



**Understanding the thermal biology of macadamia nut borer, *Thaumatotibia batrachopa*
(Meyrick) (Lepidoptera: Tortricidae): a pest of macadamia in South Africa**

Thifhelimbilu Mulateli

11612663

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Department of Biological Sciences
Faculty of Science, Engineering, and Agriculture
University of Venda
Thohoyandou, Limpopo
South Africa

Main Supervisor:

Prof. Lourens Swanepoel

University of Venda, South Africa

Co-supervisors:

Dr Elsje Joubert

Centre for Excellence, South Africa

Dr Mulalo M. Muluvhahotho

University of Venda, South Africa

Declaration

I, **Thifhelimbilu Mulateli**, declare that the thesis for the degree of Doctor of Philosophy in Life Science (Zoology) is my work and has not been previously submitted for assessment to another University or another qualification.

SIGNATURE:

DATE:

Summary

Responses and adaptation of *Thaumatotibia batrachopa* to environmental changes and the impact of how it responds and perhaps adapts to these changes and fluctuations are complex subjects. *Thaumatotibia batrachopa*'s life stages, body temperatures, and various life cycle occurrences schedules are primarily influenced by temperature and other ecological factors. There is currently no evidence on the role of temperature on the development duration of *T. batrachopa* life stages, nor the outcomes regarding developmental rates and thermal responses. Understanding the mechanisms through which the temperature affects *T. batrachopa*'s life stages should contribute to developing a model of the potential responses of this pest to climate change. Thermal biology studies should reveal the thermal breadth of *T. batrachopa*, which can be applied to current and future climate scenarios to plan and execute integrated pest management (IPM) strategies.

Therefore, we aimed to explore the thermal biology of *T. batrachopa*, to build up some knowledge on the impact of temperature on population phenology and predict the temperature-dependent population abundance at short and intermediate time scales in this study. This was achieved through the following objectives; i) To study the effects of temperature on the development rate of *T. batrachopa* life stages by monitoring temperature-dependent development duration from egg to adult stage, sex ratio, longevity, and fecundity of adults under a range of constant temperatures, ii) To determine thermal tolerance responses of *T. batrachopa* life stages, establishing critical thermal limits (CTLs) in response to acclimation, lethal limits, and heat and cold hardening, and iii) To evaluate degree day models for *T. batrachopa*, predicting generations it can complete in the orchards, and investigate linkages between variability of microclimatic data and population dynamics of *T. batrachopa* in macadamia orchards at different climate zones.

Effects of temperature on the development duration of *T. batrachopa* life stages were explored by rearing from eggs to adults at four constant temperatures. Fecundity and longevity were assessed through mated pairs of opposite sex and recording their mortality. The temperature-dependent models were derived by quantifying the development rate at each temperature, followed by fitting the nonlinear models to select the model that best estimates the thermal requirements for development. The thermal tolerance study focused

on acclimating *T. batrachopa* life stages at three temperatures and evaluating effects through CTL assays on acclimated individuals. Rapid hardening was explored by exposing *T. batrachopa* life stages to cold and warm temperatures, before assessing their responses to sublethal temperatures. The impact of climate zones on generation number, thermal stress (heat and cold), thermal safety margin (TSM), and relative fitness across four macadamia orchards was examined using phenological model setup and simulation of thermal requirements and physiological traits from Chapter 2 and Chapter 3, respectively.

The development duration of eggs, larvae, and pupae of *T. batrachopa* egg was significantly shorter in high temperatures. Mated adults lived longer at 16°C, with females depositing more eggs than at 25 and 31°C temperatures. The temperature-dependent development model that best fitted the development rate of *T. batrachopa* life stages was HarcourtYee_82 but could not estimate T_{min} . Therefore, Briere1_99 estimated the lower development temperature (T_{min}) for all life stages, and the upper development temperature (T_{max}), and optimum temperature (T_{opt}) were estimated by HarcourtYee_82. The Degree Day required for *T. batrachopa* from eggs to adults was 564.72DD.

The life stages of *T. batrachopa* showed significant variation in their thermal tolerance. The acclimation ability of *T. batrachopa* varied with acclimation temperatures and life stages, and the results were more pronounced for larval stages. The larval stages had higher critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}) than adults across all acclimation temperatures. Instar 4 larvae were the most heat and cold-tolerant after acclimation across all temperatures. Pre-exposure and exposure for two hours didn't significantly improve each life stage's survival. However, the adults had a higher %survival than larval stages after rapid heat hardening (RHH) and rapid cold hardening (RCH).

The predicted generations were high at low elevations (6.8 generations). The *T. batrachopa* population was thermally heat-stressed with the highest TSM and poor relative fitness in high-elevation climate zones.

Our findings show that *T. batrachopa* develops rapidly at warm temperatures (25 and 31°C), leading to more generations in the summer months. This might result in higher infestations and increased damage to macadamia nuts. The *T. batrachopa*'s ability to acclimate at low and high-temperature variations implies it can adapt, survive, and persist under climate change. Predictions show that Orchards in low-elevation climate zones will experience

higher infestations and greater damage to macadamia nuts due to the increased number of *T. batrachopa* generations.

Preface

Disclaimer

The work described in this thesis was conducted while registered full-time at the Department of Biological Sciences, Faculty of Science, Agriculture and Engineering, University of Venda, South Africa. This study took place from February 2022 until November 2024, under the supervision of Prof. Lourens Swanepoel, Dr Mulalo M. Muluvhahotho, the late Prof. Stefan H. Foord (Department of Biological Sciences, University of Venda), and Dr Elsje Joubert (Centre for Excellence, Levubu, South Africa). This thesis represents work originally done by the author and no assessment has been done for any qualification in a higher institution. The term “we” has been applied to acknowledge the contributions of co-authors.

Thesis format note:

This thesis has been written in a paper-based format. Therefore, references occur after each chapter, and repetition of information occurs as each chapter is written as a standalone piece.

Permission and Ethics

The Ethics committee from Faculty of Science, Engineering, and Agriculture at the University of Venda gave the permission to conduct the study (ETHICAL CLEARANCE No.FSEA/22/BS/15).

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CHAPTER 1

General introduction

Climate change is one of the significant challenges facing the global community to date (Malhi et al., 2021). Climate change is a process that involves changes in environmental factors that include temperature, humidity, precipitation, and atmospheric carbon dioxide (CO₂) concentrations for which averages have been calculated over an extended time (Shrestha, 2019; Malhi et al., 2021; Skendžić et al., 2021). For example, since 1975, the average global temperature has risen at a rate of 0.15-0.20°C per decade and is projected to increase by 1.4-5.8°C by 2021 (Mahli et al., 2021). Furthermore, CO₂ concentration rose from 315.98 ppm in 1959 to 411.43 ppm in 2019 (Mahli et al., 2021). Consequently, changes in these climate change factors significantly affect all agricultural production, e.g., projected to reduce cereal production of maize and wheat by 38% and 5.5% worldwide, respectively, and cause substantial economic losses at a global level. (Shrestha, 2019). Alongside the direct effects of climate change on crop production, global food production is threatened by the impact of insect pests on food crops (Nayak et al., 2020).

Insect pests are increasingly becoming a significant factor contributing to the decline in crop yields and are responsible for the current 38% of agricultural losses globally (Junaid and Gökçe 2024). For example, 18-20% of the annual world's production of crops (valued at > US \$470 billion/annum) is destroyed by arthropod pests (Sharma et al., 2017). Insects are poikilothermic organisms that are likely to respond to climate change, and it is projected that damage to crops by insect pests will increase due to climate change (Nayak et al., 2020). Therefore, implementing control strategies against insect pests is crucial, particularly for crop production. Traditional and current pest control relies on chemical control, which has significant environmental impacts (Guedes et al., 2016). However, the use of pesticides continues to face criticism because of the hazards they pose to non-target organisms, the health of humans, and the natural world (Aktar et al., 2009). Alternative control measures that integrate pest management have gained momentum in insect pest management but rely on detailed ecological and phenological data (Ascerno, 1991). For many insects, such information is lacking. Therefore, understanding insect-environmental responses is critical in developing integrated pest management (IPM) programs.

Literature Review

Temperature and humidity are abiotic factors that greatly regulate insect populations (Deutsch et al., 2008; Kellermann and Van Heerwaarden 2019; Motswagole et al., 2019). Temperature significantly impacts biological functions, influencing organisms at levels ranging from molecular structures to a whole ecosystem (Hochachka and Somero 2002). It is a critical abiotic factor that governs insect population dynamics by influencing key parameters such as development time of life stages, mortality, fecundity, longevity, and, ultimately, the size of populations (Honěk and Kocourek 1990; Khaliq et al., 2014; Schulte et al., 2011; Motswagole et al., 2019). The effect of temperature on development time on life stages of insects has been observed in various species, including the sycamore lace bug, *Corythucha ciliata* (Say) (Ju et al., 2011), the fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Du Plessis et al., 2020), and *Bathypocheilus distincta* (Distant) (Muluvhahotho et al., 2024). While the effect of temperature on life stages' development time may vary among species, the general trend is that development time decreases with an increase in temperature (e.g., Golizadeh et al., 2007; Aghdam et al., 2009; Andreadis et al., 2017). Therefore, understanding how temperature affects the development rate of life stages of insect pests in the context of ongoing climate change is essential for forecasting and developing management strategies against pests.

One of the important metrics of the effect of temperature on insect development is the developmental rate, which is the inverse duration needed for an organism to transition between life stages (Shi et al., 2011). Generally, the rate of development increases progressively with rising temperatures until reaching an optimal temperature (T_{opt}), beyond which it declines rapidly (Shi et al., 2011). The relationship between temperature and development rate has been described by various phenological models (linear and nonlinear) (Aghdam et al., 2009), with linear and Logan models being the most widely used. Linear models are particularly effective at describing developmental rates below T_{opt} , estimating the lower thermal threshold at which development ceases (Shi et al., 2011). However, linear models are inadequate for temperatures above T_{opt} , as they assume that developmental rates always increase with temperature and, therefore fail to account for the upper thermal threshold (Shi et al., 2011). The nonlinear variation temperature-development rate is usually

illustrated using phenological models with parameters such as critical thresholds (CT_{min} and CT_{max}) and development optimum temperature (T_{opt}) (Régnier et al., 2021). Phenological models are instrumental in determining the range of temperatures influencing development under laboratory conditions and defining life stages and species-specific thermal constants (Aghdam et al., 2009).

Consequently, they can be applied to predict how temperature influences insect phenology under field conditions, both temporally and spatially (Roy et al., 2002). Accurate predictions derived from these models are crucial for effective pest management. These developmental rate models are typically expressed through mathematical equations (Damos and Savopoulou-Soultani 2012), enabling researchers to estimate insect life stage as well as the generations (in numbers) occurring over a time frame based on the following equation:

$$K = D(T - T_0)$$

K is the thermal constant, species-specific or stage-specific, D is the development time (days), T constitutes temperature ($^{\circ}C$), and T_0 denotes the temperature at which development does not occur. The thermal constant is demonstrated in degree-days (DD) and refers to the physiological time needed to complete the development process (Damos and Savopoulou-Soultani 2012). Degree days are universally applied in estimating the temperature-dependent development of ectothermic organisms and plants, serving as the foundation for numerous phenological models for population dynamics (Roltsch et al., 1999). They are widely used in plant science, pest management, and ecology, and along with physiological traits, they offer valuable insights into plant and insect phenology and forecast pest status (Higley et al., 1986).

The insects' ability to tolerate temperature stress and sustain activity during extreme conditions is a crucial factor for the fitness and persistence of the insect population in the wild (Terblanche et al., 2007; Nyamukondiwa and Terblanche 2009). Thus, the thermal adaptation of insects influences seasonal phenology and geographical variation (Klok and Chown 2003). Data on the thermal tolerance of insects is useful for assessments of agricultural insect pests by examining their response to stressful environmental conditions (Motswagole et al., 2019).

Insects have developed adaptive mechanisms to cope with fluctuating environmental temperatures. These adaptations include behavioural and physiological mechanisms such as phenotypic plasticity and enable insects to adjust their physiological performance and thermal adaptability in response to short or prolonged temperature exposures (Nyamukondiwa et al., 2013; Motswagole et al., 2019). Therefore, the knowledge of the responses of insects to variations in temperature is fundamental to assessing their energy demands in their natural habitats and, by extension, those of the species.

Plasticity is when an organism produces different phenotypes due to changes in ecological conditions (Rodrigues and Beldade 2020). The plasticity of insects is important because it has a wide range of applications, can improve survival, enhance fitness, and shape ecological communities (Whitman and Agrawal 2008). Most plastic changes are morphological and may include physiological and biochemical changes that impact metabolism, the content and composition of protein and carbohydrates, and gene expression patterns (Sgrò et al., 2016). Plastic species are likely to withstand ecological disasters and reduce extinction rates due to their presumed broader geographic ranges, and since they have already adjusted to several environments and produce distinct phenotypes (Rodrigues and Beldade 2020). Plastic responses of insects continue to receive attention because they can allow an individual to adapt to stresses arising from environmental changes (Sgrò et al., 2016).

In many controlled laboratory experiments, insect plasticity has been assessed by exposing insects to the manipulated temperatures of their normal viable range (Sgrò et al., 2016). Modified temperature can range from minutes to hours to days or weeks (Hoffmann et al., 2003). Acclimation is the exposure of organisms within their viable temperature for days or weeks and may lead to physiological reversible or permanent changes (Hoffmann et al., 2003). Rapid Hardening is the pre-exposure and exposure of organisms for minutes or hours to sub-lethal temperatures that may lead to improved survival or mortality of the exposed organism (Cossins and Bower 1987). For instance, insects exposed to high temperatures might generate heat-shock proteins (HSPs) as molecular chaperones protecting other cellular proteins and maintaining key enzyme functions (Chidawanyika and Terblanche 2011). Brief exposure of insects to low temperatures may radically alter membrane phospholipid composition to enhance tolerance to low temperatures (Chidawanyika and Terblanche

2011). Acclimation and rapid hardening are used interchangeably in evolutionary studies to reveal an insect's plasticity under unfavourable temperature conditions (Hoffmann et al., 2003).

Critical thermal limits (CTLs) are used to measure the thermal limits of insects to a gradually increasing and decreasing temperature. However, they also serve as an essential step in understanding temperature variations and are best described by thermal performance curves (TPCs). Practical reports indicate that TPCs are generally similar in shape, i.e., performance normally increases in a relatively gradual manner from a minimum critical temperature (CT_{min}) to an optimum temperature (T_{opt}) before declining sharply to reach a critical thermal maximum (CT_{max}) (Figure 1.1) (Deutsch et al., 2008; Schulte et al., 2011). CT_{min} and CT_{max} are temperature thresholds above and below which insects lose their coordinated muscle function (Hazell and Bale 2011) and have been measured for diverse ectotherms (Motswagole et al., 2019; Mutamiswa et al., 2019; Muluvhahotho et al., 2023). They are ecologically important and continue to receive much attention because they provide researchers with an understanding of how changes in temperature shape ecological variations, distribution, and species evolution (Terblanche et al., 2007b). Several well-documented field and laboratory studies have shown that data obtained from thermal performance studies are consistent and repeatable measures of thermal tolerance of the population over time (Terblanche et al., 2007b; Vannier, 1994; Somero, 2005; Nyamukondiwa and Terblanche 2009).

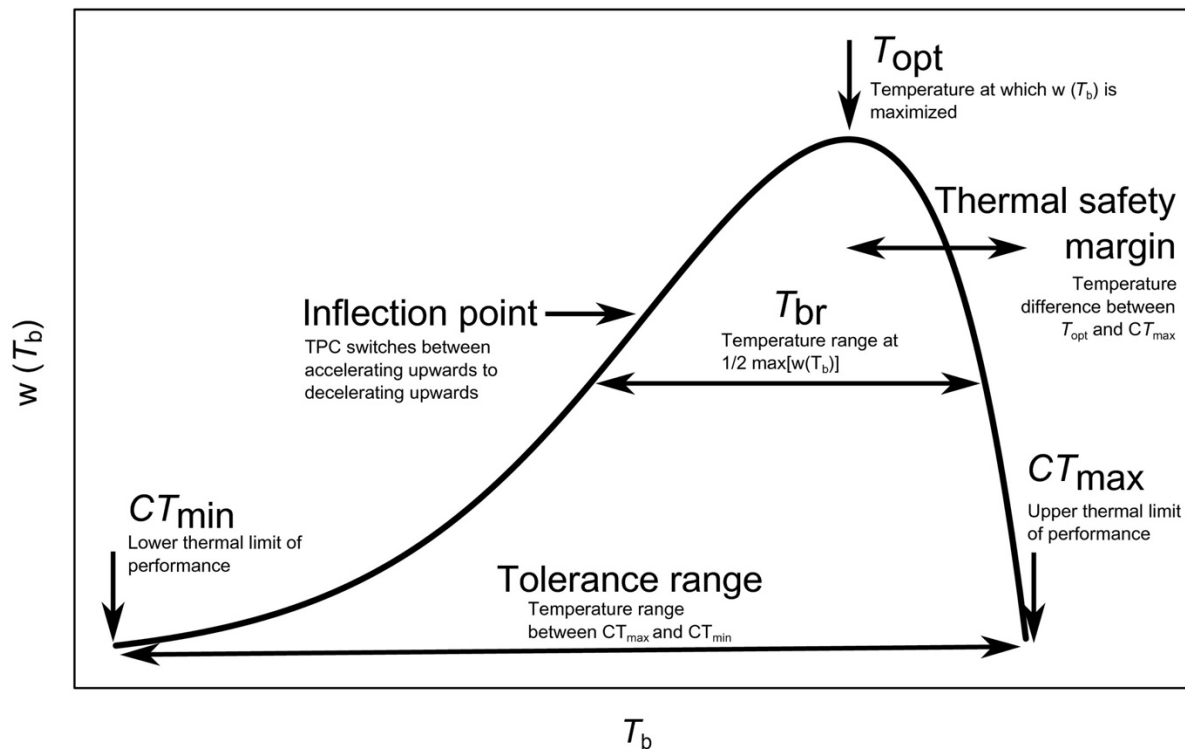


Figure 1.1 Poikilothermic Thermal performance curve (TPC) linking body temperature (T_b) to fitness $w(T_b)$ (based on (Huey and Stevenson 1979). CT_{min} - Critical thermal minimum, CT_{max} - Critical thermal maximum; T_{opt} - thermal optimum; T_{br} - thermal breath (adopted from Sinclair et al., 2016)

Macadamia farming in South Africa and its pests

Over the past five years, the South African macadamia has grown substantially with kernel production surging from 39k to 59k tonnes in 2019 and 2023, respectively (SAMAC, 2024). South Africa has six macadamia-growing regions (Figure 1.2), with Mpumalanga being the central production region (Smith et al., 2022).

Macadamia nuts represent a high-value agricultural product in South Africa, with nuts in shells selling for an average price of 71.63 ZAR (4.94 USD) /kg in 2018 (SAMAC, 2019; Smith et al., 2022). This high market value indicates the crop's economic significance and is further amplified by the rising international demand for macadamia nuts (Smith et al., 2022). In South Africa, production of macadamia is anticipated to continue to grow, supported by notable increases in annual plantings, however, the industry has several significant threats from pests including *B. distincta*, Thrips, *Thaumatotibia leucotreta* Meyrick, and diseases (Smith et al., 2022).

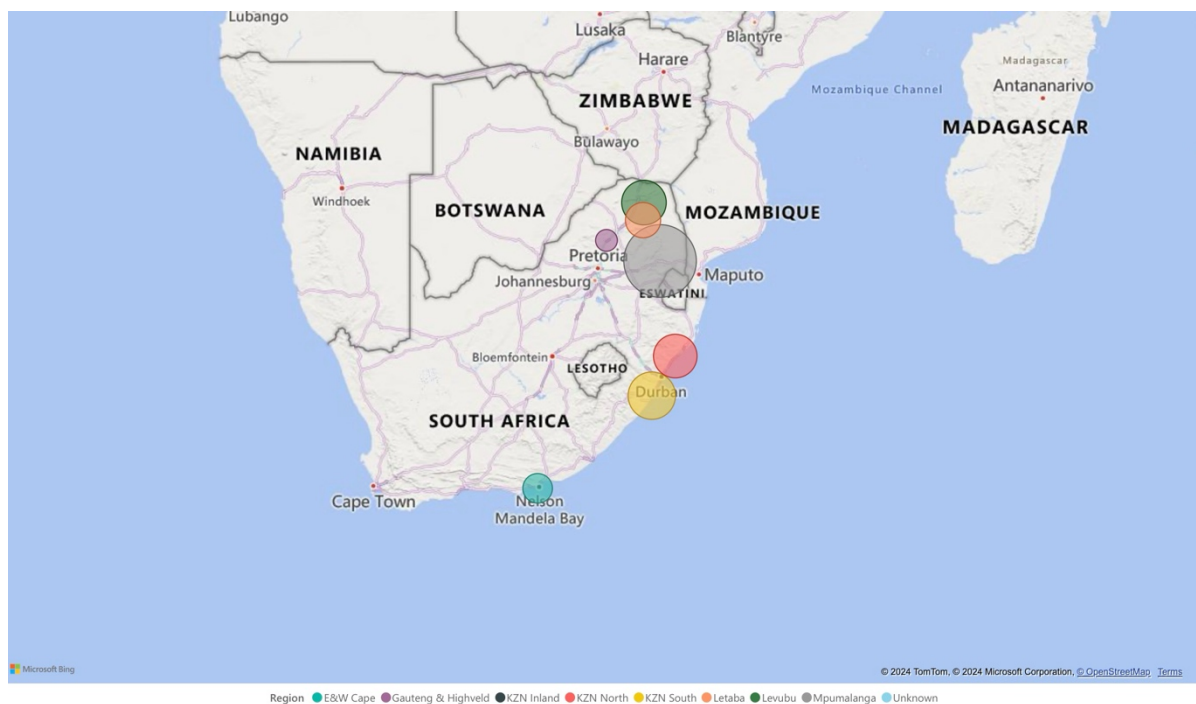


Figure 1.2 Growing regions of macadamia in South Africa, extracted from (SAMAC source: Agriculture data Analytics 2024). Different circle sizes represent macadamia-growing regions, with bigger circles showing the biggest area.

One pest that threatens the South African growing of macadamia is the macadamia nut borer *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae). *Thaumatotibia batrachopa* is an Afrotropical pest and the dominant lepidopteran accounting for about 95% of larvae recorded in macadamia nuts from 2017 to 2019 growing period in Mpumalanga, Kwazulu-Natal, and Limpopo provinces (Smith et al., 2022). Native to Africa, *T. batrachopa* was first identified in South Africa's Eastern Cape province (Rentel, 2013). Individual larvae of *T. batrachopa* were first reared in 1972 from the citrus by E.C.G. Bedford due to an unusual infestation of this citrus (de Villiers, 2001a; Rentel, 2013). *Thaumatotibia batrachopa* has a limited geographic distribution, which ranges throughout east, west, and southern Africa, and the species almost feeds exclusively on macadamia nuts (Timm et al., 2006; Smith et al., 2022). However, *T. batrachopa* has been recorded on other host plants. For example, in Malawi, *T. batrachopa* has been recorded as a serious pest of macadamia and litchi crops but was also recorded on wild trees, snot apple (*Azanza garckeana* (F. Hoffmn) Excell & Hillcoat. (Malvaceae)), the climbing shrub Mauritius thorn (*Caesalpinia decapetala*), and Feijoa (*Feijoa selwiana*). In Zimbabwe, it has been reported only in macadamia crops (La

Croix and Thindwa 1986, de Villiers, 2001a). Other reported alternative hosts of *T. batrachopa* in Malawi are the fruits of crops, *Musa paradisiaca* L. (Musaceae), *Feijoa sellowiana* Berg. (Myrtaceae), and *Litchi chinensis* Sonner (Sapindaceae), and leguminous scrambling shrub *Caesalpinia decapetala* (Roth.) Alston (Leguminosae subfam. Caesalpinioideae) (La Croix and Thindwa 1986).

Life cycle

The life cycle of *T. batrachopa* is divided into four life stages: egg, larvae, pupa, and adult moth. The female adult moth deposits eggs individually on immature macadamia nuts, particularly within the husk and in the small gaps between adjacent husks (La Croix and Thindwa 1986). The egg is flat and ovoid, and its width is 0.8 - 0.6 mm, height is 0.2 mm and has a pitted chorion (La Croix and Thindwa 1986). On average, females deposit 28 eggs, and hatching occurs within 3 - 10 days, depending on temperatures between 23 and 27°C (La Croix and Thindwa 1986; Smith, 2020). Two to three days ahead of hatching, the egg shows visible red, well-developed eyes, mandibles, and prothoracic shields (Rentel, 2013). The neonate larvae are cream to white with dark-brown head capsules measuring 0.28 mm in width (Smith, 2020). After hatching, the neonates either burrow directly into the husk or move approximately four millimeters along the exterior of a nut before burrowing in (La Croix and Thindwa 1986). Under ambient temperatures of $25 \pm 1^\circ\text{C}$, the larva undergoes four stages of development for 16 to 20 days (Smith, 2020). The fully developed larva (late instar) migrates from the nut in the husk to the soil for pupation, but other larvae can remain and pupate inside the fruit. Before pupation, the late instar larva reaches a length of 13 - 23 mm, with a cream to grey body, yellow-brown head capsules measuring around 5 mm in width, and dark pigmentation around the ocellar area (Rentel, 2013).

Freshly formed pupa is yellow-brown but changes to dark brown over time (Smith, 2020). Pupa measure between 6 and 8.5 mm in length, with the pupal stage lasting 10 to 12 days at 23°C (Smith, 2020). The mature pupa has a dark-brown frontal region with darker and more pronounced eyes (Rentel, 2013). Typically, pupae are in the soil within a cocoon made of soil or macadamia husk particles. However, no cocoon is formed when pupation occurs directly inside a nut in a shell (Timm et al., 2007). Adults of *T. batrachopa* are sexually dimorphic, with body lengths ranging from 18-22mm (Rentel, 2013). The moths are brown-grey, and

males are characterized by a dark fuscous abdomen and grey hair tufts at the base of the abdomen and hind legs (Rentel, 2013; Smith 2020). At 23°C, the adults have a lifespan of 10 – 14 days, with an average longevity of 12 days (Smith, 2020).

The feeding activity of *T. batrachopa* larvae on macadamia nuts results in immature nuts dropping, leading to crop losses and husk rot, which also increases kernel immaturity levels upon harvest and additional quality defects (Weier et al., 2019). In small immature nuts, the larvae feed entirely on the kernel, whereas in bigger nuts, the portion of this kernel may remain, except that it may be unfit for preparation (La Croix and Thindwa 1986). Also, the larvae cannot penetrate brown hardened shells but can complete larval development on the husk (La Croix and Thindwa 1986). However, this crop may not be compromised by infestations of *T. batrachopa* but can provide food to this pest's population during the following growing period (La Croix and Thindwa 1986).

Control practices

Borers are the most challenging insects to control by chemical means due to their mining and feeding habit and residing within nuts in husks by larvae (La Croix and Thingwa 1986). *Thaumatotibia batrachopa* is a notorious pest that must be controlled regularly in the orchards. Currently, the control efforts primarily rely on the chemical application of high-volume sprays such as synthetic pyrethroids and insect growth regulators, and refined products containing pheromones in combination with insecticide and products aimed to disrupt have become commercially available (de Villiers and Schoeman n.d.). However, the use of chemicals to control insect pests on food crops has been criticized because of the risks they pose to non-target organisms, humans, and unwanted risks to the environment (Aktra et al., 2009). However, the IPM programme offers a comprehensive, cost-effective, and environmentally friendly sustainable approach to control insect pest infestation (Tiwari, 2024). Integrated pest management integrates various strategies, including biological control, cultural practices, mechanical control, and selective pesticide use, to manage pest populations effectively while minimizing environmental damage (Tiwari, 2024). Implementing an IPM programme against *T. batrachopa* will allow farmers to mitigate the pest's impact while promoting environmentally friendly and economically viable agricultural practices. However, the success of an IMP programme requires a clear understanding of the impact of temperature on pest population dynamics.

The rationale of the study

Thaumatotibia batrachopa has various life stages, with its body temperature and the timing of development largely influenced by ambient temperature and other environmental factors such as humidity and precipitation. Understanding *T. batrachopa* thermal biology and thermal tolerance is critical for assessing pest population dynamics. Currently, there is a shortage of thermal information on *T. batrachopa*. Therefore, investigating how temperature impacts *T. batrachopa* development and thermal tolerance is essential for various reasons. Firstly, the population dynamics models and the accurate timing for control strategies largely depend on understanding how the temperature influences the development rate of insects. Secondly, thermal tolerance under extreme conditions is a key factor in bioclimatic and phenological models, and without including a species' thermal limits, predictions of its geographical range and abundance in future climate scenarios are limited. Lastly, understanding thermal tolerance enhances predictive modelling. Degree-days models can be refined with additional data on survival at temperature extremes and the acclimation capacity of thermal traits. Rapid hardening can improve an insect's thermal tolerance, accelerate acclimation, and increase fitness. In the long run, predictive models tested against climate data will provide critical insights into seasonal generation patterns and the impact of climate zones on *T. batrachopa* population dynamics, offering valuable management strategies for the macadamia industry.

Aims and Objectives

This study intended to give detailed information on the thermal biology of *T. batrachopa* to develop an insight into the influence of temperature on population phenology and to predict the population sizes in the macadamia-growing season in the orchards.

1. To explore the effects of temperature on the rate of development of *T. batrachopa* life stages by monitoring development duration, fecundity, longevity, and mortality under the selected constant temperatures. We hypothesized that the development of *T. batrachopa* life stages decreases with increased temperature, while fecundity, longevity, and mortality are influenced by temperature. This hypothesis is based on observed development duration

in several studies (e.g., Mironidis and Savopoulou-Soultani 2008; Duplessis et al., 2020; Muluvhahothe et al., 2024).

2. To establish which non-linear model best estimates the thermal requirements for *T. batrachopa* life stages development. We hypothesized that a specific non-linear model describes the thermal requirements and DD of *T. batrachopa* life stages. For instance, several non-linear models have been applied to describe the development rate realistically and to estimate lower and upper thresholds, and optimal temperatures for the development of life stages (Aghdam et al., 2009; Andreadis et al., 2013;

3. To examine the outcomes of acclimation on critical thermal limits (CT_{min} and CT_{max}) and of rapid hardening (heat and cold) on thermal tolerance of *T. batrachopa* life stages. We hypothesized that acclimation influences adaptability, and rapid hardening improves survival, and fitness of *T. batrachopa* life stages. This hypothesis is based on observations from other studies (e.g., Mutamiswa et al., 2019; Chidawanyika and Terblanche 2011).

4. To evaluate the degree-days model and investigate the impact of climate zones on the population dynamics of *T. batrachopa*. We hypothesized that the population dynamics of *T. batrachopa* at the orchards are influenced by the variability of microclimate conditions at different elevations. As microclimate conditions vary with elevations, we hypothesized that the differences in local climate conditions in macadamia orchards lead to variation in population phenology, fitness, and abundance of *T. batrachopa*. A study by (Kleynhans et al., 2017) has developed a better understanding on the effect of local climate on population phenology, fitness, and abundance of *Eldana saccharina* Walker in South Africa.

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CHAPTER 2

Temperature-dependent development biology at constant temperatures and thermal requirements of *T. batrachopa*

Abstract

Temperature is a significant environmental influence that shapes insect growth, particularly insect pests. Climate change-induced changes are expected to increase pest populations, which have already been observed in crops. The macadamia nut borer, *Thaumatotibia batrachopa*, is an important pest of macadamia in South Africa. The significance of quantifying the impact of temperature on development biology of *T. batrachopa* is essential, especially in the face of climate change. Here, the study aimed to quantify the effect of temperature on development biology and determine the thermal requirements of *T. batrachopa*. This experiment was conducted at constant temperatures of 16, 21, 25, and 31°C and relative humidity of 60-82%RH. Deposited eggs of *T. batrachopa* were placed in containers in rearing rooms, and the hatched larvae were inoculated into a modified false codling moth diet. Development duration was significantly faster at 25 and 31°C and longer at 16°C. Adults lived longer at 16°C and shorter at 25 and 31°C, and the fecundity was highest at 16°C and lowest at 25°C. The development of *T. batrachopa* from egg to adult required 564.72DD with thermal requirements: $T_{\min}$ of 10°C, T_{opt} of 30°C, and $T_{\max}$ of 41°C. The results suggest that the development and population growth of *T. batrachopa* is favoured in warm summer temperatures. Prolonged longevity of adults and high fecundity in low temperatures might favour population build-up of *T. batrachopa* for summer months. The temperature suitable for *T. batrachopa* development is between 10 and 41°C with an optimum of 30°C. Temperature-dependent development biology and thermal requirements for the degree day model of *T. batrachopa* are significant for phenology prediction and forecasting outbreaks in macadamia orchards.

Keywords: *T. batrachopa*, temperature, development, development rate, thermal requirements.

Introduction

Environmental variations and by harsh weather conditions due to climate change majorly affect crop production and pests (Shrestha, 2019). For example, gradually rising temperatures because of climate change have increased the population of pests and their ability to disperse, improving survival and allowing development and adaptability (Shrestha, 2019). Temperature and humidity are the basic parameters influencing insect populations (Jaworski and Hilszczański 2013). The life cycles of the insects are shortened by rising temperatures, leading to increased insect populations (Shrestha, 2019). Therefore, since temperature is a central environmental component prompting insect population dynamics, global warming is expected to broaden insect geographic range, increase survival in winter and the number of generations (Skendžić et al., 2021).

Temperature is a critical function in the development of the life stages of insects. Indeed, researchers have indicated that many biological traits of insects, such as development rate, survival, sex ratios, longevity, fecundity, and overall life cycle duration, are influenced by temperature (Ju et al., 2011). Understanding the influence of temperature on these biological traits is important because it helps to predict the timing of key life cycle events (e.g., hatching, and adult emergence), how species may respond to climate change, and for effective control of pests in agriculture. The influence of temperature can involve increased or decreased duration of each life stage and life cycle of insects (Khaliq et al., 2014; Du Plessis et al., 2020). The temperature influence in these changes is directly related to the development rate of insects, which is accelerated by high temperatures and thus leads to shortened life cycle durations. For example, the development rate of decreased life stage and life cycle duration of *Spodoptera frugiperda* (J.E Smith) was observed at 32°C (Du Plessis et al., 2021). Therefore, the development rate can influence the rate at which insects progress through the development of each life stage.

The development rate in insects is the inverse time required to grow from one life stage to another (Aghdam et al., 2009). The development rate at the lower temperature threshold (T_{min}) is zero, increasing as the temperature rises to reach its maximal development at the optimum temperature (T_{opt}), and decreases fast as it advances toward the upper-

temperature threshold ($T_{\max}$) (Aghdam et al., 2009; Jafari et al., 2012). According to Du Plessis et al., (2020), temperatures higher and lower than T_{opt} reduce the rate of insect development). The relationship between fixed temperatures and insect development rate is roughly linear at moderate temperatures but curvilinear at species optimum, and at extreme temperatures (Roy et al., 2002; Jafari et al., 2012). The importance of the relationship between the rate of development of insects and temperature has led to the formation of various temperature-dependent models that illustrate the development rate as a component of temperature (Wagner et al., 1984).

Temperature-dependent models are usually applied to forecast insect activity, seasonal population dynamics, and predators in natural environments (Roy et al., 2002). These models are applied to demonstrate the temperature's impact on the growth rate of insects (Aghdam and Nemati 2020). The linear degree-day model was initially applied to illustrate development rates estimated from laboratory studies (Briere et al., 1999). Linear models effectively explain the development rate below T_{opt} and yield a lower thermal threshold under which the development rate is equal to nil and easy to estimate (Shi et al., 2011). Nonetheless, the linear model does not include the non-linearity of high and low temperatures (Briere et al., 1999). Thus, nonlinear models are applied to illustrate the development rate at intermediate and extreme temperature values (Rebaudo and Rabhi 2018). Non-linear models explain parameters associated with different temperatures of biological significance (Régnier et al., 2022). The measured parameters are $T_{\min}$, $T_{\max}$, and T_{opt} , which are used to demonstrate species' responses to temperature or to forecast how climate warming will alter the development of insects (Liu et al., 2015; Sinclair et al., 2016). Thus, these models help to understand how species respond to temperature variations (Quinn, 2017; Sampaio et al., 2020).

So far, temperature-dependent development rate models have been successfully used to estimate the thermal requirements of development, such as developmental thresholds $T_{\min}$ and $T_{\max}$, and T_{opt} , and thermal constants of various insect pests. For example, these thermal requirements have been estimated for insect pests including *Sesamia nonagrioides* Lefèbvre (Andreadis et al., 2013), *Plutela xylostella* (Linnaeus) (Golizadeh et al., 2007), *Cydia pomonella* Lennaeus (Aghdam et al., 2009), and *Spodoptera eridania* (Stoll) (Sampaio et al., 2021). The thermal requirements estimate of insects are crucial because they indicate which

temperatures are most suitable for development, survival, and reproduction (Sampaio et al., 2021). Furthermore, the thresholds, T_{opt} , and thermal constant can help to forecast insect pests' phenological events, such as the occurrence of different stages in their natural habitat and enable farmers to select suitable times for control with greater precision (Sampaio et al., 2021).

The South African macadamia industry has grown substantially more than five years ago, including kernel production surging from 39k to 59k tonnes in 2019 and 2023, respectively (SAMAC, 2024). However, the industry faces threats of insect pests, including Lepidopteran pest species (Smith et al., 2022). One lepidopteran species of particular concern is the *T. batrachopa* (Lepidoptera: Tortricidae), which remains a crucial pest, including other pests in South Africa's macadamia orchards. The adults of *T. batrachopa* deposit their eggs on the surface of the nut in the husk onto which the newly hatched larva bore (La Croix and Thindwa 1986). The feeding by larvae causes immature nuts to drop, leading to crop losses, secondary infections such as husk rot, and more kernel quality defects (Smith, 2020). Development of management plans for *T. batrachopa* requires information on developmental biology. The impact of temperature on the development biology of *T. batrachopa* has never been evaluated. Our study aimed to 1) evaluate the effect of temperature on pre-oviposition, fecundity, and longevity of adults of *T. batrachopa*, 2) quantify the temperature-dependent development of *T. batrachopa* from eggs to adults, 3) choose nonlinear models that best illustrate the temperature-dependent development rate and thermal requirements of *T. batrachopa* life stages. We hypothesized that 1) differences in temperatures would influence the pre-oviposition, fecundity, and longevity of *T. batrachopa* adults with lower or higher temperatures, leading to changes in these reproductive and survival parameters, 2) the development of *T. batrachopa* from eggs to adults is temperature-dependent, and decreases with an increase in temperature, 3) a specific non-linear temperature-dependent development rate model best estimates thermal requirements for the development of all *T. batrachopa* life stages.

Materials and methods

Collection of infested macadamia nuts

Four macadamia-growing orchards were selected to collect infested macadamia nuts in husk. The Matari, Shifuma, Barotta, and Vhavenda (Figure 2.1) are established commercial orchards that grow different crops with different insect pest management practices. All these orchards were in the Levubu area, Vhembe district, in the Limpopo province, South Africa (Figure 2.1). The highest distance between the orchards was between Barotta and Vhavenda which was 9.3 km. The blocks on the orchards where infested macadamia nuts were collected were not treated with any chemicals to control pests. The average number of macadamia trees from each orchard was 300 per orchard. The minimum, maximum, and mean temperatures and relative humidity at the orchards are given in Table 2.1. The macadamia nuts were randomly collected from the ground and trees with infested nuts across all the orchards during the growing season from November 2023 to March 2024, just before harvest began. The number of infested macadamia nuts collected varied across the orchards because they were collected based on the availability of nuts in the orchards during collection day. The collecting effort was standardized across orchards, with the collection being done in the morning once a week until the stack crate ($53 \times 35.5 \times 29$ cm) was filled with infested macadamia nuts. The infested macadamia nuts were identified by the presence of *T. batrachopa* entry and exit holes, and frass on the surface of the husks of the nuts.



Figure 2.1 Macadamia orchards where macadamia nuts infested with *T. batrachopa* larvae were collected in Levubu, Vhembe district, Limpopo province, South Africa.

Table 2.1 The GPS coordinates (latitude, longitude), minimum, maximum, and mean temperature, and relative humidity of four macadamia orchards from the Levubu macadamia growing region in Limpopo province, South Africa. See also (Appendix A) for mean $\pm$ se of temperatures

Orchards	Latitude Longitude	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Relative humidity (%RH)
Shifuma	-23.11, 30.27	13	27	20	79
Matari	-23.07, 30.23	13	27	20	79
Vhavenda	-23.11,30.33	16	29	21	73
Barotta	-23.04,30.28	15	29	21	79

The collected infested macadamia nuts were placed in stack crates (53 × 35.5 × 29 cm) inside separate large breeding cages (90 × 60 × 60 cm) and kept at a room temperature of 25 ± 1°C and relative humidity of 65 ± 10%RH. The cages were monitored daily to collect the late-instar larvae (instar 4) that exited from the infested macadamia nuts to pupate outside. Larvae were carefully retrieved from the surfaces of the stack crates and breeding cages using tissue forceps to prevent injury and transferred to white, non-transparent plastic containers (14 × 14 × 7 cm) lined with dry paper towels and cotton wool for pupation. These containers were placed in a separate insectary room at 25°C and 64%RH and monitored daily until pupation. Temperature and relative humidity were continuously recorded hourly using ThermoChron iButtons loggers (Semiconductor Corporation, Dallas/Maxin TX and USA), placed inside the insectary room for data logging. Pupae were extracted from paper towels within 24 hours of pupation, placed individually in transparent plastic vials (10ml), and monitored daily until adults' emergence. The vials were covered with dry cotton wool to maintain moisture and prevent adults from flying away. All procedures were conducted under the same temperature and humidity conditions. Larvae, pupae, and adults were identified and verified according to the morphological characteristics of *T. batrachopa* described by (Rentel, 2013). The larvae collected in this study did not represent *T. batrachopa* populations in all macadamia-growing regions across the country since there are geographic variations in environmental conditions that will drive the evolution of the species. The main focus was to understand the influence of temperature on *T. batrachopa*'s development by determining the development duration of *T. batrachopa* life stages at constant temperatures. The use of larvae from the field to get the adults to initiate this study was because we failed to establish *T. batrachopa* colony in the insectary (See study limitations in Chapter 5). Therefore, the population in this study was a subset of temperatures experienced by some of the orchards in the Levubu region.

Pre-oviposition period and fecundity

To determine the fecundity of *T. batrachopa*, the newly emerged adults were removed from the vials and paired with the opposite sex inside small breeding cages (30 × 30 × 30 cm) and propylene transparent containers (45 × 25 × 30 cm). Each pair consisted of 10 males and 10 females per cage/container, were assigned and maintained under constant temperatures and relative humidity (see appendix B) for recorded mean ± se) with the following replicates:

four at 16°C (N= 80), 17 at 21°C (N= 340), 11 at 25°C (N= 220), and six at 31°C (N=120). The photoperiod was set to 16L:8D, which is designed for aquaculture. The pairs were given fresh macadamia nuts (five nuts per cage/container) collected from the orchards as an oviposition substrate. Before being placed into the cages/containers, the macadamia nuts were checked for eggs and entry or exit holes to prevent using infested nuts. The nuts were then rinsed with tap water and wiped with a dry paper towel to remove any dirt or contaminants. The cages and containers were observed daily to record any deposited eggs on the macadamia nuts. The macadamia nuts with eggs were removed from the cages/containers within 24 hours of deposition, and new nuts were placed. The Pre-oviposition period was defined as the number of days to deposit eggs after the adults were paired. The fecundity per female at each temperature was defined as the total number of eggs recorded divided by the total number of females per cage/container.

*Longevity of mated *T. batrachopa* adults*

The longevity of mated *T. batrachopa* adults at each temperature was determined using the pairs used to record fecundity (see previous paragraph). The pairs were observed daily to record the mortality of the mated adults. The mortality was recorded until all the pairs were dead. The dead adults were recorded and removed from the cages/containers, and no new pairs were introduced.

*Temperature-dependent development of *T. batrachopa**

To determine the temperature-dependent development of *T. batrachopa* from eggs to adults, the freshly deposited eggs on macadamia nuts from each temperature were removed from the cages/containers within 24 hours of deposition. The macadamia nuts with eggs were transferred in non-transparent plastic containers (14 × 14 × 7 cm), with each container corresponding to the date of pairing and egg deposition, and kept in similar conditions at each temperature. The total number of eggs recorded from each temperature was N = 771 at 16°C, N = 2648 at 21°C, N = 882 at 25°C, and N = 579 at 31°C. The eggs were monitored daily to record larval hatching within 24 hours.

The newly hatched larvae were carefully removed from the surfaces of the nuts and containers using a fine paintbrush and inoculated into a prepared false codling moth (FCM) diet formulated by (Moore et al., 2014) and modified by (Smith, 2020) (see Appendix C). The diet was prepared in 350ml Consol jars, with some portions from jars transferred into the

10ml plastic vials. One to 10 larvae were inoculated into the diet in vials, while 10 to 30 larvae were inoculated into the Consol jars because of the sizes of jars and vials. Each vial and Consol jar was covered with dry cotton wool for moisture and to prevent larvae from escaping. The larvae were allowed to feed on the diet until the instar 4 larvae were observed on the diet's surface and within the cotton wool. The instar 4 larvae were removed from the diet and placed into non-transparent containers as described in the collection of larvae paragraph. The dry cotton wool with larvae from the vials and Consol jars was also transferred to the non-transparent containers. The containers were monitored daily to record pupation.

Once pupation occurred, the newly formed pupae were carefully removed from the dry cotton wool and paper towel using tissue forceps to prevent injury. The pupae were subsequently placed individually into plastic vials, which were covered with dry cotton wool for moisture and to prevent the newly emerged adults from escaping. The sex ratio of emerged adults was determined by recording the number of males and females that emerged per temperature.

Temperature-dependent development models

Nonlinear models that were fitted to visualize the development of *T. batrachopa* life stages are presented in Table 2.2. These models were applied to demonstrate the relationship between the development rate of *T. batrachopa* life stages and temperature and to select the model(s) that best estimated thermal requirements based on the lowest AIC value using equations given in Table 2.2, r_T represents the development rate, and temperature is represented by T ($^{\circ}\text{C}$).

Table 2.2 Non-linear models and equations fitted to visualize the effect of temperature on development rate.

Model	Equation	Reference
Taylor_81 model	$rT = Rm \times e^{-\frac{1}{2} \times \left(\frac{T - T_m}{T_0}\right)^2}$	Taylor, 1981
Harcourt_82 model	$rT = a_0 + a_1 \times T + a_2 \times T^2 + T^2 + a_3 \times T^3$	Harcourt and Yee 1982
Kontodimas_04 model	$rT = aa \times (T - T_{min})^2 \times (T_{max} - T)$	Kontodimas et al., 2004
Damos_08 model	$rT = aa \times \left(\frac{bb - T}{10}\right) \times \left(\frac{T}{10}\right)^{cc}$	Damos and Savopoulou-Soultani 2008
Briere1_99 model	$rT = aa \times T \times (T - T_{min}) \times (T_{max} - T)^{\left(\frac{1}{2}\right)}$	Briere et al., 1999

Where aa, bb, cc, a0, a1, a2, and a3 are constants, R_m is the maximum temperature rate, T_m – is the optimum temperature, and T_0 – is the rate at which temperature deviates from T_m , T_{min} is the minimum temperature, and T_{max} is the maximum temperature.

Thaumatotibia batrachopa life stages' thermal requirements

Thermal requirements estimate: the minimum ($T_{\min}$), optimum (T_{opt}), and maximum ($T_{\max}$) temperatures for all life stages were estimated using all non-linear models. $T_{\min}$ refers to the threshold temperature under which development does not occur, T_{opt} represents the temperature at which development is at its highest rate, and $T_{\max}$ indicates the temperature beyond which development ceases. A model that best estimated thermal requirements was defined as the model that had the lowest AIC value and that revealed thresholds ($T_{\min}$, $T_{\max}$, and T_{opt}) for all life stages of *T. batrachopa*, which are significant for simulating predictive phenological models (in Chapter 4). The thermal constant, measured in degree-days (DD), quantified the cumulative thermal units required for the completion of a life stage (Herms, 2004).

The thermal constant was quantified using the equation:

$$DD = (T - T_{\min}) \times D$$

T is the temperature used in the development study, $T_{\min}$ is the lower development temperature, and D is the days (in number) for a stage of development, quantified for each life stage.

Statistical analysis

Pre-oviposition, fecundity, and longevity of T. batrachopa adults

The outliers from the data were visualized using ggplot2 (Wickham, 2016). The Interquartile range method (IQR) (Chambers, 2018) was used to remove visualized outliers identified as points 1.5 times smaller or larger than the IQR. Linear model assumptions for the pre-oviposition period, fecundity, and longevity of mated adults' data were tested by evaluating the normality and homogeneity of data using the Shapiro-Wilk test (Royston, 1995) and LeveneTest respectively (Fox, 2015). The linear model assumptions were not met, and the data were heteroscedastic. Kruskal-Wallis test (Hollander, 2013) analyzed the effect of temperature on the pre-oviposition period, fecundity, and longevity of *T. batrachopa* mated adults, as described in (Table 2.3). Significant differences in the pre-oviposition period, fecundity, and longevity of mated adults between temperatures were performed by Dunn's post-hoc using the R packages ggsignf and ggstaplot at the alpha level = 0.05 (95% confidence interval) (Ahlmann-Eltze and Patil 2021). The Rmisc package (Hope, 2022)

calculated the means, standard error, and 95% confidence intervals). The statistical analyses were done in R Studio statistical analysis software version 4.3.2 (R Core Team, 2022).

*Temperature-dependent development of *T. batrachopa**

The outliers from the data were visualized using ggplot2 (Wickham, 2016). The Interquartile range method (IQR) (Chambers, 2018) was used to remove visualized outliers identified as points 1.5 times smaller or larger than the IQR. Five outliers were removed from 25°C and 31°C tested temperatures in egg duration data. For larval duration, five outliers were removed from 25°C and one from 21°C.

Linear model assumptions of each life stage development duration data were tested by evaluating the data's normality using the Shapiro-Wilk test (Royston, 1995) and homogeneity of variance using the LeveneTest (Fox, 2015). The model assumptions were unmet, and data was heteroscedastic for egg and pupae development duration and homoscedastic for the larvae development duration. Subsequently, the Kruskal-Wallis test (Hollander, 2013) analyzed the effect of temperature on eggs, larvae, and pupae development duration. All analyzed development biology parameters are described in (Table 2.3). The significant differences in eggs, larvae, and pupae development duration between temperatures were determined by Dunn's post-hoc using ggsignif and ggstatsplot R packages (Ahlmann-Eltze and Patil 2021) at the alpha level = 0.05 (95% confidence interval). Rmisc package (Hope, 2022) calculated the means, standard error, and 95% confidence intervals.

Table 2.3 The biological parameters and their measurements analyzed using the Kruskal-Wallis test.

Parameters	Measurements	Responses variable
Pre-oviposition period	Number of days taken by females to deposit eggs after being paired with males.	Days
Fecundity	Number of eggs deposited per females.	Proportion
Adults longevity	How long the mated adults lived	Days
Egg duration	Incubation period of the egg before hatching	Days
Larval duration	Numbers of days taken to develop from first instar larvae to pupae.	Days
Pupal duration	Number of days taken to develop from pupae to adults	Days

Temperature-dependent models, thermal requirements, and degree-days

The DevRate package (Rebaudo et al., 2018) was used to fit temperature-dependent models (Briere1_99, Damos_08, HarcourtYee_82, Kontodimas_04, and Taylor_81). All models had the starting value parameters derived from the order “Lepidoptera” and family “Tortricidae” species in the DevRate package. All fitted models calculated the thresholds ($T_{\min}$ and $T_{\max}$) and T_{opt} for each life stage using the “nlsDR” DevRate function. The outliers from degree days (DD) data were removed using the Interquartile range, where outliers were identified as points 1.5 times smaller or larger than the IQR (Chambers, 2018). Six outliers were removed from the egg, four from larvae, and 15 from pupae data entries. The Shapiro-Wilk test (Royston, 1995) and LeveneTest (Fox, 2015) tested the normality and homogeneity of variance to evaluate the assumptions of the linear model. The data did not meet normality assumptions and was homoscedastic. The Kruskal-Wallis test (Hollander, 2013) analyzed degree days data, and Dunn’s post-hoc compared treatment means (life stages) where temperature significantly affected degree days.

Results

Pre-oviposition period

Observations of egg-deposition results showed that temperature had a significant effect in the pre-oviposition (i.e., time to lay eggs) ($\chi^2_{\text{Kruskal-Wallis}}(3) = 36.62, P < 0.05$). The females significantly took 2.78 and 3.60 days to deposit eggs at 25 and 31°C, respectively, and 8.20 days at 16°C (Table 2.4).

Fecundity

There were significant differences in the number of eggs (i.e., fecundity) observed per female across temperatures ($\chi^2_{\text{Kruskal-Wallis}}(3) = 21.63, P < 0.05$). The results showed that females maintained at 16 and 21°C deposited 19.27 and 15.70 eggs per female, respectively (Table 2.4). The females deposited 2.78 and 3.60 eggs at 25 and 31°C (Table 2.4).

Longevity of mated adults

The temperature had a significant impact on the longevity of mated adults ($\chi^2_{\text{Kruskal-Wallis}}(3) = 50.07$, $P < 0.000$). Mated adults significantly lived for 21.7 days at 16°C and 11.59 at 21°C, but for about 5 days at 25 and 31°C (Table 2.4).

Table 2.4 Pre-oviposition period, fecundity, and longevity of *T. batrachopa* (mean $\pm$ se) at different temperatures. Means with contrasting letters indicate significant differences after Dunn's post-hoc test with an alpha level of 95%. N represents the adult moth pairs (males and females) after removal of outliers per temperature.

Temperature (°C)	N	Pre-oviposition period	Fecundity (eggs per female count)	Longevity
16	4	8.20 $\pm$ 1.16 ^a	19.27 $\pm$ 3.62 ^{ab}	21.7 $\pm$ 1.13 ^a
21	17	6.57 $\pm$ 0.51 ^a	15.70 $\pm$ 2.61 ^a	11.59 $\pm$ 0.89 ^b
25	11	2.78 $\pm$ 0.26 ^b	8.01 $\pm$ 1.89 ^b	5.44 $\pm$ 0.59 ^c
31	6	3.30 $\pm$ 0.62 ^b	9.65 $\pm$ 2.69 ^c	5.90 $\pm$ 0.92 ^c

Variations in temperature had a significant impact on egg duration ($\chi^2_{\text{Kruskal-Wallis}}(3) = 54.11$, $P < 0.000$), larvae ($\chi^2_{\text{Kruskal-Wallis}}(3) = 89.58$, $P < 0.000$), and pupae ($\chi^2_{\text{Kruskal-Wallis}}(3) = 66.82$, $P < 0.000$) development duration. Egg stage duration was shorter at 25 and 31°C and longer at 16 and 21°C (Table 2.5). The results showed that *T. batrachopa* larvae develop faster at 25 and 31°C and slower at 16°C (Table 2.5). The pupae duration was significantly slower at 16 and 21°C and shorter at 25 and 31°C (Table 2.5). The ratio of males to females was doubled at 31°C and balanced at 21°C (Table 2.5).

Table 2.5 Development duration (days) of *T. batrachopa* eggs, larvae, and pupae, and a male-to-female ratio of emerged adults at different temperatures. The (means $\pm$ se) are summarized, with contrasting letters representing significant differences after Dunn's post-hoc test with an alpha level of 95%. The number in brackets represents larvae and pupae (in larvae and pupae duration) and adult pairs (in egg duration column) after removal of outliers.

Temperature (°C)	Egg development duration	Larvae development duration	Pupae development duration	Male: Female
16	15.8 $\pm$ 0.97 ^a (n=4)	45.50 $\pm$ 1.50 ^d (n=2)	33.00 $\pm$ 3.00 ^d (n=2)	0.0:1
21	7.69 $\pm$ 0.18 ^b (n=17)	20.90 $\pm$ 0.19 ^c (n=42)	17.30 $\pm$ 0.26 ^c (n=27)	1.05: 1
25	5.17 $\pm$ 0.15 ^c (n=11)	14.50 $\pm$ 0.17 ^b (n=49)	12.00 $\pm$ 0.19 ^b (n=50)	0.72: 1
31	4.38 $\pm$ 0.21 ^{cd} (n=6)	12.90 $\pm$ 0.40 ^a (n=16)	9.83 $\pm$ 0.54 ^a (n=13)	2: 1

Temperature-dependent development models, thermal requirements, and degree days of T. batrachopa

The HarcourtYee_82 was the best-fit model because it showed the lowest AIC value for egg, larvae, and pupae life stages (Table 2.6, Figure 2.2). However, this model, including Taylor_81, Kontodimas_04, and Damos_08, failed to estimate a lower development threshold ($T_{\min}$) for all life stages (Table 2.7). The Briere1_99 model estimated all thermal requirements for development for each life stage, however, for this study, it was only used for $T_{\min}$ because the best-fit model couldn't (Table 2.7, Figure 2.3). The $T_{\max}$ and T_{opt} for all life stages were estimated by HarcourtYee_82 (Table 2.7). The thermal constant for *T. batrachopa* from egg to adult was 564.72 DD (Table 2.7).

Table 2.6 Model parameters' estimates (estimate $\pm$ se) illustrating the relationship between developmental rate and temperature for *T. batrachopa* life stages. The statistic comparison is derived from the coefficient of determination (R^2) and Akaike Information Criterion (AIC). The model with the lowest AIC value for each life stage is highlighted in bold.

		Development stage			
Model	Parameters	Egg	Larva	Pupa	Egg-adult (all)
Taylor_81	R_m	$0.251 \pm 0.009^{***}$	$0.079 \pm 0.002^{***}$	$0.0046 \pm 0.0046^{***}$	$0.120 \pm 0.0129^{***}$
	T_m	$29.38 \pm 1.15^{***}$	$31.63 \pm 1.168^{***}$	$28.45 \pm 1.685^{***}$	$30.29 \pm 3.498^{***}$
	T_0	$8.34 \pm 1.022^{***}$	$11.658 \pm 0.963^{***}$	$8.627 \pm 1.767^{***}$	$9.231 \pm 2.887^{**}$
	R^2	0.382	0.194	0.0048	0.882
	AIC	-372.75	-864.90	-497.65	-734.28
HarcourtYee_82	a_0	0.40 ± 0.783	0.040 ± 0.0921	0.249 ± 0.455	0.1007 ± 0.773
	a_1	-0.075 ± 0.105	-0.0073 ± 0.011	-0.0393 ± 0.0589	-0.0216 ± 0.100
	a_2	0.0046 ± 0.0043	0.0005 ± 0.0005	0.00217 ± 0.00251	0.0014 ± 0.00428
	a_3	0.00007 ± 0.00006	-0.00001 ± 0.000006	-0.000034 ± 0.0000352	-0.000024 ± 0.0000602
	R^2	0.381	0.193	0.005	0.882
	AIC	-370.76	-862.90	-495.72	-732.29

Briere1_99	Aa	0.0002 ± 0.00004***	0.00003 ± 0.000006***	0.000068 ±0.000023**	0.000085± 0.0000.43
	T _{min}	11.70 ± 1.558***	4.194 ± 1.999*	9.513 ± 3.469 0**	10.39 ± 4.402*
	T _{max}	34.69 ± 1.356***	37.92 ± 1.418***	34.13 ± 1.912***	35.83 ± 4.201***
	R ²	0.384	0.193	0.0048	0.883
	AIC	-372.72	-864.89	-497.60	-734.29
Kontidimas_04	Aa	0.00006 ± 0.00002**	0.000007 ± 0.000001***	0.00002 ± 0.00001	0.0000221 ± 0.0000208
	T _{min}	9.575 ± 1.436***	4.108 ± 1.268**	8.108 ± 2.699**	8.346 ± 3.866*
	T _{max}	39.51 ± 2.437***	45.82 ± 2.612***	38.77 ± 3.536***	41.62 ± 7.653***
	R ²	0.384	0.1931	0.0047	0.883
	AIC	-372.71	-864.76	-497.53	-734.29
Damos_08	Aa	0.005 ± 0.001***	0.0034 ± 0.0002***	0.0030 ± 0.0011**	0.0031 ± 0.0016
	Bb	3.666 ± 0.186***	4.343 ± 0.265***	3.670 ± 0.301***	3.838 ±0.608***
	Cc	3.817 ± 0.448***	2.574 ± 0.207***	3.394 ± 0.679***	3.469 ± 1.0251***
	R ²	0.381	0.193	0.0049	0.8828
	AIC	-372.74	-864.88	-497.66	-734.28

Table 2.7 Thermal requirement parameters' estimates (lower: $T_{\min}$, and upper: $T_{\max}$ developmental threshold, optimum temperature: T_{opt} , and degree days of *T. batrachopa* life stages. The estimated thermal requirements for *T. batrachopa* life stages are highlighted in bold.

Development stage					
Model	Parameter (°C)	Egg	Larvae	Pupae	Egg to adult
Taylor_81	$T_{\min}$	0	0	0	0
HarcourtYee_82		-	-	-	-
Briere1_99		12	4	10	10
Kontodimas_04		-	-	-	-
Damos_08		0	0	0	0
Taylor_81	T_{opt}	29	30	28	30
HarcourtYee_82		29	30	28	30
Briere1_99		29	30	28	30
Kontodimas_04		30	30	29	30
Damos_08		29	30	28	30
Taylor_81	$T_{\max}$	-	-	-	-
HarcourtYee_82		39	44	37	41
Briere1_99		35	38	34	36
Kontodimas_04		40	46	39	42
Damos_08		37	43	37	38
Thermal constant	<i>DD</i>	60.18	316.19	188.35	564.72

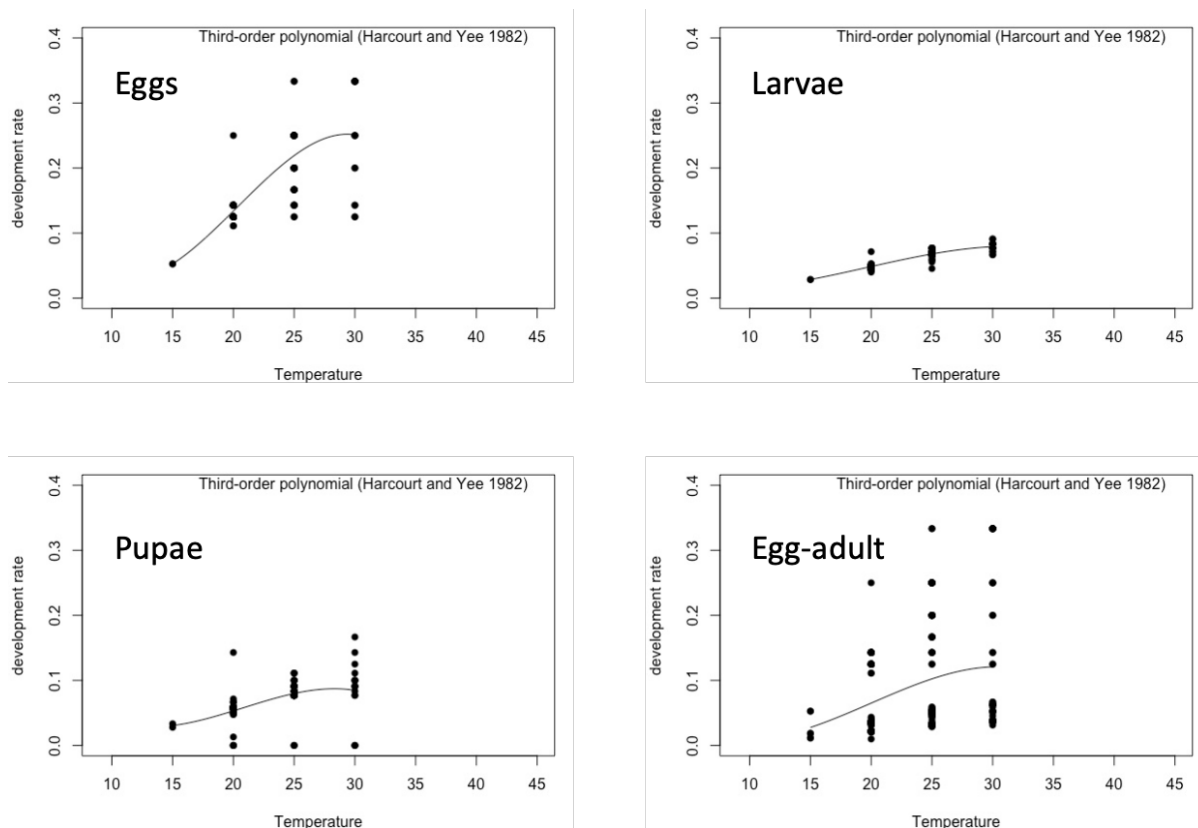


Figure 2.2 Developmental rate of *T. batrachopa* life stages at four constant temperatures. Each plot explains the best-fit model and that estimated T_{max} and T_{opt} for each life stage. The points represent rates observed.

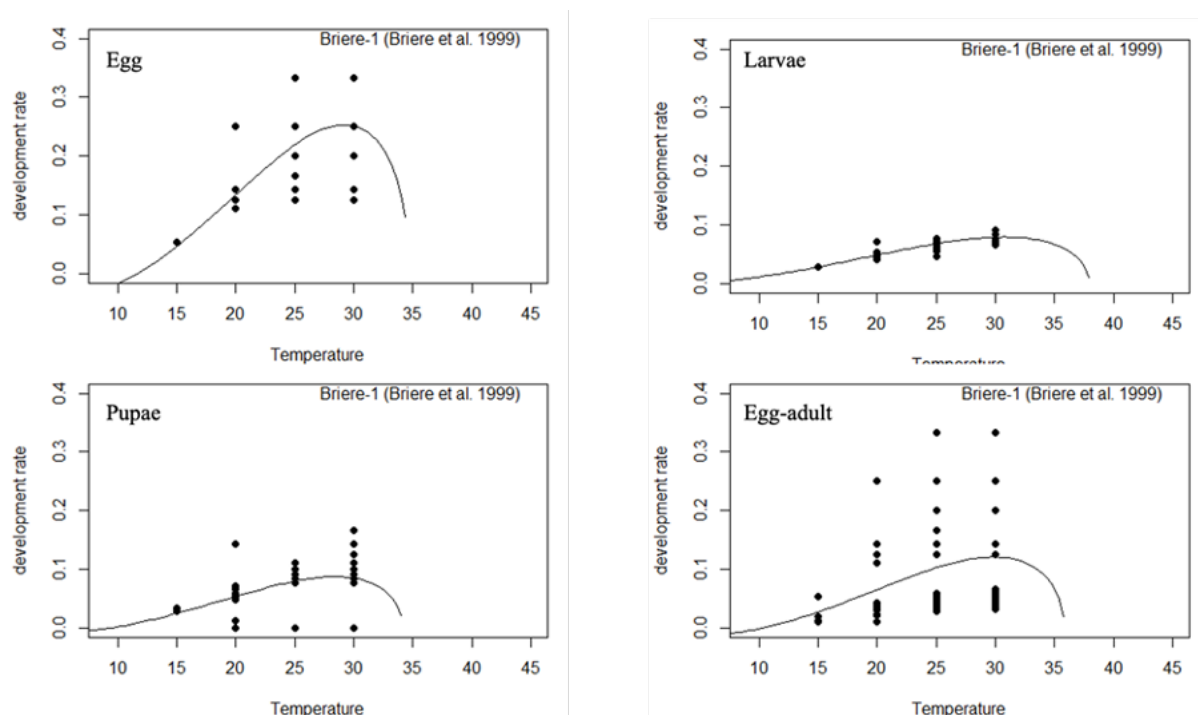


Figure 2.3. Developmental rate of *T. batrachopa* life stages at four constant temperatures. Each plot explains the model that estimated T_{min} for each life stage. The points represent rates observed.

Discussion

In this study, we aimed to evaluate the impact of temperature on the development biology of *T. batrachopa* and determine the thermal requirements for development. The results showed that the pre-oviposition period was longer at 16°C and shorter at 25 and 31°C. The results showed that female adults deposited more eggs in lower temperatures (16 and 21°C) and fewer eggs in warmer temperatures (25 and 31°C). The mated adults lived longer at 16°C and shorter at 25 and 31°C. The development duration of *T. batrachopa* from eggs to adults significantly differed across all temperatures and decreased with increased temperatures. The egg duration was longer at 16°C, and the survival rate was higher at 25°C than at other tested temperatures. The larvae and pupae developed faster at 25 and 31°C temperatures and slower at 16°C. The temperature-dependent development model that best explained the relationship between temperature and the development rate of *T. batrachopa* life stages was HarcourtYee_82, but it did not estimate T_{min} for all life stages. Therefore, the Briere1_99 model was used to estimate T_{min} for all life stages. The *T. batrachopa* required 564.72 DD to develop from egg to adult moth.

Pre-oviposition period

The findings indicated that the pre-oviposition period was long at low a temperature (16°C) and short at medium and high temperatures (25 and 31°C). Blomefield and Giliomee (2011) reported a similar pre-oviposition period, which was long (7.54 days) at 15°C for *C. pomonella*. The pre-oviposition period observed in this study suggests that temperature plays an essential role in the reproductive cycle of *T. batrachopa*, with adults taking more days to deposit eggs in cooler temperatures than in warm temperatures. The mean temperature at the orchards where infested macadamia nuts were collected is 20-21°C. Based on these temperatures, the mean pre-oviposition period in these orchards can be expected to be between 6-8 days.

Fecundity

The females deposited more eggs (19 per female) at 16°C and fewer eggs (8) at 25°C. By contrast, females of *Zeiraphera canadensis* Mut. & Free deposited 13.7 eggs at 15°C and 23.5 eggs at 25°C per female (Carroll and Quiring 1992). The differences between *T. batrachopa* and *Z. canadensis* might be attributed to species-specific responses to temperature. Although species differences can account for the variation in fecundity between *Z. canadensis* and *T. batrachopa*, observations in this study can guide the fecundity expected during the summer and winter seasons in macadamia orchards. Therefore, the mean temperature in orchards used in this study suggests that a high number of *T. batrachopa* eggs can be expected in these orchards because fecundity is favoured at cooler temperatures than in high temperatures. A high number of eggs may lead to high larval infestation on the macadamia nuts, thereby increasing the pest population at the orchards.

Longevity of mated T. batrachopa adults

The study showed that temperature affected an adult's longevity. The longevity for adults was prolonged at 16°C and short at 25 and 31°C. The effect of temperature in this study is comparable with the adult longevity of *S. nonagrioides* (Andreadis et al., 2013). *Sesamia nonagrioides* adults lived longer in cooler temperatures and shorter in warmer temperatures (Andreadis et al., 2013). The reduced longevity of *T. batrachopa* adults may be due to metabolic rate. The metabolism of insects is temperature-dependent (Andreadis et al., 2017). For example, temperature increases to the thermal optimum level accelerate the metabolism of insects (Jaworski and Hilszczański 2013). This suggests that the slow metabolic rate may allow *T. batrachopa* adults to live long and survive winter conditions. Therefore, this implies that *T. batrachopa* may have a survival advantage due to the slow metabolic rate in winter, which could result in earlier pest outbreaks in spring at the orchards.

Development duration of T. batrachopa life stages

The development duration of *T. batrachopa* eggs was longer (15.8 days) at 16°C and short (4.38 days) at 31°C. The influence of temperature on eggs' development duration in our study concurs with that of *Stethorus punctillum* Weise and its prey *Tetranychus macdanieli* McGregor (Roy et al., 2002). The *S. punctillum* eggs development duration was 14.4 days at

16°C and 3.4 days at 30°C, while *T. macdanieli* was 12.0 days at 16°C and 2.7 days at 30°C (Roy et al., 2002). The observations in our study suggest that more larvae infestations can be expected during summer since it takes about 4 days for *T. batrachopa* eggs to hatch than during winter.

The larvae and pupae of *T. batrachopa* developed slower at 16 and 21°C and faster at 25 and 30°C. The results in this study are comparable to the larval and pupal development duration of *C. pomonella* (Blomefield and Giliomee 2009). The development of *C. pomonella* larval development duration at 15°C and 30°C was 48.94 and 15.46 days, respectively, while for pupal duration days at 15°C was 56.25 and at 30°C was 13.78 (Blomefield and Giliomee 2009). The short development duration of larvae and pupae of *T. batrachopa* is expected because, in warmer conditions, the development of larvae and pupal stages is shortened (Jaworski and Hilszczański 2013). The shorter development duration of *T. batrachopa* larval and pupal stages may influence the number of generations of this pest observed at a specific geographic point. This suggests that the number of *T. batrachopa* larvae feeding on macadamia nuts and population size at the orchards may increase in summer. The adult moth sex ratio of males to females was doubled for females and males at 16°C and 31°C, respectively, and balanced at 25°C. This suggests that moderate temperatures may favour balanced adult moth sex ratios at the orchards. A balanced sex ratio implies a reproductive effort from male and female adults of *T. batrachopa* and their contribution to population growth in the orchards.

Temperature-dependent development models

The non-linear models Briere1_99 showed that the T_{min} for *T. batrachopa* is 10°C, and HarcourtYee_82 showed that the T_{max} is 41°C T_{min} and T_{opt} is 30°C from egg to adult. The T_{min} estimate for the egg stage was 12°C which is higher than that of larvae (4°C) and pupae (10°C). The T_{min} estimates from this study are comparable to *C. pomonella* which were 11.06°C, 7.89°C, and 9.89°C for egg, larvae, and pupae development stages (Blomefield and Giliomee 2009). In contrast to *T. batrachopa* estimates, the T_{min} of *Plutella xylostella* Linnaeus was 6.84°C for egg, 6.70°C, and 7.56°C for larval and pupal stages (Golizadeh et al., 2007). The differences between *T. batrachopa* and *Plutella xylostella* T_{min} estimates could be due to species-specific response, the temperature range used during development stages,

diet, genetic variation, and models used to estimate thresholds and optimum temperatures. The $T_{\min}$ estimates from this study imply that low temperatures are more unfavourable to egg development than larvae and pupae. The degree day requirements for developing *T. batrachopa* eggs were 60.18DD, 316.19DD for larvae, 188.35DD for pupae, and 564.72DD for the egg to adults. Du Plessis et al., (2020) reported the degree day requirements of *S. frugiperda* larvae as 206.7 DD and for pupae to be 150.29 DD. The degree day for *T. batrachopa* determined from this study can be used to forecast developmental life stages at the orchards.

Conclusion

Temperature is an essential factor in the development biology of *T. batrachopa*. The fecundity and longevity of adults imply that *T. batrachopa* might sustain its population in cold winters, which generally suppresses the insect population by maintaining its mating activities. However, this requires further investigation to identify possible mechanisms behind high fecundity and how the adults contribute to the overwintering of *T. batrachopa* at the orchards. This study revealed that the *T. batrachopa* eggs incubation, larvae, and pupae development duration was short at moderate to high temperatures (25 and 30°C). This implies that *T. batrachopa* in the orchards experiencing mid-high temperatures will have shortened life cycles, leading to increased populations of *T. batrachopa* during the macadamia growing season. The thermal requirements of *T. batrachopa* suggest that the pest can develop in the temperature range of 12-41°C ($T_{\min}$ and $T_{\max}$) with an optimum temperature of 30°C. This indicates that *T. batrachopa* cannot grow and persevere in habitats experiencing temperatures below 11°C and above 41°C. The degree days from our observations will provide an accurate way to predict *T. batrachopa* developmental stages based on temperature and have various implications for managing this pest. The findings from this study can be used to forecast occurrences of the life stages of *T. batrachopa* in macadamia orchards.

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Appendix A: Minimum, maximum, and mean temperature, and relative humidity (mean $\pm$ se) of four macadamia orchards from the Levubu macadamia growing region in Limpopo province, South Africa.

Orchards	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Relative Humidity %RH
Shifuma	13.47 $\pm$ 0.15	27.08 $\pm$ 0.16	19.66 $\pm$ 0.12	78.82 $\pm$ 0.37
Matari	13.47 $\pm$ 0.15	27.08 $\pm$ 0.16	19.66 $\pm$ 0.12	78.83 $\pm$ 0.37
Vhavenda	15.93 $\pm$ 0.12	28.83 $\pm$ 0.17	21.47 $\pm$ 0.12	72.63 $\pm$ 0.45
Barotta	15.17 $\pm$ 0.12	28.53 $\pm$ 0.17	20.90 $\pm$ 0.12	79.13 $\pm$ 0.42

Appendix B: Recorded constant temperatures and relative humidity (mean $\pm$ se) in rearing rooms and incubator.

Temperature ($^{\circ}\text{C}$)	Relative humidity (%RH)	Rearing room/incubator
16.02 $\pm$ 0.01	81.54 $\pm$ 0.13	Rearing room1
21.43 $\pm$ 0.05	80.98 $\pm$ 0.36	Rearing room2
25.49 $\pm$ 0.02	63.96 $\pm$ 0.19	Incubator
30.75 $\pm$ 0.35	79.47 $\pm$ 0.22	Rearing room 3

Appendix C: False codling moth diet ingredients

Ingredients	Quantity (g)
Maize meal	40
Wheat germ	4
Brewer's yeast	2
Nipagin	2
Sorbic acid	0.13
Caseins or milk power	0.73
Distilled water	50ml

CHAPTER 3

The thermal tolerance in response to acclimation, rapid cold and heat hardening of *Thaumatotibia batrachopa* Meyrick, a pest of macadamia in South Africa

Abstract

Thermal tolerance studies of insect species have proven significant because they provide valuable information on insects' thermal fitness. No study has investigated the thermal tolerance of the macadamia pest *T. batrachopa* to date. Therefore, we aimed to explore the thermal tolerance of the life stages of *T. batrachopa*. The thermal tolerance indices, such as critical thermal limits, acclimation, and rapid hardening, were used to determine the *T. batrachopa* thermal fitness. We tested the acclimation outcomes on the critical thermal limits between and within life stages using the Kruskal-Wallis test. General Linear Models analyzed the impact of rapid heat/cold hardening on the survival of *T. batrachopa* life stages. Acclimation at 20, 25, and 30°C prompted variation in CTLs between and within *T. batrachopa* life stages. Instar 4 and males showed improved cold tolerance after acclimation, while larval stages showed improved heat tolerance across all acclimation temperatures. Acclimation at 20°C improved the cold tolerance of instar 4 and male adult moths, and at 30°C improved the heat tolerance of instar 4 and male adult moths. Rapid heat/cold hardening did not improve the survival of *T. batrachopa*'s life stages. Rapid hardening did not induce plasticity in *T. batrachopa* life stages, but increased survival of adult moths. The results show the life stages of *T. batrachopa* and are likely to adapt to increasing and decreasing temperatures in the orchards.

Keywords: Temperature, acclimation, CT_{min}, CT_{max}, pre-exposure, hardening

Introduction

Insects remain a key pest that accounts for about 26% of commercial crop losses worldwide (Junaid and Gokcė 2024), and it is estimated that \$ 470 billion is lost annually to crop insect damage (Culliney, 2014). One factor that strongly influences the success and fitness of insect pests in a new habitat is their physiological tolerance to environmental strain (Nyamukondiwa and Terblanche, 2009; Le Lann et al., 2011). Although various environmental stresses have been studied (e.g. temperature, Motswagole et al., 2019; humidity, Norhisham et al., 2013), the temperature is the most important, since it profoundly affects the daily performance (Chidawanyika and Terblanche 2011). This is because insect activity and metabolism are greatly governed by temperature (Muluvhahotho et al., 2023).

Temperature affects insects mainly through processes that alter various biochemical and physiological processes (Nyamukondiwa and Terblanche 2009). For example, extremely high temperatures can harm insects by limiting their vital physiological traits, causing mortality and injuries associated with irreversible cell or tissue damage (Motswagole et al., 2019). In contrast, adverse impacts of low temperatures on insects may include reduced growth, slow development rates, and suppressed feeding, mobility, and mating (Nyamukondiwa and Terblanche 2009). Faced with extreme temperature conditions, insects employ mechanisms to cope with unfavourable conditions, including behavioural compensation and physiological mechanisms (Chidawanyika and Terblanche 2011; Mutamiswa et al., 2019). Insects may employ behavioural compensation as a first line of defence when faced with unfavourable conditions (Motswagole et al., 2019). However, if adverse conditions persist, physiological mechanisms such as phenotypic plasticity can become significant to ensure survival and fitness (Chidawanyika and Terblanche 2011).

Phenotypic plasticity is when an organism yields several phenotypes in reaction to ecological stimulations (Mutamiswa et al., 2019). The plastic response of insects can also include changes in survival and reproduction patterns, such as reduced offspring generations, prolonged longevity during winter temperatures, and modified eggs and pupae produced by insects throughout a season (Sgrò et al., 2016). Most plastic changes involve physiological

and biochemical changes that affect metabolism, the content of protein and carbohydrates, and gene expression patterns (Sgrò et al., 2016). Plastic species can survive stressful conditions and avoid species suppression due to their presumed wider geographic distribution due to various habitat-adapted phenotypes (Schlichting, 2004). Therefore, knowledge of plasticity in insects is eminent as it can allow an individual to cope with stress arising from climate change (Sgrò et al., 2016).

Various researchers have evaluated the plasticity of insects under laboratory conditions using indices including critical thermal limits (CTLs), rapid hardening, and acclimation (Terblanche et al., 2005; Nyamukondiwa and Terblanche 2009; Motswagole et al., 2019, Muluvhahotho et al., 2023). CTLs are the temperatures responsible for the coordinated muscle function loss after heating to establish the critical thermal maximum (CT_{max}) or cooling for the critical thermal minimum (CT_{min}) of an organism (Terblanche et al., 2007). CTLs are important in ecology because they show the range of temperatures in which a population remains active during severe exposure conditions (Vannier, 1994; Somero, 2005). An important consideration in CTL assays is the ramping rate (the rate at which temperature gradually increases or decreases) used, which can have a pronounced effect on CTL estimates (Chown et al., 2009). For example, a faster ramping rate improved CT_{max} for developmental stages, and a slower ramping rate improved CT_{min} for larvae of *Bactrocera dorsalis* Hendel (Motswagole et al., 2019). Results of the CTLs usually are decoupled, with further difference observed in CT_{min} than in CT_{max} (Terblanche et al., 2005). The decoupling of CTLs is also recorded in the effects of acclimation temperature studies. For example, the CT_{max} of *Chirodica chalconotus* (Coleoptera: Chrysomelidae) varied by less than 1°C while the CT_{min} varied by approximately 2.6°C (Terblanche et al., 2005). Acclimation of insects is when they are exposed for a few or more days to environments in the range of the usual optimum and may give rise to changeable and permanent physiological alterations (Hoffmann et al., 2003). There are several hypotheses as to how acclimation influences the fitness of insects. For example, the beneficial acclimation hypothesis (BAH) hypothesizes that organisms subjected to certain temperatures will enhance performance in that environment than organisms that were not (Huey et al., 1999).

Another form of phenotypic plasticity is hardening, known to improve survival to possibly lethal temperatures following a short exposure of organisms to sublethal temperatures (Chown and Nicolson 2004; Stotter and Terblanche 2009). Hardening results in reversible changes, although some insects may experience injury or have some effects that will last throughout their entire life span (Hoffmann et al., 2003). For example, hardening in *Drosophila* species results in a changed gene expression and physiology that is partially changeable (Hoffmann et al., 2003). Insects probably employ hardening to quickly alter their daily physiological tolerances (Kelty and Lee 2001). Rapid heat hardening (RHH) stimulates the heat shock response, which results in the expression of molecular chaperones (Heat shock proteins) and antioxidants (Hoffmann et al., 2003). Hardening improves survival under lethal conditions and can help an organism sustain activities, such as breeding and food consumption, notwithstanding adverse environments, increasing its fitness (Chidawanyika and Terblanche 2011). Therefore, rapid hardening quantifies insects' thermal tolerance under extreme climate events (Nyamukondiwa et al., 2010).

Studies on thermal tolerance are important in assessing insects' response to temperature variations (Nyamukondiwa et al., 2013). For example, knowledge of the plasticity of insect pests is necessary to forecast the influence of climate variation on insect fitness (Deutsch et al., 2008). Climate change postulates novel environmental niches that enable pest species to naturalize and disperse to other macro habitats and geographically spread into new regions (Skendžić et al., 2021). Thermal tolerance in pests is essential in lessening the periodical and daily outcomes of thermal variations in life stages (Mutamiswa et al., 2019). For example, acclimation studies can forecast the influence of environmental temperature variations by determining which organisms inhabit environments closer to their CTLs (Piyapongkul et al., 2018). As a result, temperature's impact on the survival and fitness of ectotherms and the transformation of this relationship over periods have gained attention (Sinclair et al., 2003; Terblanche et al., 2005; Terblanche et al., 2017; Piyaphongkul et al., 2018; Mutamiswa et al., 2019; Motswagole et al., 2019; Muluvhahothe et al., 2023).

Our aim in this study was to explore the thermal tolerance of *Thaumatotibia batrachopa*. *T. batrachopa* has several advantages for such a study. First, *T. batrachopa* is identified as a primary pest in the macadamia industry, a significant commercialized and traded crop in

South Africa (Smith et al., 2022). *T. batrachopa* inflicts damage on the placenta and the kernels of macadamia nuts (Smith, 2020) and can lead to fruit losses. Second, *T. batrachopa* is an important pest that limits macadamia production in South Africa and beyond (Bouarakia et al., 2023a). Control of *T. batrachopa* relies heavily on applying high-volume sprays such as synthetic pyrethroids and pheromones for threshold-based control (de Villiers and Schoeman n.d.). Third, there is no knowledge of the thermal tolerance of *T. batrachopa*. Therefore, we aimed to reveal the outcomes of temperature variations on the thermal adaptability of the life stages of *T. batrachopa* and how responses of life stages differ. Firstly, we investigated the impact of acclimation at different temperatures on CTLs of each *T. batrachopa* life stage and the effects of acclimation on CTLs among life stages. We hypothesized that acclimation improves tolerance to heat and cold in the life stage of *T. batrachopa* and that the response to acclimation differs for each life stage. Secondly, we determined the outcomes of pre-exposure on the persistence of life stages of *T. batrachopa* and the effects on %survival among life stages. We hypothesized that pre-exposure to cold and warm temperatures improves the survival of each *T. batrachopa* life stage, and survival differs among life stages. All acclimation trials were carried out under laboratory conditions.

Materials and methods

Larval extraction and determining larval stages

Before conducting the experiments, macadamia nuts infested with *T. batrachopa* were collected on orchards described in Chapter 2. The collected macadamia nuts were dehusked to extract larvae. The width and length of the head capsule of the larvae were measured using a microscope (Zeiss, Achromat s 0.63× FWD 107mm, Discovery.V12) to determine the larval stages of *T. batrachopa* (adopted from Go et al., 2019). The larval stages of *T. batrachopa* were estimated by fitting a nonlinear least squares function with four peaks because it is known that there are four larval stages (Smith, 2020). The values were fitted to give the mean and standard deviation of the normal probability plot, and the probability that each larva belongs to a specific larval stage was determined. Four larval stages were determined (see Appendix A and B). There was a large overlap in confidence intervals between instar 1 and instar 2 larvae; therefore, they were not used in thermal tolerance assays.

Assays in thermal tolerance

All assays in thermal tolerance were carried out using instar 3 and instar 4 larvae, and female and male adults. Therefore, the instar 1 and instar 2 larvae were kept in transparent polypropylene containers and provided with fresh macadamia husks as a food substrate and reared at $25 \pm 1^\circ\text{C}$, 65-80 % RH, and 16L: 8D until adults emerged. Adults used for thermal tolerance assays were collected in rearing containers and breeding cages (90 × 60 × 60 cm) within 24 hours after emergence and therefore considered unmated.

Acclimation

The larvae and adults were placed in 10 ml ventilated vials (one individual per vial) and acclimated at 20, 25, and 30°C , 65- 75 % RH) for 48 hours in incubators. The total number of life stages acclimated per temperature is presented in Table 3.1. This study used cold and warm acclimation temperatures that have been used for other studies (see, e.g., Chidawanyika and Terblanche 2011; Muluvhahotho et al., 2023). The larvae received fresh macadamia husks, and the adults with a 10% sucrose solution (adapted from Smith, 2020) for feeding. The larvae and adults that did not survive acclimation were removed and discarded.

Table 3.1 A total number of *T. batrachopa* life stages that were acclimated per temperature.

Life stage	20°C	25°C	30°C
Instar 3	56	27	50
Instar 4	123	119	121
Females	20	20	20
Males	20	20	20
Total	219	186	211

Critical thermal limits assay (CTLs)

CTLs of the larvae and adults of *T. batrachopa* were determined using an assay outlined by Muluvhahothe et al., (2023). Different life stages were put individually in 10ml plastic vials and were put into a series of insulated double-jacketed chambers ('organ pipes') joined to the programmable water bath (Grant, SG8 6GB; Grant instruments, Cambridge Ltd, UK, filled with water: propylene glycol ratio (1:1) to allow flow regulation at freezing temperatures. The larvae and adults were allowed to first equilibrate to the ambient temperature for 10 minutes before ramping up the temperature to determine CT_{max} or down to determine CT_{min} at a rate of $0.25^{\circ}C\ min^{-1}$. Two Thermochron ibutton loggers (Semiconductor Corporation, Dallas/Maxin TX and USA) were placed in one of the chambers to verify the temperature at 1-minute separations. Then the loss of coordinated muscle function in larvae and adults was defined as CTLs (Nyamukondiwa and Terblanche 2009).

Lethal assays and discriminating temperatures

Lethal temperatures were determined using the plunging technique summarized by Muluvhahothe et al., (2023) for *B. distincta*. The measured larvae and adults were put individually inside 6 ml vacutest tubes, placed in labelled plastic zip lock bags, and plunged into a programmable water bath filled with water 1:1 propylene glycol ratio as in CTLs assay. The larvae were plunged for 2h to acute temperatures varying from $40^{\circ}C$ to $45^{\circ}C$ for upper lethal temperature (ULT) and from 0 to $-14^{\circ}C$ for lower lethal temperature (LLT). Adults were plunged for 2h to acute temperatures that ranged from 37 to $42^{\circ}C$ for ULT and 0 to -8 for LLT. One of the ziplock bags had two ibutton loggers inside to record the temperature every minute. The plunged larvae and adults were taken to the rearing room fixed at $25 \pm 1^{\circ}C$, 60-75 %RH, 16L:8D conditions. The plunged larvae were provided with fresh macadamia husks as a source of food, and the adults with 10% sucrose solution. Survival was recorded after 24h for *T. batrachopa* larvae and adults that responded to prodded stimuli made with a paintbrush. The discriminating temperature (DT) was the temperature that caused 80% mortality of the larvae and adults.

Rapid cold and heat hardening

Rapid hardening assays of the larvae and adults of *T. batrachopa* were conducted using the plunge protocol adapted from Muluvhahotho et al., (2023). The larvae and adults were placed individually in 6ml vacutest tubes and pre-exposed for 2h at 35°C for heat and 10°C for cold hardening pre-treatment. In the control treatment, the larvae and adults were not pre-exposed and were taken directly from the rearing room. The pre-exposure temperatures used in our study were also used for rapid hardening assays for *Ceratitis capitata* and *Ceratitis rosa* (Nyamukondiwa et al., 2010) and *B. distincta* (Muluvhahotho et al., 2023). The larvae were then plunged into a water bath used in lethal assays for 2h at the discriminating temperatures of 43°C for rapid heat hardening and -11°C for rapid cold hardening. Adults were plunged at the DTs of 40°C for RHH and -5°C for RCH. Hardening was recorded as % survival after 24h at 25°C, defined as a coordinated reaction to stimulation.

Statistical analysis

Before CTLs statistical analysis was performed, data were visualized using ggplot (Wickham, 2016) and the interquartile range (IQR) method (Chambers, 2018) was used to remove the outliers from data. Outliers were identified as points 1.5 times (default value) smaller or larger than the IQR.

The assumptions of the linear model were evaluated by testing the normality and homogeneity of data using the Shapiro-Wilk test (Royston, 1995), and LeveneTest (Fox, 2015), respectively. The Rmisc R package (Hope, 2022) calculated the mean, standard deviation, standard error, and 95% confidence intervals of CTLs of each life stage for each acclimation temperature.

The Kruskal-Wallis test (Hollander, 2013) analyzed the effects of each acclimation temperature on CTLs between life stages. The R packages ggsignif and ggstatsplot R packages (Ahlmann-Eltze and Patil 2021) were used to determine significant differences between life stages and by performing multiple comparisons Dunn's post-hoc using a 95% confidence interval (the alpha level = 0.05). The Kruskal-Wallis test (Hollander, 2013) analyzed the effects of acclimation within each life stage. The comparisons between acclimation temperatures were performed using Dunn's post-hoc, where significant differences between acclimation temperatures within each life stage were determined at the

95% confidence interval (alpha level = 0.05) using ggsignif and ggstatsplot R packages (Ahlmann-Eltze and Patil, 2021). The ggplot R package (Wickham, 2016) plotted the effects of acclimation temperatures on CTLs within each life stage, and the letters above errors designate significant differences established from Dunn's post-hoc test.

The logistic regression model was fitted to the lethal assay data of larvae and adults to determine lethal temperatures: upper (ULT_{80}) and lower (LLT_{80}), which caused 80% mortality. Mean for ULT_{80} and LLT_{80} were discriminating temperatures for all larval and adult moth stages later used in rapid hardening assay.

To measure the outcomes of pre-exposure on the survival of each *T. batrachopa* life stage, we compared pre-exposure and control (no-exposure) treatment groups using GLM with a binomial distribution and logit link function. A type II Sum of Squares ANOVA was applied to evaluate the significance of the effects on survival between pre-exposure and no-exposure. The effects of life stage and pre-exposure on %survival between life stages were analyzed using GLM with a binomial distribution and logit link function. A type II Sum of Squares ANOVA was applied to assess the significance of the effects on %survival between life stages. All data were analyzed in R studio statistical analysis software version 4.3.2. (R Core Team, 2022).

Results

CTLs differences between life stages

The interaction between life stage and acclimation temperature showed a significant variation in CT_{min} among life stages after acclimation at 20 and 30°C (Table 3.2). Across all acclimation temperatures, instar 4 larvae consistently were the most cold-tolerant and had the lowest CT_{min} (Table 3.2). There was only a significant effect for adults at 20°C acclimation, where male adults were more cold-tolerant than instar 3 larvae (Table 3.3). The life stage explained significant variations in CT_{max} between life stages across all acclimation temperatures (Table 3.2) The larval stages were more heat tolerant than adults, with instar 4 larvae significantly being the most heat tolerant across all acclimation temperatures (Table 3.3).

Table 3.2. Results' summary results of the Kruskal-Wallis test showing the effects of acclimation on CTLs between life stages for each acclimation temperature. χ^2 is Chi-square, d.f represents degrees of freedom, and P-value is the P value from the test.

Acclimation temperatures	χ^2	d.f	P-value
CT _{min}			
CT20	37.96	3	<0.000***
CT25	7.62	3	0.05
CT30	15.45	3	<0.0001***
CT _{max}			
CT20	56.20	3	<0.000***
CT25	42.53	3	<0.000***
CT30	51.54	3	<0.000***

Table 3.3 CTLs (mean $\pm$ se) variation between life stages of *T. batrachopa* at acclimation temperatures. The significant differences between life stages for each temperature are indicated by contrasting letters.

Life stage	20°C	25°C	30°C
CT_{min}			
Instar 3	5.15 $\pm$ 0.17 ^a (30)	4.75 $\pm$ 0.17 ^a (6)	5.02 $\pm$ 0.15 ^{ab} (27)
Instar 4	3.72 $\pm$ 0.13 ^b (70)	3.72 $\pm$ 0.13 ^a (77)	4.57 $\pm$ 0.18 ^a (53)
Female	4.61 $\pm$ 0.13 ^{ab} (9)	4.17 $\pm$ 0.14 ^a (9)	5.35 $\pm$ 0.21 ^{ab} (10)
Male	3.65 $\pm$ 0.11 ^{bc} (10)	4.40 $\pm$ 0.16 ^a (10)	5.76 $\pm$ 0.17 ^b (10)
CT_{max}			
Instar 3	43.89 $\pm$ 0.09 ^a (19)	43.44 $\pm$ 0.21 ^a (18)	43.82 $\pm$ 0.26 ^a (22)
Instar 4	44.60 $\pm$ 0.09 ^b (52)	44.30 $\pm$ 0.16 ^a (41)	44.75 $\pm$ 0.12 ^b (68)
Female	42.15 $\pm$ 0.07 ^c (10)	42.22 $\pm$ 0.08 ^b (9)	41.80 $\pm$ 0.08 ^c (10)
Male	41.50 $\pm$ 0.00 ^{cd} (9)	41.75 $\pm$ 0.13 ^{bc} (10)	42.20 $\pm$ 0.11 ^{cd} (10)

Acclimation effects on CTLs within each life stage

Acclimation significantly affected the CT_{min} of instar 4 larvae, female and male adults (Table 3.4). Instar 4 larvae had significantly improved cold tolerance after acclimation at 20 and 25°C compared to 30°C (Figure 3.1a). Female adults had significantly improved cold tolerance after acclimation at 25°C compared to 30°C (Figure 3.1a). Male adults had significantly improved cold tolerance after acclimation at 20 and 25°C relative to 30°C (Figure 3.1a). The CT_{min} of instar 4 was lowered by 1.3°C from 30 to 20°C and from 30 to 25°C. In

adults, CT_{min} of females was reduced by $1.18^{\circ}C$ from 30 to $25^{\circ}C$, while of males was by $1.36^{\circ}C$ from 30 to $25^{\circ}C$, by $2.11^{\circ}C$ from 30 to $20^{\circ}C$, and by $0.75^{\circ}C$ from 25 to $20^{\circ}C$.

Acclimation significantly affected the CT_{max} of instar 4 larvae, female, and male adults (Table 3.4). Acclimation at $30^{\circ}C$ significantly improved the heat tolerance of instar 4 compared to at $25^{\circ}C$ (Figure 3.1b). The female adults showed reduced heat tolerance after acclimation at $30^{\circ}C$ than in 20 and $25^{\circ}C$ (Figure 3.1b). Acclimation at $30^{\circ}C$ significantly improved the heat tolerance of male adults compared to 20 and $25^{\circ}C$ (Figure 3.1b). The CT_{max} of instar 4 was increased by $0.45^{\circ}C$ from 25 to $30^{\circ}C$. Female adults had reduced CT_{max} from 30 to $25^{\circ}C$ by $0.42^{\circ}C$ and by $0.35^{\circ}C$ from 30 to $20^{\circ}C$. Male adults CT_{max} was increased by $0.7^{\circ}C$ from 20 to $30^{\circ}C$ and by $0.45^{\circ}C$ from 25 to $30^{\circ}C$.

Table 3.4 Summary results of the Kruskal-Wallis test showing the effects of acclimation on CTLs for each life stage. χ^2 - is Chi-square, d.f- represents degrees of freedom, and p-value – is the p-value from the test.

Life stage	χ^2	d.f	P-value
CT_{min}			
Instar 3	2.46	2	0.29
Instar 4	12.80	2	<0.000***
Female	12.85	2	<0.000***
Male	22.75	2	<0.000***
CT_{max}			
Instar 3	4.26	2	0.12
Instar 4	8.50	2	<0.01*
Female	10.12	2	<0.000***
Male	15.87	2	<0.000***

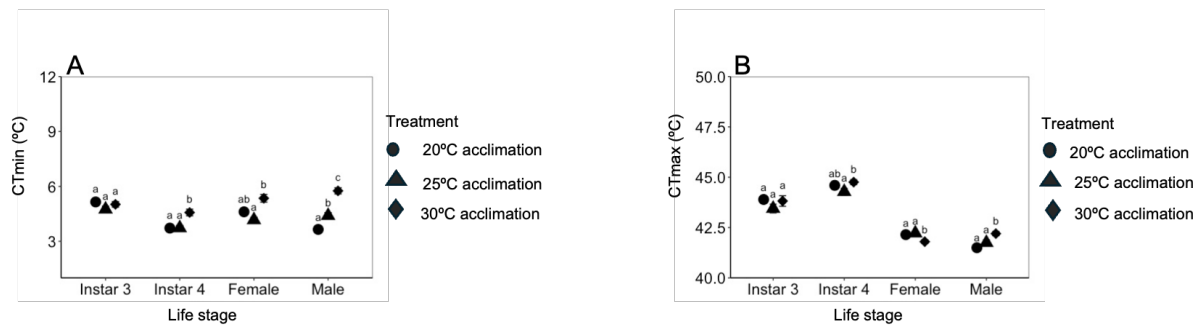


Figure 3.1 Acclimation outcomes on CT_{min} – Critical thermal minima (A) and CT_{max} – Critical thermal maxima (B) of *T. batrachopa* life stages at three acclimation temperatures. Significant differences between temperatures are shown by contrasting letters.

Lethal assays and discriminating temperatures

The discriminating temperatures, defined as temperatures at which there is 80% mortality, were determined to be 43°C and 40°C for ULT₈₀ of larvae and adults respectively, and -14°C and -5°C for LLT₈₀ of larvae and adults (Table 3.5, Figure 3.2). The DTs were used for rapid hardening assays.

Table 3.5 Summary of upper lethal temperature (ULT) and lower lethal temperature (LLT) at which different percentages of the larvae and adults died after exposure for 2 hours. Means $\pm$ error is reported.

Larval mortality (%)	LLT (°C)	ULT (°C)
10	-2.8 ± 0.71	38.99 ± 0.39
20	-4.67 ± 0.49	39.97 ± 0.25
30	-6.94 ± 0.32	41.16 ± 0.14
50	-7.88 ± 0.24	41.65 ± 0.13
90	-11.08 ± 0.24	43.33 ± 0.15
95	-14.68 ± 0.3	45.22 ± 0.21
99	-18.5 ± 0.38	47.21 ± 0.28
Adult Mortality (%)		
10	0.63 ± 1.28	37.63 ± 0.56
20	-0.72 ± 0.85	38.21 ± 0.38
30	-2.34 ± 0.50	38.91 ± 0.24
50	-3.03 ± 0.43	39.20 ± 0.19
90	-5.35 ± 0.47	40.19 ± 0.20
95	-7.96 ± 0.66	41.31 ± 0.26
99	-10.7 ± 0.85	42.49 ± 0.33

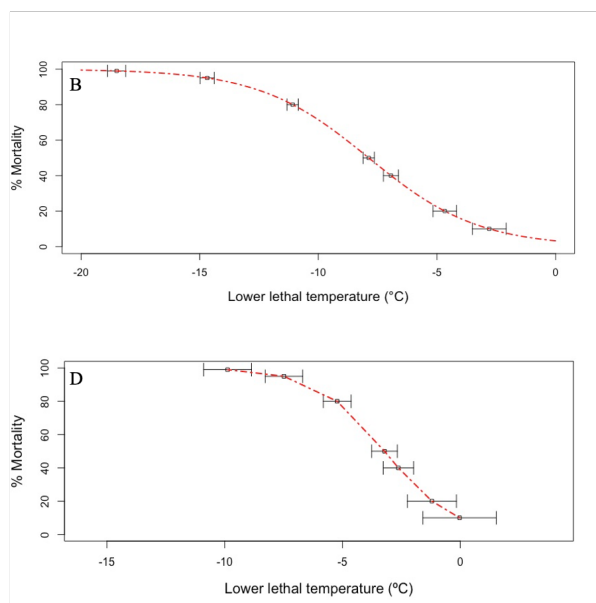
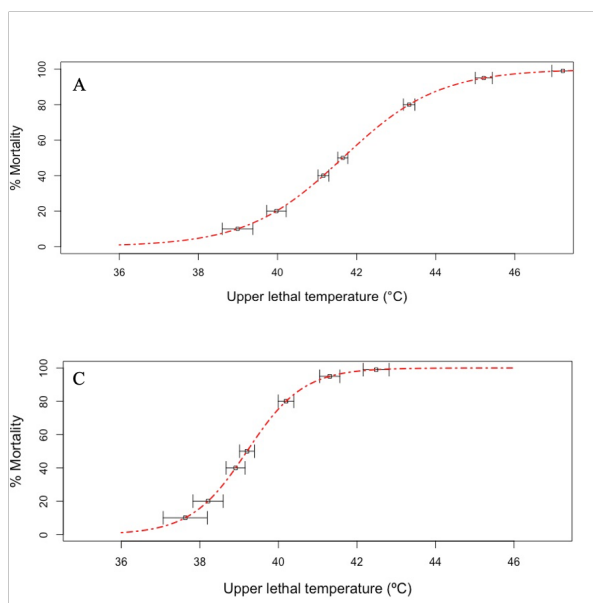


Figure 3.2 Temperatures causing 10 - 99 % mortality of *T. batrachopa* larval stages: ULT (A), LLT (B), for adults ULT (C), and LLT (D). The 95 % confidence intervals on the discriminating temperatures are indicated by error bars.

Rapid heat and cold hardening

Pre-exposure at 35°C for 2h didn't significantly improve survival of all life stages following a 2h exposure at 43 (larvae) and 40°C (adults) (Table 3.6). For RCH, pre-exposure temperature (10°C) didn't significantly improve the survival of all life stages following a 2h exposure at -11°C (larvae) and -5°C (adults) (Table 3.6). The effects of both life stage and pre-exposure on % survival among life stages of *T. batrachopa* are presented in (Table 3.7). The % survival rate for adults was higher than that of the larval stage after RHH (Figure 3.3a). The adults significantly had a higher % survival than larval stages after RCH (Figure 3.3b).

Table 3.6. General linear model (GLM) summary with (Type II) analysis, examining the effects of pre-exposure on survival of each life stage of *T. batrachopa* with a binomial distribution and identity link function.

Instar	d.f	Estimates ($\pm$ se)	χ^2	P
<i>RHH</i>				
Instar 3	1	-1.239 $\pm$ 0.66	3.261	0.07
Instar 4	1	-0.494 $\pm$ 0.40	1.409	0.23
Female	1	-1.145 $\pm$ 0.69	2.824	0.09
Male	1	-0.773 $\pm$ 0.68	1.308	0.25
<i>RCH</i>				
Instar 3	1	-1.081 $\pm$ 0.68	2.439	0.11
Instar 4	1	-0.807 $\pm$ 0.57	1.801	0.17
Female	1	0.693 $\pm$ 0.68	1.036	0.30
Male	1	0.000 $\pm$ 0.73	0.0000	1

Table 3.7. Summary of results for life stage and pre-exposure effect on %survival between life stages from rapid hardening (RHH and RCH). Significant differences are highlighted in bold.

Temperature variation effects	d.f	χ^2	P
<i>RHH</i>			
Life stage	3	13.126	<0.004**
Pre-exposure	1	7.557	<0.005**
<i>RCH</i>			
Life stage	3	86.458	<0.000***
Pre-exposure	1	0.957	0.328

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

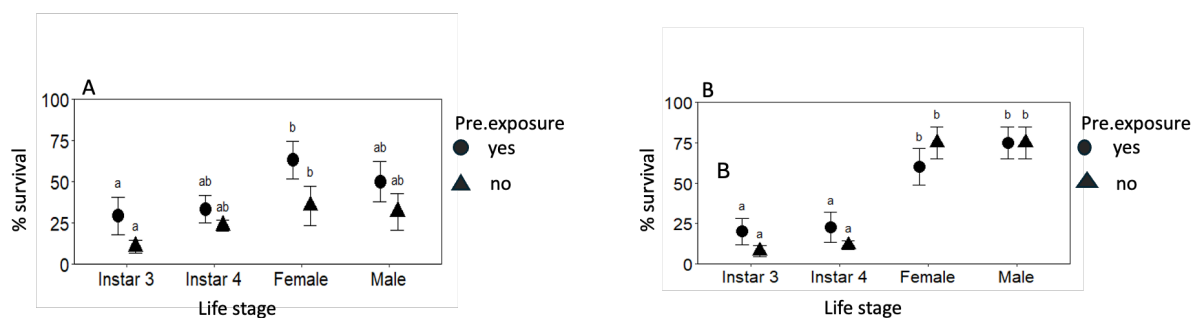


Figure 3.3 Effects of (A) life stage and pre-exposure on % survival after RHH- rapid heat hardening; (B) on % survival after RCH-rapid cold hardening of *T. batrachopa* life stages.

Discussion

The life stages of *T. batrachopa* showed significant variation in their thermal tolerance. The acclimation ability of *T. batrachopa* varied with acclimation temperatures and life stages, and the results were pronounced for larval stages. The male adults had the lowest CT_{min} followed by instar 4 larvae after acclimation at 20°C. However, instar 4 larvae were the most heat and cold-tolerant after acclimation in all temperatures. Pre-exposure for 2h and exposure for 2h didn't significantly improve each life stage's survival. However, the adults had a higher %survival than larval stages after RHH and RCH.

CTLs differences between life stages

We found that larval stages of *T. batrachopa* had the highest CT_{max} responses than adults after acclimation. This result agrees with *Pseudococcus jackbeardsleyi* Gimpel and Miller (Piyaphongkul et al., 2018). They found that the *P. jackbeardsleyi* nymphs have a higher CT_{max} than the adults after acclimation at 25 and 35°C (Piyaphongkul et al., 2018). The variation in CT_{max} in larvae compared to adults of *T. batrachopa* can be due to differences in the macroclimates they use. For example, mobile adults of *T. batrachopa* live outside of macadamia nuts and likely experience various temperatures. They can avoid temperature changes using behavioral mechanisms and move to areas with fewer temperature variations (Terblanche et al., 2017). Therefore, instead of using physiological mechanisms to survive thermal stress, they compensate behaviourally and use behaviour as a mechanism to cope with unfavourable conditions. In general, insects' sessile life stages can evolve inherent high

phenotypic plasticity during stressful conditions to compensate for the limited ability to make behavioural adjustments (Mutamiswa et al., 2019). The larvae of *T. batrachopa* live and feed inside the macadamia nut in a husk and likely employ physiological mechanisms during development (Chidawanyika and Terblanche 2011). Body size and colouration influence the ability of an insect to withstand extreme thermal conditions (Pereboom and Biesmeijer 2003). For instance, the nymphal stages are more sensitive to temperature variations than the adult stages because nymphal stages with smaller body sizes will reach equilibrium faster than adults (Piyapongkul et al., 2018). In contrast, this study revealed that adults are more sensitive to temperature variations than larval stages. These results suggest that the larval stages of *T. batrachopa* can survive the gradual increase and decrease in temperature more than the adults.

Acclimation effects on CTLs within each life stage

The results showed a more significant variation in CT_{min} than CT_{max} in response to acclimation. Acclimation at 25 and 20°C significantly improved the cold adaptability of instar 4 larvae, female and male adults. These results concur with the acclimation of *N. lugens*, where nymphs, adult females, and adult males showed improved cold tolerance after acclimation at 15°C and 23°C (Piyaphongkul et al., 2014). The improved cold tolerance of the life stages of *T. batrachopa* shows that the physiological reactions were acquired during the acclimation period. For example, *Chilo partellus* (Swinhoe) larval acclimation improved physiological tolerance to thermal strain that transferred to all life stages (Mutamiswa et al., 2019). The improved cold tolerance in response to CT_{min} suggests that the life stages of *T. batrachopa* can adapt to low environmental temperatures under ongoing climate change.

Acclimation at 30°C significantly improved the heat response of instar 4 larvae and male adults but reduced the heat tolerance of females. The CT_{max} results of males are consistent with the findings of adult male *Nilaparvata lugens* (Stal) acclimated at 30°C (Piyaphongkul et al., 2014). By contrast, acclimation at 30°C improved the heat tolerance first instar, sixth instar, and adult of *Spodoptera frugiperda* (J.E. Smith) (Phungula et al., 2023). The reduced heat tolerance of females can be attributed to damage caused by increasing temperature that denatures proteins and membranes and alters enzyme structures, water loss, or dehydration (Chown and Nicolson 2004). Elevated temperatures in the viable reach for the growth of insects will

strengthen their metabolic rate. Consequently, environmental temperatures higher than the optimum (T_{opt}) to CT_{max} will disrupt activity and are mostly permanent as those temperatures are closer to ULT (Piyaphongkul et al., 2014). Generally, males are more active fliers than females because they can fly longer distances looking for females to mate with, which may lead to them experiencing varying hot temperatures in their natural habitats. Therefore, the improved heat tolerance of males of *T. batrachopa* in this study may be due to them being exposed more to hot temperatures while looking for mating partners in the orchards.

The CTLs outcomes in this study concur with the typical decoupled observations of CT_{min} indicating more adaptation and fitness from acclimation than CT_{max} (Terblanche et al., 2005). In addition, the effect of acclimation results can be interpreted by supporting the beneficial acclimation hypothesis (BAH), which states that organisms acclimated to certain environmental conditions have a better chance of performance and survival in similar environments (Huey et al., 1999). To support this hypothesis, acclimation to low and high temperatures improved tolerance to cold and heat of acclimated individuals. This suggests that *T. batrachopa* larval stages and adults have acclimation ability. Therefore, they can adapt and survive the temperature variations caused by global climate change.

Rapid heat and cold hardening

Our study revealed that both rapid heat and cold hardening didn't induce plasticity in the life stages of *T. batrachopa*. Rapid heat hardening results in this study are comparable to that of *Ceratitis rosa* (Nyamukondiwa et al., 2010). *Ceratitis rosa* survival was not improved following a 2h exposure at 41°C (Nyamukondiwa et al., 2010). The results of rapid cold hardening showed that a 2h pre-exposure at 10°C could not enhance the survival of *T. batrachopa* life stages following a 2h exposure at -11°C (for larvae) and -5°C (for adults). By contrast, RCH has improved the survival of other species (*Ceratitis rosa* and *Ceratitis capitata* (Nyamukondiwa et al., 2010, *Frankliniella occidentalis* (Li et al., 2011), *Afrinus sp.* (Sinclair and Chown 2006). The lack of plasticity for *T. batrachopa* life stages in our results may be due to pre-exposure temperatures used (35 and 10°C) time-course during pre-exposure and exposure (2h). According to (Hoffmann et al., 2003; Chown and Nicolson 2004), an experimental approach (e.g. a range of pre-exposure temperatures) and a recovery period of

between 30 and 60 minutes in moderate temperatures between 20 and 26°C can induce a stronger plastic response. A range of pre-exposure temperatures and duration have proved to be beneficial for *C. rosa* (Nyamukondiwa et al., 2010), and a recovery period for *C. pomonella* (Chidawanyika and Terblanche 2011). This study used an experimental approach which has been used for *B. distincta* (Muluvhahotho et al., 2023), and there was no range of pre-exposure temperatures, duration, and recovery period. This study suggests that the mean temperature of the wild habitat of *T. batrachopa*, the range of pre-exposure temperatures and duration, and the recovery period should be considered in RHH and RCH experiments.

Our study shows that adults have a higher %survival than larval stages for both RHH and RCH, but the results are pronounced for RCH. The variation detected between larval stages and adults may be due to development variability and differences in their body types. The mechanism behind complexity in life stages %survival following rapid hardening in our study is poorly understood, and no study has investigated the biochemical reactions triggering variations in heat and cold responses of *T. batrachopa*. Nonetheless, life stages go through many stressors during development due to the change of seasons (Chown and Terblanche 2006; Muluvhahotho et al. 2023), which might justify the variation in %survival to extreme temperatures in our study. Furthermore, the difference in % survival between larval and adult stages of *T. batrachopa* may be due to the environments they inhabit. For instance, adults may adapt and cope with rapidly changing outside environments, whereas larvae, particularly sheltered in more stable temperature conditions, may struggle to adapt and cope with rapid temperature fluctuations.

Conclusion

The thermal tolerance of *T. batrachopa* in this study presents a foundation for comparing with temperatures expected to be experienced in macadamia orchards. It is evident from this study that different life stages of *T. batrachopa* respond differently to acclimation and hardening. The *T. batrachopa* life stages from this study showed that they can survive in temperatures that are lower and higher than viable environmental temperatures (lowest $CT_{min} = 3.65$, and highest $CT_{max} = 44.78$). This study indicates a high level of plasticity in larval

stages relative to adults after acclimation. Rapid hardening did not induce plasticity in *T. batrachopa* life stages; however, adults had a high %survival in contrast to acclimation results. The hardening results show that previously used pre-exposure temperatures may provide unclear results. Therefore, an in-depth study that includes the range of pre-exposure temperatures from its natural habitat is required. From this study, the *T. batrachopa* life stages showed adaptability to high and low-temperature variations, and this implies that the pest may thrive in macadamia orchards under ongoing climate change.

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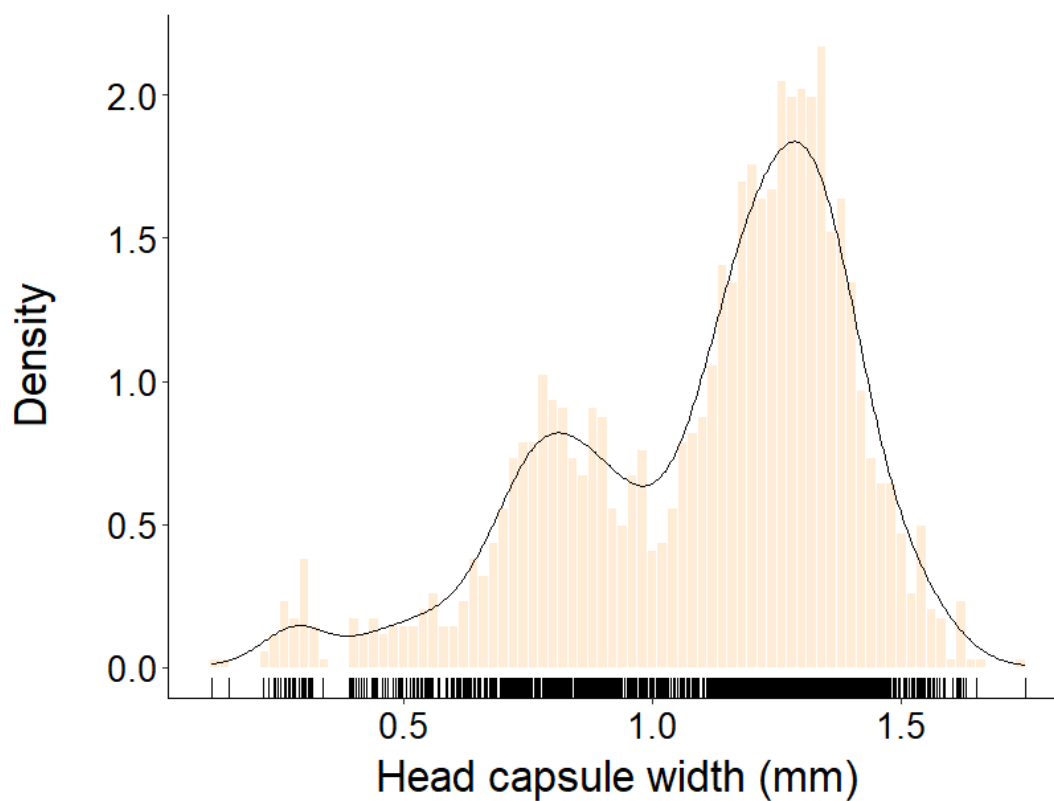
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Appendix A: Head capsule width (mean $\pm$ se) of *T. batrachopa* larval stages. ci represents confidence interval.

Instars	N	Mean $\pm$ se	Ci
Instar 1	40	0.279 $\pm$ 0.005	0.011
Instar 2	60	0.495 $\pm$ 0.010	0.020
Instar 3	476	0.831 $\pm$ 0.004	0.009
Instar 4	1129	1.283 $\pm$ 0.003	0.007

Appendix B: Frequency distribution of recorded head capsule width of *T. batrachopa* and fitted functions to head capsule data for each instar using nonlinear least square Gaussian curves.



CHAPTER 4

Influence of climate zones on population dynamics of *Thaumatotibia batrachopa* (Lepidoptera: Tortricidae) In the Levubu area, South Africa.

Abstract

Climate change has brought shifts to seasonal scheduling, geographic distribution, and population densities of many species globally. As a result, increasing temperatures allow species to expand into new climatic areas. In the Levubu production regions of Limpopo, South Africa some macadamia orchards have been established at distinctly different climatic zones along elevational gradients. Orchards planted along these gradients are susceptible to severe damage by *Thaumatotibia batrachopa* (Meyrick), one of the significant borers of macadamia nuts in South Africa. This study quantified the effects of different Koppen-Geigher identifiable climatic zones, namely the monsoon-influenced humid subtropical climate (Cwa), subtropical highland climate (Cwb), and hot semi-arid steppe climate (Bsh) on pest generation turnover, cold and heat stress, thermal safety margin (TSM), and relative fitness of *T. batrachopa* over a production season (year). The macadamia orchards selected within the climate zones were Arbor (Cwa), Highfield (Cwb), Neuhof (Cwa/Cwb/Bsh), and Welmac (Cwa/Bsh). Climate influenced the generations of *T. batrachopa* in the selected orchards. The generation number increased with decreased elevations (i.e., warmer temperatures). The *T. batrachopa* populations were not thermally cold-stressed in any orchards but experienced heat stress at the Highfield orchard. The thermal safety margin was higher at Highfield, and the relative fitness was best at Arbor and Welmac throughout the season. The increased number of generations at Arbor, Neuhof, and Welmac will likely increase the damage exerted by *T. batrachopa* in the orchards. The levitating temperatures caused by global climate variation might expand the geographical range of *T. batrachopa*.

Keywords: Climate change, increasing temperature, geographic distribution, thermal safety margin

Introduction

Climate change significantly impacts the population dynamics and the distribution of insects (Stange, 2010; Chander, 2012; Khaliq et al., 2014). Climate models have predicted that average surface temperature could increase by up to 5.8°C between 1900 and 2100 (Karuppaiah and Sujayanad 2012). Such climate and weather changes could significantly influence crop-damaging insect pests' population dynamics and status (Karuppaiah and Sujayanad 2012). Therefore, rising temperatures are estimated to accelerate the development of various insect species, increase overwinter survival rates, and cause earlier spring emergence. These changes could result in an increased annual number of generations and economic crop damage (Chander, 2012; Kiritani, 2013; Srinivasa Rao et al., 2015).

Over the past fifty years, climate change has resulted in alterations in the seasonal occurrence, geographic distribution, and population densities of numerous species globally (Parmesan, 2006). The climate is an important determinant of geographic range for many species, and increasing temperatures are expected to drive species to shift their distributions. As a result, some species may expand into new climatic areas while others face local extinction from climatically unstable habitats (Hughes, 2000). However, changes in phenology and dispersal differ in trend and size between different species (Kingsolver et al., 2013). Insects' distribution is also influenced by interactions with climatic variables (Shrestha, 2019). Therefore, an insight into the results of rapid climate change on the fitness and survival of insect populations entails examining geographic patterns in temperature variation and its magnitude (Deutsch et al., 2008).

The outcome of increasing temperatures on the fitness of insects at different locations is better understood through the thermal performance curve (TPC) (Deutsch et al., 2008). For ectotherms, TPC describes the thermal response of a genotype or species, as rising with temperature until a critical minimum temperature (CT_{min}) is reached and then peaking at an optimum temperature (T_{opt}), before falling away swiftly toward a critical thermal maximum (CT_{max}) (Kingsolver et al., 2013). Thermal performance curves (TPCs) define how the body temperature (T_b) of an ectotherm influences its physiological performance and fitness (Sinclair et al., 2016) and are widely used for foreseeing the impact of climatic changes on

ectotherms, as they account for the organisms' sensitivity to variation in temperature (Kingsolver et al., 2013; Johnson et al., 2023).

TPCs reveal geographic patterns essential for understanding local adaptation and species responses to changes in climate (Kingsolver et al., 2013). Understanding underlying mechanisms influencing temperature-related distribution patterns provides insights into current biogeographical patterns and allows predictions regarding the influence of global warming on species distribution (Somero, 2010). This enables researchers to forecast how species will adapt and thrive in the warmer habitats they may occupy in the future (Somero, 2010). A key feature of TPCs is the thermal safety margin (TSM), which represents the variance in an organism's thermal optimum (T_{opt}) and the ambient temperature (T_{hab}), expressed as $(T_{opt} - T_{hab})$ (Deutsch et al., 2008). Species inhabiting environments with temperatures near their optimal temperature are inclined to small TSMs, meaning increased temperatures can significantly reduce their performance. However, for insects, TSMs increase sharply with latitude (Deutsch et al., 2008). This is because species in low-latitude are already living in environments closer to their physiological optimum (T_{opt}), while insects in high-latitude regions are experiencing temperatures below T_{opt} (Deutsch et al., 2008).

The outcome of rising atmospheric temperatures on organisms' thermal performance represents a significant and direct biological outcome of climate change (Deutsch et al., 2008). Ectotherms, whose body temperatures (T_b) are influenced by the surrounding environment, exhibit thermal tolerance levels corresponding to the temperature variation they encounter (Angilletta, 2009). Researchers have demonstrated that the thermal adaptability of many organisms corresponds to the degree of difference in temperature in their habitat. As temperatures increase, the growing season will likely lengthen, and species will frequently be subjected to temperatures close to their optimal thermal range (Kingsolver et al., 2013). Therefore, the influence of climate shift on fitness relies upon the extent of temperature variation relative to the organism's temperature optimum and CT_{max} , with this relationship differing across mid-latitude regions (Kingsolver et al., 2013).

Borers from the order Lepidoptera are significant crop pests in Southern Africa, with considerable economic impact (Kleynhans et al., 2018). *Thaumatotibia batrachopa* Meyrick, in South Africa, is a pest of concern to commercially grown macadamia. Larval feeding

damage on nuts results in direct and indirect income forfeitures due to kernel damage and infections such as husk rot (Smith, 2020). Management strategies for *T. batrachopa* include chemical control through high-volume spray applications of synthetic pyrethroids and pheromone traps for threshold-based interventions (de Villiers and Schoeman n.d.). Understanding the population dynamics of this pest in macadamia orchards is crucial for optimizing the timing and selection of control methods. This study aimed to evaluate the influence of the climatic zones in four orchards within the Levubu macadamia growing region on the generation turnover, TSM, heat and cold stress, and relative fitness of *T. batrachopa* over one year. We hypothesized that the climatic variations across the four orchards within the Levubu macadamia growing region significantly influence the generation turnover, TSM, thermal stress (heat and cold), and relative stress of *T. batrachopa* with distinct climate zones, resulting in different effects on the pest's development and survival.

Materials and methods

Selected macadamia orchards and their climatic conditions

The study was conducted across four macadamia orchards—Arbor, Highfield, Neuhof, and Welmac—located within the Levubu macadamia-growing region of Limpopo province, South Africa. These orchards represent a range of elevations and climate zones (Table 4.1). The Arbor Boerdery orchards are in a humid subtropical climate influenced by a dry-winter monsoon (Cwa), characterized by hot summers (Beck et al., 2018). Highfield orchards are in the subtropical highland climate or Monsoon-influenced oceanic climate (Cwb) (Beck et al., 2018). Neuhof orchards are also within the Cwa zone but are positioned near the transitional areas of the hot semi-arid (steppe) climate (Bsh) and Cwb zones, experiencing dry winters and warm summers. Welmac orchards are found in the Cwa zone, adjacent to the Bsh zone, which is classified as a hot semi-dry climate with higher temperatures in summers and moderate to cool winters (Beck et al., 2018). The distances between the orchards were 20 km between Highfield and Neuhof, 13 km between Arbor and Welmac, and 9 km between Welmac and Neuhof. Temperature data for the period from 5 August 2021 to 5 August 2022 were collected from iLeaf weather stations (Hortec, Cape Town, South Africa) installed at each orchard. These stations, positioned within 38 meters of the

nearest orchard, provided hourly temperature readings, from which the season's minimum, mean, and maximum temperatures were derived (Table 4.1).

Table 4.1 Macadamia orchards in the Levubu macadamia-production region and their climatic conditions.

Orchard	Latitude Longitude	Elevation (meters a.s.l)	Climate zone	Distance (km)	Minimum temp (°C)	Mean temp (°C)	Maximum temp (°C)
Arbor	-23.0734, 30.26554	705	Cwa	13	5.1	20.0 ± 0.06	40.6
Highfield	-22.9863, 29.90229	1493	Cwb	-	2.8	17.1 ± 0.22	35.5
Neuhof	-23.0663, 30.07871	856	Cwa/Cwb/Bsh	20	4.5	20.0 ± 0.05	38.6
Welmac	-23.1186, 30.14623	812	Cwa/Bsh	9	3.2	19.3 ± 0.06	38.6

Thermal requirements and physiological traits of T. batrachopa

The thermal requirements of *T. batrachopa* were established in Chapter 2 by examining temperature-dependent development from the egg to adult moth at fixed temperatures: 16°C, 21°C, 25°C, and 31°C, under a photoperiod of 16L:8D in controlled rearing environments. The mean temperatures and relative humidity (RH) in the rearing rooms are given in Appendix A from Chapter 2. Temperature and relative humidity were monitored using two Thermochron iButtons placed in each room and incubator. Larvae were fed a modified FCM diet, while adults were given a 10% sucrose solution as a food source (Smith, 2020). Development was tracked daily to record key events, including larval emergence, pupation, and the emergence of adults. The development rate, defined as the corresponding time required by the life stage to transition from one to the next (Aghdam et al., 2009), was calculated for all life stages at each temperature. A nonlinear Briere1_99 model was used to estimate (T_{min}) and HarcourtYee_82 for T_{max} and T_{opt} for eggs, larvae, pupae, and adults (Briere et al., 1999, Harcourt and Yee 1982). Thermal constant (K), represented in degree-days (DD) was calculated using the stage-specific lower developmental threshold (T_{min}) determined via the “nlsDR” Devrate function in R (Rebaudo et al., 2018). The thermal constant for development for egg to adult moth development was quantified as:

$$DD = (T - T_{min}) \times D$$

T represents the daily mean temperature, T_{min} is the lower development temperature, and D is the duration of a life stage. The physiological traits, CT_{min} , and CT_{max} for larvae and adults were derived from the thermal tolerance study (see Chapter 3). It was assumed that the CT_{min} and CT_{max} for eggs were identical to those of the instar 3 larvae, the CT_{min} and CT_{max} for pupae were the same as those of the instar 4 larvae, and the CT_{min} and CT_{max} for adults were the average values for females and males. We modeled four life stages, which corresponds to the findings in Chapter 2 of the thermal biology study.

Table 4.2 Thermal requirements, Degree-days (DD, accumulated (AccDD), thresholds; lower developmental temperature ($T_{\min}$), upper developmental temperature ($T_{\max}$), optimum temperature (T_{opt}), and physiological traits (Critical thermal minimum ($CT_{\min}$) and Critical thermal maximum ($CT_{\max}$) of *T. batrachopa* life stages for model simulation.

	Developmental stage			
Thermal requirements and physiological traits	Eggs	Larvae	Pupae	Adults
DD	60.18	316.19	188.35	203.8
$T_{\min}$	11.7	4.1	9.5	10.3
$T_{\max}$	34.7	38	34.2	35.9
T_{opt}	29.2	29.9	28.4	29.9
$CT_{\min}$	4.75	3.72	3.72	4.28
$CT_{\max}$	43.44	44.3	44.3	41.98

Phenology model setup and simulation

A procedure-built phenology model by (Barton and Terblanche, 2014) was employed to evaluate number of generations, heat and cold stress, TSM, and relative fitness over one growing season, from 5 August 2021 to 5 August 2022, across four macadamia orchards. The model integrated daily minimum, maximum, and mean ambient temperatures from weather station data, thermal requirements, and physiological traits of *T. batrachopa*. The model output parameters were the number of generations (including life stage transition throughout the year), heat and cold stress, TSM, and relative fitness. The thermal requirements and physiological traits (Table 4.2) were assumed to remain constant for all orchards. To model population responses throughout the year, the body temperatures (T_b) for life stages of *T. batrachopa* were considered equivalent to the atmospheric temperature. The model was set to begin accumulating DD for eggs on the 5th of August 2021 if the female moth deposited eggs during the first week. The model calculated the DD number at each hourly time step based on the ambient temperature (T_b) above LDT for each life stage. Subsequently, the model used the ambient temperature to calculate the DD units required to return to the egg stage, resetting the accumulated DD to zero in preparation for the next generation (Barton and Terblanche 2014). These were modelled for 365 days, producing

outputs for generation number, heat, and cold stress, TSM, and each life stage's relative fitness (Kleynhans et al., 2018). Heat and cold stress were quantified as the sum of hours for the period where the T_b exceeded the CT_{max} or fell below the CT_{min} , with a threshold of five hours. Degree days (DD) were computed for each life stage based on the temperatures above T_{min} and below T_{max} . The TSM was calculated as the difference between the thermal optimum (T_{opt}) and T_b for each life stage ($TSM = T_{opt} - T_b$). Relative fitness was derived as the inverse of TSM, using the equation $Fitness = 1/TSM$. The R studio statistical analysis software version 4.3.2 was used to do all modelling (R Core Team, 2022).

Statistical analysis

The interquartile range method was used to identify outliers that were points 1.5 times smaller or larger than the IQR (Chambers, 2018). The assumptions of the linear model were determined by assessing the normality of data using the Shapiro-Wilk test (Royston, 1995) and homogeneity of variance using the LeveneTest, respectively (Fox, 2015). The Rmisc package (Hope, 2022) calculated averages, standard deviations, standard error, and 95% confidence intervals. Values for the number of generations from the Rmisc package did not match the number of predicted generations. Therefore, statistical analysis was not performed for this parameter. The outcomes of climate zones in heat and cold stress, TSM, and relative fitness were tested using the Kruskal-Wallis tests (Hollander, 2013). Multiple comparisons Dunn's test was done to determine significant differences in cold and heat stress, TSM, and relative fitness between orchards using ggsignif and ggstatsplot R packages (Ahlmann-Eltze and Patil 2021) on the 95% confidence interval (alpha level = 0.05). R studio statistical analysis software version 4.3.2 was used to perform all data analyses (R core team, version 4.3.2., 2022).

Results

Climate zones had a significant influence on thermal heat stress ($\chi^2_{Kruskal-Wallis}(3) = 110.54$, $P < 0.000$), TSM ($\chi^2_{Kruskal-Wallis}(3) = 133.30$, $P < 0.001$), and relative fitness ($\chi^2_{Kruskal-Wallis}(3) = 37.19$, $P < 0.001$). The number of predicted *T. batrachopa* generations was significantly higher at Arbor, followed by Welmac and Neuhof relative to Highfield across the season (Table 4.3). The predicted generations were highest in low-elevation orchards (Arbor = 6.8). The life stage

transitions were higher in Arbor, Neuhof, and Welmac than in Highfield Orchards (Figure 4.1B). The *T. batrachopa* populations in Arbor, Neuhof, and Welmac had six complete transitions from egg to adult stage, while Highfield had five complete transitions (Figure 4.1B). The *T. batrachopa* populations were thermally heat-stressed high-elevation climate zone (Highfield) but were not thermally stressed in any of the climatic zones across the season (Table 4.3). The TSM was significantly higher in Highfield than Arbor, Neuhof, and Welmac (Figure 4.1C, Table 4.3). However, TSM did not differ between Arbor, Neuhof, and Welmac (Table 4.3). Relative fitness of *T. batrachopa* populations significantly varied with climate zone across the season. The relative fitness was highest in low-elevation climate zone (Arbor) and lowest in high-elevation (Highfield) (Figure 4.1D, Table 4.3).

Table 4.3 Results of the effect of climate zones on the generations number, heat stress, cold stress, thermal safety margin (TSM), and relative fitness of *T. batrachopa* from the model simulation. Significant differences ($P < 0.05$) between the means of climate zones are represented by contrasting letters.

Model predictions					
Orchard (Climate zone)	Generations	Heat stress (h)	Cold stress (h)	TSM	Relative fitness
Arbor (Cwa)	6.8	0.00 ± 0.00^a	0.00 ± 0.00	9.87 ± 0.17^a	0.22 ± 0.04^a
Highfield (Cwb)	5.0	0.17 ± 0.03^b	0.00 ± 0.00	13.2 ± 0.22^b	0.13 ± 0.01^b
Neuhof (Cwa/Cwb/Bsh)	6.0	0.00 ± 0.00^a	0.00 ± 0.00	9.91 ± 0.20^a	0.21 ± 0.03^b
Welmac (Cwa/Bsh)	6.0	0.00 ± 0.00^a	0.00 ± 0.00	10.2 ± 0.20^a	0.14 ± 0.02^{ab}

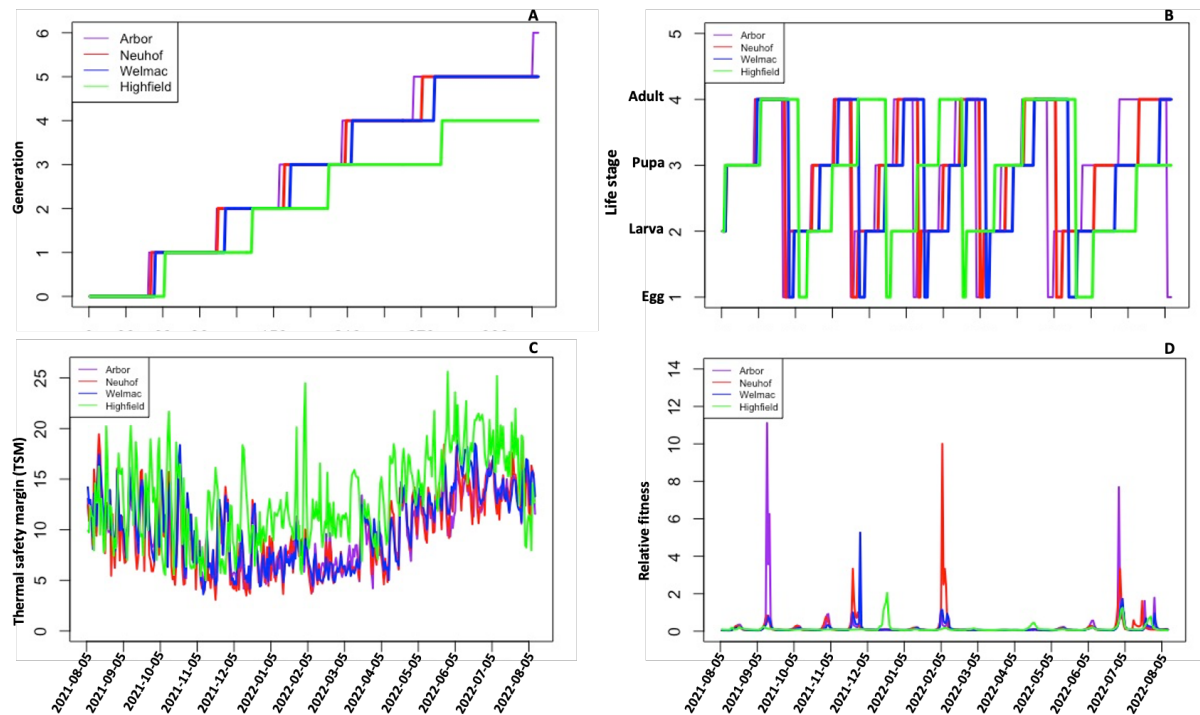


Figure 4.1 The effect of climate zones on the generations number (A), life stage transitions (B), Thermal safety margin (TSM) (C), and relative fitness (D) for *T. batrachopa* in four macadamia orchards.

Discussion

Model predictions showed significant variations across orchards and climate zones. The predicted generations were high at the low-elevation climate zone (6.8 generations). The *T. batrachopa* population was thermally heat-stressed only in Highfield but was not cold-thermally stressed in any of the orchards. The TSM was the highest in Highfield, however, the relative fitness was poor.

Elevated temperatures are presumed to quicken various insect development, which might increase the generation's number and subsequent harm to crops annually (Srinivasa Rao et al., 2015). The findings from this study indicate that higher maximum temperatures in the Arbor, Neuhoof, and Welmac orchards are likely to accelerate the development of *T. batrachopa*, which may, in turn, increase the annual number of generations in these orchards. In contrast, the five generations predicted in Highfield orchards can be attributed to the high elevation of 1493 m a.s.l. and an average daily temperature of 17.1°C, which slows the development of *T. batrachopa* (see Appendix A). These variations in daily mean

temperatures across different climatic zones could explain observed differences in the number of generations, with Arbor having 6.8, Neuhof and Welmac having six generations, while Highfield showed five generations.

The universal mean surface temperature has been projected by the Intergovernmental Panel on Climate Change (IPCC) to increase by 1.4 to 5.8°C from 1900 to 2100 (Karuppaiah and Sujayanad 2012). If temperatures rise by 2°C in the next century, the adverse impacts of global warming will likely affect most regions worldwide (Kiritani, 2006). According to Yamura and Kiritani (1998), a temperature increase of 2°C can give rise to an additional one or more generations of multivoltine insects. This implies that temperatures increasing by 2°C globally could give rise to more than six complete generations of *T. batrachopa* in macadamia orchards across various climatic zones. Model simulations for the sugarcane stalk borer *Eldana saccharina* Walker demonstrated that the third generation was completed at warmer sites, while only one generation occurred at cooler sites (Kleynhans et al., 2018). In our study, the Arbor orchard, which was the warmest, having a maximum of 40.6°C and a 20.0°C average temperature, also showed a similar trend. The differences between the number of generations predicted for *E. saccharina* and *T. batrachopa* may be attributed to differences in their respective thermal physiological requirements and adaptations.

Climate change is anticipated to significantly affect macadamia crops due to *T. batrachopa* not being thermally cold-stressed in orchards across all climatic zones and only thermally heat-stressed in Highfield orchard. Extreme temperature fluctuations in climatic zones where *T. batrachopa* populations are not subjected to heat and cold thermal stress could influence population dynamics due to the species' thermal plasticity. The capacity of any species to withstand thermal stress and remain active is an integral part of fitness (Terblanche et al., 2007). However, the degree of this impact will likely rely on the time of the extreme temperature conditions. Acclimation of the *T. batrachopa* study (see Chapter 3) showed the pest plasticity following acclimation, however, no plastic response to hardening. Acclimation is one of the plasticity traits expected to increase climate change adaptation by improving fitness (Terblanche and Hoffmann 2020). *Thaumatotibia batrachopa* not being under heat and cold stress in some orchards indicates fitness and could adapt and survive unfavourable hotter and colder temperatures. Conversely, the *T. batrachopa* populations in Highfield

orchard being thermally heat-stressed suggests that they are susceptible to unexpected heatwaves.

Fluctuations in insect populations are caused by a combination of non-living and living factors, including changes in biotic (e.g., host plants) and abiotic conditions (Nyamukondiwa et al., 2013). Macadamia nuts (fruits of host plants) set, growth, and development to maturity start from August to February (SAMAC, 2024). The limited availability of macadamia nuts during harvest and winter is expected to reduce *T. batrachopa* populations, while the increased food availability in summer might promote pest abundance.

Insect populations can be affected by winter weather conditions. Low winter temperatures can cause increased mortality, suppressing insect populations and abundance in the following season (Skendžić et al., 2021). Low temperatures (e.g., 16.02°C) slow the development of *T. batrachopa*, with the egg to adult moth development taking roughly 90 days (see Appendix A). This suggests that *T. batrachopa* likely survives the winter through diapause, which may occur in either the active larval stage, the inactive pupal stage, or both. The overwintering stages, particularly the larval and pupal stages of *T. batrachopa*, are likely critical for survival during winter when macadamia nuts are scarce. The success of overwintering directly influences the population densities of the following season (Skendžić et al., 2021). Increased overwintering success can lead to a larger over-wintering population, resulting in higher insect abundance during warmer periods (Skendžić et al., 2021). Thus, global warming could facilitate the buildup of insect populations, leading to earlier infestations and more significant crop damage by insect pests (Kocmánková et al., 2011). The successful overwintering of *T. batrachopa* may result in higher population densities in macadamia orchards during the warmer months.

The anticipated global temperature rise projected by the IPCC will likely shorten the overwintering duration of insects (Kiritani, 2013). Research by Huang and Li (2015) demonstrated that warmer winter temperatures led to a quicker onset of wintering pupal eclosion and a late end to the eclosion period for *Helicoverpa armigera* (Hübner), by 3.23 and 1.82 days, respectively. The increased temperature ultimately enhanced the over-winter survival of *H. armigera* (Huang and Li 2015). Similarly, global warming could affect the

overwintering success of *T. batrachopa* by shortening its overwintering duration. This would likely result in an earlier increase in *T. batrachopa* populations during the warmer months, leading to higher infestations and more severe damage to macadamia nuts in the orchards.

The high thermal safety margin (TSM) of *T. batrachopa* from this study suggests this pest may expand its geographic range, as it does not inhabit environments close to its optimum temperature (T_{opt}). Species living near their physiological optimum typically exhibit smaller TSMs, and as a result, increased temperatures can negatively affect their performance (Deutsch et al., 2008). Conversely, TSMs generally increase with latitude in insects (Deutsch et al., 2008). This is because species in low latitudes are already living in environments closer to their physiological optimum (T_{opt}), while insects in high latitude regions are experiencing temperatures below T_{opt} (Deutsch et al., 2008). Low-latitude regions are characterized by warm temperatures, and therefore, warmer temperatures will likely reduce the fitness of insects. The TSM results of *T. batrachopa* from all orchards in this study suggest that the populations' growth rates and performance will increase as the temperature rises, particularly in Highfield orchards where the daily mean temperature is lower (17.1°C).

Simulation results for one-year environmental conditions show that mean relative fitness decreased with increases in low-elevation orchards except for Welmac and decreased in high-elevation orchards. The relative fitness of *T. batrachopa* was highest in September, November to December 2021, February, and July to August 2022 in the Arbor, Welmac, and Neuhof orchards. This suggests that *T. batrachopa* thrives during warmer months when macadamia nuts are available, providing food for larval feeding and supporting population growth. The reduced fitness observed in Highfield orchard may be attributed to the reduced daily mean temperature of 17.1°C in the climatic zone, which reduces reproduction and development and ultimately suppresses population densities (see Table 4.1). Low fitness between March and July is expected due to harvesting in the orchards, leading to limited food availability. High fitness observed in Arbor, Neuhof, and Welmac orchards is expected due to warmer temperatures that fasten reproduction and life cycle and the availability of nuts to feed on. In comparison, the relative fitness of *E. saccharina* was greater at the warmer location in Malelane compared to the cooler location in Eston (Kleynhans et al., 2018). The outcomes from this study reveal that *T. batrachopa* is more active and performs

better in warmer months when macadamia nuts are available, potentially increasing damage from the pest's feeding life stages in the macadamia orchards.

Conclusion

Thaumatotibia batrachopa population dynamics are affected by elevation and climate zones. Our findings suggest that Arbor, Neuhof, and Welmac orchards are susceptible to more macadamia nut damage due to the increased number of predicted generations in low-elevated climatic zones orchards. *Thaumatotibia batrachopa* is not living closer to its T_{opt} of 30°C and its geographical range can be expected to expand beyond any macadamia growing regions that experience temperature variations below the T_{opt} of this pest. In addition, this study indicates that *T. batrachopa* is expected to occupy habitats that have temperatures below their critical thermal limits (less than 43.5°C in macadamia orchards. The *T. batrachopa* populations are fit during warmer summer months, and therefore, all infested macadamia orchards will experience more damage to the nuts from this pest due to its abundance since the development takes 31.67 and 27.11 days from egg to adult moth at 25 and 31°C (see appendix A) respectively, and its fitness.

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Appendix A: Development duration (days) of *T. batrachopa*'s eggs, larvae, and pupae at different temperatures. Mean $\pm$ se are summarized, with different letters indicating significant differences after Dunn's post-hoc test with an alpha level of 95%.

Temperature and humidity	Eggs	Larval stage duration	Pupal stage duration	Total development duration (days)
16.02 $\pm$ 0.71°C; 81.54 $\pm$ 0.13%RH	15.8 $\pm$ 0.97 ^a	45.50 $\pm$ 1.50 ^d	33.00 $\pm$ 3.00 ^d	94.3
21.43 $\pm$ 0.04°C; 80.98 $\pm$ 0.36%RH	7.69 $\pm$ 0.18 ^b	20.90 $\pm$ 0.19 ^c	17.30 $\pm$ 0.26 ^c	45.89
25.49 $\pm$ 0.02°C; 63.96 $\pm$ 0.19%RH	5.17 $\pm$ 0.15 ^c	14.50 $\pm$ 0.17 ^b	12.00 $\pm$ 0.19 ^b	31.67
30.75 $\pm$ 0.35°C; 79.47 $\pm$ 0.22%RH	4.38 $\pm$ 0.21 ^{cd}	12.90 $\pm$ 0.40 ^a	9.83 $\pm$ 0.54 ^a	27.11

CHAPTER 5

General conclusions and recommendations

Introduction

This chapter reports the main findings and discusses how they support the aims and objectives. Limitations of the study and recommendations for future work are also stated.

Thermal biology studies are essential and useful for understanding insect population dynamics and forecasting pest outbreaks (Nyamukondiwa et al., 2013). Insect population dynamics are shaped by various biotic and abiotic factors and their interactions (Motswagole et al., 2019). Among these factors, are temperature and humidity, with the temperature being the primary factor that affects the development, biochemical, behavioural, and physiological processes of insect pests, and this has been well documented (e.g., Nyamukondiwa et al., 2013; de Campos et al., 2021; Parra et al., 2022). Understanding the impact of temperature on these biological parameters is particularly significant for developing cost-effective control measures against the pest (Motswagole et al., 2019). Therefore, empirical data on the impact of temperature on the development biology and thermal tolerance of *T. batrachopa*, particularly concerning climate change is essential for developing an integrated pest management (IPM) programme for this pest. Establishing an IPM programme tailored to specific agroecosystems requires foundational data on the development and fitness of pests relating to weather parameters. This foundational information helps to determine the optimal timing for interventions and appropriate effective control strategy.

Degree day models can be necessary for projecting the development of insects and programming plans for IPM. These models are significant and help forecast pest phenological events, such as reproduction days and emergence (first or peak adult emergence) (Ebbenga et al., 2022). This allows researchers and decision-makers to determine suitable times of control and appropriate effective control strategies. Quantifying degree days requires development thresholds (upper and lower), quantified using temperature-dependent development rate models.

This study investigated the thermal biology of *T. batrachopa*, a South African macadamia substantial pest, by assessing the temperature's impact on development, fecundity, longevity, and fitness. *Thaumatotibia batrachopa* development and thermal tolerance studies were conducted under controlled conditions, and the influence of climate zones on population dynamics was an extension of findings from Chapter 2 and Chapter 3. Knowledge of the thermal biology of *T. batrachopa* is necessary for developing cost-effective management strategies such as IPM programme. To address this, we investigated three subjects that form part of the thermal biology of insect pests. Chapter 2 established the temperature-dependent development biology, fecundity, longevity, and non-linear model(s) that best describes the development rate of *T. batrachopa* and the thermal requirements for development. In Chapter 3, the thermal tolerance (phenotypic plasticity) responses of *T. batrachopa* to extreme temperatures was investigated. An implication of *T. batrachopa* phenology for population turnover and fitness across climate zones was examined in Chapter 4. The overall study developed an understanding of temperature's influence on the population dynamics of *T. batrachopa*, which can encourage the build-up of IPM programmes against the pest.

Research findings

The primary aim of this study was to provide detailed information on the thermal biology of *T. batrachopa* to bring insight into the influence of temperature on population phenology and to predict generations at a short and intermediate period level in the orchards. This aim was addressed by exploring four main objectives.

The first objective was to determine the impact of temperature on the development biology of *T. batrachopa* by monitoring fecundity, longevity, and the development duration of each life stage under the selected constant temperatures. The temperature-dependent development duration of *T. batrachopa* life stages was essential for fitting temperature-dependent development rate models and determining thermal requirements for this pest. Temperature-dependent development is generally used to understand several aspects of insect biology (Sampaio et al., 2021), such as development, fecundity, longevity, and survival. We observed a long and short pre-oviposition period at low and high temperatures,

respectively, however, fecundity per female high low temperatures. The longevity of mated *T. batrachopa* adults was highly influenced by temperatures, with adults living significantly longer at low temperatures than in high temperatures. The development duration of each life stage was long at low temperatures and short at high temperatures.

The second objective was to determine the nonlinear model(s) that best estimated the thermal requirements for development. This has been done for other species, such as *S. eridania* (Sampaio et al., 2021), and *B. distincta* (Muluvhahotho et al., 2024). The Briere1_99 model estimated $T_{\min}$ and HarcourtYee_82 estimated $T_{\max}$ and T_{opt} for *T. batrachopa* life stages. Furthermore, Briere1_99 and HarcourtYee_82 models revealed that the temperatures suitable for *T. batrachopa* from egg to adult were between 10 and 41°C. This study revealed that *T. batrachopa* develops quicker from egg to adult and slower at high and low temperatures, respectively, with an optimum temperature (T_{opt}) of 30°C. Our temperature-dependent development duration findings are consistent with other studies that suggested that high temperatures accelerate development and shorten life cycles (e.g., Aghdam et al., 2009; Du Plessis et al., 2020). However, our study expands this knowledge and highlights specific development duration and thermal requirements for *T. batrachopa* life stages. The accelerated development and short life cycle of *T. batrachopa* life in this study suggest that there will be an increased pest infestation in the summer months at the orchards. Global warming projections indicate that temperature will increase from 1 to 5.8°C in the following fifty years (Karuppaiah and Sujayanad 2012). Although the increased temperature will differ in each region, the Levubu macadamia-growing region is also expected to experience increased temperatures due to global warming. Therefore, increasing temperatures in the Levubu region could increase pest infestations and populations of *T. batrachopa* in the macadamia orchards, thereby increasing damage to the macadamia nuts. *Thaumatotibia batrachopa* does not develop from egg to adult in temperatures below 10 and above 41°C. This suggests that *T. batrachopa* populations will be stable at temperatures within their optimal range. Findings in this study show that *T. batrachopa* populations could be suppressed in colder temperatures (below 10°C) and heatwave summers (above 41°C). These findings imply that existing control measures against *T. batrachopa* in South African macadamia-growing regions may have to be altered to account for its short life cycle and temperatures suppressing its population, particularly in climate change-induced environmental variations.

The third objective of this study was to investigate thermal tolerance responses to acclimation and rapid hardening (heat and cold) of *T. batrachopa*'s life stages. Understanding how *T. batrachopa* life stages respond to unfavourable environmental conditions gave an insight into how this pest survives in macadamia orchards under the ongoing climate change.

The thermal tolerances study revealed *T. batrachopa* life stages' ability to adapt and survive unfavourable temperature variations. However, *T. batrachopa* could not survive after RHH and RCH. Previous studies have indicated that acclimation and rapid hardening can improve the thermal tolerance of insects, particularly insect pests (e.g., Chidawanyika and Terblanche 2011; Muluvhahotho et al., 2023). However, this study demonstrated specific CTLs of *T. batrachopa* after acclimation ($CT_{\min} = 4.48^{\circ}\text{C}$, $CT_{\max} = 44.13^{\circ}\text{C}$ for larvae, $CT_{\min} = 5.16^{\circ}\text{C}$, $CT_{\max} = 41.94^{\circ}\text{C}$ for adults) and further showed that rapid hardening didn't improve survival. These findings have revealed the possible mechanisms that are used by *T. batrachopa* to cope with and survive unfavourable temperatures in the orchards. The higher CTLs of *T. batrachopa* larvae, as compared to adults, imply that they are more plastic and respond better to fluctuating temperatures in the orchards. Rapid hardening findings suggest the *T. batrachopa* life stages do not respond to quick, sudden temperature changes (unexpected cold fronts and heat waves). With temperatures rising due to climate change, more temperature fluctuations will occur, but intense and extreme temperatures could also be expected. Therefore, *T. batrachopa* may survive temperature fluctuations but could be severely affected by frequent extreme temperatures. This implies that the IPM programme should pay attention to alleviating the impact of these findings in the orchards. This includes monitoring weather patterns from weather stations closer to the orchards to predict extreme weather change such as cold fronts and heat waves, together with cost-effective control measures.

The fourth and final objective was to investigate the impact of climate zones of *T. batrachopa* by simulating thermal requirements and physiological traits (quantified in Chapters 2 and 3, respectively) of the pest under field conditions using temperature time data from the weather station in different climate zones at Levubu area. Understanding the influence of temperature on the population dynamics of *T. batrachopa* will help to develop a

timely, cost-effective IPM programme for this pest in macadamia orchards. The phenological model predicted more generations of *T. batrachopa* in low-elevation climatic zones orchard (Arbor). The *T. batrachopa* population was thermally heat-stressed only in the high-elevation climate zone (Highfield), with the highest TSM and poor relative fitness was poor.

Our findings suggest that orchards in low-elevation climate zones (e.g., Arbor) will have high numbers of *T. batrachopa* generations. Maximum and mean temperatures in Arbor are 40.6 and 20°C, respectively. The orchards where infested macadamia nuts were collected had maximum and mean temperatures ranging from 27 to 28°C and 19 to 21°C, respectively. Therefore, the global rise in temperature will likely occur in these orchards, leading to increased population size during each macadamia growing season. The thermal safety margin of *T. batrachopa* was high (between 9.87 and 13.2), particularly in high-elevation climate zone orchards (Highfield). This suggests that this pest is inhabiting environments with temperatures below its T_{opt} . This implies that *T. batrachopa* can spread beyond its current geographical range in South Africa, provided that macadamia nuts are available in new growing regions. The thermally heat-stressed *T. batrachopa* with low relative fitness in Highfield suggests that the population may survive but with compromised fitness in environments with similar temperatures. However, macadamia-growing regions in low-elevation climate zones orchards such as Arbor, Neuhof, and Welmac will have persistent populations of *T. batrachopa*.

Orchards in low-elevation climate zones may experience outbreaks during summer months, resulting in increased damage to macadamia nuts. *Thaumatotibia batrachopa* not experiencing thermal stress in Arbor, Neuhof, and Welmac suggests that increased generations will thrive under varying temperature conditions because it can adapt and cope with unfavourable environmental conditions. The IPM programme should consider control measures that consistently target orchards at low-elevation climate zones in summer months because of predicted generations and *T. batrachopa*'s shortened life cycle. In the case of high-elevation climate zones such as in the Highfield orchard, control measures should target months when the relative fitness is relatively lower to induce a population crash of *T. batrachopa*.

Limitations

One of the initial objectives was to establish a *T. batrachopa* colony in the laboratory. Several diets such as FCM (modified and from X Sterile Insect Technique (XSIT), macadamia husk, and soybean diet, were used in an effort to establish the colony. However, the colony could not be established due to high larval mortality. Establishing a *T. batrachopa* colony would have helped to minimize the reliance on orchards-collected individuals. Using *T. batrachopa* eggs deposited by adults initially reared from the laboratory for temperature-dependent development biology would have provided less bias and consistency and reduced the external variability experienced in the orchards. Furthermore, field-collected individuals may carry pathogens, parasites, and diseases that could affect reproduction and survival. Using *T. batrachopa* individuals reared in the laboratory where the environment is consistently controlled for thermal tolerance assays would have reduced the risk of external factors or contaminants on the fitness outcomes.

The development duration of *T. batrachopa* was investigated under controlled temperatures in the laboratory, which does not mirror the fluctuating environmental temperatures at the orchards. While the results are informative, a study using fluctuating temperatures experienced in the orchards could reveal how they affect the development of *T. batrachopa* in their natural habitats.

This study was also limited by the exclusion of instar 1 and instar 2 life stages when assessing the thermal tolerance responses of *T. batrachopa* due to the overlapping results of the two life stages' head capsule width. Information on the thermal tolerance of *T. batrachopa* life stages with instars 1 and instar 2 included would provide more insight into how all life stages respond to varying temperatures. In addition, the pupal stage was omitted from the study because it is the most sessile life stage; therefore, it would have been unable to provide accurate CTLs and rapid hardening outcomes. A thermal tolerance study of all life stages would have provided information on which life stages are more resistant and sensitive to temperature fluctuations. This could help decide which key life stages to target for control to prevent pest infestations in the orchards.

Future recommendations

This thesis established the relationship between temperature and the biology *T. batrachopa* life stages under controlled environments, but future investigations are required. While the temperature-dependent development of *T. batrachopa* life stages was established, determining the influence of temperature on the transition to each larval stage will be useful in constructing the life table of this pest. The life tables provide the most comprehensive summary of a species' survivorship, development, and reproduction (Carey, 2001)

The development duration study used an FCM diet for larval stages. The diet had ingredients that did not include macadamia nuts and husks, which they feed on in their natural habitat. Therefore, future research should investigate on the impact of different temperatures on the development of *T. batrachopa* using different diets (e.g., FCM, semi-natural- placenta diet). This might reveal how the different diets influence the survival of *T. batrachopa* larval stages since they are feeding and damaging the life stage.

Thermal tolerance of *T. batrachopa* was investigated using instar 3, instar 4, female and male adults only. While these life stages have provided insight into the plasticity of *T. batrachopa*, a future investigation should include instar 1 and instar 2 life stages. *Thaumatotibia batrachopa* larval stages are the feeding and most damaging life stages. Therefore, including all larval stages of *T. batrachopa* will provide a broad understanding of the plasticity of this pest, which will be informative for control decision-makers and farmers. Furthermore, thermal tolerance studies of *T. batrachopa* should also use laboratory-reared individuals. Using laboratory-reared *T. batrachopa* life stages will reduce the influence of external variability such as diseases, parasites, and acclimatization on the thermal tolerance of this pest.

Conclusion

The management of *T. batrachopa* infestations at the orchards is a challenge since the environment, crop damage, and thermal biology of the pest need to be considered to ensure a successful control strategy is developed. The control of *T. batrachopa* currently relies on the chemical application of high-volume sprays such as synthetic pyrethroids and insect

growth regulators, and refined products containing pheromones in combination with insecticide. Therefore, data on the thermal biology of *T. batrachopa* will help improve the management of this pest and forecast the impact of climate change in macadamia orchards. For example, the thermal tolerance results from this study may be significant for the sterile insect technique (SIT) programme, as it highlights how short-term temperature variations, the rate of temperature change, and both the magnitude and duration of temperature exposure affect the survival of *T. batrachopa*. Furthermore, this study will aid in developing resilient control practices against *T. batrachopa*, such as the IMP programme.

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