L}/\text{cm}^2$). We ensured that the liquid completely covered all of the diet surface and allowed the diet to dry, checking that untreated diet was not exposed as it dried. We then randomly assigned recently molted fourth instar larvae from the temperature treatment to either the “*Bt* exposure” condition or the “control” condition, transferred larvae accordingly to the relevant treated or control containers using paintbrushes and returned them to the same treatment incubator. In each combination of temperature regime and *Bt* exposure condition, we had 35–45 fourth instar larvae. We monitored survivorship and instar/ development stage for each larva in the assay daily at 24-hour intervals from the time of transfer. We monitored surviving larvae through the pupal stage until the date of adult eclosion, and weighed and sexed each surviving pupa one day following pupation to prevent damage to the newly formed pupal cuticle.

Data analysis

Final sample sizes from the main experiment are in **Supplemental Table 4-2**. While this experiment was replicated, we did not analyze data from the second experimental run due to elevated mortality and a high proportion of prepupal and pupal abnormalities among control larvae in that cohort (**Supplemental Fig. 4-5**).

Our first goal was to determine how temperature mean and fluctuation modified the impact of exposure to a moderate *Bt* concentration on *S. frugiperda* larval survival to the pupal and adult stages. We used logistic regression models with binomial error distributions and logit link functions to analyze survivorship to successive developmental stages. To understand the potential interactive effect of temperature and *Bt* exposure, we included mean temperature, temperature fluctuation, exposure condition and all two- and three-way interactions as predictors in the initial models. Because each combination of mean and variance corresponded to an experimental group and since we expect the general shape of temperature-performance functions

to be nonlinear, mean temperature and temperature fluctuation were specified as factor variables in our models. We could only identify sex at pupation, so we could not include sex as a predictor of survival since many individuals died in the larval stage.

In three of four temperature regimes, all unexposed control larvae survived to pupation, which led to complete separation in the logistic regression model for survival to pupation. Since a similarly high proportion (98%) of controls pupated in the remaining temperature regime ($30 \pm 6^\circ\text{C}$), we removed exposure condition from this model and focused on predicting survival to pupation among *Bt*-exposed larvae as a function of mean temperature, temperature fluctuation and their interaction. Complete separation did not occur for our model of survival to eclosion, so we proceeded with the initial model.

Our second goal was to understand whether and how temperature variation altered development time between hatch and pupation, pupal mass and the duration of the pupal stage in those that survived initial infection with *Bt*. We first conducted likelihood ratio tests to investigate whether *Bt* exposure affected average pupal mass and development times to pupation within each temperature regime. There was little variation in the duration of the pupal stage among individuals in each temperature and exposure condition, therefore we did not conduct statistical comparisons of this response between exposure conditions. Because final sample sizes and the shapes of the outcome distributions differed considerably between control and *Bt*-exposed larvae (**Supplemental Table 4-2**), we then evaluated the independent and interactive effects of mean temperature and temperature fluctuation on outcomes separately for *Bt*-exposed larvae and controls (for comparison).

We analyzed the number of days between hatch and pupation and the duration of the pupal stage using Conway-Maxwell Poisson GLMs (glmmTMB package with family = `compois(link = "log")`; Brooks et al., 2017), since development time was measured in discrete units (days) and since these data were underdispersed. We modelled development time between hatch and pupation as a function of mean temperature, temperature fluctuation and their interaction. We did not include sex in our final models of time to pupation because the average difference between male and female individuals was negligible (for *Bt*-exposed larvae) or minor (0.3 days or about 7 hours for unexposed larvae) and was not modified by temperature. For pupae that had been exposed to *Bt* as larvae, we modelled the duration of the pupal stage in days

as a function of mean temperature, temperature fluctuation, sex and the interaction between mean temperature and temperature fluctuation. On average, across all treatments, males surviving the pupal stage took 0.5–1 day longer than females to reach eclosion (**Table 4-1**).

Our models of pupal mass assumed a Gaussian error distribution and our analyses excluded individuals with missing sex identification or pupal abnormalities. We initially modeled mass at pupation as a function of mean temperature, temperature fluctuation, sex and all two-way and three-way interactions, to account for potential differences between males and females in responses to pathogen and/ or thermal stress. However, for individuals exposed to *Bt*, our sample size did not support a model of this complexity. Therefore, for this group, we removed the three-way interaction and the interaction between sex and mean temperature since these did not appear to be supported by the data and since doing so did not sacrifice explanatory power (the reduced model still explained about 18% of the variation in pupal mass among those exposed to *Bt*).

All statistical analyses were conducted in R version 4.4.1 (R Core Team, 2024). We evaluated models using the residual diagnostics tools from the DHARMa R package (Hartig, 2022) and tested hypotheses about model predictors using the Anova function from the car R package (Fox and Weisberg, 2019). We evaluated simple effects associated with the interactions between temperature variables, exposure condition and sex using the emmeans R package (Lenth, 2024).

Results

Survival to pupation

The concentration of *Bt* spore-protein mixture used in the main experiment (0.06% volume/volume) elevated larval mortality under all temperature regimes, but a small to substantial percentage of larvae exposed as fourth instars survived and pupated in each temperature treatment: ranging from only 19% in constant 26°C to 75% in constant 30°C (**Fig. 4-1**).

Among control larvae followed in this study, mortality was close to zero in all temperature regimes. Among larvae reared on diet treated with *Bt*, most larval deaths occurred within 2 to 5 days from exposure (**Supplemental Fig. 4-6**).

Table 4-1. Duration of larval instars within each temperature regime and *Bt* exposure condition.

Temperature regime	Exposure	Median duration, instar 4 (IQR)	Median duration, instar 5 (IQR)	Median duration, instar 6 (and instar 7) (IQR)
26 ± 0°C	<i>Bt</i>	4 (4–5)	3 (3–4.25)	9 (8.75–10.25)
26 ± 0°C	Control	2 (2–2)	2 (2–2)	4 (4–4)
26 ± 6°C	<i>Bt</i>	5 (4–5)	3 (3–3)	8 (7–8)
26 ± 6°C	Control	2 (2–2)	2 (2–2)	4 (4–4.5)
30 ± 0°C	<i>Bt</i>	3 (3–3)	2 (2–3)	5 (3–5)
30 ± 0°C	Control	2 (2–2)	1 (1–2)	3 (3–3)
30 ± 6°C	<i>Bt</i>	3 (3–4)	2.5 (2–3)	6 (4–6)
30 ± 6°C	Control	1 (1–2)	2 (1.75–2)	4 (3–4)

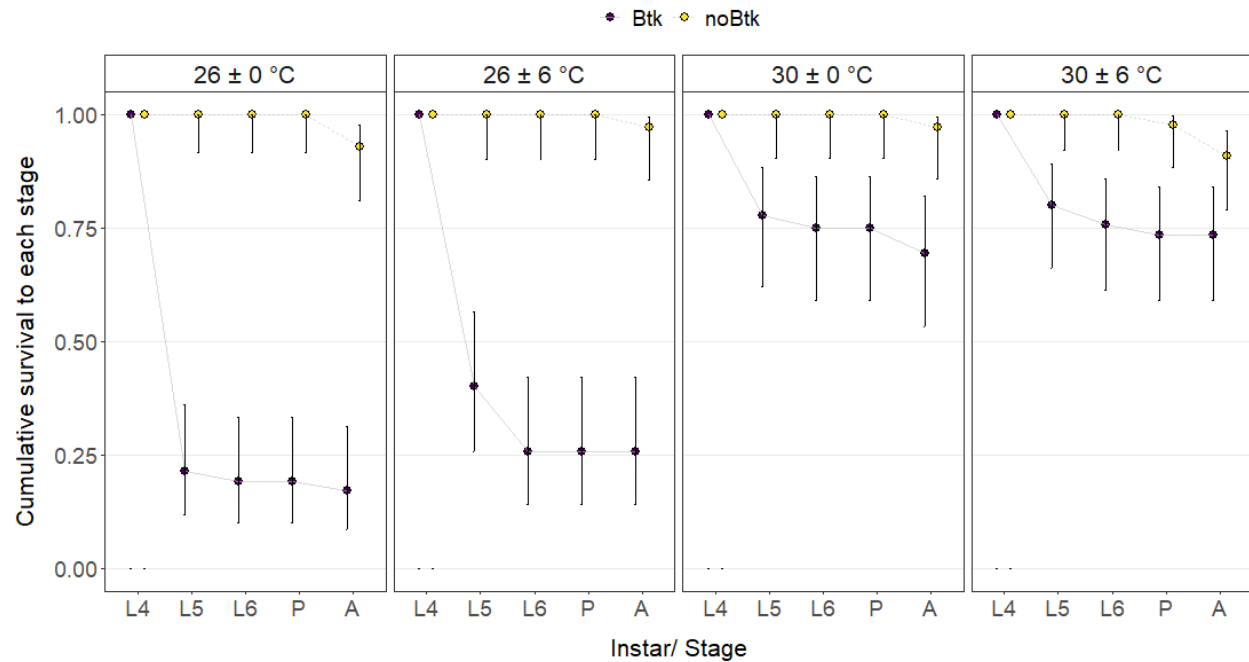


Figure 4-1. Cumulative survival of fourth instar larvae to successive life stages in each temperature and *Bt* exposure condition. Points represent the proportion of all starting fourth instars in a temperature and exposure condition that survive to a subsequent instar or life stage. Error bars indicate Wilson 95% confidence intervals for the proportions. L5 and L6 represent molts to the fifth and sixth larval instars, and P and A represent pupation and adult eclosion respectively.

Overall, our model of larval stage survivorship among those exposed to *Bt* explained 20.8% of the variation in survivorship (**Supplemental Table 4-3**). Analyses of deviance revealed that mean temperature significantly altered survival to pupation following exposure to *Bt* ($p < 0.0001$). However, we did not detect a statistically significant independent or interactive effect of temperature fluctuation on survival (**Supplemental Table 4-4**). *Bt*-exposed larvae held at constant 30°C were 3.9 times as likely to survive to pupation as *Bt*-exposed larvae held at constant 26°C. Under fluctuating temperatures, *Bt*-exposed larvae were 2.9 times as likely to survive to pupation in conditions with a mean temperature of 30°C relative to conditions with a mean temperature of 26°C.

Survival to adult eclosion

For individuals exposed to *Bt*, mortality in the pupal stage was low relative to larval mortality. Yet, the overall proportion of these individuals that survived from the fourth instar to adult eclosion was still lower than the same proportion among controls in every temperature regime and ranged from 17% in constant 26°C to 73% in the fluctuating treatment with mean 30°C (**Fig. 4-1**). Our overall model of survival to adult eclosion explained 36.7% of the deviance in survivorship. Analyses of deviance revealed a significant interaction between mean temperature and exposure, suggesting that the extent to which *Bt* exposure reduced survival depended on mean temperature ($p = 0.003334$; **Supplemental Table 4-5**). However, interactions involving temperature fluctuation were not supported ($p > 0.27$), suggesting that temperature fluctuations did not significantly alter the effect of *Bt* on survival at either mean temperature (26°C or 30°C). When we removed interactive effects that were not supported from the model, the reduced model still explained 36% of the variation in survivorship.

Treatment with *Bt* significantly reduced the probability of surviving to adult eclosion relative to unexposed controls at both mean temperatures ($p \leq 0.0006$; **Supplemental Table 4-6**). However, *Bt*-exposed larvae reared under constant or fluctuating temperatures with a mean of 30°C were 3.4 times as likely to survive to eclosion as *Bt*-exposed larvae reared in conditions with a mean of 26°C ($p < 0.0001$). We did not find a significant difference in survival probability between unexposed controls reared in mean 26°C versus those reared in mean 30°C ($p = 0.7543$). In all temperature regimes, between 91–97% of control larvae reached maturity.

Development time from hatch to pupation

Exposure to *Bt* during the fourth instar significantly delayed development time from hatch to pupation in all temperature treatments ($p < 0.0001$; **Supplemental Table 4-7**). In both treatments with a mean temperature of 30°C, larvae that survived exposure to *Bt* took 40–43% longer to reach pupation relative to unexposed controls. This delay was more pronounced in the constant 26°C treatment, with those surviving *Bt* exposure taking 70% longer to reach pupation than unexposed larvae. However, under fluctuating temperatures with the same mean ($26 \pm 6^\circ\text{C}$), time to pupation was only 48% longer in *Bt*-exposed larvae relative to controls. Larval development time was more variable for *Bt*-exposed individuals that survived the larval stage, ranging from 12 to 30 days across temperatures, whereas development times for unexposed controls ranged from 9 to 16 days across temperatures (**Fig. 4-2**).

Analyses of deviance revealed significant interactive effects of mean temperature and temperature fluctuation on development time to pupation in both *Bt*-exposed larvae and

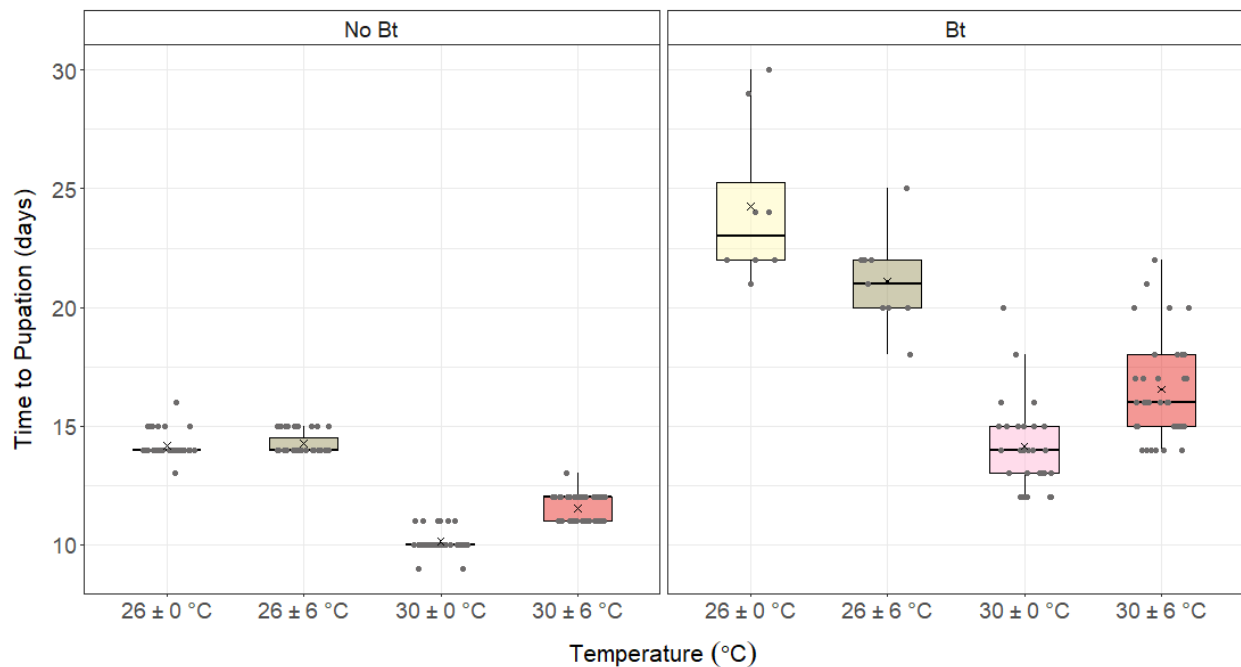


Figure 4-2. Number of days from hatching to pupation for surviving pupae in each temperature and *Bt* exposure treatment. Jittered points represent individual development times. Boxes and dark lines represent inter-quartile ranges and medians, respectively, for each group. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range. Crosses (×) represent group means.

unexposed control larvae ($p < 0.0001$; **Supplemental Tables 4-8, 4-9**). On average, control larvae reared in conditions with a mean temperature of 30°C reached pupation in fewer days than those reared in conditions with a mean temperature of 26°C (10–12 days versus 14 days; $p < 0.0001$). For these larvae, temperature fluctuations around 26°C did not alter larval development time compared to constant 26°C ($p = 0.4455$), whereas fluctuations around 30°C delayed larval development time by 1.5 days on average relative to constant 30°C ($p < 0.0001$; **Supplemental Table 4-10**).

For individuals exposed to *Bt* during the fourth instar that successfully pupated, the effects of both increasing mean temperature and increasing fluctuation were generally more pronounced. For these larvae, increasing the mean temperature from 26°C to 30°C significantly reduced time to pupation by 42% in constant temperatures ($p < 0.0001$) and by 22% under fluctuations ($p < 0.0001$). Temperature fluctuations around both mean temperatures significantly altered time to pupation, but in opposite directions at 26°C and at 30°C. Around mean 26°C, temperature fluctuation reduced the average number of days to pupation by about 13% ($p = 0.007$). Around mean 30°C, temperature fluctuation increased the average number of days to pupation by about 17% ($p < 0.0001$; **Supplemental Table 4-11**).

Table 4-1 shows the median duration of each larval instar for all temperature treatments.

Mass at pupation

Likelihood ratio tests indicate that larval exposure to *Bt* does not affect the expected pupal mass of surviving individuals under most temperature conditions. However, pupae held at constant 26°C that had been exposed to *Bt* were lighter by about 27 mg on average than control pupae at the same temperature ($p = 0.02901$; **Supplemental Table 4-12**).

Among unexposed controls in this experiment, expected pupal mass did not vary significantly across temperature regimes or between males and females. None of the predictors in this model had statistically significant independent or interactive effects on pupal mass (**Supplemental Table 4-13**).

For pupae surviving exposure to *Bt*, the effect of temperature fluctuation on pupal mass does not depend on mean temperature as we had predicted. However, analysis of variance revealed a statistically significant interaction between temperature fluctuation and sex ($p =$

0.046; **Supplemental Table 4-14**). Specifically, among those that survived to pupation, *Bt*-exposed males in fluctuating temperature treatments weighed more on average than *Bt*-exposed males in constant temperature treatments ($p = 0.0019$, **Supplemental Table 4-15**). On the other hand, for *Bt*-exposed females, expected pupal mass was consistent across fluctuation treatments ($p = 0.63$, **Supplemental Table 4-15**) and across mean temperatures (**Fig. 4-3**). However, given the low number of surviving pupae in the *Bt* condition, particularly for treatments with a mean of 26°C, these results should be interpreted cautiously. Our reduced model of pupal mass for the *Bt*-exposed group explained about 18% of the variation in this response.

Duration of the pupal stage

For those that survived to adult eclosion, pupal stage durations did not differ between exposure conditions in most temperature regimes (**Fig. 4-4**). At constant 26°C, pupae that were exposed to *Bt* as larvae took a day longer to eclose on average than unexposed controls, although there were few survivors in this exposure condition.

For those in the control condition, pupal stage duration decreased from 8–9 days in treatments with mean 26°C to 6–7 days in treatments with mean 30°C (**Fig. 4-4**). For this group, temperature fluctuations did not appear to impact pupal stage durations, although females in 30 ± 6°C spent 1 fewer day in the pupal stage than females in constant 30°C.

For surviving individuals that had been exposed to *Bt* as larvae, temperature fluctuation modified the effect of mean temperature on pupal stage duration ($p = 0.02339$; **Supplemental Table 4-16**). Pupae in this exposure condition took 1–3 fewer days to eclose in treatments with mean 30°C relative to treatments with mean 26°C ($p < 0.0001$; **Supplemental Table 4-17**). For this group, fluctuations around mean 26°C reduced pupal stage durations by 1 day on average ($p < 0.0001$; **Supplemental Table 4-17**).

Discussion

Our results partially support our hypothesis that the level of mortality induced by an intermediate concentration of *B. thuringiensis subsp. kurstaki* (0.06% volume/volume) in *S. frugiperda* would depend heavily on environmental temperature. As expected, increasing mean temperature of the rearing environment substantially increased larval survivorship

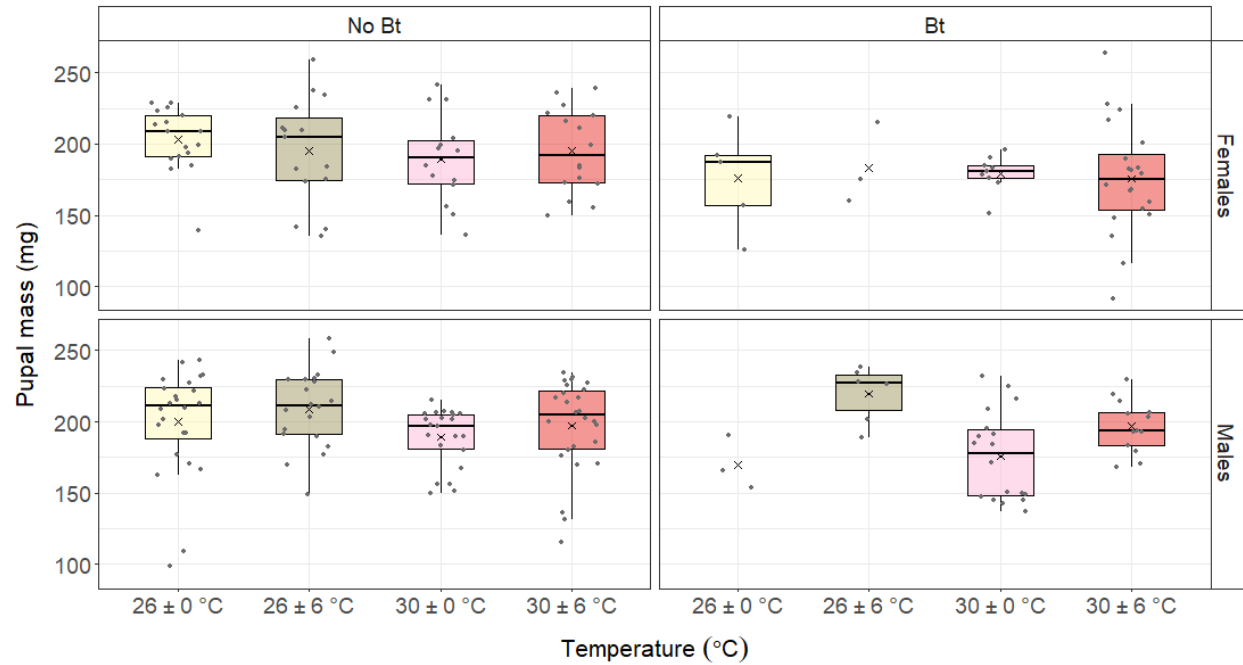


Figure 4-3. Distributions of pupal mass within each temperature regime and *Bt* exposure condition. Jittered points represent individual pupal masses. Boxes and dark lines represent inter-quartile ranges and medians, respectively, for each group. Whiskers extend to the most extreme data value that is within 1.5 times the inter-quartile range. Crosses (×) represent group means.

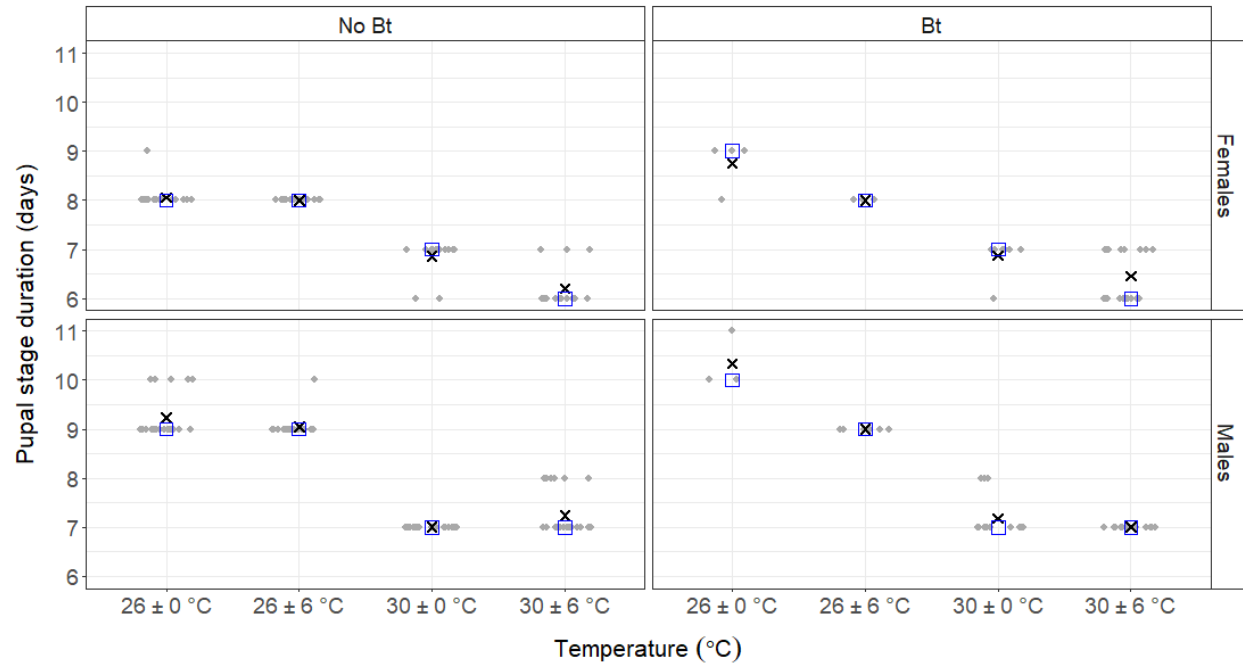


Figure 4-4. Pupal stage durations for males and females that survived to eclosion in each temperature and *Bt* exposure treatment. Jittered points represent individual stage durations. Black crosses (×) represent group means. Blue open squares show group medians.

following *Bt* exposure. These results align with some findings from other lepidopteran and dipteran species (van Frankenhuyzen et al., 2008; Lysyk and Selinger, 2012), but contradict other observations that insects fed *Bt*-contaminated diet experienced higher mortality at warmer temperatures (Li et al., 1995; Katbeh-Bader et al., 1999). The latter relationship between temperature and *Bt* toxicity has been attributed to higher feeding rates at warmer temperatures, which lead to larvae receiving higher pathogen doses – indeed, tightly controlling the dose ingested eliminated the effect of rearing temperature on overall larval mortality in at least one species of lepidoptera (*Choristoneura fumiferana*; van Frankenhuyzen and Nystrom, 1987). Warmer temperatures could also increase rates of bacterial cell multiplication within the affected insect, which may quicken the progression of septicemia and death in an individual receiving an adequate dose (van Frankenhuyzen, 1994). However, despite not controlling the exact pathogen dose ingested by larvae, we found an inverse relationship between mortality and temperature in infected larvae. Secondly, median time to death in our study was comparable across most treatments (though 2 days shorter at constant 30°C). The pattern observed in this study may instead be mediated by changes in the effectiveness of host defenses and in larval development rates (Lysyk and Selinger, 2012). Warmer temperatures may enhance the activation of immune responses involved in recovery from *Bt* (Thomas and Blanford, 2003). By developing more rapidly to later instars, larvae at mean 30°C may more quickly become less susceptible to the pathogen despite continuing to feed on contaminated diet; in general, later instar larvae require higher lethal doses (van Frankenhuyzen et al., 2008). Sustained exposure to higher temperatures may also cause the biopesticide overlay to lose efficacy over a shorter timescale.

Contrary to our predictions, however, ambient temperature fluctuations had little impact on overall survivorship to pupation or adult eclosion in individuals exposed to *Bt* as larvae. While *Bt*-exposed larvae reared in the cooler fluctuating temperature regime molted to the next larval instar at higher rates than those in the corresponding constant mean temperature (**Fig. 4-1**), the difference in survival had diminished by the sixth instar. Therefore, any effect of daily temperature variation on this outcome is minor relative to the effect of the overall average environmental temperature. In our previous study (see Chapter 2), we found large fluctuations around 30°C to reduce survival to eclosion in uninfected larvae, likely because these larvae were regularly exposed to stressful temperatures near their upper performance threshold. In the current

study, we did not find lower survivorship to subsequent life stages among control larvae in this temperature regime. Here, however, we monitored a smaller subset of individuals that were all alive by the fourth instar, were similarly sized and at roughly the same point in development. The impacts of fluctuations in this temperature range may possibly be more salient in larvae infected with low doses of *Bt* in early instars, which may show more variation in growth and development trajectories and thermal tolerance.

In survivors of larval exposure to *Bt*, outcomes such as development times and pupal mass did not show the same pattern across temperatures as we had observed for survivorship. Of these, temperature had the largest impact on development time to pupation in infected individuals. While long delays in larval development times are an expected outcome of sublethal *Bt* exposure in *S. frugiperda* since larvae may pause feeding after initial exposure (Sousa et al., 2016; Botha et al., 2019), less attention has been paid to the temperature-dependence of this response in variable thermal environments. In line with our hypothesis, temperature fluctuations modified the effect of mean temperature on time to pupation in *Bt* survivors. Fluctuations around mean 30°C delayed time to pupation relative to constant 30°C – an expected outcome when ambient fluctuations include physiologically stressful temperatures (Colinet et al., 2015). We observed similar delays in larval development time with fluctuations in this temperature range in uninfected larvae, in this study and our previous experiment (see Chapter 2). Meanwhile, fluctuations around mean 26°C shortened time to pupation relative to constant 26°C in *Bt* survivors. In contrast, fluctuations around 26°C did not alter larval development times in uninfected larvae in either study. Diel fluctuations could possibly ameliorate the stressful effects of *Bt* exposure at this temperature, though not to the extent of decreasing acute mortality. For instance, periodic exposure to unfavorable temperatures over time could decrease the toxic activity of *Bt* in the insect body or in the diet, and fluctuations may potentially stimulate a faster, if not larger, immune reaction – either of which could speed up recovery in surviving larvae.

Infection with *Bt* directly inhibits digestion and recovery imposes additional energetic costs, both of which could constrain mass gain during the larval stage. Previous studies have found that *S. frugiperda* larvae reared on leaves from transgenic crops producing *Bt* Cry toxins have lower pupal mass (Hardke et al., 2011; Botha et al., 2019). However, in our study, we did not find differences between pupal mass in *Bt*-exposed and unexposed controls in most

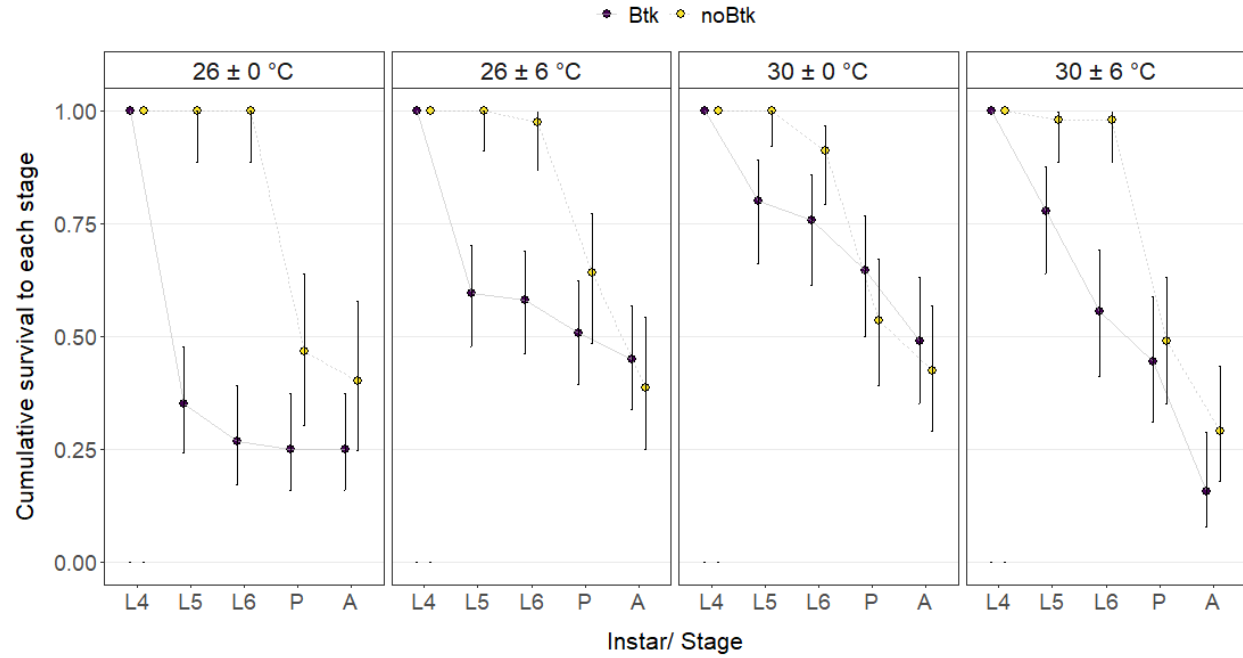
temperature regimes. Characteristics of the dietary exposure may explain this difference (*Bt* corn leaves versus meridic diet with a one-time surface application of a biopesticide that could degrade in the environment). Nevertheless, we should be mindful of potential survivorship bias in these results. For instance, it is possible that only larvae with sufficient energetic reserves weighing above a certain mass threshold may have been able to pupate successfully in the *Bt* treatments and that this threshold may have been higher for males under thermal fluctuations. Monitoring larval mass at multiple points throughout larval development could allow us to compare growth trajectories between those that pupate, those that survive initial infection but fail to pupate and those that do not survive.

In the current study, as in our previous work (see Chapter 2), we found differences in how fluctuating temperatures affected larval and pupal development times, with both temperature and *Bt* exposure having relatively subtle effects on pupal stage duration. Unlike larval development, pupal development in those exposed to *Bt* as larvae was faster under fluctuations relative to constant mean temperatures at both mean 26°C and at mean 30°C. This suggests that the thermal performance curve for development in pupae may be right-shifted relative to that for larvae, such that large fluctuations around 30°C are less stressful for pupae. Our observation that *Bt* infection affected pupal maturation rates to a smaller extent than larval development rates follows expectations given the latter's dependence on larval nutrition and agrees with other observations from *S. frugiperda* infected with other bacteria during the larval stage (Zhang et al., 2018).

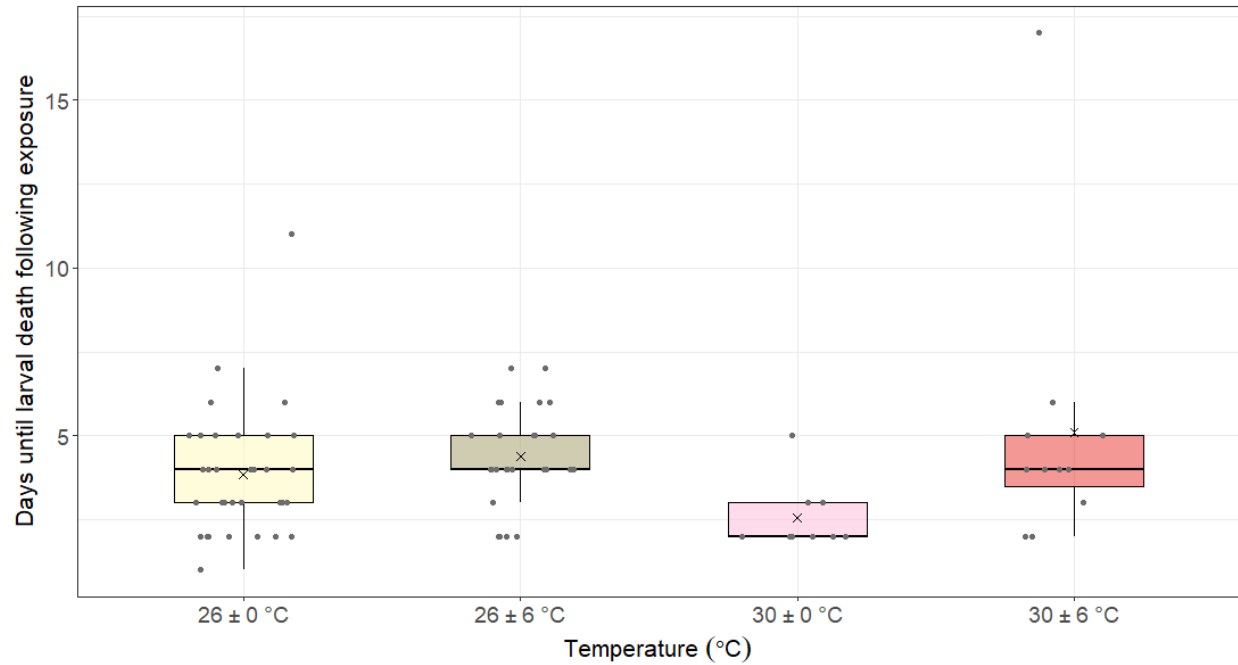
In summary, warmer average temperatures appear to reduce the negative effects of *Bt* on *S. frugiperda*, with declines in *Bt*-induced mortality following exposure being the most obvious effect. Though the efficacy of *Bt* and its toxins as biocontrol agents will depend on the specific *S. frugiperda* population, given variable levels of resistance in natural populations (Monnerat et al., 2006; Ingber et al., 2018; Loto et al., 2019), our results suggest that the relative dose needed to control populations would be larger at higher temperatures. Additionally, the relative delays in development time with sublethal *Bt* exposure may be smaller under warmer mean temperatures. The amplitude of diurnal temperature variability may have minimal impacts on larval and pupal survival following *Bt* exposure. However, increasing temperature fluctuations could still impact population-level outcomes by altering development times, with the nature of these impacts depending on mean environmental temperature. In the field, delays in larval development due to

sublethal *Bt* infection and environmental temperature could result in substantial additional larval mortality from prolonged exposure to threats such as intraspecific and interspecific competition, predation by natural enemies and additional pesticides.

Appendix A: Supplementary figures



Supplemental Figure 4-5. Cumulative survivorship data from the second experimental run. We excluded data from this run from the analyses due to unusually poor survival among control larvae under benign temperatures and a high rate of pupal and prepupal abnormalities (50% among control larvae).



Supplemental Figure 4-6. Number of days between *Bt* exposure and larval death for all individuals in the *Bt* treatment condition that died in the larval stage. Jittered points indicate time until death for each individual.

Appendix B: Supplementary tables

Supplemental Table 4-2. Sample sizes for each temperature regime and *Bt* exposure. Table shows the number of individuals assigned to each temperature and *Bt* exposure condition at the beginning of the experiment (L4) and the number reaching each successive life stage. L5 and L6 are the number molting to instars 5 and 6, respectively, Pupa is the number pupating, and Adult is the number eclosing.

Temperature regime	Exposure	L4 (initial)	L5	L6	Pupa	Pupa (female)	Pupa (male)	Adult
26 ± 0°C	No <i>Bt</i>	42	42	42	42	17	24	39
26 ± 0°C	<i>Bt</i>	42	9	8	8	5	3	7
26 ± 6°C	No <i>Bt</i>	35	35	35	35	15	20	34
26 ± 6°C	<i>Bt</i>	35	14	9	9	3	6	9
30 ± 0°C	No <i>Bt</i>	36	36	36	36	14	22	35
30 ± 0°C	<i>Bt</i>	36	28	27	27	9	18	25
30 ± 6°C	No <i>Bt</i>	44	44	44	43	16	26	40
30 ± 6°C	<i>Bt</i>	45	36	34	33	20	13	33

Supplemental Table 4-3. Final regression model and coefficients table for survival to pupation following exposure to *Bt*-treated diet.

Term	Estimate	Standard Error	z	p
(Intercept)	-1.44692	0.392953	-3.68217	0.000231
Mean 30°C	2.545531	0.550054	4.627783	3.7E-06
Fluctuation (high)	0.386047	0.551348	0.700188	0.48381
Mean 30°C: Fluctuation (high)	-0.47306	0.752176	-0.62892	0.529401

Number of observations = 158; Residual deviance = 173.48; Residual degrees of freedom = 154; Null deviance = 218.93; Null degrees of freedom = 157

Supplemental Table 4-4. Analyses of deviance results for survival to pupation following exposure to *Bt*-treated diet.

	χ^2	df	p
Mean Temperature	44.13117	1	3.07E-11
Temperature Fluctuation	0.123608	1	0.725154
Mean Temperature: Temperature Fluctuation	0.396834	1	0.528729

Supplemental Table 4-5. Analyses of deviance results for survival to adult eclosion.

	χ^2	df	p
Mean Temperature	32.25417	1	1.35E-08
Temperature Fluctuation	0.419526	1	0.517174
Exposure	104.2765	1	1.76E-24
Mean Temperature: Temperature Fluctuation	1.118827	1	0.290171
Mean Temperature: Exposure	8.614983	1	0.003334
Temperature Fluctuation: Exposure	0.488822	1	0.484453
Mean Temperature: Temperature Fluctuation: Exposure	1.195432	1	0.274237

Supplemental Table 4-6. Simple effects of mean temperature and *Bt* exposure on survival to adult eclosion.

Contrast	Mean Temperature	Exposure	Odds Ratio	SE	Null	z	p
<i>Bt</i> / No <i>Bt</i>	26	-	0.014474	0.008488	1	-7.22259	5.1E-13
<i>Bt</i> / No <i>Bt</i>	30	-	0.167499	0.087745	1	-3.41085	0.000648
Mean 30°C / Mean 26°C	-	No <i>Bt</i>	0.805387	0.556987	1	-0.31295	0.754315
Mean 30°C / Mean 26°C	-	<i>Bt</i>	9.320134	3.491783	1	5.958042	2.55E-09

Supplemental Table 4-7. Likelihood ratio tests of the effect of *Bt* exposure on development time between hatching and pupation.

Temperature Regime	Full model	Nested model	Difference in degrees of freedom	χ^2	p
26 ± 0°C	Days to Pupation ~ Exposure	Days to Pupation ~ 1	1	118.7651	1.17894E-27
26 ± 6°C	Days to Pupation ~ Exposure	Days to Pupation ~ 1	1	107.1455	4.1365E-25
30 ± 0°C	Days to Pupation ~ Exposure	Days to Pupation ~ 1	1	86.62795	1.30983E-20
30 ± 6°C	Days to Pupation ~ Exposure	Days to Pupation ~ 1	1	112.8610	2.31441E-26

Supplemental Table 4-8. Analyses of deviance results for development time between hatching and pupation in control individuals (i.e., not exposed to *Bt* during the fourth instar).

	χ^2	df	p
Mean Temperature	1889.241	1	0
Temperature Fluctuation	86.56206	1	1.35E-20
Mean Temperature: Temperature Fluctuation	92.27099	1	7.56E-22

Supplemental Table 4-9. Analyses of deviance results for development time between hatching and pupation in individuals exposed to *Bt*-treated diet during the fourth instar.

	χ^2	df	p
Mean Temperature	156.5698	1	6.36E-36
Temperature Fluctuation	5.751839	1	0.016471
Mean Temperature: Temperature Fluctuation	23.01294	1	1.61E-06

Supplemental Table 4-10. Simple effects of mean temperature and temperature fluctuation on days to pupation among control larvae (i.e., not exposed to *Bt* during the fourth instar).

Contrast	Temperature Fluctuation	Mean Temperature	Ratio	SE	Null	z	p
Mean 30°C / Mean 26°C	Constant	-	0.715686	0.006261	1	-38.2391	0
Mean 30°C / Mean 26°C	Fluctuation (high)	-	0.80906	0.007523	1	-22.7877	6.1E-115
Fluctuation (high) / Constant	-	26	1.006387	0.008398	1	0.762942	0.445498
Fluctuation (high) / Constant	-	30	1.137687	0.010992	1	13.35107	1.17E-40

Supplemental Table 4-11. Simple effects of mean temperature and temperature fluctuation on days to pupation among larvae exposed to *Bt*-treated diet during the fourth instar.

Contrast	Temperature Fluctuation	Mean Temperature	Ratio	SE	Null	z	p
Mean 30°C / Mean 26°C	Constant	-	0.583429	0.025955	1	-12.1122	9.1E-34
Mean 30°C / Mean 26°C	Fluctuation (high)	-	0.783732	0.033308	1	-5.73392	9.81E-09
Fluctuation (high) / Constant	-	26	0.870561	0.04478	1	-2.69485	0.007042
Fluctuation (high) / Constant	-	30	1.169443	0.039475	1	4.637083	3.53E-06

Supplemental Table 4-12. Likelihood ratio tests of the effect of *Bt* exposure on pupal mass (mg).

Temperature Regime	Full model	Nested model	Difference in degrees of freedom	Difference in Residual Sums of Squares	F	p
26 ± 0°C	Pupal Mass ~ Exposure	Pupal Mass ~ 1	1	-4977.3	5.0734	0.02901
26 ± 6°C	Pupal Mass ~ Exposure	Pupal Mass ~ 1	1	-149.19	0.1495	0.7009
30 ± 0°C	Pupal Mass ~ Exposure	Pupal Mass ~ 1	1	-2360.6	3.6468	0.06088
30 ± 6°C	Pupal Mass ~ Exposure	Pupal Mass ~ 1	1	-2866.5	2.7261	0.103

Supplemental Table 4-13. Analyses of variance results for mass (mg) at pupation of individuals in the control condition (i.e., not exposed to *Bt* as larvae).

	Sum of Squares	df	F	p
Mean Temperature	3325.885	1	3.603606	0.059628
Temperature Fluctuation	806.2088	1	0.873529	0.351523
Sex	343.5805	1	0.37227	0.542718
Mean Temperature: Temperature Fluctuation	224.1809	1	0.242901	0.622859
Mean Temperature: Sex	182.3309	1	0.197556	0.657359
Temperature Fluctuation: Sex	837.0561	1	0.906953	0.342498
Mean Temperature: Temperature Fluctuation: Sex	540.7028	1	0.585853	0.445263
Residuals	134748.2	146		

Supplemental Table 4-14. Analyses of variance results for mass (mg) at pupation of individuals that were exposed to *Bt* as larvae.

	Sum of Squares	df	F	p
Mean Temperature	626.3504	1	0.721208	0.398602
Temperature Fluctuation	4207.574	1	4.844793	0.030982
Sex	2435.54	1	2.804392	0.098406
Mean Temperature: Temperature Fluctuation	1398.298	1	1.610064	0.208627
Temperature Fluctuation: Sex	3567.879	1	4.108219	0.046432
Residuals	61661.61	71		

Supplemental Table 4-15. Simple effects of temperature fluctuation and sex on the mass (mg) of pupae that were exposed to *Bt*-treated diet as larvae.

Contrast	Sex	Temperature Fluctuation	Estimate	SE	df	t	p
Fluctuation (high) - Constant	f		5.307903	10.97833	71	0.483489	0.630236
Fluctuation (high) - Constant	m		33.84261	10.51668	71	3.217993	0.001946
Male - Female	-	Constant	-4.16508	10.50154	71	-0.39662	0.69284
Male - Female	-	Fluctuation (high)	24.36963	9.376191	71	2.599097	0.011361

Supplemental Table 4-16. Analyses of deviance results for pupal stage duration in individuals that were exposed to *Bt* as larvae.

	χ^2	df	p
Mean Temperature	370.5454	1	1.42E-82
Temperature Fluctuation	17.24449	1	3.29E-05
Sex	33.73114	1	6.33E-09
Mean Temperature: Temperature Fluctuation	5.139336	1	0.02339

Supplemental Table 4-17. Simple effects of mean temperature and temperature fluctuation on pupal stage duration in individuals exposed to *Bt* as larvae.

Contrast	Temperature Fluctuation	Mean Temperature	Ratio	SE	Null	z	p
Mean 30°C / Mean 26°C	Constant	-	0.732895	0.017698	1	-12.8685	6.77E-38
Mean 30°C / Mean 26°C	Fluctuation (high)	-	0.784855	0.013571	1	-14.01	1.35E-44
Fluctuation (high) / Constant	-	26	0.901768	0.022029	1	-4.23268	2.31E-05
Fluctuation (high) / Constant	-	30	0.965702	0.01667	1	-2.02183	0.043194

CHAPTER 5

CONCLUSIONS

In this dissertation, I explored the impacts of temperature variability across a range of average temperatures on *Spodoptera frugiperda* life history, demography, and susceptibility to the toxic effects of a pathogen and primary biocontrol agent. Here, I summarize my findings from each phase of this research and conclude with some final themes and takeaways.

First, I used controlled experiments to expose *S. frugiperda* individuals to combinations of different average environmental temperatures and different magnitudes of temperature fluctuation, with treatments representing ambient fluctuations within the species' range. For these individuals, I monitored life-history traits across the life cycle, which enabled me to consider potential time-dependent physiological effects of fluctuations. I used these data to parametrize matrix population models and analyze long-term population growth rates and demographic drivers of temperature effects at the population level. From these studies, I concluded that temperature fluctuations can influence survival, development rates, reproductive output, and population growth rates, with these effects generally being more salient and more negative at higher temperatures that approach upper performance thresholds (close to where performance is maximized for some traits). Furthermore, the effects of fluctuations vary substantially by trait and life stage, with more pronounced consequences for reproductive parameters.

Integrating effects at the population level, I found similar interactive effects of temperature means and variability: fluctuations around 30°C are expected to reduce λ for this population while fluctuations around intermediate temperatures may increase λ . Across mean temperatures, changes in fecundity, the longevity of reproductively active females and egg hatch rates explained these differences. Therefore, improving our understanding of how populations respond to warming and thermal variability may require more studies that focus on insect reproduction, since current efforts focus heavily on lethal limits.

Finally, I investigated the effects of temperature fluctuations at intermediate (26°C) and elevated temperatures (30°C) on outcomes in *S. frugiperda* larvae exposed to sublethal doses of *Bacillus thuringiensis* subsp. *kurtsaki* spore-protein mixtures. Here, I found survival following exposure to *Bt* to be largely predicted by mean temperature, with fluctuations having little impact

on survivorship to subsequent life stages. However, temperature fluctuations noticeably modified delays in larval development following *Bt* exposure in survivors, including at the intermediate mean temperature (26°C). Since population growth in this species may be particularly elastic to changes in survival and development in the larval stage (see Chapter 3), reductions in *Bt*-induced mortality with warming and changes in any sublethal effects with increasing variability could have significant consequences at the population level.

Taken together, a few key themes emerge from this dissertation. The first is that insect thermal performance can be complex and highly variable, within individual lifespans and across different environmental contexts. Although there is much interest in finding optimal temperatures for insect development, a more complex story emerges when we examine a larger suite of traits across the lifespan. This is especially true in thermally variable environments where organisms are exposed to multiple stressors and in the context of strongly interacting species, where temperature variability may alter which participant is favored in a given environment (Ferguson and Sinclair, 2020).

Second, temperature variability seems to have larger effects on thermal performance curves towards the tails of the curve, at least when we consider individual responses to temperature alone. Therefore, organismal responses to heat waves and other extreme events will be modified by characteristics of thermal variability during these events. These characteristics include fluctuation amplitude (the focus of this dissertation), but also fluctuation symmetry (for instance, disproportionate increases in nighttime temperatures) and predictability. Focusing our attention on measuring responses towards the extremes of thermal performance curve for traits other than critical thermal limits will help clarify these effects.

REFERENCES

- Abatzoglou, J.T., Dobrowski, S.Z., Parks, S.A., Hegewisch, K.C., 2018. TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958–2015. *Sci Data* 5, 170191. <https://doi.org/10.1038/sdata.2017.191>
- Adamo, S.A., 2012. The effects of the stress response on immune function in invertebrates: An evolutionary perspective on an ancient connection. *Hormones and Behavior, Special Issue: The Neuroendocrine-Immune Axis in Health and Disease* 62, 324–330. <https://doi.org/10.1016/j.yhbeh.2012.02.012>
- Adan, M., Tonnang, H.E.Z., Kassa, C.E.F., Greve, K., Borgemeister, C., Goergen, G., 2024. Combining temperature-dependent life table data into Insect Life Cycle Model to forecast fall armyworm *Spodoptera frugiperda* (J.E. Smith) distribution in maize agro-ecological zones in Africa. *PLOS ONE* 19, e0299154. <https://doi.org/10.1371/journal.pone.0299154>
- Ali, A., Luttrell, R.G., Schneider, J.C., 1990. Effects of Temperature and Larval Diet on Development of the Fall Armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 83, 725–733. <https://doi.org/10.1093/aesa/83.4.725>
- Angilletta, M.J., 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*, Thermal Adaptation. Oxford University Press.
- Angilletta, M.J., 2006. Estimating and comparing thermal performance curves. *Journal of Thermal Biology* 31, 541–545. <https://doi.org/10.1016/j.jtherbio.2006.06.002>
- Ardia, D.R., Gantz, J.E., C, B., Schneider, Strebel, S., 2012. Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. *Functional Ecology* 26, 732–739. <https://doi.org/10.1111/j.1365-2435.2012.01989.x>
- Ashok, K., Balasubramani, V., Kennedy, J.S., Geethalakshmi, V., Jeyakumar, P., Sathiah, N., 2021. Effect of elevated temperature on the population dynamics of fall armyworm, *Spodoptera frugiperda*. *J. Environ. Biol.* 42, 1098–1105. [https://doi.org/10.22438/jeb/42/4\(SI\)/MRN-1525a](https://doi.org/10.22438/jeb/42/4(SI)/MRN-1525a)
- Awde, D.N., Řeřicha, M., Knapp, M., 2023. Increased pupal temperature has reversible effects on thermal performance and irreversible effects on immune system and fecundity in adult ladybirds. *Commun Biol* 6, 1–7. <https://doi.org/10.1038/s42003-023-05196-0>

- Barfield, C.S., Ashley, T.R., 1987. Effects of Corn Phenology and Temperature on the Life Cycle of the Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The Florida Entomologist 70, 110–116. <https://doi.org/10.2307/3495097>
- Barfield, C.S., Mitchell, E.R., Poeb, S.L., 1978. A Temperature-Dependent Model for Fall Armyworm Development. Annals of the Entomological Society of America 71, 70–74. <https://doi.org/10.1093/aesa/71.1.70>
- Bathiany, S., Dakos, V., Scheffer, M., Lenton, T.M., 2018. Climate models predict increasing temperature variability in poor countries. Science Advances 4, eaar5809. <https://doi.org/10.1126/sciadv.aar5809>
- Botha, A.S., Erasmus, A., du Plessis, H., Van den Berg, J., 2019. Efficacy of Bt Maize for Control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in South Africa. J Econ Entomol 112, 1260–1266. <https://doi.org/10.1093/jee/toz048>
- Bozinovic, F., Bastías, D.A., Boher, F., Clavijo-Baquet, S., Estay, S.A., Angilletta, M.J., 2011. The Mean and Variance of Environmental Temperature Interact to Determine Physiological Tolerance and Fitness. Physiological and Biochemical Zoology 84, 543–552. <https://doi.org/10.1086/662551>
- Bozinovic, F., Catalan, T.P., Estay, S.A., Sabat, P., 2013. Acclimation to daily thermal variability drives the metabolic performance curve. Evolutionary Ecology Research 15, 579–587.
- Brooks, M.E., Kristensen, K., Benthem, K.J. van, Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9, 378–400.
- Buckley, L.B., Cannistra, A.F., John, A., 2018. Leveraging Organismal Biology to Forecast the Effects of Climate Change. Integr Comp Biol 58, 38–51. <https://doi.org/10.1093/icb/icy018>
- Buckley, L.B., Huey, R.B., 2016. Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. Global Change Biology 22, 3829–3842. <https://doi.org/10.1111/gcb.13313>

- Buckley, L.B., Huey, R.B., Ma, C.-S., 2025. How Damage, Recovery, and Repair Alter the Fitness Impacts of Thermal Stress. *Integrative and Comparative Biology* icaf019. <https://doi.org/10.1093/icb/icaf019>
- Capinera, J.L., 2017. Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae). University of Florida/ Institute of Food and Agricultural Sciences.
- Carrington, L.B., Armijos, M.V., Lambrechts, L., Barker, C.M., Scott, T.W., 2013. Effects of Fluctuating Daily Temperatures at Critical Thermal Extremes on *Aedes aegypti* Life-History Traits. *PLOS ONE* 8, e58824. <https://doi.org/10.1371/journal.pone.0058824>
- Carter, A.W., Sheldon, K.S., 2020. Life stages differ in plasticity to temperature fluctuations and uniquely contribute to adult phenotype in *Onthophagus taurus* dung beetles. *Journal of Experimental Biology* 223. <https://doi.org/10.1242/jeb.227884>
- Caswell, H., 2019. Sensitivity Analysis: Matrix Methods in Demography and Ecology, Demographic Research Monographs. Springer International Publishing, Cham, Switzerland. <https://doi.org/10.1007/978-3-030-10534-1>
- Caswell, H., 2001. Matrix Population Models: Construction, Analysis, and Interpretation, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.
- Caswell, H., 1989. Analysis of life table response experiments I. Decomposition of effects on population growth rate. *Ecological Modelling* 46, 221–237. [https://doi.org/10.1016/0304-3800\(89\)90019-7](https://doi.org/10.1016/0304-3800(89)90019-7)
- Chen, H., Zheng, X., Luo, M., Guo, J., Solangi, G.S., Wan, F., Zhou, Z., 2018. Effect of short-term high-temperature exposure on the life history parameters of *Ophraella communa*. *Sci Rep* 8, 1–11. <https://doi.org/10.1038/s41598-018-32262-z>
- Chen, Y.-C., Chen, D.-F., Yang, M.-F., Liu, J.-F., 2022. The Effect of Temperatures and Hosts on the Life Cycle of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects* 13, 211. <https://doi.org/10.3390/insects13020211>
- Cheng, L.-Y., Chang, Y., Chen, Z.-Z., Xu, Y.-Y., 2018. Effects of constant and fluctuating temperatures on development and reproduction of *Megoura crassicauda* and *Aphis craccivora* (Hemiptera: Aphididae). *Entomologica Fennica* 29, 1–12. <https://doi.org/10.33338/ef.70036>

- Clavijo A, S., Fernandez Badillo, A., Ramirez A, A., Delgado, A., Lathullerie, J.M. (Venezuela U.C., 1991. Influence of temperature on the development of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). *Agronomia Tropical* (Venezuela).
- Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am Nat* 177, 738–751. <https://doi.org/10.1086/660021>
- Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in Fluctuating Thermal Environments. *Annual Review of Entomology* 60, 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>
- Cunha, F.M., Wanderley-Teixeira, V., Torres, J.B., Teixeira, Á.A.C., Alves, T.J.S., Brayner, F.A., 2013. Impact of Bt cotton on the immune system and histology of the midgut of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Animal Biol* 63, 185–197. <https://doi.org/10.1163/15707563-00002405>
- Damos, P., Savopoulou-Soultani, M., 2012. Temperature-Driven Models for Insect Development and Vital Thermal Requirements. *Psyche: A Journal of Entomology* 2012, 123405. <https://doi.org/10.1155/2012/123405>
- Denny, M., 2019. Performance in a variable world: using Jensen’s inequality to scale up from individuals to populations. *Conserv Physiol* 7. <https://doi.org/10.1093/conphys/coz053>
- Denny, M., 2017. The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen’s inequality. *Journal of Experimental Biology* 220, 139–146. <https://doi.org/10.1242/jeb.140368>
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *PNAS* 105, 6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate. *Science* 361, 916–919. <https://doi.org/10.1126/science.aat3466>
- Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., Oberg, E., Dunn, R.R., 2012. Who likes it hot? A global analysis of the climatic, ecological, and

- evolutionary determinants of warming tolerance in ants. *Global Change Biology* 18, 448–456. <https://doi.org/10.1111/j.1365-2486.2011.02542.x>
- Díaz-Álvarez, E.A., Martínez-Zavaleta, J.P., López-Santiz, E.E., de la Barrera, E., Larsen, J., del-Val, E., 2023. Climate change can trigger fall armyworm outbreaks: a developmental response experiment with two Mexican maize landraces. *International Journal of Pest Management* 69, 184–192. <https://doi.org/10.1080/09670874.2020.1869347>
- Dinh, K.V., Janssens, L., Stoks, R., 2016. Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. *Glob Chang Biol* 22, 3361–3372. <https://doi.org/10.1111/gcb.13415>
- Du Plessis, H., Schlemmer, M.-L., Van den Berg, J., 2020. The Effect of Temperature on the Development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects* 11. <https://doi.org/10.3390/insects11040228>
- Du Plessis, H., van den Berg, J., Ota, N., Kriticos, D.J., 2018. *Spodoptera frugiperda*. CSIRO-InSTePP Pest Geography.
- Dubovskiy, I.M., Krukova, N.A., Glupov, V.V., 2008. Phagocytic activity and encapsulation rate of *Galleria mellonella* larval haemocytes during bacterial infection by *Bacillus thuringiensis*. *Journal of Invertebrate Pathology, Special Issue for SIP 2008* 98, 360–362. <https://doi.org/10.1016/j.jip.2008.03.011>
- Duffy, K., Gouhier, T.C., Ganguly, A.R., 2022. Climate-mediated shifts in temperature fluctuations promote extinction risk. *Nat. Clim. Chang.* 12, 1037–1044. <https://doi.org/10.1038/s41558-022-01490-7>
- Early, R., González-Moreno, P., Murphy, S.T., Day, R., 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. *NeoBiota* 40, 25–50. <https://doi.org/10.3897/neobiota.40.28165>
- Englehart, P.J., Douglas, A.V., 2005. Changing behavior in the diurnal range of surface air temperatures over Mexico. *Geophysical Research Letters* 32. <https://doi.org/10.1029/2004GL021139>
- Enriquez, T., Colinet, H., 2017. Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. *PeerJ* 5, e3112. <https://doi.org/10.7717/peerj.3112>

- Estay, S.A., Clavijo-Baquet, S., Lima, M., Bozinovic, F., 2011. Beyond average: an experimental test of temperature variability on the population dynamics of *Tribolium confusum*. *Population Ecology* 53, 53–58. <https://doi.org/10.1007/s10144-010-0216-7>
- Estay, S.A., Lima, M., Bozinovic, F., 2014. The role of temperature variability on insect performance and population dynamics in a warming world. *Oikos* 123, 131–140. <https://doi.org/10.1111/j.1600-0706.2013.00607.x>
- Ezard, T.H.G., Bullock, J.M., Dalglish, H.J., Millon, A., Pelletier, F., Ozgul, A., Koons, D.N., 2010. Matrix models for a changeable world: the importance of transient dynamics in population management. *Journal of Applied Ecology* 47, 515–523. <https://doi.org/10.1111/j.1365-2664.2010.01801.x>
- Fatoretto, J.C., Michel, A.P., Silva Filho, M.C., Silva, N., 2017. Adaptive Potential of Fall Armyworm (Lepidoptera: Noctuidae) Limits Bt Trait Durability in Brazil. *J Integr Pest Manag* 8. <https://doi.org/10.1093/jipm/pmx011>
- Ferguson, L.V., Sinclair, B.J., 2020. Thermal Variability and Plasticity Drive the Outcome of a Host-Pathogen Interaction. *The American Naturalist* 195, 603–615. <https://doi.org/10.1086/707545>
- Fleming, J.M., Carter, A.W., Sheldon, K.S., 2021. Dung beetles show metabolic plasticity as pupae and smaller adult body size in response to increased temperature mean and variance. *Journal of Insect Physiology* 131, 104215. <https://doi.org/10.1016/j.jinsphys.2021.104215>
- Folguera, G., Bastías, D.A., Caers, J., Rojas, J.M., Piulachs, M.-D., Bellés, X., Bozinovic, F., 2011. An experimental test of the role of environmental temperature variability on ectotherm molecular, physiological and life-history traits: Implications for global warming. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 159, 242–246. <https://doi.org/10.1016/j.cbpa.2011.03.002>
- Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, Third. ed. Sage, Thousand Oaks, CA.
- Garcia, A.G., Ferreira, C.P., Godoy, W.A.C., Meagher, R.L., 2019. A computational model to predict the population dynamics of *Spodoptera frugiperda*. *J Pest Sci* 92, 429–441. <https://doi.org/10.1007/s10340-018-1051-4>

- Garcia, A.G., Godoy, W.A.C., Thomas, J.M.G., Nagoshi, R.N., Meagher, R.L., 2018. Delimiting Strategic Zones for the Development of Fall Armyworm (Lepidoptera: Noctuidae) on Corn in the State of Florida. *Journal of Economic Entomology* 111, 120–126.
<https://doi.org/10.1093/jee/tox329>
- García-Carreras, B., Reuman, D.C., 2013. Are Changes in the Mean or Variability of Climate Signals More Important for Long-Term Stochastic Growth Rate? *PLOS ONE* 8, e63974.
<https://doi.org/10.1371/journal.pone.0063974>
- García-Robledo, C., Baer, C.S., 2021. Demographic Attritions, Elevational Refugia, and the Resilience of Insect Populations to Projected Global Warming. *The American Naturalist* 198, 113–127. <https://doi.org/10.1086/714525>
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R.A., Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews*.
<https://doi.org/10.1111/brv.12588>
- Greenspan, S.E., Bower, D.S., Roznik, E.A., Pike, D.A., Marantelli, G., Alford, R.A., Schwarzkopf, L., Scheffers, B.R., 2017. Infection increases vulnerability to climate change via effects on host thermal tolerance. *Scientific Reports* 7, 9349.
<https://doi.org/10.1038/s41598-017-09950-3>
- Grizanova, E.V., Dubovskiy, I.M., Whitten, M.M.A., Glupov, V.V., 2014. Contributions of cellular and humoral immunity of *Galleria mellonella* larvae in defence against oral infection by *Bacillus thuringiensis*. *Journal of Invertebrate Pathology* 119, 40–46.
<https://doi.org/10.1016/j.jip.2014.04.003>
- Guedes, R.N.C., Walse, S.S., Throne, J.E., 2017. Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science, Pests and resistance * Behavioural ecology* 21, 47–53. <https://doi.org/10.1016/j.cois.2017.04.010>
- Haahr, M., 2025. RANDOM.ORG: True Random Number Service [WWW Document]. True Random Integer Generator. URL <https://www.random.org/integers/> (accessed 3.8.25).
- Hardke, J.T., Leonard, B.R., Huang, F., Jackson, R.E., 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus*

- thuringiensis* Cry proteins. Crop Protection 30, 168–172.
<https://doi.org/10.1016/j.cropro.2010.10.005>
- Harrison, J., Woods, H.A., Roberts, S., 2012. Ecological and Environmental Physiology of Insects, Ecological and Environmental Physiology Series. Oxford University Press, Oxford, New York.
- Hartig, F., 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.
- Henderson, Chas.F., Tilton, Elvin.W., 1955. Tests with Acaricides against the Brown Wheat Mite. Journal of Economic Entomology 48, 157–161. <https://doi.org/10.1093/jee/48.2.157>
- Herrero, S., Bel, Y., Hernández-Martínez, P., Ferré, J., 2016. Susceptibility, mechanisms of response and resistance to *Bacillus thuringiensis* toxins in *Spodoptera* spp. Current Opinion in Insect Science 15, 89–96. <https://doi.org/10.1016/j.cois.2016.04.006>
- Huang, L.-L., Xue, F.-S., Chen, C., Guo, X., Tang, J.-J., Zhong, L., He, H.-M., 2021. Effects of temperature on life-history traits of the newly invasive fall armyworm, *Spodoptera frugiperda* in Southeast China. Ecology and Evolution 11, 5255–5264.
<https://doi.org/10.1002/ece3.7413>
- Ingber, D.A., Mason, C.E., Flexner, L., 2018. Cry1 Bt Susceptibilities of Fall Armyworm (Lepidoptera: Noctuidae) Host Strains. Journal of Economic Entomology 111, 361–368.
<https://doi.org/10.1093/jee/tox311>
- Jeffs, C.T., Leather, S.R., 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. Entomologia Experimentalis et Applicata 150, 240–249. <https://doi.org/10.1111/eea.12160>
- Ju, R.-T., Zhu, H.-Y., Gao, L., Zhou, X.-H., Li, B., 2015. Increases in both temperature means and extremes likely facilitate invasive herbivore outbreaks. Scientific Reports 5, 15715.
<https://doi.org/10.1038/srep15715>
- Jurat-Fuentes, J.L., Heckel, D.G., Ferré, J., 2021. Mechanisms of Resistance to Insecticidal Proteins from *Bacillus thuringiensis*. Annual Review of Entomology 66, 121–140.
<https://doi.org/10.1146/annurev-ento-052620-073348>

- Karl, I., Stoks, R., De Block, M., Janowitz, S.A., Fischer, K., 2011. Temperature extremes and butterfly fitness: conflicting evidence from life history and immune function. *Global Change Biology* 17, 676–687. <https://doi.org/10.1111/j.1365-2486.2010.02277.x>
- Katbeh-Bader, A., Khyami-Horani, H., Mohsen, Z.H., 1999. Effect of temperature on the susceptibility of *Culiseta longiareolata* (Macquart) (Dipt., Culicidae) to two standard strains of biocontrol bacteria. *Journal of Applied Entomology* 123, 629–631. <https://doi.org/10.1046/j.1439-0418.1999.00414.x>
- Kefford, B.J., Ghalambor, C.K., Dewenter, B., Poff, N.L., Hughes, J., Reich, J., Thompson, R., 2022. Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Global Change Biology* 28, 6872–6888. <https://doi.org/10.1111/gcb.16453>
- Kenis, M., Benelli, G., Biondi, A., Calatayud, P.-A., Day, R., Desneux, N., Harrison, R.D., Kriticos, D., Rwomushana, I., van den Berg, J., Verheggen, F., Zhang, Y.-J., Agboyi, L.K., Ahissou, R.B., Ba, M.N., Wu, K., 2023. Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomologia Generalis* 187–241. <https://doi.org/10.1127/entomologia/2022/1659>
- Kingsolver, J.G., Arthur Woods, H., Buckley, L.B., Potter, K.A., MacLean, H.J., Higgins, J.K., 2011. Complex Life Cycles and the Responses of Insects to Climate Change. *Integrative and Comparative Biology* 51, 719–732. <https://doi.org/10.1093/icb/ucr015>
- Kingsolver, J.G., Buckley, L.B., 2017. Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372, 20160147. <https://doi.org/10.1098/rstb.2016.0147>
- Kingsolver, J.G., Diamond, S.E., Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology* 27, 1415–1423. <https://doi.org/10.1111/1365-2435.12145>
- Kingsolver, J.G., Higgins, J.K., Augustine, K.E., 2015. Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. *Journal of Experimental Biology* 218, 2218–2225. <https://doi.org/10.1242/jeb.120733>

- Kingsolver, J.G., Woods, H.A., 2016. Beyond Thermal Performance Curves: Modeling Time-Dependent Effects of Thermal Stress on Ectotherm Growth Rates. *The American Naturalist* 187, 283–294. <https://doi.org/10.1086/684786>
- Kremer, C.T., Fey, S.B., Arellano, A.A., Vasseur, D.A., 2018. Gradual plasticity alters population dynamics in variable environments: thermal acclimation in the green alga *Chlamydomonas reinhardtii*. *Proceedings of the Royal Society B: Biological Sciences* 285, 20171942. <https://doi.org/10.1098/rspb.2017.1942>
- Lawson, C.R., Vindenes, Y., Bailey, L., Pol, M. van de, 2015. Environmental variation and population responses to global change. *Ecology Letters* 18, 724–736. <https://doi.org/10.1111/ele.12437>
- Lazzaro, B.P., Little, T.J., 2009. Immunity in a variable world. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364, 15–26. <https://doi.org/10.1098/rstb.2008.0141>
- Lee, Seongkyun, Park, Y., Hwang, C., Park, A., Lee, Seokho, Kim, J., 2022. Prediction of Growth Characteristics and Migration Period of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) According to Temperature. *Insects* 13, 897. <https://doi.org/10.3390/insects13100897>
- Lehmann, P., Ammunét, T., Barton, M., Battisti, A., Eigenbrode, S.D., Jepsen, J.U., Kalinkat, G., Neuvonen, S., Niemelä, P., Terblanche, J.S., Økland, B., Björkman, C., 2020. Complex responses of global insect pests to climate warming. *Frontiers in Ecology and the Environment* 18, 141–150. <https://doi.org/10.1002/fee.2160>
- Leith, N.T., Miller, E.A., Fowler-Finn, K.D., 2024. Thermoregulation enhances survival but not reproduction in a plant-feeding insect. *Functional Ecology* 38, 1344–1356. <https://doi.org/10.1111/1365-2435.14546>
- Lenth, R., 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means.
- Li, S., De Mandal, S., Xu, X., Jin, F., 2020. The Tripartite Interaction of Host Immunity–*Bacillus thuringiensis* Infection–Gut Microbiota. *Toxins* 12, 514. <https://doi.org/10.3390/toxins12080514>
- Li, S.Y., Fitzpatrick, S.M., Isman, M.B., 1995. Effect of temperature on toxicity of *Bacillus thuringiensis* to the obliquebanded leafroller (Lepidoptera: Tortricidae). *The Canadian Entomologist* 127, 271–273. <https://doi.org/10.4039/Ent127271-2>

- Loto, F. del V., Carrizo, A.E., Romero, C.M., Baigorí, M.D., Pera, L.M., 2019. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Strains from Northern Argentina: Esterases, Profiles, and Susceptibility to *Bacillus thuringiensis* (Bacillales: Bacillaceae). *flen* 102, 347–352. <https://doi.org/10.1653/024.102.0209>
- Lysyk, T.J., Selinger, L.B., 2012. Effects of Temperature on Mortality of Larval Stable Fly (Diptera: Muscidae) Caused by Five Isolates of *Bacillus thuringiensis*. *Journal of Economic Entomology* 105, 732–737. <https://doi.org/10.1603/EC11359>
- Ma, C.-S., Ma, G., Pincebourde, S., 2021. Survive a Warming Climate: Insect Responses to Extreme High Temperatures. *Annual Review of Entomology* 66, 163–184. <https://doi.org/10.1146/annurev-ento-041520-074454>
- Malekera, M.J., Acharya, R., Mostafiz, M.M., Hwang, H.-S., Bhusal, N., Lee, K.-Y., 2022. Temperature-Dependent Development Models Describing the Effects of Temperature on the Development of the Fall Armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Insects* 13, 1084. <https://doi.org/10.3390/insects13121084>
- Marenco, R.A., Saunders, J.L., 1993. Parasitoides del gusano cogollero, *Spodoptera frugiperda* (Lepidoptera:Noctuidae) en maíz, en Turrialba Costa Rica. *Manejo Integrado Plagas* 27, 18–23.
- Marshall, K.E., Anderson, K.M., Brown, N.E.M., Dytneriski, J.K., Flynn, K.L., Bernhardt, J.R., Konecny, C.A., Gurney-Smith, H., Harley, C.D.G., 2021. Whole-organism responses to constant temperatures do not predict responses to variable temperatures in the ecosystem engineer *Mytilus trossulus*. *Proceedings of the Royal Society B: Biological Sciences* 288, 20202968. <https://doi.org/10.1098/rspb.2020.2968>
- Marshall, K.E., Sinclair, B.J., 2012. The impacts of repeated cold exposure on insects. *Journal of Experimental Biology* 215, 1607–1613. <https://doi.org/10.1242/jeb.059956>
- Massey, M.D., Fredericks, M.K., Malloy, D., Arif, S., Hutchings, J.A., 2022. Differential reproductive plasticity under thermal variability in a freshwater fish (*Danio rerio*). *Proc. R. Soc. B.* 289, 20220751. <https://doi.org/10.1098/rspb.2022.0751>
- Massey, M.D., Hutchings, J.A., 2021. Thermal variability during ectotherm egg incubation: A synthesis and framework. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 335, 59–71. <https://doi.org/10.1002/jez.2400>

- Meagher, R.L., Nuessly, G.S., Nagoshi, R.N., Hay-Roe, M.M., 2016. Parasitoids attacking fall armyworm (Lepidoptera: Noctuidae) in sweet corn habitats. *Biological Control* 95, 66–72. <https://doi.org/10.1016/j.biocontrol.2016.01.006>
- Mironidis, G.K., Savopoulou-Soultani, M., 2008. Development, Survivorship, and Reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) Under Constant and Alternating Temperatures. *env* 37, 16–29. [https://doi.org/10.1603/0046-225X\(2008\)37\[16:DSAROH\]2.0.CO;2](https://doi.org/10.1603/0046-225X(2008)37[16:DSAROH]2.0.CO;2)
- Monnerat, R., Martins, E., Queiroz, P., Ordúz, S., Jaramillo, G., Benintende, G., Cozzi, J., Real, M.D., Martinez-Ramirez, A., Rausell, C., Cerón, J., Ibarra, J.E., Rincon-Castro, M.C.D., Espinoza, A.M., Meza-Basso, L., Cabrera, L., Sánchez, J., Soberon, M., Bravo, A., 2006. Genetic Variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) Populations from Latin America Is Associated with Variations in Susceptibility to *Bacillus thuringiensis* Cry Toxins. *Appl. Environ. Microbiol.* 72, 7029–7035. <https://doi.org/10.1128/AEM.01454-06>
- Montezano, D.G., Specht, A., Sosa-Gómez, D.R., Roque-Specht, V.F., Malaquias, J.V., Paula-Moraes, S.V., Peterson, J.A., Hunt, T.E., 2019. Biotic Potential and Reproductive Parameters of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). *Journal of Agricultural Science* 11, 13.
- Murdock, C.C., Paaijmans, K.P., Bell, A.S., King, J.G., Hillyer, J.F., Read, A.F., Thomas, M.B., 2012. Complex effects of temperature on mosquito immune function. *Proc Biol Sci* 279, 3357–3366. <https://doi.org/10.1098/rspb.2012.0638>
- Musolin, D.L., 2007. Insects in a warmer world: ecological, physiological and life-history responses of true bugs (Heteroptera) to climate change. *Global Change Biology* 13, 1565–1585. <https://doi.org/10.1111/j.1365-2486.2007.01395.x>
- Nagoshi, R.N., Meagher, R.L., 2008. Review of Fall Armyworm (Lepidoptera: Noctuidae) Genetic Complexity and Migration. *The Florida Entomologist* 91, 546–554.
- Niehaus, A.C., Angilletta, M.J., Sears, M.W., Franklin, C.E., Wilson, R.S., 2012. Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology* 215, 694–701. <https://doi.org/10.1242/jeb.058032>

- Nyamukondiwa, C., Kleynhans, E., Terblanche, J.S., 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitidis capitata*). *Ecological Entomology* 35, 565–575. <https://doi.org/10.1111/j.1365-2311.2010.01215.x>
- Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology* 19, 2373–2380. <https://doi.org/10.1111/gcb.12240>
- Pannuti, L.E.R., Baldin, E.L.L., Hunt, T.E., Paula-Moraes, S.V., 2016. On-Plant Larval Movement and Feeding Behavior of Fall Armyworm (Lepidoptera: Noctuidae) on Reproductive Corn Stages. *Environ Entomol* 45, 192–200. <https://doi.org/10.1093/ee/nvv159>
- Parratt, S.R., Walsh, B.S., Metelmann, S., White, N., Manser, A., Bretman, A.J., Hoffmann, A.A., Snook, R.R., Price, T.A.R., 2021. Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nat. Clim. Chang.* 11, 481–484. <https://doi.org/10.1038/s41558-021-01047-0>
- Phungula, S.M., Krüger, K., Nofemela, R.S., Weldon, C.W., 2023. Developmental diet, life stage and thermal acclimation affect thermal tolerance of the fall armyworm. *Physiological Entomology* 48, 122–131. <https://doi.org/10.1111/phen.12414>
- Pigot, A.L., Merow, C., Wilson, A., Trisos, C.H., 2023. Abrupt expansion of climate change risks for species globally. *Nat Ecol Evol* 7, 1060–1071. <https://doi.org/10.1038/s41559-023-02070-4>
- Pincebourde, S., Suppo, C., 2016. The Vulnerability of Tropical Ectotherms to Warming Is Modulated by the Microclimatic Heterogeneity. *Integrative and Comparative Biology* 56, 85–97. <https://doi.org/10.1093/icb/icw014>
- Pitre, H.N., Mulrooney, J.E., Hogg, D.B., 1983. Fall Armyworm (Lepidoptera: Noctuidae) Oviposition: Crop Preferences and Egg Distribution on Plants. *J Econ Entomol* 76, 463–466. <https://doi.org/10.1093/jee/76.3.463>
- Prasad, T.V., Rao, M.S., Rao, K.V., Bal, S.K., Muttapa, Y., Choudhary, J.S., Singh, V.K., 2022. Temperature-based phenology model for predicting the present and future establishment and distribution of recently invasive *Spodoptera frugiperda* (J. E. Smith) in India.

- Bulletin of Entomological Research 112, 271–285.
<https://doi.org/10.1017/S0007485321000882>
- Prasanna, B.M., Huesing, J.E., Eddy, R., Peschke, V.M., 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management. CIMMYT, Mexico.
- Pureswaran, D.S., Neau, M., Marchand, M., De Grandpré, L., Kneeshaw, D., 2019. Phenological synchrony between eastern spruce budworm and its host trees increases with warmer temperatures in the boreal forest. *Ecology and Evolution* 9, 576–586.
<https://doi.org/10.1002/ece3.4779>
- R Core Team, 2024. R: A Language and Environment for Statistical Computing.
- Ragland, G.J., Kingsolver, J.G., 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*. *Evol Ecol Res* 10, 29–44.
- Ramirez-Cabral, N.Y.Z., Kumar, L., Shabani, F., 2017. Future climate scenarios project a decrease in the risk of fall armyworm outbreaks. *The Journal of Agricultural Science* 155, 1219–1238. <https://doi.org/10.1017/S0021859617000314>
- Raun, E.S., Sutter, G.R., Revelo, M.A., 1966. Ecological factors affecting the pathogenicity of *Bacillus thuringiensis* var. *thuringiensis* to the European corn borer and fall armyworm. *Journal of Invertebrate Pathology* 8, 365–375. [https://doi.org/10.1016/0022-2011\(66\)90052-8](https://doi.org/10.1016/0022-2011(66)90052-8)
- Rebaudo, F., Rabhi, V.-B., 2018. Modeling temperature-dependent development rate and phenology in insects: review of major developments, challenges, and future directions. *Entomologia Experimentalis et Applicata* 166, 607–617.
<https://doi.org/10.1111/eea.12693>
- Rebolledo, A.P., Sgrò, C.M., Monro, K., 2021. Thermal Performance Curves Are Shaped by Prior Thermal Environment in Early Life. *Front. Physiol.* 12.
<https://doi.org/10.3389/fphys.2021.738338>
- Reshma, R., Sagar, D., Subramanian, S., Kalia, V.K., Kumar, H., Muthusamy, V., 2023. Transgenerational effects of thermal stress on reproductive physiology of fall armyworm, *Spodoptera frugiperda*. *J Pest Sci* 96, 1465–1481. <https://doi.org/10.1007/s10340-023-01660-2>

- Roh, J.Y., Choi, J.Y., Li, M.S., Jin, B.R., Je, Y.H., 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. *J Microbiol Biotechnol* 17, 547–559.
- Ruel, J.J., Ayres, M.P., 1999. Jensen's inequality predicts effects of environmental variation. *Trends Ecol Evol* 14, 361–366. [https://doi.org/10.1016/s0169-5347\(99\)01664-x](https://doi.org/10.1016/s0169-5347(99)01664-x)
- Schulte, P.M., Healy, T.M., Fanguie, N.A., 2011. Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology* 51, 691–702. <https://doi.org/10.1093/icb/icr097>
- Sheldon, K.S., Dillon, M.E., 2016. Beyond the Mean: Biological Impacts of Cryptic Temperature Change. *Integr Comp Biol* 56, 110–119. <https://doi.org/10.1093/icb/icw005>
- Sheldon, K.S., Huey, R.B., Kaspari, M., Sanders, N.J., 2018. Fifty Years of Mountain Passes: A Perspective on Dan Janzen's Classic Article. *The American Naturalist* 191, 553–565. <https://doi.org/10.1086/697046>
- Sheldon, K.S., Padash, M., Carter, A.W., Marshall, K.E., 2020. Different amplitudes of temperature fluctuation induce distinct transcriptomic and metabolomic responses in the dung beetle *Phanaeus vindex*. *Journal of Experimental Biology* 223. <https://doi.org/10.1242/jeb.233239>
- Shinner, R., Terblanche, J.S., Clusella-Trullas, S., 2020. Across-stage consequences of thermal stress have trait-specific effects and limited fitness costs in the harlequin ladybird, *Harmonia axyridis*. *Evol Ecol* 34, 555–572. <https://doi.org/10.1007/s10682-020-10045-1>
- Simmons, A.M., 1993. Fall Armyworm Symposium: Effects of Constant and Fluctuating Temperatures and Humidities on the Survival of *Spodoptera Frugiperda* Pupae (Lepidoptera: Noctuidae). *Florida Entomologist* 333–340.
- Simmons, A.M., Marti, O.G., Jr, 1992. Mating by the Fall Armyworm (Lepidoptera: Noctuidae): Frequency, Duration, and Effect of Temperature. *Environmental Entomology* 21, 371–375. <https://doi.org/10.1093/ee/21.2.371>
- Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* 19, 1372–1385. <https://doi.org/10.1111/ele.12686>

- Slein, M.A., Bernhardt, J.R., O'Connor, M.I., Fey, S.B., 2023. Effects of thermal fluctuations on biological processes: a meta-analysis of experiments manipulating thermal variability. *Proceedings of the Royal Society B: Biological Sciences* 290, 20222225. <https://doi.org/10.1098/rspb.2022.2225>
- Sokame, B.M., Subramanian, S., Kilalo, D.C., Juma, G., Calatayud, P.-A., 2020. Larval dispersal of the invasive fall armyworm, *Spodoptera frugiperda*, the exotic stemborer *Chilo partellus*, and indigenous maize stemborers in Africa. *Entomologia Experimentalis et Applicata* 168, 322–331. <https://doi.org/10.1111/eea.12899>
- Sousa, F.F., Mendes, S.M., Santos-Amaya, O.F., Araújo, O.G., Oliveira, E.E., Pereira, E.J.G., 2016. Life-History Traits of *Spodoptera frugiperda* Populations Exposed to Low-Dose Bt Maize. *PLOS ONE* 11, e0156608. <https://doi.org/10.1371/journal.pone.0156608>
- Souza, C.S.F., Silveira, L.C.P., Pitta, R.M., Waquil, J.M., Pereira, E.J.G., Mendes, S.M., 2019. Response of field populations and Cry-resistant strains of fall armyworm to Bt maize hybrids and Bt-based bioinsecticides. *Crop Protection* 120, 1–6. <https://doi.org/10.1016/j.cropro.2019.01.001>
- Sparks, A.N., 1979. A Review of the Biology of the Fall Armyworm. *The Florida Entomologist* 62, 82. <https://doi.org/10.2307/3494083>
- Stocker, C.W., Bamford, S.M., Jahn, M., Mazué, G.P.F., Pettersen, A.K., Ritchie, D., Rubin, A.M., Noble, D.W.A., Seebacher, F., 2024. The Effect of Temperature Variability on Biological Responses of Ectothermic Animals—A Meta-Analysis. *Ecology Letters* 27, e14511. <https://doi.org/10.1111/ele.14511>
- Stoks, R., Verheyen, J., Van Dievel, M., Tüzün, N., 2017. Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world. *Current Opinion in Insect Science* 23, 35–42. <https://doi.org/10.1016/j.cois.2017.06.008>
- Stott, I., Hodgson, D., Townley, S., 2025. popdemo: Demographic Modelling Using Projection Matrices.
- Stubben, C., Milligan, B., 2007. Estimating and Analyzing Demographic Models Using the popbio Package in R. *Journal of Statistical Software* 22, 1–23. <https://doi.org/10.18637/jss.v022.i11>

- Tessnow, A.E., Nagoshi, R.N., Meagher, R.L., Fleischer, S.J., 2023. Revisiting fall armyworm population movement in the United States and Canada. *Front. Insect Sci.* 3. <https://doi.org/10.3389/finsec.2023.1104793>
- Thomas, M.B., Blanford, S., 2003. Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution* 18, 344–350. [https://doi.org/10.1016/S0169-5347\(03\)00069-7](https://doi.org/10.1016/S0169-5347(03)00069-7)
- Vajedsamiei, J., Melzner, F., Raatz, M., Morón Lugo, S.C., Pansch, C., 2021. Cyclic thermal fluctuations can be burden or relief for an ectotherm depending on fluctuations' average and amplitude. *Functional Ecology* 35, 2483–2496. <https://doi.org/10.1111/1365-2435.13889>
- van Frankenhuyzen, K., 1994. Effect of temperature on the pathogenesis of *Bacillus thuringiensis* Berliner in larvae of the spruce budworm, *Choristoneura fumiferana* clem. (Lepidoptera: Tortricidae). *The Canadian Entomologist* 126, 1061–1065. <https://doi.org/10.4039/Ent1261061-4>
- van Frankenhuyzen, K., Nystrom, C.W., 1987. Effect of temperature on mortality and recovery of spruce budworm (Lepidoptera: Tortricidae) exposed to *Bacillus thuringiensis* Berliner. *The Canadian Entomologist* 119, 941–954. <https://doi.org/10.4039/Ent119941-10>
- van Frankenhuyzen, K., Régnière, J., Bernier-Cardou, M., 2008. Response of *Lymantria dispar* L. (Lepidoptera: Lymantriidae) to *Bacillus thuringiensis* subsp. *kurstaki* at different ingested doses and temperatures. *Journal of Invertebrate Pathology* 99, 263–274. <https://doi.org/10.1016/j.jip.2008.06.011>
- van Heerwaarden, B., Sgrò, C.M., 2021. Male fertility thermal limits predict vulnerability to climate warming. *Nat Commun* 12, 2214. <https://doi.org/10.1038/s41467-021-22546-w>
- Vangansbeke, D., Audenaert, J., Nguyen, D.T., Verhoeven, R., Gobin, B., Tirry, L., Clercq, P.D., 2015. Diurnal Temperature Variations Affect Development of a Herbivorous Arthropod Pest and its Predators. *PLOS ONE* 10, e0124898. <https://doi.org/10.1371/journal.pone.0124898>
- Vasseur, D., DeLong John P., Gilbert Benjamin, Greig Hamish S., Harley Christopher D. G., McCann Kevin S., Savage Van, Tunney Tyler D., O'Connor Mary I., 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proceedings*

- of the Royal Society B: Biological Sciences 281, 20132612.
<https://doi.org/10.1098/rspb.2013.2612>
- Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth Edition. ed, Statistics and Computing. Springer, New York, NY. <https://doi.org/10.1007/978-0-387-21706-2>
- Verheyen, J., Delnat, V., Theys, C., 2022. Daily temperature fluctuations can magnify the toxicity of pesticides. Current Opinion in Insect Science 51, 100919.
<https://doi.org/10.1016/j.cois.2022.100919>
- Wagner, D.L., Fox, R., Salcido, D.M., Dyer, L.A., 2021. A window to the world of global insect declines: Moth biodiversity trends are complex and heterogeneous. Proc Natl Acad Sci U S A 118, e2002549117. <https://doi.org/10.1073/pnas.2002549117>
- Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A.R., 2019. The Impact of Climate Change on Fertility. Trends in Ecology & Evolution 34, 249–259. <https://doi.org/10.1016/j.tree.2018.12.002>
- Wang, Y., Zhang, H., Wang, X., 2021. Effects of Delayed Mating on the Reproductive Performance and Longevity of the Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Neotrop Entomol 50, 622–629. <https://doi.org/10.1007/s13744-021-00877-z>
- Williams, C.M., Marshall, K.E., MacMillan, H.A., Dzurisin, J.D.K., Hellmann, J.J., Sinclair, B.J., 2012. Thermal Variability Increases the Impact of Autumnal Warming and Drives Metabolic Depression in an Overwintering Butterfly. PLOS ONE 7, e34470.
<https://doi.org/10.1371/journal.pone.0034470>
- Wojda, I., 2017. Temperature stress and insect immunity. Journal of Thermal Biology, SI:Ectotherms: Performance 68, 96–103. <https://doi.org/10.1016/j.jtherbio.2016.12.002>
- Wojda, I., Taszłow, P., 2013. Heat shock affects host-pathogen interaction in *Galleria mellonella* infected with *Bacillus thuringiensis*. J Insect Physiol 59, 894–905.
<https://doi.org/10.1016/j.jinsphys.2013.06.011>
- Woods, H.A., 2013. Ontogenetic changes in the body temperature of an insect herbivore. Functional Ecology 27, 1322–1331. <https://doi.org/10.1111/1365-2435.12124>

- Wu, P., Head, M.L., Liu, C., Haseeb, M., Zhang, R., 2022. The high invasion success of fall armyworm is related to life-history strategies across a range of stressful temperatures. *Pest Management Science* 78, 2398–2404. <https://doi.org/10.1002/ps.6867>
- Yang, C.-L., Meng, J.-Y., Zhou, L., Yao, M.-S., Zhang, C.-Y., 2021. Identification of five small heat shock protein genes in *Spodoptera frugiperda* and expression analysis in response to different environmental stressors. *Cell Stress and Chaperones* 26, 527–539. <https://doi.org/10.1007/s12192-021-01198-1>
- Zhang, L., Ringbauer, J.A., Goodman, C. I, Reall, T., Jiang, X.-F., Stanley, D., 2018. Prostaglandin-mediated recovery from bacteremia delays larval development in fall armyworm, *Spodoptera frugiperda*. *Archives of Insect Biochemistry and Physiology* 97. <https://doi.org/10.1002/arch.21444>
- Zhao, J., Li, S., Gao, X.-W., Zhang, F., Wang, S., 2015. Comparison of Life Tables of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae) Under Laboratory and Greenhouse Conditions. *Journal of Economic Entomology* 108, 1700–1707. <https://doi.org/10.1093/jee/fov178>
- Zheng, J., Cheng, X., Hoffmann, A.A., Zhang, B., Ma, C.-S., 2017. Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress? *Journal of Insect Physiology* 102, 36–41. <https://doi.org/10.1016/j.jinsphys.2017.09.004>

VITA

Anchal Padukone was born in Mumbai, India, and spent her formative years in Sri Lanka, Tanzania and India. She graduated *magna cum laude* from Princeton University in 2016 with a Bachelor of Arts (A.B.) degree in Ecology and Evolutionary Biology and a Certificate in Global Health and Health Policy. While at Princeton, Anchal completed a senior thesis examining how the distribution of an insect vector of Chagas disease in Central Panama (*Rhodnius pallescens*) depends on local microhabitat characteristics. She also completed field courses and conducted fieldwork at various Smithsonian Tropical Research Institute facilities in Panama and Kenya. These experiences solidified her interest in the population ecology and ecophysiology of insects in heavily managed ecosystems, such as croplands and rangelands. After graduation, Anchal coordinated science-based outreach and educational programs at the Mpala Research Centre as the 2016–2017 Princeton in Africa Fellow. She stayed at Mpala until 2018 and managed field data collection for a study of ant community dynamics and genetic diversity on *Acacia drepanolobium* trees for members of the Pierce Lab in Harvard University's Department of Organismic and Evolutionary Biology.

Anchal began her PhD in 2018 with Dr. Kimberly Sheldon at the University of Tennessee, Knoxville. Using fall armyworm moths (*Spodoptera frugiperda*) as her study system, her PhD research examined the effects of temperature variability on organismal life-histories, population dynamics and insect host-pathogen interactions. While at the University of Tennessee, she pursued further training and opportunities in science education and outreach through the Center for the Integration of Research, Teaching, and Learning (CIRTL). She received a department award for Outstanding Teaching by a Graduate Student in 2023 and a UT Graduate Student Senate Teaching Award in 2025.