Thermal biology of the two-spotted stink bug, *Bathycoelia distincta* (Hemiptera: Pentatomidae) a major pest of macadamia

by

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Declaration

I, **Mulalo Meriam Muluvhahothe**, declare that the dissertation for the degree of Doctor of Philosophy in Zoology is my original work and has not been submitted for any degree at any other university or institution. The dissertation does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signature Muluvhahothe M. Date 19 July 2023

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Abstract

The physiological functions of insects are mainly dictated by temperature because they rely on the external environment to regulate their body temperatures. This temperature dependence drives their performance, with profound implications on abundance and distribution. Daily environmental temperature fluctuations may outpace an insect's thermal tolerance capacity, which requires physiological plastic mechanisms to survive. In the context of global warming, insects may be vulnerable to temperature variations, and may ultimately determine their population dynamics. The two-spotted stink bug, *Bathycoelia distincta* (Distant) (Hemiptera: Pentatomidae) is an important pest of macadamia in South Africa. It causes damage by direct feeding on the kernel and comprises more than 80 % of the shield bugs in the orchards. Increased crop losses due to stink bug damage threaten macadamia nut production. Modern pest management practices require an understanding of the biology of pests and ecology. An important question to pose is, how does an organism's thermal plastic traits affect its ecological population dynamics? First, this study aimed to quantify the effects of temperature on biological parameters of *B. distincta* life stages, such as the development rate, development duration, survival, adult longevity, pre-oviposition period, oviposition period, and life table parameters, to determine its thermal requirements and population growth at constant temperatures ranging from 19 to 29 °C. In addition, the effect of diet (macadamia nut and sweetcorn) on development, survival, and sex ratio was investigated at 25 °C (Chapter 2). Second, to quantify the phenotypic plasticity of *B. distincta* life stages. Two thermal tolerance indices were explored: rapid hardening (rapid heat hardening: RHH and cold hardening: RCH) and acclimation (critical thermal maximum: CT_{max} and minimum: CT_{min}). RHH and RCH were determined by exposing *B. distincta* life stages to extreme temperatures of 41 and -8 °C, respectively. Acclimation effects on CT_{max} and CT_{min} were quantified by exposing *B. distincta* life stages to 48 h at 20, 25, and 30 °C. Temperature was ramped up and down at a rate of 0.2 min^{-1} to score survival at high (CT_{max}) and low (CT_{min}) critical temperature points (Chapter 3). Thirdly, the development rate was monitored (on the host plant) at temperatures ranging from 18 to 40 °C to acquire the total heat required to complete development (degree-days) and thermal requirements of each life stage of *B. distincta*.

Lastly, the physiological traits, degree-days, and thermal requirements were used to predict seasonal generation turn-over, heat and cold thermal stress, thermal safety margin (TSM), and relative fitness in macadamia orchards along an elevational gradient (705 - 1493 m a.s.l.) to determine the effects of climatic zones (Cwa climate zone**:** Monsoon-influenced humid subtropical climate, Cwb: Subtropical highland climate or Monsoon-influenced temperate oceanic climate, and Bsh: Hot semi-arid steppe climate) (Chapter 4). *B. distincta* developed at a wide range of temperatures on sweetcorn (18 to 29 °C) and macadamia nut (18 to 35 °C). The survival rate was high (51 to 100 %) between temperatures with a monotonic increase of population growth from 19 to 29 °C. The total number of heat units required to complete development was 783 DD. All life stages of *B. distincta* displayed thermal plasticity, but instar 2 was the most plastic stage except in response to cold acclimation. Response to extremes varied more at low extreme temperatures compared to high extremes. As expected, the number of generations decreased with increasing elevations from the Cwa (Arbor: 2.4 generations) to the Cwb climate zone (Highfield: 1.1 generations). None of the life stages experienced thermal stress. TSM and relative fitness were highest at the Cwb climate zone of the highest elevation. These findings suggest that *B. distincta* will potentially cause more damage in response to global warming because of its estimated population growth rate at elevated temperatures. Although relative fitness was highest in the Cwb climate zone, damage could be expected in the Cwa zone due to increased number of generations. This study can also help identify macadamia orchards in climate zones vulnerable to climate-related consequences such as outbreaks. Climatic data combined with the DD model can be used to predict the phenology of *B. distincta* and timing of chemical applications. The impacts of global warming on crop losses due to insect pests are evident worldwide, and this study has shown that macadamia orchards in certain climatic zones (e.g., Cwa climate zone) could be at risk of increased abundance of *B. distincta.* Thus, integrated pest management strategies should be of priority to macadamia farmers for effective management of *B. distincta*. Given that South Africa is the largest producer of macadamia globally and its major pest is thermal plastic, linking the physiological traits of *B. distincta* life stages to climatic conditions of all macadamia growing regions in South Africa will help understand its distribution limits.

Keywords: temperature, development, life stage, phenotypic plasticity, elevation

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Dedication

Dedicated to my daughter Muelelwa Muluvhahothe and my late brother Tshimangadzo Muluvhahothe

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CHAPTER 1

General introduction

Temperature is the main abiotic factor affecting insects (Bale 2002) because they have limited means of regulating their body temperatures (Stevenson 1985). Temperature determines all cellular and physiological functions within an insect's life cycle, including metabolism, development, growth, movement, and reproduction, with profound implications at organismal and ecosystem levels (Grigaltchik et al. 2012). Extreme body temperatures, both high and low, in the absence of specific mechanisms for thermotolerance, can cause injury or death (Chown and Nicolson 2004). Insects are, therefore, vulnerable to changes in ambient temperature, especially those associated with climate change (Mech et al. 2018; Weaving et al. 2022).

Insects can compensate for environmental temperature variations by adjusting their physiological tolerances to extremes within a single generation or evolving enhanced tolerance over several generations (Chown and Nicolson 2004; Chown et al. 2011). As a result, insect tolerance to extremes can vary dramatically throughout the life cycle (Mutamiswa et al. 2019) due to differences in microclimate conditions they experience (Bowler and Terblanche 2008), because some life stages can use behavioural strategies to escape harsh conditions (Marais and Chown 2008; Alford et al. 2014). Thermal tolerance is, therefore, essential for organisms or insect life stages that cannot cope with changing conditions through dispersal or behavioural adjustments (Fernandez et al. 2018). In addition, tolerance to temperature variations for both immobile and mobile insect life stages is vital for coping with extremes posed by the current climate change (Klockmann et al. 2017).

Phenotypic plasticity

Insects can survive extreme temperatures using plastic physiological mechanisms known as phenotypic plasticity (Whitman and Agrawal 2009). For example, the winter survival of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is induced through plastic responses to seasonal cues (Shearer et al. 2016). Nyamukondiwa et al. (2010) showed that phenotypic plasticity contributes to the invasion success of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) into novel thermal habitats. Phenotypic plasticity is ecologically important because it (i) allows organisms to adaptively match to their changing environment (DeWitt and Langerhans 2004) (ii) can extend niche breadth and geographical range, and may influence dispersal (Price et al. 2003, Schlichting 2004, Pigliucci et al. 2006) and (iii) generates adaptive genetic change crucial for a long-term persistence (Whitman and Agrawal 2009). It is suggested that plastic species have a chance to withstand ecological disruptions because they can rapidly adapt to different environmental conditions (Schlichting 2004).

Insects can respond to extreme temperatures through rapid hardening and acclimation (Lee 2010; Chidawanyika and Terblanche 2011). Rapid hardening is a form of phenotypic plasiticity, which is a rapid improvement in survival at acute temperatures after brief pre-treatment to a sub-lethal temperature, while acclimation is survival up to a critical point after exposure to desired temperatures for days to weeks (Nyamukondiwa et al. 2010; Teets and Denlinger 2013). Insects employ a rapid hardening mechanism to quickly alter their physiological tolerances during the day to increase mating and feeding time (Fasolo and Krebs 2004) and it can be critical for survival when exposed to sudden, unpredictable extremes. For example, low temperatures are regarded as one of the most significant challenges insects experiences in winter, as their small bodies can readily lose heat to their environment and, thus, must be able to tolerate extreme temperatures (Teets and Denlinger 2013). Proof of rapid hardening is evident in insects (Nyamukondiwa et al. 2010; Chidawanyika and Terblanche 2011; Muluvhahothe et al. 2023), but not all insect species have inducible tolerance, especially over short time-scales. For example, little evidence for rapid cold hardening has been observed in *Glossina pallidipes* (Wiedemann) (Diptera: Glossinidae) (Terblanche et al. 2008), although this species shows seasonal adjustments in low-temperature tolerance (Terblanche et al. 2006). Furthermore, of the insect species that show physiological phenotypic plasticity, such as rapid cold hardening, considerable variation in the magnitude of the plasticity exists within and among species (Nyamukondiwa et al. 2010) and between life stages (Klockmann et al. 2017).

Similar to rapid hardening, differences in response to acclimation between species and life stages have been reported (Klockmann et al. 2017; Mutamiswa et al. 2019). However, our understanding of acclimation includes several perspectives, including the beneficial acclimation hypothesis (BAH), deleterious acclimation hypothesis (DAH), and inverse acclimation (IA). The BAH argues that exposure to certain thermal conditions enhances insect fitness (Leroi et al. 1994). The DAH predicts a fitness decline due to a pre-exposure to extreme temperatures relative to intermediate temperatures (Loeschcke and Hoffmann 2002; Wilson and Franklin 2002). The IA predicts that exposure to low temperatures improves fitness benefits relative to warmer temperatures, "colder is better". In contrast, exposure to warmer temperatures improves fitness benefits relative to low temperatures, "hotter is better" (Deere and Chown 2006).

Phenotypic plasticity forms such as rapid hardening and acclimation can be linked to an organism's thermal habitat across space and time (Terblanche and Hoffmann 2020). For example, Coulin et al. (2019) investigated the impacts of a 10-day acclimation by measuring critical thermal limits to predict the distribution limits of *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). This study found that the southern geographic limit of *W. auropunctata* was defined by a minimum temperature of 4.2 °C, similar to the results obtained in the laboratory. Recently, a meta-analysis of 102 species on acclimation has shown weak plasticity in insects' critical limits, suggesting their vulnerability to climate-related temperature extremes (Weaving et al. 2022). The latter study showed that the critical thermal maximum increased by 0.09 °C for every 1 °C of the acclimation temperature while the critical thermal minimum decreased by 0.15 °C. This study further indicated that critical thermal limits plasticity would be less beneficial in response to climate change, but juveniles were more plastic than adults (Weaving et al. 2022).

Thermal performance curves (TCPs)

The thermal performance curves (TPCs) (Fig 1.1) are useful tools for understanding thermal physiology and adaptation of ectotherms (Sinclair et al. 2016). The physiological consequences of temperature variations can be examined by how the temperature affects performance or fitness at an organismal level (Huey and Slatkin 1976).

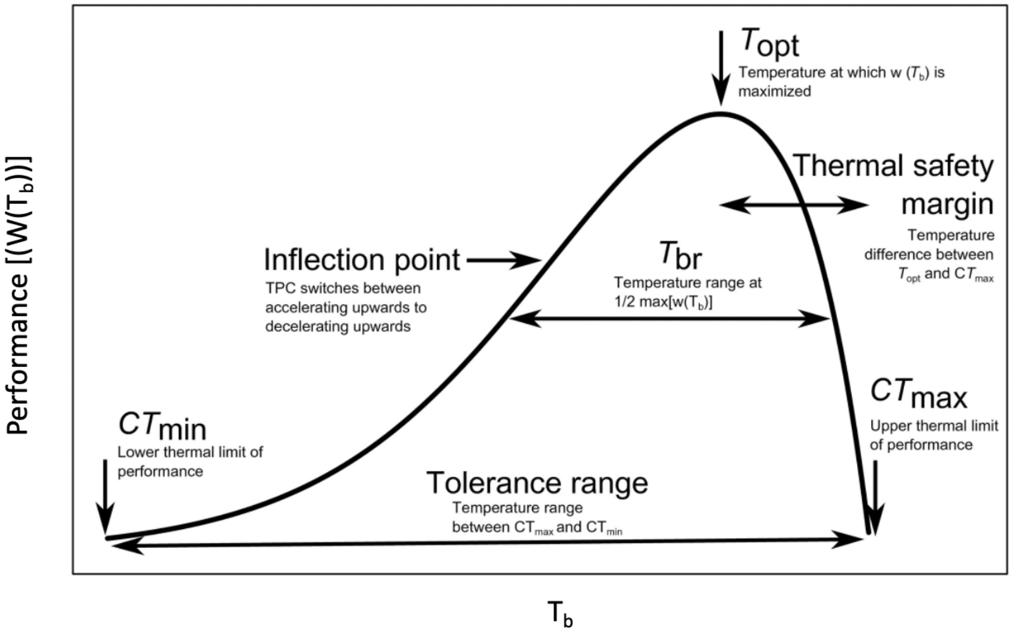


Figure 1.1 A general thermal performance curve of ectotherms based on Huey and Stevenson (1979), adopted from Sinclair et al. (2016).

In TPCs, the performance (w (T_b)) of an organism increases gradually from the CT_{min} (critical thermal minimum) up to the T_{opt} (optimum temperature) and drops abruptly at CT_{max} (critical thermal maximum). Thus, the performance is limited at CT_{min} and CT_{max} and maximized at T_{opt} with the thermal safety margin (TSM), showing how close the organismal T_b is to its CT_{max} (Fig 1.1). Although TCPs are important for understanding the ecological consequences of temperature, they have limitations (Sinclair et al. 2016; Khelifa et al. 2019). Fitness measured using TPC in figure 1.1 is restricted to CT_{max} and CT_{min}, which are arguably not survival limits of all ectotherms (Sinclair et al. 2016). For example, Klok and Chown (1997) showed that the movement of *Pringleophaga marioni* (Viette) (Lepidoptera: Tineidae) stopped at around -0.6 °C but died below -7.5 °C.

Annual warm periods are shorter at higher latitudes and altitudes, shifting the TPCs horizontally towards a lower temperature range, suggesting slower development at lower temperatures, or vertically towards overall higher performance, suggesting faster development at higher latitudes and elevations (Yamahira et al. 2007). However, due to the complexity of life stage responses to temperature, it has been suggested that TPCs should consider stage-specific ecological models rather than assuming that TPCs are fixed during ontogeny (Sinclair et al. 2006). Furthermore, TPCs are mainly determined using constant temperatures, but recent studies have shown their limitations regarding response to winter temperatures because fluctuating temperatures below CT_{min} affect the development rate (Khelifa et al. 2019).

Insects as pests

Agriculture plays an important role in food security and economic growth. However, the agricultural industry is expected to face increased crop losses due to climate-related outbreaks of insect pests (Skendžić et al. 2021). Elevated temperatures affect the mobility of pests, metabolism, metamorphosis, and the availability of hosts with profound consequences for their population dynamics (Shrestha 2019). The main predictions are increased generations, overwintering survival rates, geographical range expansion, and plant disease outbreaks transmitted by insect (Skendžić et al. 2021). For example, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) could not complete one generation annually before 1984, but the number of generations has increased from one to two whole generations due to global warming (Jaramillo et al. 2009). The same pest also expanded its range in Southwestern Ethiopia (Mendesil et al. 2003). *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) has also expanded its range with predictions that it will threaten other crops in the Western United States. In addition, the winter survival of this pest has increased (Diffenbaugh et al. 2008).

In African countries, the pest pressure of crops such as cotton, maize, cowpea, and tomatoes is expected to increase in response to global warming. These pests include the *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Maruca vitrata* (Fabricius) (Lepidoptera: Pyralidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), and *Sodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Van den Berg et al. 2022). Due to global warming, the pest damage among the eight major field crops such as the pulses, coarse cereals, wheat (*Triticum aestivum* L.), groundnut (*Arachis hypogea* L.), cotton (*Gossypium* spp.), rapeseed-mustard (*Brassica* spp.), and rice, maize, sugarcane (*Saccharum* spp.) accounts for 17.5 % (US$17.28 billion) (Dhaliwal et al. 2004, 2010; Sharma 2014).

Integrated pest management (IPM)

The management of insect pests is vital for crop production. In many instances, chemicals are used to manage pests to reduce yield losses, which may lead to environmental contamination and put human health at risk (Rani et al. 2021). Without any form of environmentally friendly strategies to control pests (e.g., biological control), more chemicals will be applied because the number of generations of insect pests is expected to rise (Skendžić et al. 2021).

Integrated pest management (IPM) is a form of pest management that combines chemical, cultural, biological, and physical methods (Dent and Binks 2020). Part of IPM gathers information on the insect pest life cycles and how they interact with the environment to manage pests with less chemical applications. The ectothermic nature of insect pests allows for phenological predictions using field temperature data. One common approach to understanding and predicting insect phenology is using the degree-day model (DD) (Roltsch et al. 1999; Herms 2004). This model uses the heat accumulated by each life stage to transition into the next stage (Herms 2004). The DD model has been used to predict the effects of winter temperatures by timing the egg hatch of the tent caterpillar, *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae) on its two major hosts in response to warming (Uelmen et al. 2016). This study showed that climate warming could influence insect-host plant interaction through changes in phenology patterns. Although the DD model can predict important phenological events such as adult emergence (Herms 2004), it has also been used to project the geographical range of the four significant maize pests in North American grain productions (Diffenbaugh et al. 2008). The DD model can therefore be used to determine areas that are suitable for insect pests, potential distribution, and outbreaks. In South Africa, the DD model was developed for important pests such as the *Spodoptera frugiperda* (Smith) (Lepidoptera:

Noctuidae) and (Du Plessis et al. 2020) of *Nysius natalensis* (Evans) (Hemiptera: Orsillidae) (Du Plessis et al. 2014).

The macadamia industry

The macadamia tree is native to Australia and belongs to the *Proteaceae* family. There are four species of macadamia, of which two (*Macadamia integrifolia and M. tetraphylla*) produce nuts that are suitable for human consumption, while the other two (M. *ternifolia and M. jansenii*) produce toxic nuts (Mast et al. 2008; Carrillo et al. 2017). South Africa and Australia are the top global producers, accounting for half of the world's macadamia production in 2018 (Quiroz et al. 2019). The production of these two countries was estimated at 68 840 and 48 847 tonnes in 2022, respectively (Macadamias South Africa NPC, SAMAC 2021).

The value of macadamia nuts

The macadamia kernel is highly nutritious, it comprises nutrients such as minerals, vitamins, dietary fibre, and carbohydrates. It also contains 75 % of fats (specifically monounsaturated fats) and 8 % of protein (Carrillo et al. 2017; Lara et al. 2017; Mereles et al. 2017). The kernel can be used in ice cream toppings and consumed raw, salted, or fried (Piza and Moriya 2014). The high lipid content of the macadamia kernel allows for oil extraction used as a cooking oil (Navarro and Rodrigues 2016). The oil contains linoleic and phytosterols acid that prevent inflammation, recover skin, and retain moisture (Rafia 2013; Lin et al. 2017). Studies in the pharmaceutical industry have shown that the high content of monounsaturated fatty acids in the oil can reduce serum cholesterol and low-density lipoprotein (Navarro and Rodrigues 2016). The most abundant fatty acid in macadamia oil is oleic acid, followed by palmitoleic acid, which is essential for human metabolism (Hu et al. 2019). Furthermore, it has been shown that macadamia oil can be used for biodiesel production as an alternative fuel for diesel engines (Rahman et al. 2016). As a result, the macadamia nut is among the most expensive nuts in the world because it is globally in demand (Mason 2000, Macadamias South Africa NPC, SAMAC 2021).

Insect pest species associated with macadamia

Insect pest species are among the major global challenges macadamia growers face (Prasannath et al. 2022, Schoeman 2013). Nut damage due to pest species depends on climatic conditions, diversity and abundance of pest species, and management strategies (Kawate and Tarutani 2004). Macadamia-growing countries are associated with various pest species responsible for annual crop losses. For example, the major pests of macadamia in Australia are the *Kuschelorhynchus macadamiae* (Jennings) (Coleoptera:

Curculionidae) (Khun et al. 2021) and *Hypothenemus obscurus* (Fabricius) (Coleoptera: Scolytidae) (Mitchell and Maddox 2010). In Hawaii, the reported major pests include the *Cryptophlebia illepida* (Butler) (Lepidoptera: Eucosmidae), *Cryptophlebia ombrodelta* (Lower) (Lepidoptera: Tortricidae), tropical *Hypothenemus obscurus* (Fabricius) (Coleoptera: Scolytidae), and *Nezara viridula* (Linnaeus) (Heteroptera: Pentatomidae) (Jones 2002). In Brazil the *Elaphria hypophaea* (Hampson) (Lepidoptera: Noctuidae), *Gymnandrosoma aurantianum* (Lima) (Lepidoptera: Tortricidae), and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) were identified as the major pests (de Matos et al. 2019). In South Africa, *Bathycoelia distincta* (Distant) (Schoeman 2018), *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) and *Ryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) are major pests (Weier et al. 2019).

South African macadamia industry and its major pest

Approximately ninety-eight percent of macadamia nuts produced in South Africa are for the export market. The global demand has resulted in an increase in macadamia trees planted annually, with 6 235 hectares planted in 2021 (Macadamias South Africa NPC, SAMAC 2021). The annual macadamia production has increased from R32 million in 1996 to approximately R5.1 billion in 2021, making it one of the fastest-growing crops in South Africa. Macadamia nuts are mainly produced in the Limpopo, Mpumalanga, and Kwazulu-Natal provinces, and to a limited extent in the Eastern Cape, Western Cape, and Gauteng provinces in South Africa (Fig 1.2) (Macadamias South Africa NPC, SAMAC 2021).

Several pests are responsible for annual nut losses (Schoeman 2020), with stink bugs considered the most significant pest. Sonnekus et al. (2022) reported twenty-one stink bug species associated with macadamia in the Limpopo, Mpumalanga, and Kwazulu-Natal Province. The annual damage caused by stink bugs has been estimated at R200 million (US$15.23 million) (Taylor et al. 2018). *B. distincta* (Fig 1.3) dominates these assemblages and is responsible for most of the damage in South African orchards. *B. distincta* is native to East and Southern Africa. It was first recorded in 1984 in the Levubu valley in the north-eastern corner of the country (Schoeman 2018), and genetic diversity analysis showed that its populations are not structured to macadamia orchards only (Pal et al. 2022).

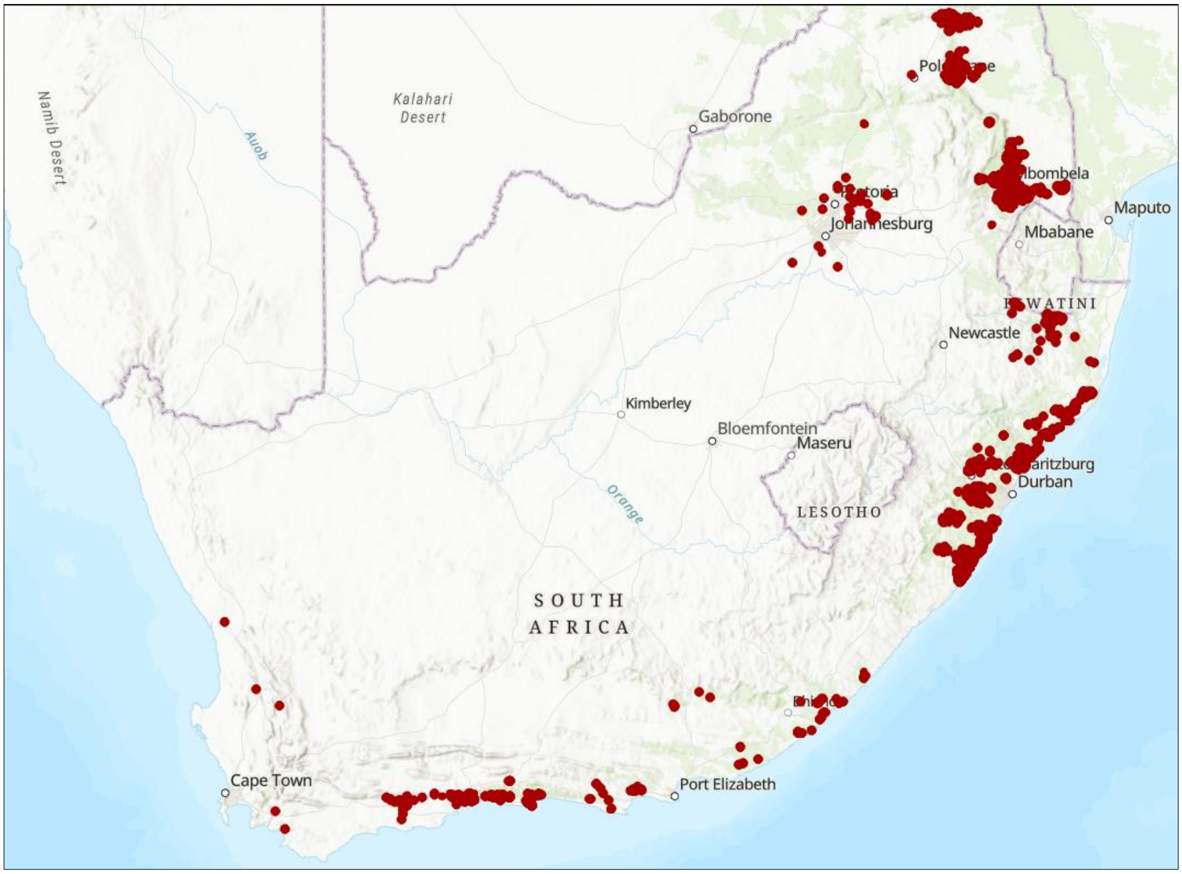


Figure 1.2 Recently mapped macadamia orchards (red dots) in South Africa (adopted from the University of New England, 2023).

B. distincta causes kernel damage by direct feeding on macadamia nuts using its long proboscis (Bruwer et al. 2021). The length of the proboscis appears to be responsible for the ability of *B. distincta* to cause further damage even on mature nuts (Schoeman 2020; Bruwer et al. 2021). The average *B. distincta* adult proboscis length is 13.6 mm, exceeding the maximum length of 10.4 mm of the nut in husk measured 26 weeks after the fruit set (Bruwer et al. 2021). Fluids are extracted from the nuts, resulting in fungus and mould development on the kernel, causing premature nut drop and poor-quality nuts that are not marketable (Golden et al. 2006; Schoeman 2009). *B. distincta* undergoes five nymphal stages before becoming adults (Fig 1.3). Sexually matured females produce barrel-shaped eggs laid in groups or rows glued firmly to each other on the stems or leaves of macadamia trees. Hot spots develop in the orchards because the ability of the flightless nymphs to disperse is limited (Schoeman and De Villiers 2015). Individuals are numerous during summer when developing nuts are available and vulnerable to damage.

The life cycle of *B. distincta* corresponds with the phenology of the macadamia nuts, and its length depends on ambient temperature (Schoeman and De Villiers 2015).

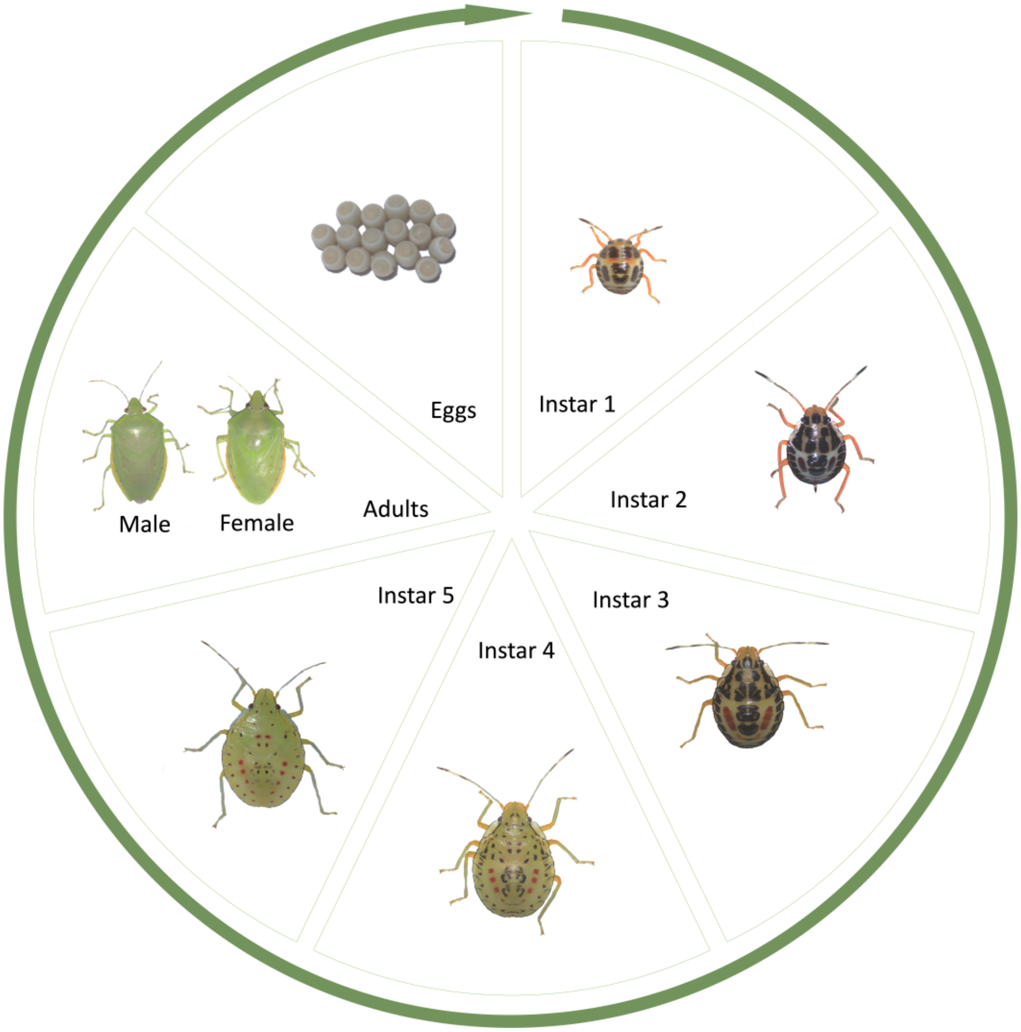


Figure 1.3 The life cycle of the two-spotted stink bug, *B. distincta*.

Management of <u>B. distincta</u>

Chemical control is deemed the most effective control strategy for *B. distincta* (Schoeman 2011). However, pests are known to develop resistance due to frequent application of chemicals (Wilson and Tisdell 2001). It is speculated that *B. distincta* has evolved resistance against some chemicals (e.g., synthetic pyrethroids) used for its management (Schoeman 2014; Schoeman 2018). A disadvantage of chemical application is that it contaminates the environment and kills non-targeted insects, such as crop-beneficial insects (Karuppuchamy and Venugopal 2016).

Rationale

Understanding the basic thermal biology and variation in thermal tolerance of *B. distincta* has important implications for pest population dynamics. The insect's ability to undergo dramatic and rapid changes in temperature tolerance deserves thorough investigation for several reasons, three of which are most critical for the success of integrated pest management (IPM). Firstly, since the thermal tolerance of a species forms an integral component of bioclimatic and phenology, modeling without knowledge of a species' temperature tolerance constraints any prediction of a pest's geographic distribution and abundance under future climate change scenarios is limited. Secondly, population dynamic models and the accurate timing of pest control efforts rely heavily on understanding the insect developmental rate influenced by temperature. Finally, trait plasticity adds value to predictive modeling. Indeed, degree-days models may be improved through additional information on survival at thermal extremes, acclimation capability of thermal trait responses. The ability of an insect species to undergo rapid thermal hardening or improve its temperature tolerance quickly could lead to faster acclimation and greater fitness. Predictive modeling will ultimately be employed and tested against climatic data to provide a clear understanding of seasonal generation patterns and the role of climatic zones on *B. distincta* population dynamics and provide management recommendations to the macadamia industry.

Main objectives

This study aims to understand the thermal biology of *B. distincta* life stages across different temperatures. Specifically, chapter 2 explores whether temperature regimes influence the development and survival of *B. distincta* life stages. A range of biological parameters will be investigated, including the effects of diet on development and survival, effects of temperature on adult life span and life table parameters. Jointly, these parameters provide an estimation of the total amount of heat required to complete (degree-days) a life cycle and the rate of population growth across different temperatures. In chapter 3, the phenotypic plasticity of *B. distincta* is quantified to understand each life stage's ability to tolerate extreme temperatures (rapid hardening and acclimation). Chapter 4 combines the developmental biology at constant temperatures and phenotypic plasticity to model climate-related population dynamics.

CHAPTER 2

The population growth of *Bathycoelia distincta* (Hemiptera: Pentatomidae) peaks at higher temperatures

Abstract

Temperature is the most influential condition affecting insect development and population dynamics. Understanding its impact and other important factors such as diet and relative humidity could provide fine-scale predictions of species abundance and distribution in space and time. The two-spotted stink bug, *Bathycoelia distincta* Distant (Hemiptera: Pentatomidae) is a major pest of macadamia in South Africa for which limited information on developmental biology exists. Here, for the first time, variation of *B. distincta* biological key developmental parameters such as the developmental duration, survival rate, sex ratio, developmental rate, lower (T_{min}), optimum (T_{opt}), and upper (T_{max}) developmental thresholds, thermal constant, adults longevity, pre-oviposition period, oviposition period, fecundity, and life table parameters were quantified at five constant temperatures (18, 21, 22, $25_{high\ RH}$, $25_{low\ RH}$, and 29 °C) and a photoperiod of 16L: 8D. In addition, the effect of diet (macadamia nut and sweetcorn) on the developmental duration, survival rate, and sex ratio were quantified at 25 °C. The developmental duration from egg to adult decreased significantly with increasing temperature from 21 (60 days) to 29 °C (32 days). Survival rate was high (< 69 %) and only varied for instar 2 between temperatures. Nymphs (instar 2 and 4) developed faster on the sweetcorn than on macadamia, while the sex ratio was female-biased (1: 1.1) on macadamia nut. Development from egg to adult required 783 degree days (DD) with a T_{min} of 13.5 °C, T_{opt} of 29.5 °C, and T_{max} of 38 °C for development. The net reproductive rate (R_0) was highest at 25 °C$_{low\ RH,}$ and intrinsic rate of increase (r_m) peaked at 29 °C (0.08). The ongoing global warming will increase the population growth rates of *B. distincta* and increase damage to macadamia nuts. An understanding of the developmental biology and thermal requirements for the DD model of *B. distincta* is fundamental for predicting its phenology and outbreaks in macadamia orchards.

Keywords: development, survival, thermal requirements, macadamia, global warming

Introduction

Temperature is a major environmental factor determining the development and survival of insects and ultimately affecting population size and geographical distribution (Bale 2002; Bale et al. 2002). It is essential to understand an organism's development and survival in the face of climate change, especially insect pests. Climate change is expected to increase pest number generations, overwintering survival, and could expand their geographical range (Skendžić et al. 2021). Crops are increasingly vulnerable to insect pest damage (Deutsch et al. 2018; Skendžić et al. 2021), resulting in a growing concern for crop protection, production, and food security (Sharma 2014). There is an increasing need to understand the effects of temperature on pests in order to improve the management of these pests (Skendžić et al. 2021; Tonnang et al. 2022).

Although temperature interacts with other abiotic and biotic factors, the effects of temperature on insects are mostly quantified in the laboratory (e.g., Dhillon and Hasan 2017; Govindan and Hutchison 2020; Savaris et al. 2022) because of the difficulties associated with tracking parameters such as survival, and reproduction in field conditions (Gabre et al. 2005). Investigating the influence of temperature is fundamental to understanding pest management. For example, knowing when an insect lays eggs, the developmental durations for each life stage, and adult emergence has important implications for the timing of pesticide application (Herms 2004). Inappropriate applications can be costly and reduce natural enemy populations without affecting target pests (Herms 2004; Herrera et al. 2005). In addition, some insect pests are difficult to detect (Herms 2004), and monitoring is time consuming and expensive.

Due to the ectothermic nature of insects, growers use phenology models to time spraying (Herms 2004). Phenology models require information on the developmental parameters of pests, such as the lower developmental threshold (T_{min}) and the thermal constant for each life stage expressed in degree days (DD) (Nietschke et al. 2007). If these parameters have not been determined, they can be derived from the literature based on that of a closely related species (Nietschke et al. 2007). However, closely related species can differ in terms of biological parameters depending on the geographical area. For example, the *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in the United States had a T_{min} of 15 °C (Nielsen et al. 2008), while the closely related *Antestiopsis thunbergii* (Leston) (Hemiptera: Pentatomidae) in Tanzania had a T_{min} of 12 °C (Azrag et al. 2017). Thus, determining the species-specific biological parameters can add value to effective management and optimising pesticide applications (Herrera et al. 2005).

Insects develop at a linear rate at intermediate temperatures but non-linearly at a wide range of temperatures, with a slow development at low temperatures increasing at higher temperatures before decreasing again (Damos and Savopoulou-Soultani 2008). Other than temperature, factors such as humidity and diet also influence the developmental rate of insects (Hagstrum and Milliken 1988; Tochen et al. 2016). Development is therefore driven by a combination of multiple factors that influence other important physiological functions, such as reproduction (Chanthy et al. 2015), ultimately affecting species fitness, distribution, and abundance (Kingsolver and Huey 1989; Kaspari et al. 2000; Deutsch et al. 2008).

Life tables provide the most comprehensive summary of a species survivorship, development, and reproduction (Carey 2001). Life table parameters such as the intrinsic rate of increase (r_m) are used to predict a species population growth under given environmental conditions (Birch 1948; Southwood and Henderson 2000). Effects of temperature on the intrinsic rate of increase (r_m) have been widely tested because of its biological significance and the ability to measure population fitness (e.g., Govindan and Hutchison 2020). Additionally, it has proven to be essential in population response to climate change (Jaramillo et al. 2009).

The two-spotted stink bug, *Bathycoelia distincta* (Distant) (Hemiptera: Pentatomidae), is a major pest in the South African macadamia industry. Regardless of its major pest status, no systematically gathered developmental study exists for this species except for a degree-day model based on field temperatures in macadamia orchards in South Africa (Schoeman 2011). *B. distincta* is mainly controlled by pesticide application using calendar dates (Schoeman 2013). Comprehensive knowledge of its developmental biology at different temperatures is needed to improve the degree-day model. This study aims to 1) quantify the effects of temperature (18, 20, 22.5, 25, 27.5, and 30 °C) and diet (macadamia nuts and sweetcorn at 25 °C) and humidity (at 25 °C) on the developmental duration, sex ratio, and survival of *B. distincta* life stages, 2) fit and evaluate temperature-dependent development models for *B. distincta* life stages 3) to determine the thermal requirements (lower developmental threshold: T_{min}, optimum developmental threshold: T_{opt}, upper developmental threshold: T_{max}, and thermal constant) of *B. distincta* life stages, 4) assess the effect of temperature on longevity, pre-oviposition period, oviposition period, and fecundity of *B. distincta* adults and 5) construct a life table across different temperatures. All experiments were done under controlled laboratory conditions.

Materials and methods

Establishment of B. distincta colony and rearing

B. distincta eggs were collected from a commercial macadamia farm in Levubu (23°4'0.96"S, 30°4'31.07"E), Limpopo, South Africa, and a colony was established under controlled conditions in a laboratory at the University of Venda, Limpopo, South Africa. The eggs were collected in packets from October 2020 to February 2021. Upon arrival at the laboratory, four to five egg clusters (≈ 56 eggs) were placed on a paper towel (Fig 2.1A) lined on the bottom of a polypropylene plastic container (45 x 25 x 30 cm) covered with a fine net for ventilation. A small piece of cotton wool moistened with distilled water was placed in each container to increase humidity (Geng and Jung 2018) and provide water for instar 1 nymphs (Dingha and Jackai 2017). All nymphal stages were reared in smaller transparent polypropylene containers (45 x 25 x 30 cm) (Fig 2.1B) and transferred to larger containers (33 cm x 24 cm x 16 cm) when they transitioned to the adult stage for mating and oviposition. As eggs are oviposited with a glue-like material that attaches them to the oviposition surface, a few drops of water were added to egg clusters and removed with a scalpel. Fresh macadamia nuts were initially provided as a food source (Fig 2.1C), but this was later changed to sweetcorn (*Zea mays*) kernels on the cob (Fig 2.1D) so that colonies could be maintained throughout the year. Nuts collected from the orchard were rinsed thoroughly before feeding the colony, and sweetcorn was bought on the same day or a day before feeding. Water and food were replenished two to three times per week. Rearing containers were cleaned twice weekly with a mixture of 50% water and 50% ethanol to prevent fungal infection. The colony was supplemented with wild eggs in 2022 from macadamia orchards to reduce the inbreeding effect. The temperature was kept at 25 ± 0.0 °C, at a relative humidity (RH) of 71.8 ± 0.1 %, verified and recorded at hourly intervals using Thermocron iButtons (Semiconductor Corporation, Dallas/Maxin TX and USA) and a photoperiod of 16L:8D.

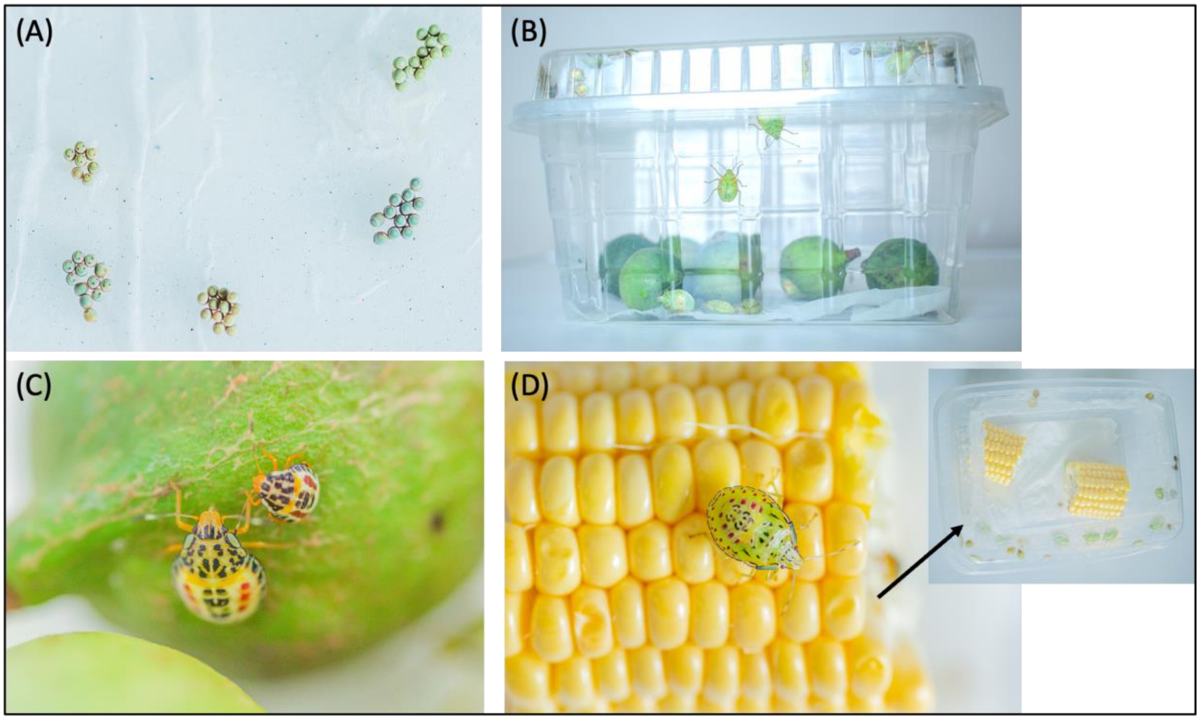


Figure 2.1 Egg packets of *B. distincta* on a paper towel (A), size of the container used to rear nymphal instars (B), instars 2 and 4 on a macadamia nut (C), instar 5 on the sweetcorn (D).

Effect of temperature on the developmental duration, sex ratio, and survival rate

Three to four newly laid egg packets (N ≈ 42) were collected within 24 h from the stock colony, placed in transparent polypropylene containers (45 x 25 x 30 cm), and kept at 18, 20, 22.5, 25, 27.5, and 30 °C. This was replicated six times in each temperature treatment. The actual temperatures and RH in the growth chambers varied as follows: 18.3 ± 0.0 °C and 28.8 ± 0.3 % RH, 20.8 ± 0.0 °C and 91.2 ± 0.1 % RH, 22.2 ± 0.0 °C and 29.8 ± 0.2 % RH, 25.3 ± 0.0 °C and 64.1 ± 0.1 % RH (high RH), 25.4 ± 0.0 °C and 25.3 ± 0.1 % RH (low RH, and 29.2 ± 0.0 °C and 22.7 ± 0.3 % RH. The developmental duration was determined by recording the completion time of each life stage. The total number of live and dead were recorded daily. The presence of exuvia signaled the completion of each life stage. Upon adult eclosion, individuals were sexed to determine the sex ratio. Cleaning of the containers, food replacement, and water supply were similar to the rearing methods, except at 29 °C, where food was changed three times a week. Containers with instar 1 were not cleaned until they reached instar 2 to allow for their aggregation behaviour.

Effect of diet on the developmental duration, sex ratio, and survival rate

The developmental duration, sex ratio, and survival rate were quantified at 25 °C on the macadamia nut (25.3 ± 0.0 °C and 64.1 ± 0.1 % RH) and sweetcorn diet (25 ± 0.0 °C and 71.8 ± 0.1 % RH) under the photoperiod of 16L: 8D. The rearing containers were observed daily as mentioned above.

Temperature-dependent models

Four non-linear models: Taylor-81 (Taylor 1981), HarcourtYee-82 (Harcourt and Yee 1982), Kontodimas-04 (Kontodimas et al. 2004), and Damos (Damos and Savopoulou-Soultani 2008) were fitted to the observed developmental rates of *B. distincta* life stages. However, linear models have been used more often because of their ease of analysis (Dhillon and Hasan 2017; Manzoor et al. 2020). Linear models remain valid because they allow for the calculation of T_{min} and thermal constant (Jarošík et al. 2002). In contrast, some non-linear models can provide stage-specific T_{min}, T_{opt}, and T_{max} thermal requirements and more realistic development rate estimations at a wider range of temperatures (Taylor 1981; Kontodimas et al. 2004). In all the model equations, rT is the mean developmental rate (1/day), and T (°C) is the temperature.

The Taylor-81 model is calculated as:

$$rT \ = R_m \times e^{-\frac{1}{2} \times (\frac{T - T_m}{T_o})2}$$

where R_m is the maximum temperature rate, T_m - optimum temperature, and T_o - rate at which temperature falls away from T_m.

The HarcourtYee-84 model is calculated as:

$$rT = ao + a1 \ \times \ T + a2 \ \times T^2 + a3 \ \times T^3$$

where $a0$, $a1$, $a2$, and $a3$ are constants.

The Kontodimas-04 model is calculated as:

$$rT \ = aa \ \times (T - \ T_{min})^2 \ \times \ (T_{max} - T)$$

where aa is a constant, T_{min} is the minimum temperature, and T_{max} is the maximum temperature.

The Damos-08 model is calculated as:

$$rT = aa \times \left(\frac{bb - T}{10}\right) \times \left(\frac{\mathrm{T}}{10}\right)^{cc}$$

where *aa*, *bb*, and *cc* are constant empirical parameters.

Models with the lowest AIC value were considered the best fit for the observed data (Rebaudo et al. 2017, Geng and Jung 2018) and further confirmed by observations in the laboratory.

Thermal requirements of <u>B. distincta</u> life stages

Non-linear models were used to estimate the T_{min}, T_{opt}, and T_{max}. The T_{min} describes the temperature below which there is no development, T_{opt} is the temperature where development is maximized, and T_{max} is the temperature above where development stops. The thermal constant expressed in degree-days (DD) is the total amount of thermal units required to complete a life stage (Herms 2004).

The thermal constant was calculated as:

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

where *T* represents the examined constant temperatures, T_{min} is the lower developmental threshold temperature, and *D* is the duration of the stage in days, calculated for each life stage.

Effect of temperature on longevity, pre-oviposition period, oviposition period, and fecundity

Newly emerged adults were paired in transparent polypropylene containers (45 x 25 x 30 cm) from the stock colony (25 °C, 64 % RH) on the sweetcorn diet. Pairs were replicated fifteen times (N = 15) and assigned to five constant temperatures (21, 22, $25_{\text{high RH}}$, $25_{\text{low RH}}$, 29 °C) on the same day of emergence. Daily observations were made for the presence of egg packets and dead adults. Whenever a male died, it was replaced, and only the first male was considered for longevity.

Longevity was calculated as the total number of days lived by adults, pre-oviposition period as the number of days it took the females to start laying eggs, oviposition period as the number of days over which females produced eggs, and fecundity as the total number of eggs laid per female throughout their lifetime. All the growing conditions were maintained as described above.

Life table parameters

Female-based life table parameters of *B. distincta* estimated were net reproductive rate (R_0), mean generation time (T), finite rate of increase (λ), intrinsic rate of increase (r_m), and doubling time (DT). The life table was constructed based on the l_x and m_x parameters, where l_x was determined as the proportion of individuals alive at the start of stage x and m_x as the number of eggs produced by females during the age interval x.

R_0 is described as the number of offspring that a female contributes to the next generation and can be calculated as:

$$R_0 = \sum_{x=0}^{w} l_x \, m_x$$

T is the average time from when female egg is laid until she starts laying eggs herself, and calculated as:

$$T = \frac{lnR_0}{r_m}$$

The finite rate of increase (λ) is the total number of female offspring added to the population per female per day calculated as:

$$\lambda = e^{r_m}$$

The intrinsic rate of increase (r_m), defined as the intrinsic capacity of increase, was calculated as:

$$r_m = \frac{lnR_0}{T}$$

The doubling time (DT) is the time it takes a population to double in size and was calculated as:

$$DT = \frac{ln(2)}{r_m}$$

Statistical analysis

All analyses were done in R version 4.2.1 (R core team, version 4.2.1, 2022). Outliers were identified and removed using the Interquartile Range (IQR) method before analysis. Means, standard deviation, standard error, and 95% confidence intervals were calculated using the Rmisc package (Hope 2022). The mean for the developmental duration from one stage to another was calculated as a weighted mean. Model assumptions were evaluated by testing for the normality of model residuals using the Shapiro-Wilk and Levene's tests for homogeneity of variance. The effect of temperature on developmental duration and survival rate for normally distributed and homoscedastic data were analysed using one-way ANOVA followed by Tukey HSD multiple post-hoc tests, models with non-normal or heteroscedastic residuals were analysed using a Kruskal-Wallis test followed by Duncan’s multiple post-hoc test. Post hoc tests were set to a confidence interval level of 0.05. The effect of temperature on the sex ratio was analysed using Fisher's exact test. The sex ratio within each temperature was compared to an expected 1:1 (male: female) ratio using the Chi-Square test of independence. The effect of diet on developmental duration, sex ratio, and survival rate at 25 °C was analysed using student's t-test. Temperature-dependent models (Campbell-74, Taylor-81, HarcourtYee-84, Kontodimas-04, and Damos-08) were fitted using the DevRate package (Rebaudo et al. 2018). The start value parameters of all models were obtained from the "Hemiptera" and "Lepidoptera" families in the DevRate package. The "nlsDR" DevRate function was used to calculate the T_{min}, T_{opt}, and T_{max} from the four non-linear models for each life stage. Variations in female longevity, pre-oviposition period, and fecundity in response to temperatures (21, 22, $25_{high\ RH}$, $25_{low\ RH}$, 29 °C) were analysed using the Kruskal-Wallis test, male longevity with the linear model, and oviposition period with Welchs ANOVA. Analysis of variation was used to test the effects of temperature on the following life table parameters: finite (λ) and intrinsic (r_m) rates of increase while the net reproductive rate (R_0), generation time (T), and doubling time (DT) were analysed using the Kruskal-Wallis test. Multiple comparisons for both linear and Kruskal-Wallis were the same as previously described.

Results

Effect of temperature on the developmental duration, sex ratio, and survival rate

The mean (weighted) developmental durations of all *B. distincta* life stages varied significantly in response to temperature (eggs: ANOVA: $F_{5,28} = 88.9$, $P < 0.001$; instar 1: ANOVA: $F_{5,26} = 53.7$, $P < 0.001$; instar 2: $\chi^2_{\text{Kruskal-Wallis}}$ (23.4) = 4, $P < 0.001$; instar 3: ANOVA: $F_{4,23} = 8.2$, $P < 0.001$; instar 4: $\chi^2_{\text{Kruskal-Wallis}}$ (18.1) = 4, P = 0.001; instar 5: ANOVA: $F_{4,22} = 21.6$, $P < 0.001$). At 18 °C, individuals only survived up to instar 2 (Table 2.1). The longest developmental duration was recorded at 18 °C for egg (15 days) and shortest at 29 °C for instar 1 (4.1 days). Total developmental duration from egg to adult varied significantly between temperatures (ANOVA: $F_{4,22} = 21.4$, $P < 0.001$) with 60.5 days at 20 °C, 62.5 days at 22 °C, 38.4 days at 25 °$C_{\text{high RH}}$, 41.6 days at 25 °$C_{\text{low RH}}$, and 32.0 days at 29 °C (Table 2.1). The sex ratio did not vary significantly in response to temperatures (F_{fisher} (4,24) = 1.27, P = 0.31) while ratios did not differ significantly from 1:1 within each temperature (Table 2.1). The survival rate of all life stages did not vary between temperatures except for instar 2 (ANOVA: $F_{4,24} = 2.91$, $P < 0.05$), with the lowest survival of 51 % at 22 °C and the highest rate of 82 % at 25 °$C_{\text{low RH}}$ (Table 2.2).

Effect of diet on the developmental duration, sex ratio, and survival rate

Diet had a significant impact on the developmental duration of instar 2 (t_{Student} (10) = -3.1, P = 0.01)) and instar 4 (t_{Student} (10) = -2.5, P = 0.03). Both instars 2 and 4 developed faster on the sweetcorn diet (Table 2.1). No significant differences were observed for eggs, instars 1, 3, 5, and total development from egg to adult (Table 2.1). The sex ratio did not differ significantly between the diets (t_{Student} (10) = -0.8, P = 0.4) but was biased towards females on the macadamia nut diet (χ^2 = 18.2, df = 10, P = 0.05) (Table 2.1). The survival rate did not differ significantly between diets for all the life stages (Table 2.2).

Temperature-dependent models

The linear model (Campbell-74) had the highest AIC value for all life stages, and all the non-linear models converged (Table 2.3). Of all the evaluated models, Kontodimas-04 was the best fit (lowest AIC) for the egg, instars 2 and 5, Damos-08 for instars 1 and 4, and the Taylor-81 model for instar 3 (Fig 2.2, Table 2.3).

Thermal requirements of B. distincta life stages

Non-linear models successfully estimated all developmental parameters (T_{min}, T_{opt}, and T_{max}) for instars 2,3,4, and 5 (Table 2.4). The HarcourtYee-84 model failed to estimate the T_{min} for egg, and instar 1 and Taylor-81 model the T_{max} for egg. Laboratory-based observations suggest that the Kontodimas-04 provided plausible estimates of T_{min} for all life stages and HarcourtYee model for instars 2 and 3. The best model with the lowest estimate of T_{min} was Damos-08 (instar 4), and the highest was Kontodimas-04 (instar 2). T_{opt} ranged from 27.1 (Taylor-81 model) to 31.8 °C (Damos-08 model) and T_{max} from 38 (HarcourtYee-82, Kontodimas-04, and Damos-08 models) to 46.9 °C (Taylor-81). The Taylor-81 model overestimated the values of T_{max} for all life stages. The Kontodimas-04 was the best model for egg to adult T_{min} (13.5 °C), T_{opt} (29.5 °C) and T_{max} (38.0 °C). The eggs thermal constant was 62.9 DD: instar 1: 58.9 DD, instar 2: 99.8 DD, instar 3: 134.1 DD, instar 4: 225.7 DD, and instar 5: 201.3 DD. The thermal constant from egg to adult was 783 DD (Table 2.4).

Table 2.1 Weighted mean developmental durations (days, mean ± SE) of *B. distincta* from egg to adult stage at different temperatures. Mean values with different letters indicate significant differences between temperatures in columns ($P < 0.05$). The number of individuals (N) that transitioned to the next stage is shown in brackets.

		Developmental stage							
Temperature, Relative humidity	**N**	**Egg**	**Instar 1**	**Instar 2**	**Instar 3**	**Instar 4**	**Instar 5**	**Egg to adult**	**Sex ratio (♂: ♀)**
18 °C, 29 %	241	15.0 ± 0.1^{a} (222)	14.2 ± 0.3^{a} (146)	-	-	-	-	-	-
21 °C, 91 %	225	10.4 ± 0.1^{b} (192)	8.5 ± 0.1^{b} (158)	12.6 ± 0.3^{a} (101)	8.1 ± 0.3^{a} (83)	9.1 ± 0.4^{ab} (73)	11.8 ± 0.6^{a} (46)	60.3 ± 0.6^{a}	$1: 1^{a}$
22 °C, 30 %	232	7.8 ± 0.1^{c} (232)	7.8 ± 0.1^{b} (199)	12.2 ± 0.3^{a} (99)	8.3 ± 0.5^{a} (90)	10.6 ± 0.4^{a} (79)	15.7 ± 0.6^{b} (60)	62.5 ± 0.6^{a}	$1: 1^{a}$
25 °C$_{\text{high RH}}$, 64 %	241	5.4 ± 0.1^{d} (232)	5.3 ± 0.1^{c} (214)	5.6 ± 0.1^{b} (158)	5.2 ± 0.1^{b} (128)	6.4 ± 0.2^{bc} (111)	10.4 ± 0.3^{ac} (82)	38.4 ± 0.3^{b}	$1: 0.8^{a}$
25 °C$_{\text{low RH}}$, 25 %	248	5.0 ± 0.0^{d} (243)	5.9 ± 0.1^{c} (204)	7.2 ± 0.1^{ab} (167)	5.3 ± 0.2^{b} (142)	7.4 ± 0.2^{abc} (121)	10.7 ± 0.2^{ac} (93)	41.6 ± 0.2^{b}	$1: 1.3^{a}$
29 °C, 23 %	209	4.4 ± 0.1^{d} (194)	4.1 ± 0.1^{c} (157)	4.6 ± 0.1^{b} (98)	4.9 ± 0.1^{b} (94)	5.2 ± 0.2^{c} (90)	8.7 ± 0.2^{c} (77)	32.0 ± 0.4^{c}	$1: 0.7^{a}$
		Macadamia nut and sweetcorn diet comparison							
25 °C, 71 % (Macadamia nut)	194	6.2 ± 0.0^{a} (194)	5.2 ± 0.0^{a} (187)	7.2 ± 0.1^{a} (156)	6.3 ± 0.1^{a} (150)	7.0 ± 0.1^{a} (143)	10.6 ± 0.1^{a} (126)	43.3 ± 0.2^{a}	$1: 1.1^{a}$
25 °C, 64 % (Sweetcorn)	241	5.4 ± 0.1^{a} (232)	5.3 ± 0.1^{a} (214)	5.6 ± 0.1^{b} (158)	5.2 ± 0.1^{a} (128)	6.4 ± 0.2^{b} (111)	10.4 ± 0.3^{a} (81)	38.5 ± 0.3^{a}	$1: 0.8^{a}$

Table 2.2 Survival rates (%, mean ± SE) of *B. distincta* life stages at different temperatures. Mean values with different letters indicate significant differences in survival between temperatures ($P < 0.05$).

	Developmental stage					
Temperature, Relative humidity	**Egg**	**Instar 1**	**Instar 2**	**Instar 3**	**Instar 4**	**Instar 5**
18 °C, 29 %	88 ± 0.0^{a}	69 ± 0.1^{a}	-	-	-	-
21 °C, 91 %	95 ± 0.1^{a}	76 ± 0.1^{a}	66 ± 0.1^{ab}	80 ± 0.1^{a}	87 ± 0.0^{a}	72 ± 0.1^{a}
22 °C, 30 %	100 ± 0.0^{a}	86 ± 0.0^{a}	51 ± 0.1^{a}	88 ± 0.1^{a}	88 ± 0.0^{a}	76 ± 0.1^{a}
25 °C$_{\text{high RH}}$, 64 %	96 ± 0.0^{a}	92 ± 0.0^{a}	73 ± 0.1^{ab}	77 ± 0.1^{a}	86 ± 0.0^{a}	77 ± 0.1^{a}
25 °C$_{\text{low RH}}$, 25 %	98 ± 0.0^{a}	84 ± 0.1^{a}	82 ± 0.1^{b}	83 ± 0.0^{a}	83 ± 0.0^{a}	81 ± 0.1^{a}
29 °C, 23 %	93 ± 0.0^{a}	81 ± 0.0^{a}	64 ± 0.0^{ab}	95 ± 0.0^{a}	97 ± 0.0^{a}	84 ± 0.1^{a}
	Macadamia nut and sweetcorn diet comparison					
25 °C, 71 % (Macadamia nut)	95 ± 0.0^{a}	96 ± 0.0^{a}	83 ± 0.1^{a}	96 ± 0.0^{a}	95 ± 0.0^{a}	88 ± 0.1^{a}
25 °C, 64 % (Sweetcorn)	96 ± 0.0^{a}	92 ± 0.0^{a}	73 ± 0.1^{a}	77 ± 0.1^{a}	86 ± 0.0^{a}	77 ± 0.1^{a}

Table 2.3 Estimated parameters (estimate ± SE) of the models fitted to the relationship between developmental rate and temperature of *B. distincta* life stages and statistical comparisons based on the coefficient of determination (R^2) and Akaike Information Criterion (AIC). Models with the lowest AIC values are shown in bold for each life stage.

		Developmental stage					
Model	**Parameters**	**Egg**	**Instar 1**	**Instar 2**	**Instar 3**	**Instar 4**	**Instar 5**
Campbell-74	aa	-0.057 ± 0.05	-0.05 ± 0.05	0.04 ± 0.07	0.05 ± 0.07	0.04 ± 0.05	0.04 ± 0.03
	bb	0.0085 ± 0.00**	0.0084 ± 0.00**	0.00069 ± 0.00*	0.004 ± 0.00	-0.0035 ± 0.00	0.0016 ± 0.00
	R^2	0.08	0.02	0.11	0.04	0.13	0.27
	AIC	-92	-91.5	-75.4	-73.7	-89.1	-119.0
Taylor-81	R_m	0.23 ± 0.01***	0.23 ± 0.01***	0.21 ± 0.01***	0.21 ± 0.01***	0.17 ± 0.01***	0.11 ± 0.00***
	T_m	27.9 ± 0.58***	28.4 ± 0.73***	28.4 ± 0.68***	27.1 ± 0.63***	28.0 ± 0.78***	27.5 ± 0.45***
	T_o	-5.60 ± 0.48***	6.1 ± 0.59***	-4.84 ± 0.58***	5.24 ± 0.72***	6.13 ± 0.79***	6.36 ± 0.55***
	R^2	0.04	0.005	0.006	0.02	0.007	0.03
	AIC	-143.1	-131.4	-114.8	**-105.8**	-118.5	-148.0
HarcourtYee-84	a0	0.83 ± 0.41.	9.96 ± 0.05*	0.011 ± 0.046*	0.011 ± 0.01	0.038 ± 0.04	0.0044 ± 0.025
	a1	-0.13 ± 0.04**	-0.01 ± 0.00**	-0.018 ± 0.00**	-0.0047 ± 0.00	-0.0073 ± 0.00	-0.0021 ± 0.00
	a2	0.0073 ± 0.00***	0.00079 ± 0.00***	0.00088 ± 0.00***	0.00037 ± 0.00	0.00043 ± 0.00*	0.00017 ± 0.00
	a3	-0.00011 ± 0.00***	-0.000013 ± 0.00***	-0.00001 ± 0.00***	-0.0000056 ± 0.00*	-0.0000069 ± 0.00**	-0.000031 ± 0.00*
	R^2	0.02	0.02	0.04	0.01	0.003	0.02
	AIC	-145.3	-137.3	-114.8	-103.7	-123.0	-152.5
Kontodimas-04	aa	0.00001 ± 0.00***	0.0000097 ± 0.00***	0.000012 ± 0.00***	0.0000084 ± 0.00***	0.0000069 ± 0.00***	0.0000036 ± 0.00***
	T_{min}	0.011 ± 0.06 ***	0.013 ± 0.07***	0.015 ± 0.07***	0.011 ± 0.01***	0.012 ± 0.01***	0.011 ± 0.01***

	T_{max}	0.038 ± 0.04***	0.038 ± 0.05***	0.038 ± 0.05***	0.037 ± 0.06***	0.038 ± 0.06***	0.038 ± 0.06***
	R^2	0.02	0.02	0.04	0.005	0.003	0.02
	AIC	**-147.0**	-138.3	**-116.2**	-105.2	-125.0	**-154.2**
Damos-08	aa	0.003 ± 0.00***	0.0024 ± 0.00***	0.001 ± 0.00*	0.0042 ± 0.00*	0.0029 ± 0.00**	0.002 ± 0.00**
	bb	4.01 ± 0.03***	3.80 ± 0.03***	3.79 ± 0.03***	3.79 ± 0.06***	3.80 ± 0.04***	3.80 ± 0.05***
	cc	3.98 ± 0.15***	4.46 ±± 0.27***	5.13 ± 0.40***	3.83 ± 0.40***	4.02 ± 0.33***	3.61 ± 0.31***
	R^2	0.03	0.02	0.07	0.003	0.02	0.01
	AIC	-145.6	**-141.9**	-114.2	-102.8	**-125.9**	-153.9

Signif. codes: 0 *** 0.001 ** 0.01 * 0.05 . 0.1 1. aa is the intercept of the line, bb: slope of the line, R_m: maximum temperature rate, $T_{m:}$ optimum temperature, T_o: rate at which temperature falls away from T_m, a0,a1,a2,a3: constant, aa,bb,cc: constant, T_{min}: minimum temperature, T_{max}: maximum temperature.

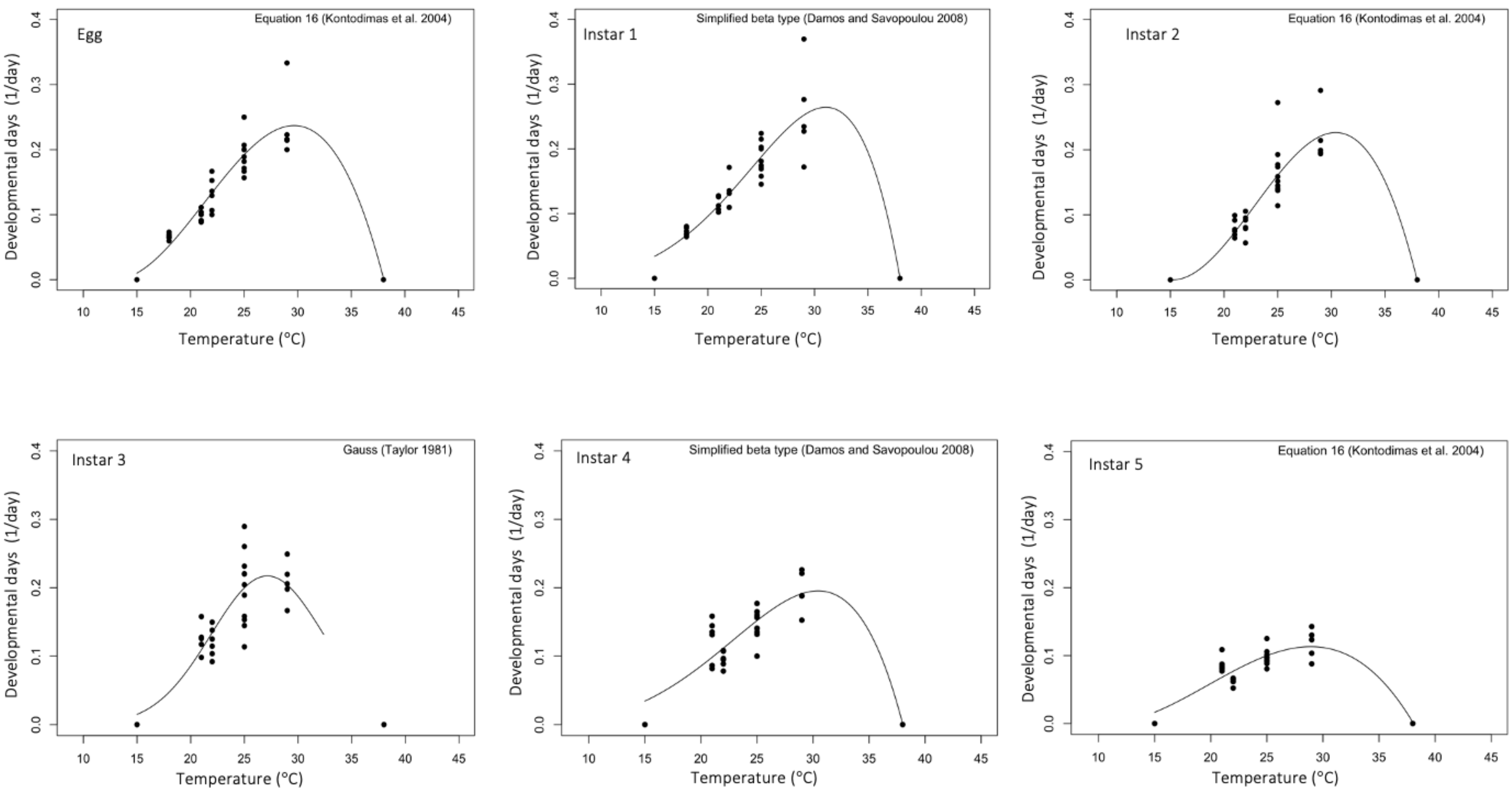


Figure 2.2 Developmental rate of *B. distincta* life stages at constant temperatures. Each plot describes the best model for each life stage with dots showing the observed rates.

Effect of temperature on longevity, pre-oviposition period, oviposition period, and fecundity

Longevity of sexes varied significantly in response to temperatures (females: $\chi^2_{\text{Kruskal-Wallis}}$ (4) = 12.28, P = 0.02, male: ANOVA: $F_{4,70}$ = 4.1, P = 0.004) (Fig 2.3A, B, Appendix A). The longevity was longest at 22 °C for both female and male, 42.1 and 50.7 days, respectively, and shortest at 29 °C 18.6, and 21.8 days, respectively (Fig 2.3A, B, Appendix A). Female pre-oviposition period significantly decreased with increasing temperature ($\chi^2_{\text{Kruskal-Wallis}}$ (4) = 35.59, P < 0.001). Females began laying eggs after 19.6 days at 21 °C and 7.7 days at 29 °C (Fig 2.3C, Appendix A). Oviposition period did not vary significantly between temperatures (F_{welch} (4,17) = 0.96, P = 0.46) (Fig 2.3D, Appendix A). Temperature significantly influenced fecundity (ANOVA: $F_{4,70}$ = 4.3, P = 0.003), with the most of eggs laid at 25°$C_{\text{low RH}}$ and lease at 29 °C (Fig 2.3E, Appendix A).

Life table parameters

Life table statistics varied significantly between temperatures, net reproductive rate, R_0: $\chi^2_{\text{Kruskal-Wallis}}$ (4) = 16.17, P < 0.001, generation length, T: $\chi^2_{\text{Kruskal-Wallis}}$ (4) = 43.98, P < 0.001, finite rate of increase, λ: $F_{4,49}$ = 56.63, P < 0.001, intrinsic rate of increase, r_m: $F_{4,49}$ = 58.16, P < 0.001, doubling time, DT: $\chi^2_{\text{Kruskal-Wallis}}$ (4) = 40.14, P < 0.001 (Table 2.5, Appendix B). The net reproductive rate (R_0) peaked at 25 °$C_{\text{low RH}}$ and differed significantly between 21 and 29 °C (Table 2.5, Appendix B). Generation length (T) was significantly longer at 22 °C (95.86 days) and shortest at 29 °C (58.88 days). The finite (λ) and intrinsic (r_m) rate of increase peaked at 29 °C but did not differ significantly from 25 °$C_{\text{low RH}}$ (Table 2.5, Appendix B). Doubling time (DT) of 21 and 22 °C were significantly longer than $25_{\text{high RH}}$, $25_{\text{low RH}}$, and 29 °C (Table 2.5, Appendix B).

Table 2.4 Estimated thermal requirement parameters (lower: T_{min}: $T_{opt,}$ and upper developmental threshold: T_{max}) from the non-linear models using the "nlsDR" function from the DevRate package and thermal constant in degree days (DD) for each life stage of *B. distincta* estimated by the linear model.

		Developmental stage						
Model	**Parameter (°C)**	**Egg**	**Instar 1**	**Instar 2**	**Instar 3**	**Instar 4**	**Instar 5**	**Egg to adult**
Taylor-81	T_{min}	10.9	10.0	13.7	**11.2**	9.4	8.1	11.0
HarcourtYee-82		NE	NE	16.1	15.3	12.9	14.1	14.1
Kontodimas-04		**13.9**	13.6	**16.1**	12.8	12.9	**11.6**	**13.5**
Damos-08		7.9	**7.9**	9.6	6.2	**6.8**	5.6	7.2
Taylor-81	T_{opt}	27.9	28.4	28.4	**27.1**	28.0	27.5	27.9
HarcourtYee-82		29.7	29.9	30.4	28.9	29.3	28.7	29.5
Kontodimas-04		**29.6**	29.9	**30.4**	29.2	29.3	**29.8**	**29.5**
Damos-08		30.9	**31.1**	31.8	30.1	**30.5**	29.8	30.6
Taylor-81	T_{max}	NE	46.9	43.1	**43.1**	46.7	46.8	44.9
HarcourtYee-82		38.1	38.1	38.0	37.9	38.1	38.1	38.0
Kontodimas-04		**38.1**	38.2	**38.0**	37.9	38.1	**38.1**	**38.0**
Damos-08		38.0	**38.0**	38.0	37.9	**38.0**	38.0	38.0
Linear	DD	62.9	58.9	99.8	134.1	225.7	201.3	783.0

The values in bold represent the thermal requirements estimated by the best models.

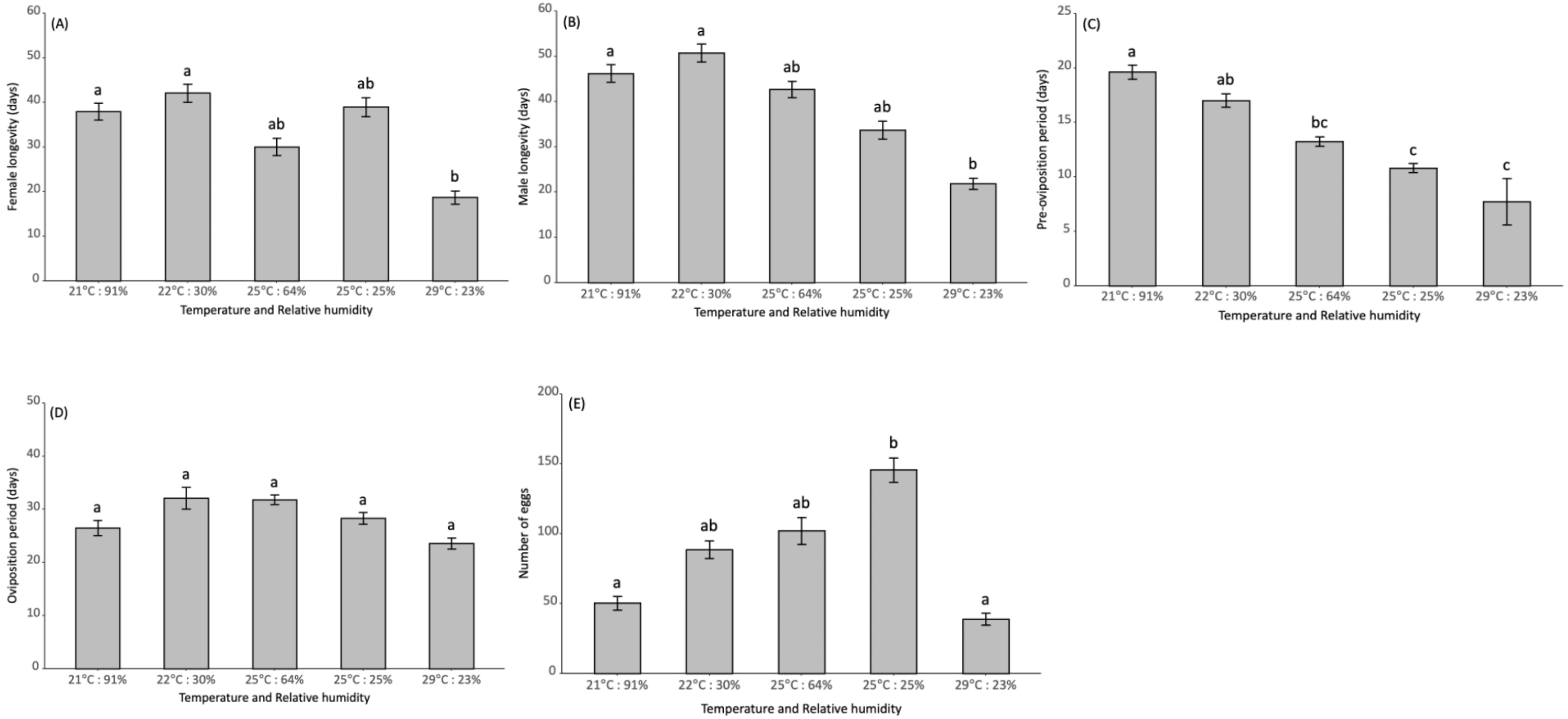


Figure 2.3 Effect of temperature on female (A) and male (B) longevity, pre-oviposition period (C), oviposition period (D), and fecundity (E) (mean ± SE) of *B. distincta.* Different letters indicate significant differences between temperatures ($P < 0.05$).

Table 2.5 Effects of temperature on the net reproductive rate (R_0), mean generation time (T), finite rate of increase (λ), intrinsic rate of increase (r_m), and doubling time (DT) (mean ± SE) of *B. distincta*. Mean values with different letters annotate significant differences between temperatures in columns ($P < 0.05$).

	Parameter				
Temperature and relative humidity	R_0	T	λ	r_m	DT
21 °C, 91 %	46.57 ± 7.62^{a}	94.18 ± 1.75^{a}	1.04 ± 0.00^{a}	0.04 ± 0.00^{a}	18.00 ± 0.68^{a}
22 °C, 30 %	70.47 ± 13.00^{ab}	95.82 ± 2.12^{a}	1.04 ± 0.00^{a}	0.04 ± 0.00^{a}	16.12 ± 0.41^{a}
25 °C$_{\text{high RH}}$, 64 %	104.62 ± 15.82^{b}	66.41 ± 1.35^{b}	1.07 ± 0.00^{b}	0.07 ± 0.00^{b}	10.32 ± 0.32^{b}
25 °C$_{\text{low RH}}$, 25 %	126.29 ± 18.25^{b}	67.84 ± 0.61^{b}	1.07 ± 0.00^{bc}	0.07 ± 0.00^{bc}	9.91 ± 0.26^{b}
29 °C, 23 %	70.82 ± 16.50^{a}	50.88 ± 0.83^{b}	1.08 ± 0.00^{c}	0.08 ± 0.00^{c}	9.02 ± 0.32^{b}

Discussion

The developmental duration of all *B. distincta* life stages and total development (egg to adult) significantly decreased with increasing temperature while the sex ratio did not differ between temperatures. The survival rate for all life stages was high but decreased significantly at 22 °C (51 %) and peaked at 25 °Clow RH (82 %) for instar 2. Relative to macadamia nuts, the sweetcorn diet significantly sped up the developmental duration of instars 2 and 4. The sex ratio did not differ between the diets but was significantly female-biased on the macadamia nut diet. Temperature-dependent models that best fitted the developmental rate of *B. distincta* life stages were Kontodimas-04 and Damos-08 and Taylor-81 for instar 3. The Kontodimas-04 model estimated a thermal range of 13.5 °C (T_{min}) to 38.0 °C (T_{max}) and an optimum developmental rate of 29.5 (T_{opt}) for the total development. *B. distincta* required 783 DD to develop from egg to adult stage. Males lived longer than females on average, but both survived longest at 22 °C. The Pre-oviposition period significantly decreased with increasing temperature, but the oviposition period was almost similar between temperatures. Fecundity peaked at 25°C low RH and was lowest at 29 °C. Generation length (T) and doubling time (DT) decreased, while the net reproductive rate (R_0), finite rate (λ), and intrinsic rate of increase (r_m) increased with increasing temperature.

The narrow temperature range of *B. distincta'*s total development (21 to 29 °C) mirrors that of *Chlorochroa uhleri* (Stål) (Hemiptera: Pentatomidae) and *Thyanta pallidovirens* (Stål) (Hemiptera: Pentatomidae) of 20 to 30 °C (Daane et al. 2022). In contrast, other stink bug species had a wider temperature range, 17 to

36 °C of *H. halys* (Govindan and Hutchison 2020) and 25 to 36 °C of *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) (Chanthy et al. 2015). These differences between species depend on the temperatures examined, climatic conditions within their geographical area, and genetic variations. The wider temperature of *H. halys* is probably related to the larger variation in summer climatic conditions of Minnesota, United States (Govindan and Hutchison 2020). Nevertheless, stink bug species show some similar nymphal and total development trends. Notably, The instar 5 of *B. distincta*, *N. viridula,* and *H. halys* had the longest developmental duration of all temperatures examined (Nielsen et al. 2008; Chanthy et al. 2015). Development of sexual organs in instar 5 could explain its longest developmental duration. In addition, *B. distincta* (25 $°C_{\text{low RH}}$) and *H. halys* (Nielsen et al. 2008) took 42 days to complete their development at 25 °C.

Non-linear models suggest that *B. distincta* will develop above 13.5 (T_{min}) and below 38 (T_{max}), with an optimal developmental rate at 29.5 °C (T_{opt}). These thermal requirements varied greatly between models and life stages. Similarly, Dhillon and Hasan (2017) and Geng and Jung (2018) reported large variations in thermal requirements estimated by non-linear models. However, the T_{min} and T_{opt} of *B. distincta* are consistent with the 13 and 13.2 °C (T_{min}) and 29.1 and 28.4 °C (T_{opt}) for *T. pallidovirens* and *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae), respectively (Da Silva and Daane 2014; Daane et al. 2022). In contrast, the T_{max} of 30.3 and 33.4 °C of the latter studies are lower than 38 °C of *B. distincta*, which may be due to California's cooler climatic conditions.

Although diet influences the development of stink bugs (Hagstrum and Milliken 1988), the total developmental duration of *B. distincta* did not differ between the macadamia nut and sweetcorn diets. However, the development of instars 2 and 4 was significantly faster on the sweetcorn diet. Dingha and Jackai (2017) reported improved development of *H. halys* reared on sweetcorn compared to 15 other diets at 27 °C. It has been shown that stink bug growth can be facilitated by a combination of different diets (Funayama 2006; Dingha and Jackai 2017). Despite the insignificant total developmental duration between diets, *B. distincta* seems to be polyphagous with adults feeding on a range of different plants, including green tea, sunflower, almond, carrot, *Xylopia* sp., *Bridelia* sp, *Lauracease family*, *Celtis africana*, *Pinus roxburghii*, and *Flindersia* sp. (Fourie et al. 2022). Macadamia nuts take about eight months to fully ripe which could explain the prevalence of *B. distincta* in this crop relative to other crops.

Diet and temperature did not affect survival, except for instar 2. In addition, the survival rate was high and did not seem to decrease with temperature in contrast to the survival rate of *N. viridula* nymphal stages that declined with increasing temperature (Chanthy et al. 2015). High survival rates are associated with the aggregation behaviour of newly emerged instar 1 nymphs (McPherson and McPherson 2000). Stink bug instar 1 nymphs acquire symbionts by tapping the egg shells using their mouth parts after hatching (Taylor et al. 2014). In addition, Lockwood and Storey (1986) found that the aggregations of *N. viridula* instar 1 nymphs increased humidity, thereby reducing mortality. As a result, it has been suggested that laboratory bioassay starts with instar 2 to allow instar 1 nymph to aggregate (Lockwood and Storey 1986; Dingha and Jackai 2017). This could account for the high survival rates of *B. distincta* in this study as the newly emerged instar 1 nymphs were allowed to aggregate.

The survival rate of instar 2 at 22 °C could have been lowered by handling the individuals while transferring them to clean-rearing containers with a soft paintbrush. This could suggest an alternative method of cleaning and observing the development of instar 2 because opening the containers daily while counting and removing exuviae and dead individuals may have negatively impacted this stage. In contrast, instar 2 was observed to be the most thermally plastic stage of *B. distincta* in response to heat and cold hardening and heat acclimation (Muluvhahothe et al. 2023), which could be related to its morphology and behaviour. The dark pigmentation of this stage could provide more heat absorption when they start searching for food (Stuart-Fox et al. 2017, Muluvhahothe et al. 2023).

Adults lived longest at 22 °C, but males lived longer than females. Longevity was expected to decrease with increasing temperature. However, adults lived longer at 22 °C than 21 °C, which could have resulted from high relative humidity at 21 °C. A considerable amount of mold development was observed at 21 °C, which may have indirectly affected their survival. The prolonged longevity at cooler temperatures suggests that they develop slower and therefore live longer. The latter implies that adults may survive low temperatures but start mating and reproducing when the temperature gets warmer.

B. distincta at 25 °C$_{\text{high and}}$ 25 °C$_{\text{low RH}}$ had longer pre-oviposition periods (13.2 and 11 days) than *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) (8 days) (Santos et al. 2011). However, Nielsen et al. (2008) reported a similar pre-oviposition period of 13.3 days for *H. halys* at 25 °C. It has been suggested that the females of *B. distincta* first migrate back into the orchards after winter, looking for oviposition sites where the progeny will have a food source (Schumann and Todd 1982). Therefore, the estimated pre-oviposition periods obtained here can be used to time pesticide applications.

While oviposition periods were almost similar at different temperatures, fecundity varied significantly. The average fecundity of 146 eggs at 25 °C$_{\text{high RH}}$ of *B. distincta* matched the fecundity of *N. viridula* (146 eggs) at the same temperature of 25 °C (Chanthy et al. 2015). Fecundity between stink bug species is incomparable because it varies with the number of eggs per egg packet. *B. distincta* lays ~14 eggs per packet, *H. halys* ~28 eggs (Skillman and Lee 2017), and *N. viridula* ~60 to 90 eggs (Geerinck et al. 2022). However, most stink bug species exhibit high fecundity at 25 °C (e.g., Medeiros et al. 2003; Chanthy et al. 2015). At low temperatures, insects deplete their energy reserves (Renault et al. 2003), which could explain the low fecundity at cooler temperatures. Thus, a lack of energy could have prevented the adults to mate or lead to insufficient energy to produce viable eggs at 21 and 22 °C despite living longer at 22 °C. On the other hand, high temperatures can temporarily or permanently cause sterility or deactivate sperms stored in the spermatheca resulting in decreased fertility (Riordan 1957). With regard to humidity, *B. distincta* adults at 25 °C$_{\text{high RH}}$ produced more eggs than those at 25 °C$_{\text{low RH}}$. Similarly, an investigation of the effects of both the temperature and relative humidity showed that 40 % RH improved the fecundity of *N. viridula* compared to 80 % RH at 25 °C (Chanthy et al. 2015).

The intrinsic rate of increase (r_m) is an important population parameter that integrates the net reproductive (R_0) and generation length (T) into one metric (Carey 1993). The intrinsic rate of increase (r_m) of *B. distincta* population was highest at 29 °C (r_m: 0.08) followed by 25 °C (r_m: 0.07). The same intrinsic rate of increase of 0.07 at 25 °C was reported for *H. halys* (Nielsen et al. 2008) while that of the *Brachynema germari* (Kolenati) (Hemiptera: Pentatomidae) was 0.06 (Yazdanpanah et al. 2019). Apart from the examined temperatures and rearing conditions, population increase in the field may be affected by multiple interactions, such as disease, precipitation, predators, and fluctuating temperatures (Daane et al. 2022). In conjunction with other important biological parameters, the population of *B. distincta* at 29 °C will be positively influenced by T_{opt} which was highest at 29 °C with high survival rates (< 64 %) of nymphal instars and a shorter pre-oviposition period. However, fecundity and female longevity were lower at this temperature which was compensated by the shorter generation length. At lower temperatures, a generation length (T) of almost three months at 21 (94 days) and 22 °C (96 days) appears to explain the low abundance of *B. distincta* during post-harvest period from mid-April to mid-August during winter. The latter implies that lower temperatures reduce the developmental rate, ultimately, low fecundity.

In conclusion, The orchard ambient mean temperature (five years) is 20 °C with a minimum temperature of 1.0 °C and a maximum temperature of 42 °C for a period of five years (Muluvhahothe et al. 2023). This farm is situated in a dry-winter subtropical highland climate with noticeable dry winters and rainy summers and sometimes experiences extremely hot summers. Thus, *B. distincta* population in macadamia orchards will grow rapidly in warmer temperatures with survival rates not decreasing at temperatures as high as 29 °C. The thermal range of 13.5 (T_{min}) to 38 °C (T_{max}) with an optimum developmental rate of 29.5 °C (T_{opt}) implies that this species develops at a wide range of temperatures, but their reproductive rate has a narrow range. Given the intrinsic rate of increase (r_m) of 0.08 at 29 °C, the projected global warming will certainly benefit *B. distincta,* shortening the doubling time and resulting in increased damage at warmer temperatures, while lower humidity associated with drier conditions could result in a further increase in growth.

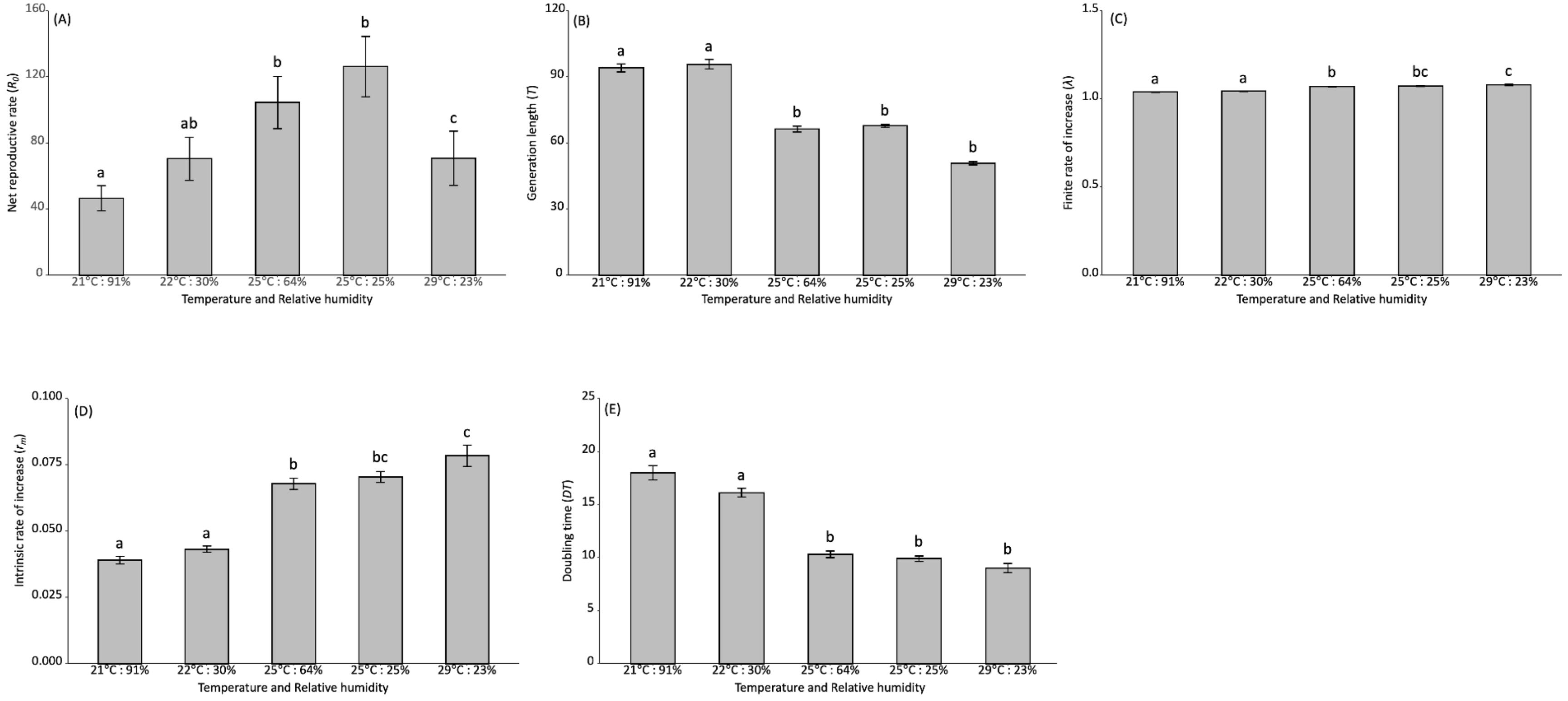
The thermal constant (783 DD) reported here can be used to improve the previous degree-day model of 515 DD and 807 DD calculated using a T_{min} of 10 °C (Schoeman and Pieter Haasbroek, unpublished data). This study provides insights into the temperature-dependent models (e.g., Kontodimas-04) that can be used for other stink bug species to develop degree days models. The life-stage specific T_{min} values can be applied to field-based temperatures to obtain degree days that are reliable for macadamia farmers. In-depth knowledge of this pest dispersal between macadamia orchards and the surrounding matrix would also improve the degree day model. The reported biology of *B. distincta* will be crucial in decision-making as part of integrated pest management (IPM) to mitigate its damage in the face of global warming.

Appendices

Appendix A: Longevity, pre-oviposition period, oviposition period, and fecundity (mean ± SE) of *B. distincta* at four constant temperatures. Mean values with different letters annotate significant differences in columns ($P < 0.05$).

	Parameters				
Temperature	**Female longevity**	**Male longevity**	**Pre-oviposition Period**	**Oviposition period**	**Fecundity**
21 °C, 91 %	37.9 ± 1.9^{a}	46.2 ± 1.9^{a}	19.6 ± 0.7^{a}	26.5 ± 1.4^{a}	50.2 ± 4.8^{a}
22 °C, 30 %	42.1 ± 2.0^{a}	50.7 ± 1.9^{a}	17.0 ± 0.6^{ab}	32.1 ± 2.0^{a}	88.7 ± 6.3^{ab}
25 °C$_{\text{high RH}}$, 64 %	30.0 ± 1.9^{ab}	42.7 ± 1.8^{ab}	13.2 ± 0.4^{bc}	31.8 ± 0.9^{a}	102 ± 9.7^{ab}
25 °C$_{\text{low RH}}$, 25 %	38.9 ± 2.1^{ab}	33.6 ± 2.0^{ab}	11.0 ± 0.4^{c}	28.3 ± 1.0^{a}	146 ± 8.8^{b}
29 °C, 23 %	18.6 ± 1.5^{b}	21.8 ± 1.2^{b}	7.7 ± 2.1^{c}	23.6 ± 1.0^{a}	38.8 ± 4.4^{a}

Appendix B: Effects of temperature on the net reproductive rate: R_0) (A), mean generation time: T (B), finite rate of increase: λ (C), intrinsic rate of increase: r_m (D), and doubling time: DT (E) (mean ± SE) of *B. distincta*. Mean values with different letters annotate significant differences between temperatures ($P < 0.05$).



CHAPTER 3

Thermal tolerance responses of the two-spotted stink bug, *Bathycoelia distincta* (Hemiptera: Pentatomidae), vary with life stage and the sex of adults

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Abstract

Temperature tolerance is an essential component of insect fitness, and its understanding can provide a predictive framework for their distribution and abundance. The two-spotted stink bug, *Bathycoelia distincta* Distant, is a significant pest of macadamia. The main goal of this study was to investigate the thermal tolerance of *B. distincta* across different life stages. Thermal tolerance indices investigated included critical thermal maximum (CT$_{max}$), critical thermal minimum (CT$_{min}$), effects of acclimation on CT$_{max}$ and CT$_{min}$ at 20, 25, and 30◦C, and rapid heat hardening (RHH), and rapid cold hardening (RCH). The Kruskal-Wallis test was used to explore the effects of life stage and acclimation on CT$_{max}$ and CT$_{min}$ and Generalized Linear Models (GLM) for the probability of survival after pre-exposure to RHH at 41 ◦C for 2 h and RCH at 8 ◦C for 2 h. CT$_{max}$ and CT$_{min}$ varied significantly between life stages at all acclimation temperatures, but CT$_{min}$ (3.5 ◦C) varied more than CT$_{max}$ (2.1 ◦C). Higher acclimation temperatures resulted in larger variations between life stages for both CT$_{max}$ and CT$_{min}$. A significant acclimation response was observed for the CT$_{max}$ of instar 2 (1.7 ◦C) and CT$_{min}$ of females (2.7 ◦C) across acclimation temperatures (20–30 ◦C). Pre-exposure significantly improved the heat and cold survival probability of instar 2 and the cold survival probability of instar 3 and males. The response between life stages was more variable in RCH than in RHH. Instar 2 appeared to be the most thermally plastic life stage of *B. distincta*. These results suggest that the thermal plastic traits of *B. distincta* life stages may enable this pest to survive in temperature regimes under the ongoing climate change, with early life stages (except for instar 2) more temperature sensitive than later life stages.

Keywords: CT$_{max}$, CT$_{min}$, acclimation, RHH, RCH

Introduction

Climatic factors such as temperature and humidity strongly influence insects (Jaworski and Hilszczański 2013; Fisher et al. 2021). As a result of ongoing climate change, the intensity and variability of these climatic factors are increasing (Diffenbaugh et al. 2005), resulting in variable temperatures in diel and seasonal cycles (Tarusikirwa et al. 2020). Because insects are ectotherms, their activity and metabolism are greatly influenced by temperature, with high temperature stimulating activity and low temperature suppressing it (Mellanby 1939). Thus, changes in climatic factors may present physiological challenges and, ultimately, directly impact their thermal fitness (Nyamukondiwa et al. 2018). Therefore, thermal tolerance is of fundamental importance in determining the survival of insects in their distinct microhabitats (Rodrigues and Beldade 2020).

Phenotypic plasticity is the ability of ectotherms to withstand temperatures outside their optimal zone (Sommer 2020) through physiological, morphological, and behavioural strategies when introduced to a novel environment (Chidawanyika and Terblanche 2011; Gray 2013). Physiologically, insects may respond to temperature variation through processes such as acclimation and hardening (Zhang et al. 2021). Phenotypic plasticity improves the survival of many insect species, including pests such as *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)*, C. rosa* (Karsch) (Diptera: Tephritidae) (Nyamukondiwa et al. 2010) and *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) (Chidawanyika and Terblanche 2011). This trait can be evaluated in the laboratory using thermal tolerance indices such as critical thermal limits (Motswagole et al. 2019), acclimation (Jumbam et al. 2008), and rapid hardening (Teets et al. 2020).

Critical thermal limits (CTLs) are temperatures at which organisms lose coordinated muscle function after a gradual increase, highest (CT_{max}), or decrease, lowest (CT_{min}) temperature. CTLs are ecologically relevant because they can resemble natural environmental conditions through gradual ramping of temperature (Terblanche et al. 2007). Slow ramping rates (less than 0.5 min^{-1}) are considered more ecological as compared to very slow ramping rates (less than 0.1 min^{-1}), which would result in the hardening of organisms (Worland 2005; Terblanche et al. 2007; Escribano-Álvarez et al. 2022). CTLs have been used to investigate the thermal fitness, sensitivity, and vulnerability to extreme temperatures of insects (Piyaphongkul et al. 2012; Nyamukondiwa et al. 2018; Horne et al. 2019). The response pattern of insects to CTLs is decoupled such that there is generally more variation in CT_{min} than CT_{max} (Nyamukondiwa et al. 2010; Chanthy et al. 2012). CTLs may be determined independently or used to quantify the effects of acclimation (Chidawanyika and Terblanche 2011). Interestingly, the decoupling of CT_{max} and CT_{min} is also evident under acclimation temperatures (Terblanche et al. 2017).

Acclimation is a type of phenotypic plasticity where organisms adjust to changes in environmental conditions. The beneficial acclimation hypothesis (BAH) states that an organism previously acclimated to a certain temperature will perform better than a non-acclimated organism (Leroi et al. 1994). Thus, the capacity of an organism to acclimate may be advantageous for coping with climatic variability (Terblanche and Hoffmann 2020). Beneficial acclimation has been observed in the laboratory (Chidawanyika and Terblanche 2011; Piyaphongkul et al. 2018) and under field conditions (Thomson et al. 2001). Although the BAH has been criticized (Deere and Chown 2006; Ramniwas et al. 2020), acclimatory capacity is still being investigated for different taxa (Ruthsatz et al. 2022; Sentis et al. 2022).

Rapid hardening is a quick adaptive phenotypic plasticity with brief pre-exposure to sub-lethal temperatures providing protection from injury while enhancing stress tolerance (Teets et al. 2020). Rapid cold hardening (RCH) initially received considerable attention in insects’ ability to survive extreme winter temperatures (Chown and Nicolson 2004). However, the rising global average temperatures (Diffenbaugh et al. 2005), has resulted in the increased relevance of rapid heat hardening (RHH) studies of insects (Moghadam et al. 2019; Sørensen et al. 2019; Zhu et al. 2022). These traits can be induced within minutes or hours after exposure to certain sub-lethal temperatures (Nyamukondiwa et al. 2010; Sørensen et al. 2019). For example, a 30-minute pre-exposure to sub-lethal temperatures improved the cold of *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) adults (Yi et al. 2007), while the heat survival of *Nysius groenlandicus* (Zetterstedt) (Hemiptera: Lygaeidae) was improved following a pre-exposure of 15 to 60 minutes (Sørensen et al. 2019).

Thermal tolerance studies are essential in evaluating insects' thermal sensitivity to temperature changes (Braschler et al. 2021). Thermal tolerance indices have helped build forecast models (Nyamukondiwa et al. 2013; Kleynhans et al. 2018) and management strategies (Nyamukondiwa et al. 2013) of pests. Plastic response of agricultural insect pests is important because it can interact with climate change resulting in increased outbreaks and abundance (Skendžić et al. 2021). There is a projected rise of 25% in yield losses per global degree of mean surface warming for wheat in temperate zones due to pests (Deutsch et al. 2018). As a result, the physiological responses of insect pests to temperature have gained renewed recognition as part of integrated pest management (Hallman and Denlinger 2019). However, temperature response may vary throughout a single life cycle (Marais et al. 2009) and within a generation (Nyamukondiwa and Terblanche 2010). Anticipating how changing environmental conditions will affect insect pest populations will rely on understanding the thermal tolerance of different life stages (Radchuk et al. 2013; Zhao et al. 2019).

The thermal tolerance of the macadamia major pest, the two-spotted stink bug *Bathycoelia distincta* (Distant) (Hemiptera: Pentatomidae), was investigated with the objectives to 1) quantify CTLs differences between *B. distincta* life stages, 2) evaluate the effects of acclimation on CTLs at different temperatures, and 3) investigate if rapid heat hardening (RHH) and rapid cold hardening (RCH) can enhance survival of different life stages of *B. distincta.*

Materials and methods

Origin of B. distincta and rearing

A colony of *B. distincta* was initiated from eggs that were hand-collected on a commercial macadamia farm between August 2020 and February 2021 in Levubu (23°4'0.96"S, 30°4'31.07"E), Limpopo province, South Africa. Eggs and instars were reared in transparent polypropylene containers (45 x 25 x 30 cm). The newly emerged adults were placed in transparent polypropylene containers (33 cm x 24 cm x 16 cm) for reproduction. All container lids were covered with a fine net and lined with toweling paper on the bottom. Small rounds of cotton moistened with distilled water were randomly placed inside the containers to maintain humidity (Geng and Jung 2018) and as a source of water for instar 1 nymphs (Dingha and Jackai 2017). To prevent fungal infection, individuals were transferred to prepared containers with fresh macadamia nuts twice a week. The colony was reared at a temperature of 25 ± 1 °C, 71.8 % relative humidity, and a long day photoperiod of 16L: 8D. Temperature and relative humidity were recorded using Thermocron iButtons (Semiconductor Corporation, Dallas/Maxin TX and USA), at hourly intervals for the entire period of rearing.

CTLs differences between life stages and effects of acclimation at three temperatures

CTLs assays were conducted using a water bath (Grant, SG8 6GB; Grant Instruments, Cambridge Ltd, UK) filled with 1:1 water: propylene glycol to allow sub-zero temperatures, connected to an insulated system of 11 chambers that enables the fluid to circulate around the chambers. Ten newly emerged individuals per life stage (1 - 2 days old) were selected and placed individually into the chambers. The temperature was increased at a constant rate of 0.2 °C min^{-1} from 25 to 65 °C for the determination of the critical thermal maximum (CT_{max}) and decreased from 25 to -5 °C for the determination of the critical thermal minimum (CT_{min}) (Fig 3.1). To ensure an accurate temperature reading at which individuals reached their CTLs, two Thermocron iButton loggers were inserted into one chamber to record temperature at one-minute intervals. To determine thermal tolerance differences between life stages and changes at three temperatures (20, 25, and 30 °C), individuals were acclimated at 20 and 30 °C (Memmert Peltier-cooled incubator) for 48 h under the photoperiod of 16L: 8D prior exposure to CT_{max} and CT_{min} (Fig 3.1). The trials were repeated three times (N = 30) for each life stage. CT_{max} and CT_{min} were identified as the temperature at which an individual loses coordinated muscle function, typically accompanied by loss of movement or neuromuscular control (Nyamukondiwa and Terblanche 2010). To identify the endpoints of the assays, individuals were prodded gently with a soft paintbrush (Kleynhans et al. 2014).

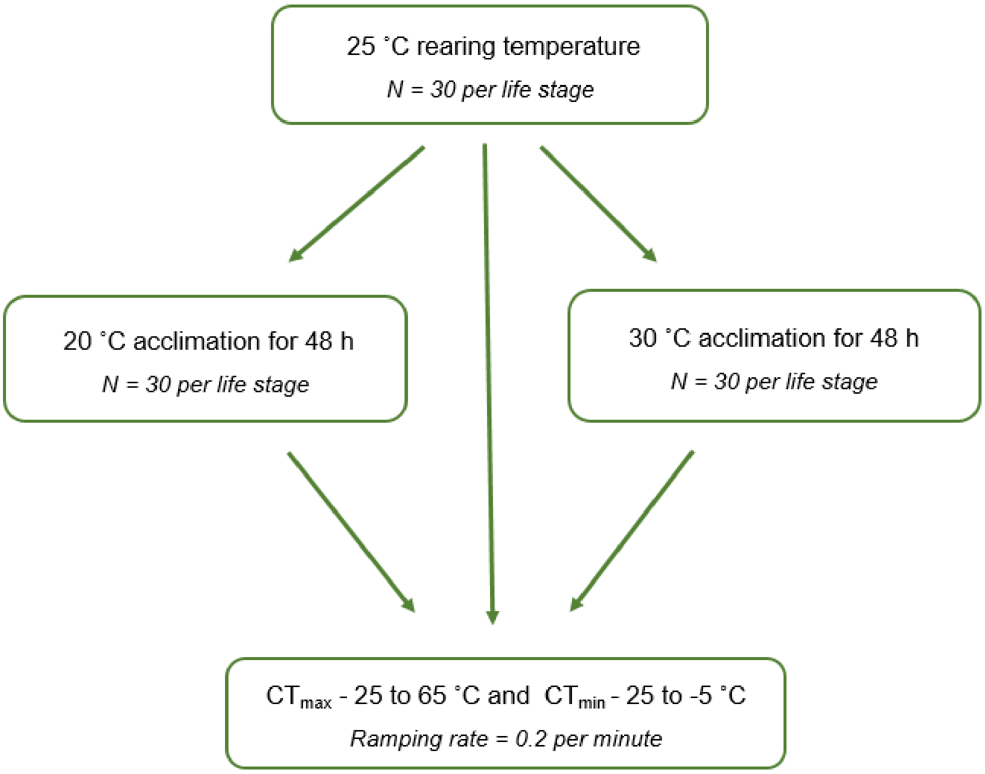

Figure 3.1 Schematic diagram of CT_{max} - critical thermal maximum and CT_{min} - critical thermal minimum and effects of acclimation experiments.

Determining the discriminating temperature

Discriminating temperature is defined as a temperature that causes approximately 80 % mortality (Nyamukondiwa et al. 2010). To establish the discriminating temperature, newly emerged individuals (1 - 2 days old) were exposed to upper lethal temperatures (ULT) and lower lethal temperatures (LLT). Pilot experiments revealed that temperature and the number of individuals per 60 ml glass vial influenced mortality. Therefore, vials with instars 1 to 3 contained five individuals, and those with instars 4 to adults had two individuals (N = 20). These vials were placed in plastic zip lock bags and exposed to acute temperatures in 1 °C increments ranging from 39 to 43 °C for ULT and -5 to -10 °C for LLT using a standard "plunge" protocol (Terblanche et al. 2008) for 2 h in a programmable water bath. This was achieved by partly submerging the plastic zip-lock bags in the programmable water bath fluid (1:1 water: glycol). Each plastic zip lock bag had two Thermocron iButton loggers recording temperature every minute. Individuals were returned to rearing temperature, and survival was recorded as the total number of individuals that responded to stimuli when prodded with a paintbrush after 24 h.

Rapid hardening

Rapid heat hardening (RHH) and rapid cold hardening (RCH) assays were assessed using the previously described “plunge” protocol. Treatment groups per life stage (N = 20), housed in transparent polypropylene containers (45 x 25 x 30 cm) were transferred from rearing conditions to 35 °C (RHH) and 10 °C (RCH) for 2 h (Nyamukondiwa et al. 2010). Thereafter, the control group and treatment group were exposed to discriminating temperatures of 41 °C (RHH) and -8 °C (RCH) for 2 h (Fig 3.2). Upon completion of each assay, the control and treatment groups were transferred back to the rearing temperature. Similar methods to those described in determining discriminating temperature were used to score survival.

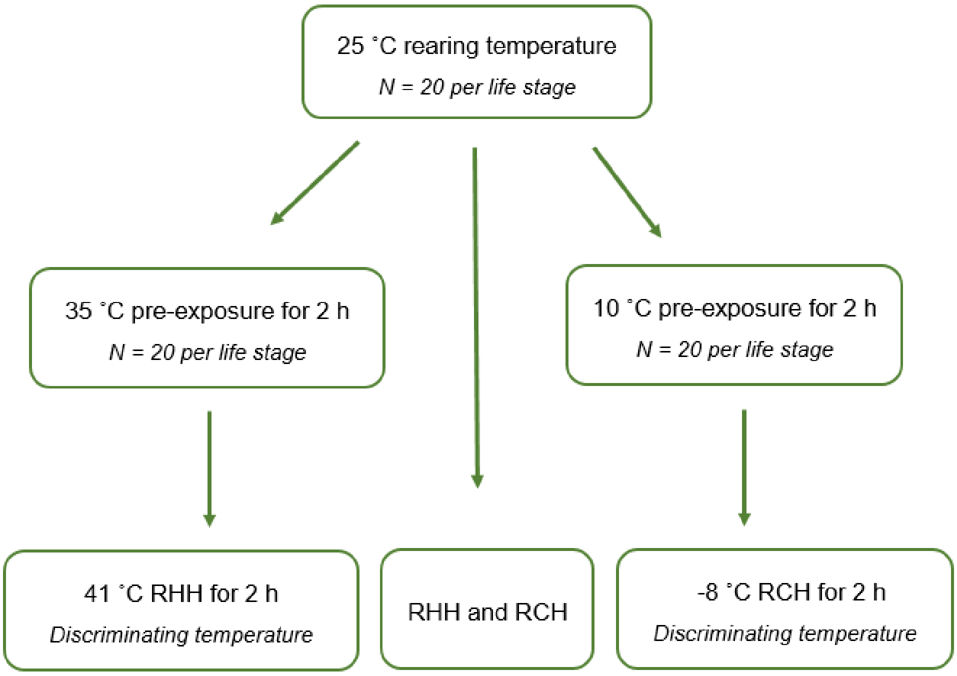

Figure 3.2 Schematic diagram of RHH - rapid heat hardening and RCH - rapid cold hardening experiments.

Statistical analysis

CTLs data per acclimation temperature were tested for normality and homogeneity of variance on the model residuals using the Shapiro-Wilk and Levene tests respectively. Outliers identified as residuals were removed using the cook’s distance by removing any values that were three times greater than the mean (Cook 1977). CTLs did not meet the linear model assumptions and the groups were homoscedastic. The treatment effects (interaction) between acclimation temperatures and life stages were analysed using the Generalized Linear Model (GLM) assuming a Gaussian distribution and an identity link function with a type III Sum of Squares Anova. Differences between life stages per acclimation temperature were analysed using the Kruskal-Wallis test and comparisons between life stages were performed using Dunn's post-hoc where significant differences were determined on the alpha level = 0.05 (95% confidence interval) using the ggsignif (Ahlmann-Eltze 2021) and ggstatsplot R packages (Patil 2021). The latter tests were used for the effects of acclimation temperatures for each life stage. CTLs of each life stage per acclimation temperature were averaged and the standard deviation, standard error, and 95% confidence intervals were calculated using the Rmisc R package (Hope 2022). Data were plotted per life stage using the ggplot2 R package (Wichham 2016) with significant differences indicated by letters above the error bars based on the post-hoc test. To determine the upper (ULT_{80}) and lower (LLT_{80}) lethal temperatures that resulted in 80 % mortality, a logistic regression model was fitted to the mortality data per life stage. A mean average for ULT_{80} and LLT_{80} was used as a discriminating temperature for all the stages. A GLM model was fitted to the survival data using a binomial distribution and logit link function to determine the survival probability of *B. distincta* life stages (Marais et al. 2009). All statistical analyses were performed in R, version 4.1.1 (R core team, 2022).

Results

CTLs differences between life stages at three acclimation temperatures

Life stages, acclimation temperatures, and their interaction explained a significant amount of variation in CT_{max} (GLZ: $\chi^2 = 131.8$, d.f. = 12, $P < 0.001$) and CT_{min} (GLZ: $\chi^2 = 307$, d.f. = 12, $P < 0.001$). Life stage had a significant effect on CT_{max} at 20 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 77.09, $P < 0.0001$), 25 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 48.4, $P < 0.0001$), and 30 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 69.8, $P < 0.0001$). At 20 °C, the multiple comparisons of Dunn's test showed that instars 1 and 2 had a significantly lower CT_{max} than all other life stages (Table 3.1). At 25 °C, instar 5 and males had significantly higher CT_{max} than the first three instars (Table 3.1). At 30 °C, the males and instar 2 had a significantly higher CT_{max}, except for instars 4 and 5, while instar 1 was significantly lower than all life stages except for instar 3 and females (Table 3.1). Similarly, life stage had a significant effect on CT_{min} at 20 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 124.7, $P < 0.0001$), 25 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 94.1, $P < 0.0001$), and 30 °C (ANOVA: $\chi^2_{\text{kruskal-Wallis}}$ (6) = 109.1, $P < 0.0001$). The Dunn's test showed that instars 1 and 2 were significantly higher than all other stages at 20 °C (Table 3.1). At 25 °C, instar 3 had the lowest CT_{min}, followed by males, with instar 2 with the highest CT_{min} (Table 3.1).

At 30 °C, instars 3 to 5 had significantly lower CT_{min} than the first two instars, females and males (Table 3.1). In general, the response of the life stages to acclimation, measured as the trait effects CT_{max} and CT_{min}, seemed to get more complex with increasing acclimation temperature (Table 3.1).

Table 3.1 Multiple comparisons results of the CT_{max} - critical thermal maximum and CT_{min} - critical thermal minimum (mean ± se) between life stages in response to acclimation at 20, 25, and 30 °C. Means with different letters indicate significant differences per acclimation temperature (Dunn test, $P < 0.05$).

Life stage	20 °C	25 °C	30 °C
CT_{max}			
Instar 1	44.3 ± 0.2^{a}	45.4 ± 0.1^{ac}	44.8 ± 0.1^{a}
Instar 2	44.5 ± 0.1^{a}	45.1 ± 0.1^{a}	46.2 ± 0.1^{b}
Instar 3	45.8 ± 0.2^{b}	45.4 ± 0.1^{ac}	45.2 ± 0.1^{ac}
Instar 4	45.8 ± 0.1^{b}	45.4 ± 0.2^{ab}	46.0 ± 0.1^{b}
Instar 5	45.9 ± 0.1^{b}	46.1 ± 0.2^{bd}	45.9 ± 0.1^{bd}
Female	45.8 ± 0.2^{b}	45.8 ± 0.1^{bcd}	45.4 ± 0.1^{acd}
Male	46.0 ± 0.2^{b}	46.3 ± 0.1^{d}	46.4 ± 0.2^{b}
CT_{min}			
Instar 1	3.6 ± 0.1^{a}	2.7 ± 0.2^{ab}	4.1 ± 0.1^{a}
Instar 2	4.2 ± 0.1^{a}	3.4 ± 0.2^{a}	2.9 ± 0.2^{be}
Instar 3	0.7 ± 0.1^{b}	0.6 ± 0.1^{d}	1.8 ± 0.1^{d}
Instar 4	1.3 ± 0.1^{b}	2.3 ± 0.1^{b}	2.3 ± 0.1^{bd}
Instar 5	1.1 ± 0.1^{b}	2.0 ± 0.1^{bc}	1.6 ± 0.1^{cd}
Female	0.8 ± 0.2^{b}	2.0 ± 0.2^{bc}	3.5 ± 0.1^{ae}
Male	1.1 ± 0.1^{b}	1.6 ± 0.2^{c}	3.0 ± 0.2^{be}

Effects of acclimation on CTLs

Acclimation had a significant impact on the CT_{max} of instar 1 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 21.5, P < 0.0001), instar 2 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 45.5, P < 0.0001), instar 3 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 7.3, P = 0.03), and instar 4 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 9.03, P = 0.01), and females (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 6.5, P = 0.04) while instar 5 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 0.89, P = 0.6), and males (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 3.7, P = 0.2) did not differ across acclimation temperature (Fig 3.3A). Dunn's test multiple comparisons showed that the CT_{max} of instar 1 increased by 1.1 °C from 20 and 25 °C and decreased by 0.6 °C from 25 to 30 °C. The CT_{max} of instar 2 increased by 0.9 °C from 20 °C to 25 °C, by 1.1 °C from 25 to 30 °C, and by 1.7 °C from 20 to 30 °C. Instar 3 CT_{max} decreased by 0.6 °C from 20 to 30 °C. The CT_{max} of instar 4 increased by 0.6 °C from 25 to 30 °C (Fig 3.3A). In response to CT_{min}, acclimation had a significant impact on all life stages; instar 1 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 26.4, $P < 0.0001$), instar 2 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 19.8, $P < 0.0001$), instar 3 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 30.6, $P < 0.0001$), instar 4 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 25.8, $P < 0.0001$), instar 5 (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 15.9, $P < 0.0001$), female (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 42.5, $P < 0.0001$), and male (ANOVA: $\chi^2_{kruskal\text{-}Wallis}$ (2) = 37.2, $P < 0.0001$) (Fig 3.3B). The CT_{min} of instar 1 decreased from 20 to 25 °C by 0.9 °C and increased by 1.4 °C from 25 to 30 °C. The CT_{min} of instar 2 increased by 0.8 °C from 20 to 25 °C and from 20 to 30 °C it decreased by 1.3 °C. Instar 3 CT_{min} increased by 1.1 °C from 20 to 30 °C and also increased by 1.2 °C from 25 to 30 °C. The CT_{min} of instar 4 increased from 20 to 25 and 30 °C by 1 °C. The CT_{min} of instar 5 increased by 0.9 from 20 to 25 °C and decreased by 0.4. The CT_{min} of females increased from 20 to 25 °C by 1.2 °C, from 25 to 30 °C by 1.5 °C, and from 20 to 30 °C by 2.7 °C. The CT_{min} of males increased from 20 to 25 and 30 °C by 0.5 and 1.9 respectively (Fig 3.3B).

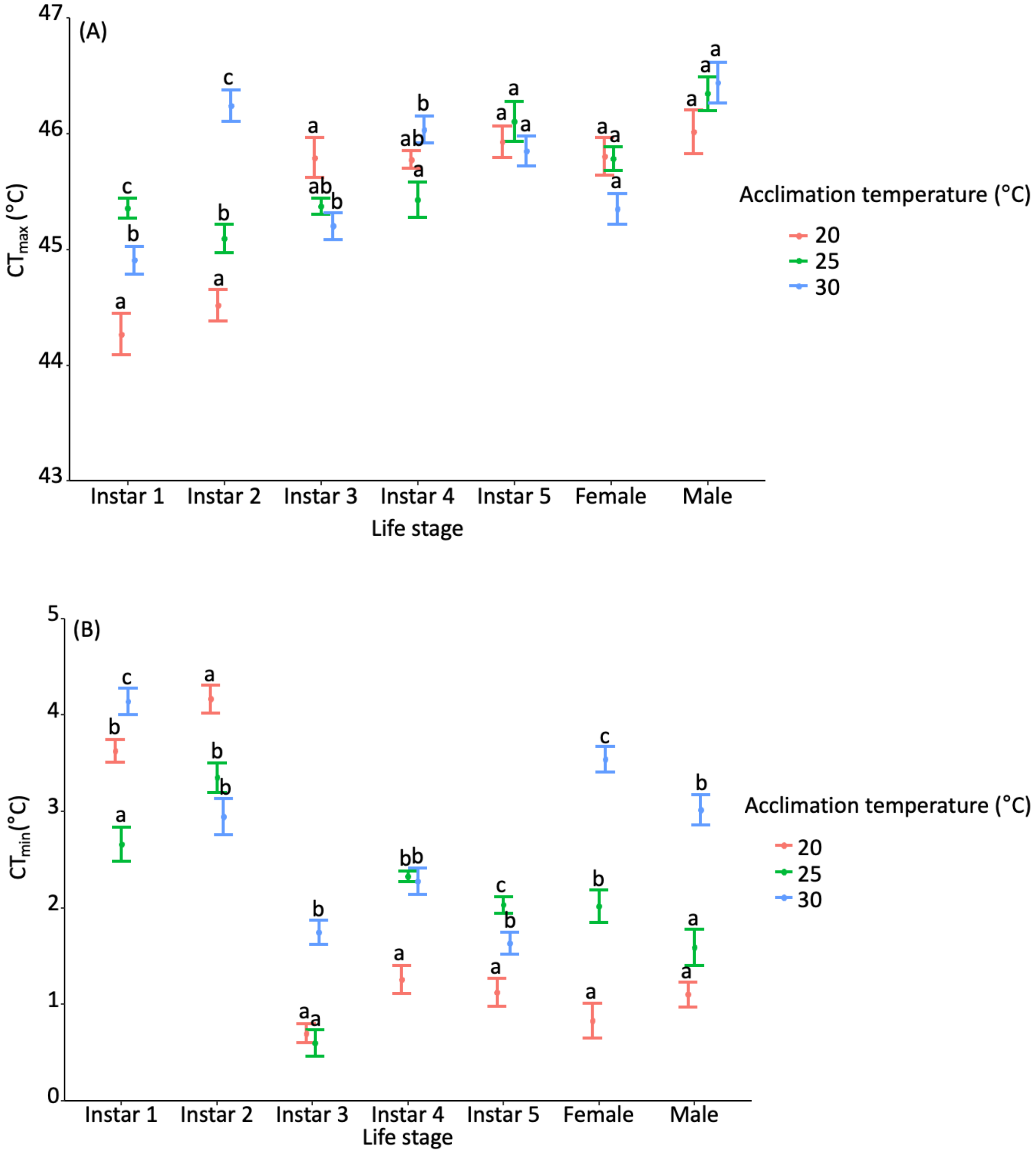

Figure 3.3 Effects of acclimation at three temperatures (mean ± se) in response to CT$_{max}$ - critical thermal maximum (A) and CT$_{min}$ - critical thermal minimum (B). Letters indicate significant differences between temperatures for each life stage.

Discriminating temperatures

After exposure to acute temperatures for 2 h, 80 % mortality for each life stage ranged from 40.4 to 41.5 °C for ULT_{80} and -7.7 to -9.5 °C for LLT_{80} (Table 3.2, Fig 3.4). ULT_{80} and LLT_{80} of all life stages were averaged and used as the discriminating temperatures for rapid hardening assays.

Table 3.2 Logistic regression results for the ULT - upper lethal temperature and LLT - lower lethal temperature (mean ± 95% confidence intervals) that resulted in 80 % mortality for each life stage after exposure to acute temperatures for 2 h.

Life stage	ULT_{80} (°C)	LLT_{80} (°C)
Instar 1	41.4 ± 1.2	-9.0 ± 1.0
Instar 2	40.4 ± 1.3	-7.9 ± 1.2
Instar 3	41.2 ± 0.3	-8.5 ± 0.9
Instar 4	41.1 ± 0.9	-9.5 ± 1.5
Instar 5	41.0 ± 1.6	-8.7 ± 0.9
Female	41.4 ± 0.8	-7.8 ± 1.1
Male	41.5 ± 0.8	-7.7 ± 0.9

Rapid hardening

Pre-exposure had a significant impact on heat survival ($P < 0.001$) of all *B. distincta* life stages (Table 3.3). However, the survival probability of all life stages except for the females decreased relative to instar 1. (Table 3.3, Fig 3.5A). In response to RCH, pre-exposure also had a significant impact on cold survival ($P < 0.001$) (Table 3.3). The survival probability of all life stages decreased relative to instar 1 (Table 3.3, Fig 3.5B). Among all life stages, pre-expose improved both the heat and cold survival probability of instar 2 (Fig 3.5A, B).

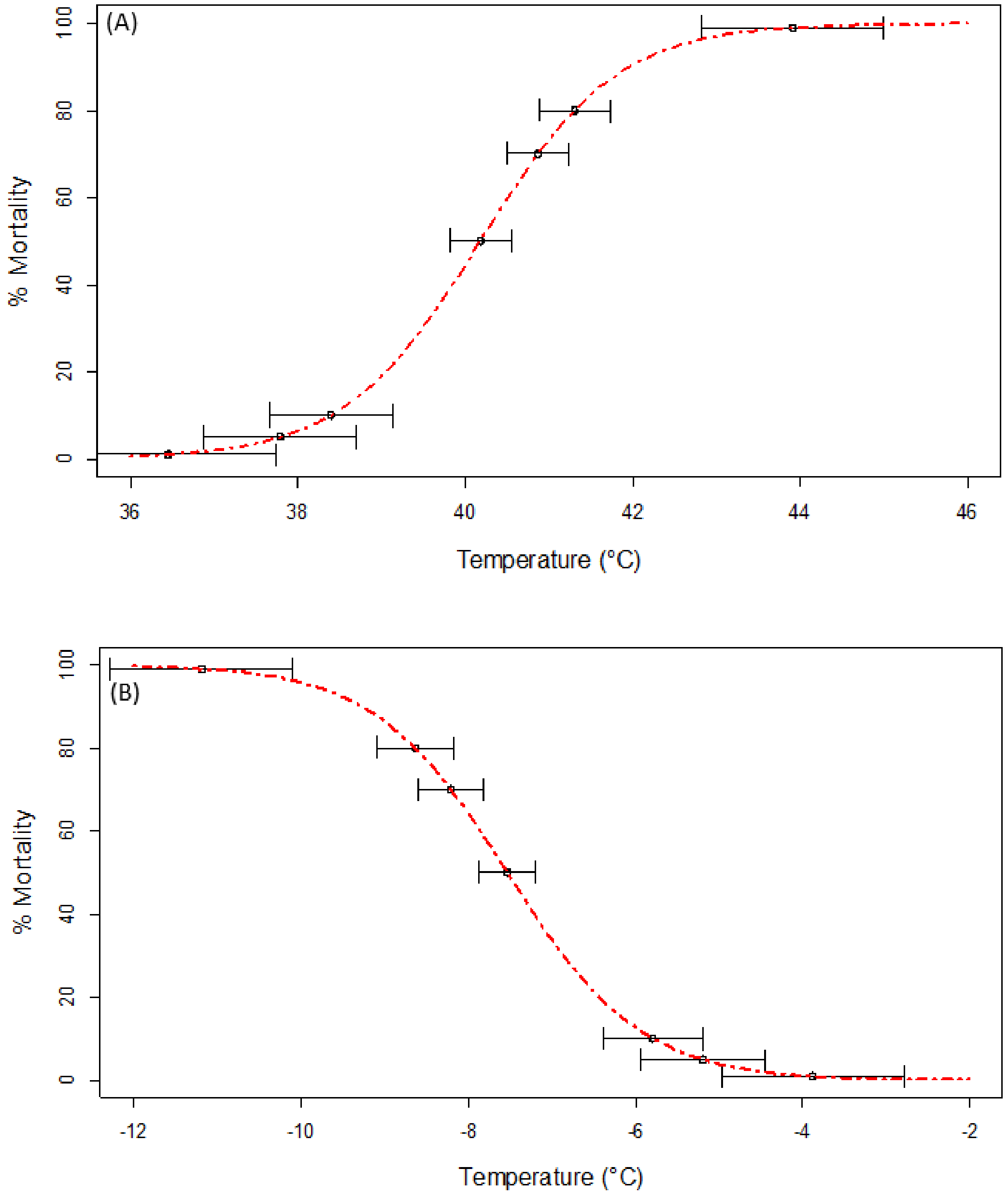

Figure 3. 4 Fitted logistic regression for temperatures that caused 10 - 99 % mortality of *B. distincta* life stages (nymphal and adults) for the determination of UDT - upper discriminating temperature (A) and LDT - lower discriminating temperature (B). Error bars represent 95 % confidence intervals on the discriminating temperatures.

Table 3.3 Results of the Generalized Linear Model (GLM) with a binomial distribution and logit link function for the effects of RHH - rapid heat hardening and RCH - rapid cold hardening on the survival of *B. distincta* life stages after pre-exposure for 2 h.

Life stage	Estimate	Std. Error	Z value	P value
RHH				
Intercept	2.71	3.63	0.74	0.45
Pre-exposure	1.00	2.62	3.84	**<0.001** ***
Instar 2	-1.55	4.92	-3.14	**<0.001** **
Instar 3	-7.75	4.78	-1.62	0.10
Instar 4	-4.54	4.78	-0.94	0.34
Instar 5	-1.18	4.86	-0.24	0.80
Female	2.51	5.02	0.50	0.61
Male	-2.17	4.90	0.00	1.00

Survival probability

$$= \frac{e^{2.72+Pre-exposure\ temperature-1.55Instar2-7.75Instar3-4.54Instar4-1.18Instar5+2.51Female-2.17Male}}{1+e^{2.72+Pre-exposure\ temperature-1.55Instar2-7.75Instar3-4.54Instar4-1.18Instar5+2.51Female-2.17Male}}$$

RCH				
Intercept	1.02	0.43	2.35	0.01 *
Pre-exposure	1.34	0.26	5.01	**<0.001** ***
Instar 2	-1.69	0.54	-3.10	**0.001** **
Instar 3	-1.80	0.54	-3.30	**<0.001** ***
Instar 4	-1.12	0.54	-2.04	0.04 *
Instar 5	-2.03	0.54	-3.69	**<0.001** ***
Female	-2.14	0.55	-3.89	**<0.001** ***
Male	-2.26	0.55	-4.08	**<0.001** ***

Survival probability

$$= \frac{e^{1.02+1.34Pre-exposure\ temperature-1.69Instar2-1.80Instar3-1.12Instar4-2.03Instar5-2.14Female-2.26Male}}{1+e^{1.02+1.34Pre-exposure\ temperature-1.69Instar2-1.80Instar3-1.12Instar4-2.03Instar5-2.14Female-2.26Male}}$$

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

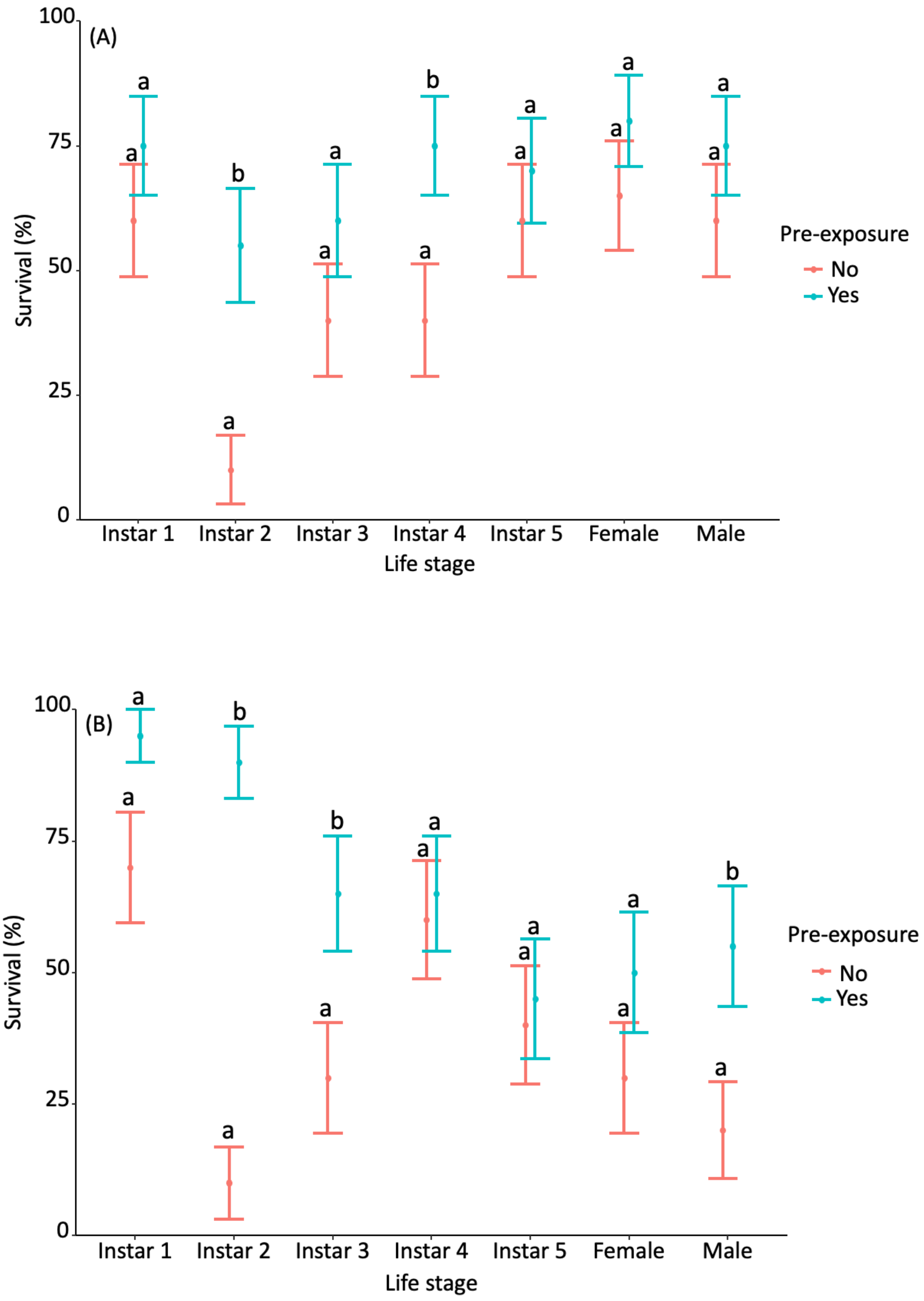

Figure 3. 4 Survival (mean ± se) of *B. distincta* life stages in response to RHH - rapid heat hardening (A) and RCH - rapid cold hardening (B) after exposure to discriminating temperatures of 41 and -8 °C for 2 h, respectively.

Discussion

Thermal tolerance varied significantly with *B. distincta* life stages and sex. The variation between life stages increased with increasing acclimation temperatures. The results were more pronounced for colder temperatures for both CTLs and rapid hardening. However, the divergence between life stages was less for rapid hardening compared to CTLs. Thermal plasticity for CT_{max} and rapid hardening peaked in instar 2 while females were the most plastic for CT_{min}. Instar 1 was identified as the most temperature-sensitive while later life stages generally were more stress-resistant than earlier life stages.

Thermal tolerance variation of *B. distincta* is consistent with that observed between life stages of other taxa such as *Paractora dreuxi* (Séguy) (Diptera: Helcomyzidae) (Marais et al. 2009) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Mutamiswa et al. 2019). Instar 1 was heat-intolerant among all other life stages and this is similar to the instar 1 nymphs of *Nilaparvata lugen*s (Stål) (Hemiptera: Delphacidae) (Piyaphongkul et al. 2012). Dehydration is a major factor determining survival, and improvement in evaporative cooling may have resulted in heat resistance in larger-bodied later life stages relative to instar 1 (Chown et al. 2011; Le Lann et al. 2011; Johnson and Stahlschmidt 2020). Different species of ants also showed higher CT_{max} in relation to their larger bodies (Baudier et al. 2015; Johnson and Stahlschmidt 2020). Extreme environmental temperatures can therefore threaten smaller-bodied individuals (Piyaphongkul et al. 2012). Consequently, instar 1 of *B. distincta* will be the most susceptible to heat waves.

In contrast, instar 1 nymphs of *Pseudococcus jackbeardsleyi* (Gimpel and Miller) (Hemiptera: Pseudococcus) (Piyaphongkul et al. 2018), and field-collected third instar larvae of *P. dreuxi* (Klok and Chown 2001) had significantly higher CT_{max} than the adults. Additionally, the immobile pupal stage of *Bicyclus anynana* (Butler) (Lepidoptera: Nymphalidae) was more heat tolerant than other life stages (Klockmann et al. 2017). In early life stages, mobility may be absent or limited, resulting in a lack of alternative behavioural strategies. This would then make early life stages more resistant to environmental temperature changes (Bowler and Terblanche 2008). The instar 1 of *B. distincta* remains on egg clusters after hatching and the lower CT_{max} could be related to the cooler microhabitats provided by macadamia trees. Additionally, instar 1 does not feed (Rivera and Mitchell 2020) as the energy gained from feeding may influence physiological tolerance (Hofmann and Todgham 2010; Rogers et al. 2021).

The CT_{max} (44.3 - 46.4 °C) was less variable between life stages than CT_{min} (0.6 - 4.1 °C). Between the nymphal life stages of *Blaptica dubia* (Serville) (Blattodea: Blaberidae), the CT_{max} ranged from 44. 8 to 49.9 °C, while the CT_{min} ranged from -2 to -3.1 °C (Wu et al. 2017). Contrastingly, the CT_{max} of the *Bombus impatiens* (Cresson) (Hymenoptera: Apidae) was more variable than the CT_{min}, ranging from 42 to 65 °C and 1.4 to 8 °C, respectively (Oyen and Dillon 2018). Observed CTLs for *B. distincta* are comparable with those recorded previously for other laboratory-reared insects with larger variation in CT_{min} and less variation in CT_{max} (Klok and Chown 2001; Käfer et al. 2020). The remarkably limited plasticity in response to high temperatures could result from the irreversible cell damage caused by water loss as the temperature increases (Hallman and Denlinger 2019). Klock et al. (2004) noted that fewer physiological factors contribute to heat tolerance than cold tolerance leading to more variation in $CT_{min.}$ For example, tolerance to high temperatures is mainly controlled at cellular levels, whereas low tolerance can be improved by increasing poly cryoprotectants that prevent body fluids from crystallization (Sinclair et al. 2003). Variations in CT_{max} and CT_{min} may also result from the methodology used (Terblanche et al. 2007). Using different ramping may yield different results (Terblanche et al. 2008). However, the current study used a slower ramping rate of 0.2 min^{-1} because of its ecological relevance (Escribano-Álvarez et al. 2022). Furthermore, Chown et al. (2009) and Terblanche et al. (2006) showed that slower ramping rates may result in more plasticity, though the opposite might be the case for other organisms (Terblanche 2007).

Poor cold tolerance of instars 1 and 2 indicates that they cannot cope with gradual temperature decreases compared to other stages. The latter is not surprising as *B. distincta* is more abundant in summer when macadamia nuts are available and declines in winter during postharvest (Schoeman 2018). While scouting surveys have detected both nymphs and adults in winter (Schoeman and Mohlala 2012), this study did not specify which nymphal stages were present. However, as macadamia nuts begin to develop in early summer, only adults return to the macadamia orchards (Schoeman and Mohlala 2012; Fourie et al. 2022). Consequently, the cold tolerance variation between the life stages of *B. distincta* reflects the seasonal timing of developmental stages.

The effects of acclimation on CTLs have been shown to differ between life stages and the magnitude of acclimation temperatures (Mutamiswa et al. 2019). The latter is consistent with the differences observed between the life stages of *B. distincta* across acclimation temperatures for both CT_{max} and $CT_{min.}$ However, acclimation revealed a high level of plasticity in cold tolerance in contrast to heat tolerance between life stages. Except for the CT_{max} of instar 2 and CT_{min} of females, minor acclimation effects in response to CTLs were also reported for *B. impatiens* (Oyen and Dillon 2018) and *Zygogramma bicolorata* (Pallister) (Coleoptera: Chrysomelidae) (Chidawanyika et al. 2017). Weak responses to acclimation might suggest that most life stages of *B. distincta* compensate for environmental variations behaviourally.

B. distincta starts feeding with the emergence of instar 2, which could have led to improved tolerance of this stage through its nutritional status. In addition, instar 2 is darkly pigmented, which has been shown to absorb more heat (Stuart-Fox et al. 2017). Thus, this stage may have had an advantage in acclimating faster than other life stages because of its smaller body size resulting in a more robust response to acclimation. Rohr et al. (2018) highlighted the relationship between acclimation duration and organismal body size. This study suggested that short acclimation periods might favor smaller organisms over larger ones as they may have time to fully acclimate because of their body size.

In contrast, female' CT_{min} improved across acclimation temperatures implying that they may respond better to environmental cues. *B. distincta* adults may be expected to show more plastic responses to cold temperatures as they go through diapause in winter (Schoeman and Mohlala 2012). Indeed, a study of diapause in the seasonal cycle of stink bugs from the temperate zone confirmed that most bugs overwinter as adults surviving low temperatures through freeze avoidance (Saulich and Musolin 2012). Diapause has been shown to improve the cold tolerance of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) both in laboratory and field conditions (Cira et al. 2018). Therefore, stink bug adults may be cold resistant due to the low temperatures they encounter while overwintering. The adults of *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) were also cold-tolerant after a seven-day acclimation period (Chanthy et al. 2012). Thus, diapause may be an important factor in determining cold tolerance of stink bug adults under extremely low temperatures.

The physiological mechanisms underlying enhanced stress tolerance might be linked to the production of heat shock proteins activated under stress to protect against the denaturation of proteins under extreme temperatures (Farahani et al. 2020; Harvey 2020). The upregulation of heat shock proteins is exhibited in response to heat and cold tolerance and has been shown to differ among insect life stages (Rinehart et al. 2006; Teets et al. 2019). The larvae of *Belgica antarctica* (Jacobs) (Diptera: Chironomidae) produced more heat shock proteins than adults under temperature stress (Rinehart et al. 2006). Similarly, heat and cold tolerance of the developmental life stages of the larvae of *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) was confirmed to be associated with the upregulation of two heat shock proteins (HSP70 and HSP90) (Farahani et al. 2020). Thus, some life stages of *B. distincta* (instar 2 and females) could upregulate heat shock protein synthesis under stress better than other stages depending on the intensity of the temperature.

The lack of plastic response to RHH found here was also the case for *C. capitata and rosa* (Nyamukondiwa et al. 2010). This outcome may be owing to the time interval (2 h) used to induce rapid hardening for *B. distincta* life stages. Different time intervals and pre-exposure temperatures prior to heat knockdown have effects on survival (Marais et al. 2009; Nyamukondiwa et al. 2010; Pieterse et al. 2017). A short-time interval of 45 minutes successfully induced RHH of *Nysius groenlandicus* (Dallas) (Hemiptera: Lygaeidae) (Sørensen et al. 2019)*,* while a 2 h time interval and different temperatures resulted in poorer RHH response in fruit flies (Nyamukondiwa et al. 2010). Short-time intervals have also been shown to be beneficial in improving the survival of *Drosophila* (Hoffmann et al. 2003). Using the same time interval (2 h) and pre-exposure temperature (35 °C) as in the current study, RHH was successfully induced for the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Li et al. 2011). Therefore, this could mean that environmental temperatures of *B. distincta* should be considered when determining the pre-exposure temperature rather than using the time intervals of other insects from the literature.

The complex response of *B. distincta* life stages to RCH is evident in other taxa, with considerable variation between life stages (Lee et al. 2006; Terblanche et al. 2007). RCH has also decreased with age (Czajka and Lee 1990) and corresponded with what was observed for *B. distincta* life stages. In contrast, adults of *P. dreuxi* did not respond to RCH (Terblanche et al. 2007). Life stages undergo multiple stressors associated with seasonal changes during development, which explains the variable response to extreme temperatures (Chown and Terblanche 2007*).* While possible mechanisms for RCH remains poorly understood and disputed among authors, an increase in hemolymph osmolarities, changes in phospholipid composition, and production of cold-induced proteins and polyols play an essential role (Košt’ál et al. 2001) but vary between species (Chown and Terblanche 2007).

Overall, CT_{max} of *B. distincta* life stages was considerably higher than temperatures observed for RHH, while RCH was much lower (> 5 °C) than CT_{min}. CTLs and rapid hardening represent different aspects of the thermal tolerance indices (Terblanche et al. 2011). CTLs experiments took more than 2 h to complete, which could have given individuals enough time to harden, resulting in higher CT_{max} and may explain the significant variation between CTLs and rapid hardening. The ecological implications of the differential thermal tolerance of *B. distincta* to CTLs and rapid hardening require further investigation because the underlying mechanisms are still poorly understood. These results demonstrate the effects of temperature, developmental stage, and sex on thermal tolerance.

The methodological approach used may have affected the obtained results because no prior experiments (i.e., testing of different ramping rates, time intervals, and temperature for rapid hardening) were conducted due to the small size of the colony. Consequently, ramping rates for CTL and time intervals for rapid hardening assays were adopted from the literature. Importantly, *B. distincta* was reared on a host plant eliminating the effects of an alternative diet on the development (Dingha and Jackai 2017) and, ultimately, thermal tolerance.

Finally, there seems to be more plasticity in response to cold tolerance, particularly for instar 2 and adults. *B. distincta* life stages perform better in response to gradual increases than temperature decreases, but they perform worse when exposed to sudden increases in temperature. Thus, high mortality rates in *B. distincta* populations may be expected with sudden extreme high temperatures and gradual temperature decreases. In contrast, sudden temperature decreases could improve survival at even colder temperatures. In the field, *B. distincta* is exposed to temperatures that are close to their thermal tolerances estimates. The mean average temperature recorded from October 2018 to March 2022 was 20.48 °C with a maximum of 41.95 °C and a minimum of 1.1 °C. The highest and lowest CT_{max} recorded were 46.4 and 44.3 °C, and for CT_{min} were 4.1 and 0.6 °C, respectively. In hardening responses, *B. distincta* life stages survived 41 and -8 °C for 2 h. CTLs and rapid cold hardening estimates exceeded the maximum and minimum ambient temperatures inside and outside the macadamia orchards. In conclusion, this study shows that the thermal plastic traits of *B. distincta* life stages coupled with behavioural strategies may enable this pest to survive temperature changes associated with the ongoing climate change, with instar 2 and adults being temperature resistant and early life stages temperature sensitive.

CHAPTER 4

Climatic zones influence the turnover in generations of *Bathycoelia distincta* (Hemiptera: Pentatomidae) in the Levubu area, South Africa

Abstract

Understanding pest population dynamics requires knowledge of the thermal physiology of a species and its climatic conditions. Climate change affects insects physiologically and climatic conditions globally, with mountainous agricultural landscapes most threatened. The macadamia orchards near Levubu, Limpopo Province, South Africa are situated in different climatic zones along elevational gradients. Orchards in this area are prone to pest damage by the two-spotted stink bug, *Bathycoelia distincta* (Distant) (Hemiptera: Pentatomidae), a major pest of macadamia in South Africa. This study aims to quantify the effects of three climate zones namely, the Cwa **(**Monsoon-influenced humid subtropical climate), Cwb (Subtropical highland climate or Monsoon-influenced temperate oceanic climate), and Bsh (Hot semi-arid steppe climate) on generation turnover, heat and cold thermal stress, thermal safety margin (TSM), and relative fitness of *B. distincta* throughout an annual cycle. Four macadamia orchards were selected along an elevational gradient within the three climate zones (Arbor: Cwa at 705 m a.s.l, Welmac: Cwa/Bsh at 812 m a.s.l, Neuhof: Cwa/Cwb/Bsh at 856 m a.s.l, and Highfield: Cwb at 1493 m a.s.l). Overall, climate zones along the elevational gradient significantly impacted the populations of *B. distincta*. The number of generations decreased with increasing elevation: Arbor (2.4 generations), Welmac (2 generations), Neuhof (2.3 generations), and Highfield (1.1 generations). The model suggest that *B. distincta* populations were not thermally (heat and cold) stressed and encountered high TSM and relative fitness at Highfield throughout the season. Collectively, macadamia orchards at lower elevations in the Cwa climate zone will probably experience more damage because of the increased number of generations. The consequences of global warming could result in expanding the geographical range of *B. distincta* and pose management challenges.

Keywords: physiology, global warming, thermal safety margin, relative fitness, thermal stress

Introduction

The relative seasonal occurrence of pests is primarily driven by the climatic conditions (Kleynhans et al. 2018). Temperature among these climatic conditions strongly influences the ecology of organisms, especially that of ectotherms, because their body temperatures depend on the ambient temperature (Kingsolver and Buckley 2020). Knowledge of insect physiology in relation to their climatic conditions in agricultural landscapes is important for understanding pest distribution limits (Battisti and Larsson 2015). For example, *Rhagoletis mendax* (Curran) (Diptera: Tephritidae), cannot survive throughout the full range of its host *Vaccinium* species due to low winter temperatures in Canada (CFIA, 2014).

Identifying the climate zones in agricultural landscapes is important because climatic conditions can vary across short distances (Bouma 2003). The Köppen-Geiger is an extensively used climate classification that provides information on the climatic conditions of a region (Beck et al. 2018). Climates are grouped based on a combination of factors that track how conditions vary across different regions, such as mean monthly precipitation and air temperatures (Beck et al. 2018). The Köppen-Geiger classification has been acknowledged for its ecological relevance (Chen and Chen 2013). Current research has projected shifts and expansion of these zones with a negative influence on agricultural landscapes along elevational gradients (Wang et al. 2022).

Weather stations monitor daily conditions such as the minimum and maximum temperatures, relative humidity, rainfall, precipitation, etc (de Sá Júnior et al. 2012; Santos et al. 2021). This information can improve the management and predictions of insect pests because their body temperatures match that of the surrounding ambient temperatures (Azrag et al. 2018). The seasonal patterns of insect pests are correlated with temperature with low abundance associated with low temperature and limited resources (Scherrer et al. 2016). Understanding how local climates influences pest abundance through their thermal physiology can provide insight into their population dynamics. For example, the sugarcane pest, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) have more generations at warmer sites than colder sites, with higher relative fitness of the overwintering pupae at the colder sites (Kleynhans et al. 2018).

The geographical range of insects is primarily driven their thermal physiology because they survive within a specific range of temperatures (Roitberg and Mangel 2016). As a result of the current warming, the geographical range, abundance, and winter survival of insect pests are expected to increase (Kiritani 2006; Skendžić et al. 2021). However, the effects of warming will vary depending on the thermal physiology of a pest and its geographical location.

Deutsch et al. (2008) investigated the fitness effects of climate change around the optimal temperature (T_{opt}) of terrestrial insects relative to their latitudinal position. Their study concluded that if habitat temperatures (T_{hab}) rise above T_{opt} and nearer to insects' critical thermal maximum (CT_{max}: the upper limit of organism performance), the relative fitness of the organisms is likely to decrease. By contrast, insects living at low environmental temperatures, such as those in regions nearer the poles, are likely to experience increased fitness since an increase in T_{hab} will place them nearer to their T_{opt}. Insects living close to T_{opt}, such as those in sub-tropical areas, will perform worse if T_{hab} were to increase in their environment by decreasing the amount of buffer temperature between T_{hab} and CT_{max}. Thus, tropical insects could be more vulnerable to climate change-related warming while higher latitude species are likely to experience elevated fitness (Deutsch et al. 2008).

Thermal performance curves (TPCs) can be used to quantify the fitness of ectotherms in relation to their habitats and climate change (Huey and Slatkin 1976; Sinclair et al. 2016). The fitness of insect pests is important because it provides information on the ecological consequences of the environmental temperatures (Kleynhans et al. 2018). In addition, seasonal temperature variations have implications on the insect life cycle fitness (Kingsolver and Buckley 2020) which is relevant for understanding the changes in the pest's biology. Indeed, a good understanding of pest population dynamics in response to its environment regarding the primary physiology remains extremely important since fitness is declining in temperate zones due to climate change (Kingsolver et al. 2013).

The two-spotted stink, *Bathycoelia distincta* (Distant) (Hemiptera: Pentatomidae) is a dominant pest of macadamia in South Africa (Sonnekus et al. 2022) occupying different climate zones along elevational gradients near the Levubu area of the Limpopo Province, South Africa. The thermal physiology of *B. distincta* is well documented (Muluvhahothe et al. 2023, Chapter 2) and the climatic conditions of macadamia orchards in the Levubu area are monitored. This study aims to quantify the effects of three climatic zones along an elevational transect across four orchards on generation turnover, heat and cold thermal stress, thermal safety margin (TSM), and relative fitness of *B. distincta* for one season from August 2021 to August 2022.

Climatic conditions

Four macadamia orchards (Arbor at 705 m a.s.l, Welmac at 812 m a.s.l, Neuhof at 856 m a.s.l, and Highfield at 1493 m a.s.l) in the macadamia growing region of Levubu, along an elevation gradient and in different climate zones were selected near Levubu in the Vhembe district of the Limpopo Province, South Africa (Fig 4.1, Table 4.1). Arbor Boerdery orchards (23°4'25.38"S, 30°15'51.88"E) are situated in a dry-winter subtropical highland climate with noticeable hot summers (Cwa climate zone) (Beck et al. 2018). Welmac orchards (23°3'58.68"S, 30° 4'43.36"E) are situated in the Cwa climate zone and next to the Bsh climate zone with a hot semi-arid climate that sometimes has extremely hot summers and warm to cool winters (Beck et al. 2018). Neuhof orchards (23°3'59.02"S, 30°4'41.73"E) are situated within the Cwa climate zone, closer to the Bsh climate zone and the Cwb climate zone that experiences dry winters and warm summers. Highfield orchards are in the Cwb climate zone (Beck et al. 2018). The distance between the Arbor and Welmac orchard is thirteen kilometers, Welmac to Neuhof is nine kilometers, and Neuhof to Highfield is twenty kilometers. The minimum, mean, and maximum temperatures were calculated for one season (5th August 2021 to 5th August 2022) based on the hourly temperature data recorded on iLeaf weather stations (Hortec, Cape Town, South Africa) at each orchard (Table 4.1). Weather stations were less than 38 meters away from the nearest orchard.

Table 4.1 The climatic conditions of the four macadamia orchards in the Levubu macadamia growing region.

Orchard	Latitude, Longitude	Elevation (m 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
Welmac	-23.1186, 30.14623	812	Cwa/Bsh	9	3.2	19.3 ± 0.06	38.6
Neuhof	-23.0663, 30.07871	856	Cwa/Cwb/Bsh	20	4.5	20.0 ± 0.05	39.1
Highfield	-22.9863, 29.90229	1493	Cwb	-	2.8	17.1 ± 0.22	35.5

Cwa - Monsoon-influenced humid subtropical climate

Cwb - Subtropical highland climate or Monsoon-influenced temperate oceanic climate

Bsh - Hot semi-arid (steppe) climate

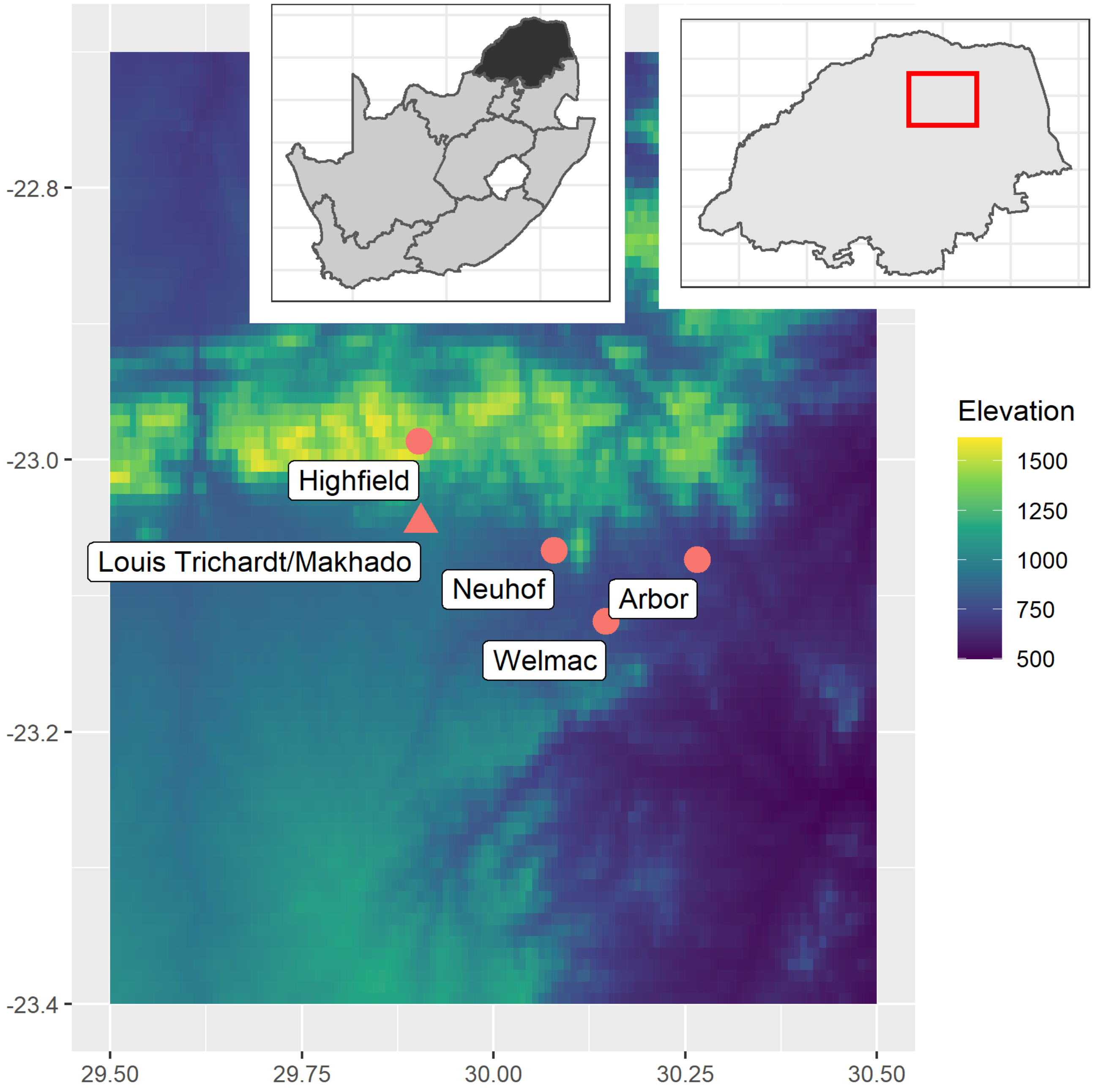

Figure 4.1 A topographical map of the four macadamia orchards near Levubu showing the elevational gradient.

Thermal requirements and physiological traits of B. distincta

Thermal requirements were obtained by quantifying the development of *B. distincta* (from the egg stage to adult emergence) at 5, 10, 15, 18, 20, 25, 30, 35, and 40 °C in controlled growth chambers. Two Thermocron iButtons (Semiconductor Corporation, Dallas/Maxin TX and USA) recorded temperature at hourly intervals within each chamber. The actual temperatures and relative humidity (% RH) were: 6.9 ± 0.1 °C and 31.1 ± 0.3 % RH, 11.0 ± 0.0 °C and 87.4 ± 0.0 % RH, 14.6 ± 0.0 °C and 25.8 ± 0.2 % RH, 18.4 ± 0.0 °C and 18.3 ± 0.1 % RH, 19.1 ± 0.1 °C and 60.7 ± 0.3 % RH, 25 ± 0.0 °C and 71.8 ± 0.1 % RH, 30.3 ± 0.0 °C and 32.1 ± 0.3 % RH, 34.8 ± 0.0 °C and 43.9 ± 0.1 % RH, and 39.5 ± 0.0 °C and 41.3 ± 0.6 % RH. The photoperiod was 16L: 8D in all incubators and macadamia nuts served as a food source. Freshly collected nuts were replaced twice weekly and rinsed thoroughly before feeding. Development was monitored daily by counting the total number of alive and dead individuals and those that transition into the next stages. Where high mortality of eggs occurred, newly emerged nymphs collected within 24 h (instar 1 to instar 5) were used to monitor further development. Developmental rates from egg to adult were included in the analysis of population turnover and climatic stress. The development rate, corresponding to the inverse of the number of days to complete a life stage (1/day), was fitted to all temperatures examined except at 5, 10, and 15 °C where no development was observed. The highest temperature (40 °C) was excluded because development from egg to adult was not observed (only instars 2 - 4 transitioned to the next stage) (Appendix A). A linear model was used to calculate the thermal constant (K) expressed in degree-days (DD) of *B. distincta* using the stage-specific lower developmental thresholds (T_{min}) obtained using the "nlsDR" DevRate function in R (Rebaudo et al. 2018) (Table 4.2). The linear model for egg to instar 5 was calculated as $DD = (T - T_{min}) \times D$. The thermal constant for the adults was calculated using the average longevity of females and males at 25 °C, as $DD = 25 - T_{\min} \times (average\ adult\ longevity)$ (Cullen and Zalom 2000). Developmental thresholds ($T_{min,}$ optimum : T_{opt}, and upper: T_{max}) were estimated from the non-linear models determined by the lowest AIC values (Table 4.2) (Rebaudo et al. 2018). The physiological traits were the critical thermal minimum (CT_{min}) and maximum (CT_{max}) temperatures obtained from Muluvhahothe et al. (2023) at 25 °C (Table 4.2). The CT_{min} and CT_{max} for eggs were assumed to be the same as those of instar 1, and those for the adults were calculated as the average CT_{min} and CT_{max} of females and males, respectively.

Table 4.2 Thermal requirements (DD: degree days, acc (DD): cumulative degree days, T_{min}: lower, T_{opt}: optimum, and T_{max}: upper developmental thresholds) and physiological traits (CT_{min}: critical thermal minimum and CTmax: critical thermal maximum) of *B.distincta* life stages for model simulations.

	Developmental stage						
Thermal requirements	Egg	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Adult
DD	75	20	90	62	77	104	305
Acc (DD)	75	95	185	246	323	427	732
T_{min}	14	14	15	15	15	15	18
T_{max}	39	37	44	38	44	44	38
T_{opt}	31	28	27	25	26	27	25
CT_{min}	3	3	3	1	2	2	3
CT_{max}	45	45	45	45	45	46	46

Phenology model setup and simulations

A process-based phenology model (Barton and Terblanche 2014) was used to determine the generation turnover, heat and cold stress, thermal safety margin (TSM), and relative fitness for one season, starting from the 5th August 2021 to 5th August 2022, for all the four macadamia orchards. The model was set to consolidate the weather station data, thermal requirements, and physiological traits to determine generation turnover, heat and cold thermal stress, TSM, and relative fitness. Thermal requirements and physiological traits (Table 4.1) were kept constant for the orchards. The ambient temperature was assumed to be similar to *B. distincta* life stages body temperature (T_b) for modeling population responses throughout the season. The model calculated the number of degree-days (DD) units obtained at each hourly time step according to the ambient temperature (T_b) for each life stage. The model was set to start accumulating DD for eggs assuming that the females lay eggs during the first week in August (Schoeman 2013). The model then used the ambient temperature to calculate the number of degree-days (DD) units at hourly intervals. At adult emergence, the model remained constant for returning to the egg stage by resetting the accumulated DD to zero to start another generation (Barton and Terblanche 2014). These were modelled throughout a 365-day simulation period giving an output of the generation turnover, heat and cold thermal stress, and adult fitness experienced by each life stage (Kleynhans et al. 2018).

Heat and cold thermal stress were calculated as the total number of hours in which the T_b increased above CT_{max} and decreased below CT_{min} at a threshold of five hours. Degree days (DD) units were calculated above T_{min} and below T_{max} for each life stage. TSM was calculated as $TSM = T_{opt} - T_b$, where T_{opt} is the stage-specific T_{opt} (Table 4.1), and T_b is body temperature. Relative fitness was calculated as an inverse of TSM as $Fitness = 1/TSM$. R statistical software (R core team, version 4.2.1, 2022) was used for all modeling.

Statistical analysis

Outliers were identified and removed using the Interquartile Range (IQR) method before analysis. The normality of model residuals was tested using the Shapiro-Wilk and Levene's tests for homogeneity of variance. The Rmisc R package calculated the means, standard deviation, standard error, and 95% confidence intervals (Hope 2022). The effect of climate zones on cold stress, TSM, and relative fitness was tested using the Kruskal-Wallis test. Comparisons between life stages were performed using Dunn's post-hoc where significant differences were determined on the alpha level = 0.05 (95% confidence interval) using the ggsignif (Ahlmann-Eltze, 2021) and ggstatsplot R packages (Patil 2021). Statistical analysis was not performed for the number of generations because the mean values calculated by Rmisc package did not represent the total number of generations. All analyses were done in R version 4.2.1 (R core team, version 4.2.1, 2022).

Results

Climate zones had a significant effect on the model predictions (TSM: $\chi^2_{\text{Kruskal-Wallis}}$ (3) = 78.8, $P < 0.0001$, and relative fitness: $\chi^2_{\text{Kruskal-Wallis}}$ (3) = 52.6, $P < 0.0001$) except for cold stress ($\chi^2_{\text{Kruskal-Wallis}}$ (3) = 3.7, $P = 0.4$) (Table 4.3). The number of generations decreased significantly from the Cwa (Arbor) to the Cwb climatic (Highfield) zone (Table 4.3). The number of generations decreased with increasing elevation from the Cwa to the Cwb climate zone, Arbor, 2.4 generations, Welmac, 2 generations and Neuhof, 2.3 generations, and Highfield, 1.1 generations (Fig 4.2A, Table 4.3). Arbor had significantly more instar transitions, followed by the Neuhof orchard (Fig 4.2B). *B. distincta* populations were not thermally (heat and cold) stressed throughout the season at any of the macadamia orchards (Table 4.2). TSM significantly peaked at Highfield and differed from Arbor, Welmac, and Neuhof. TSM did not differ significantly between Arbor, Welmac, and Neuhof (Fig 4.2C, Table 4.3). Relative fitness was significantly higher at Highfield and did not differ between Arbor and Welmac (Fig 4.2D, Table 4.3).

Table 4. 3 The effect of climate zones on generation turnover, heat stress, cold stress, TSM, and relative fitness of *B. distincta* from the model simulation. Mean values with different letters indicate significant differences between climate zones in columns ($P < 0.05$).

	Model predictions				
Orchard (climate zone)	Generations	Heat stress (h)	Cold stress (h)	TSM	Relative fitness
Arbor (Cwa)	2.4	0.00 ± 0.00	0.00 ± 0.00^{a}	7.55 ± 0.15^{a}	0.22 ± 0.02^{a}
Welmac (Cwa/Bsh)	2.0	0.00 ± 0.00	0.00 ± 0.00^{a}	7.35 ± 0.18^{a}	0.24 ± 0.03^{b}
Neuhof (Cwa/Cwb/Bsh)	2.3	0.00 ± 0.00	0.00 ± 0.00^{a}	7.70 ± 0.21^{a}	0.23 ± 0.02^{ab}
Highfield (Cwb)	1.1	0.00 ± 0.00	0.01 ± 0.01^{a}	10.01 ± 0.25^{b}	0.29 ± 0.04^{c}

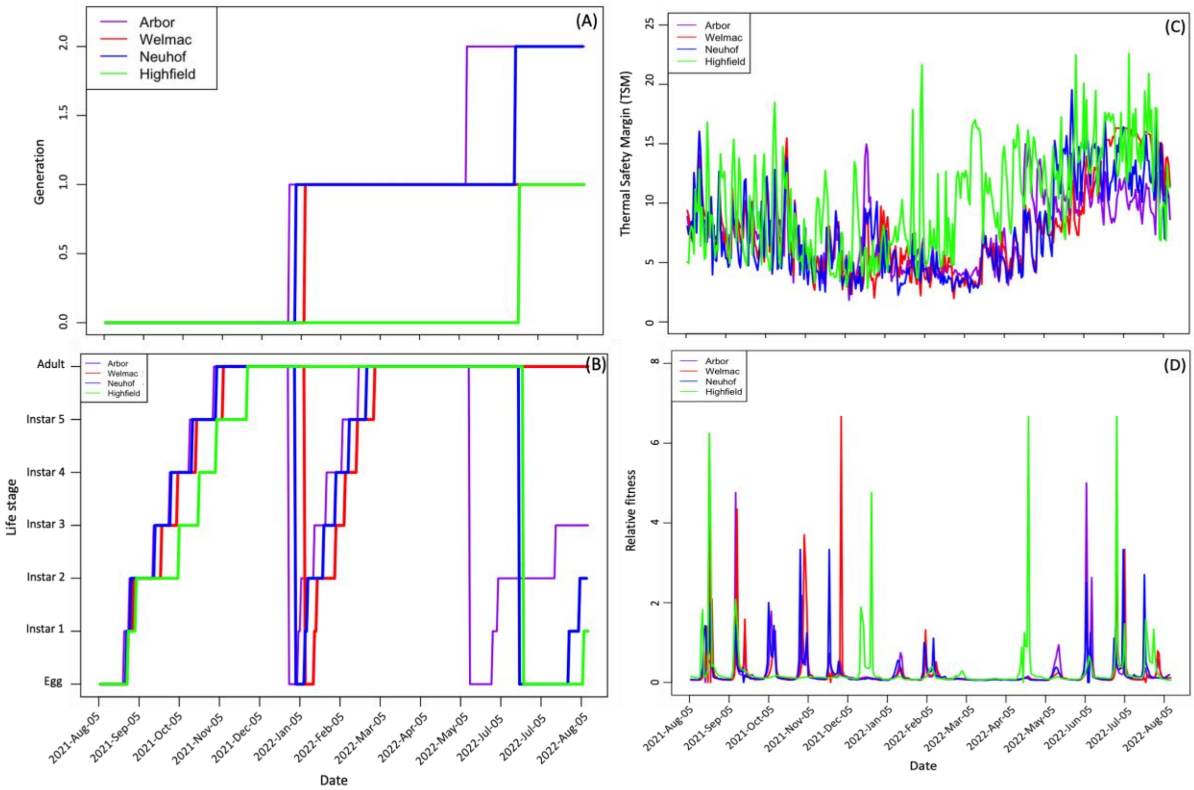

Figure 4.2 The effect of climate zones on the number of generations (A), life stage transitions (B), TSM (C), and relative fitness (D) for *B. distincta* in four macadamia orchards.

Discussion

The climate zone had a significant impact on the model predictions. The number of generations decreased with increasing elevation from 2.4 (Arbor) to 1.1 (Highfield). *B. distincta* was not heat, nor cold stressed throughout the season. The population TSM and relative fitness were highest at Highfield but decreased towards March 2022 in all orchards.

The decreased number of generations from the Cwa to the Cwb climate zone is due to the seasonal temperatures that decreased with increasing elevation. Seasonal number of generations may depend on the developmental rate of each life stage, with warmer temperatures speeding up development and cooler temperatures slowing it down (Reed et al. 2017). It is evident that temperature variations in the Cwa accelerate the development of *B. distincta* compared to those in the Cwb climatic zone. Low abundance of *B. distincta* would be expected at the Cwb climate zone because the Highfield orchard is situated at a higher elevation (1493 m a.s.l) where the temperatures are considerably lower compared to the orchards in the Cwa climate zone (705 to 856 m a.s.l). The mean temperatures at lower elevations ranged from 19.3 °C to 20 °C decreasing to 17.1 °C at the highest elevation. The difference of 0.7 °C in the mean annual temperature in the Cwa climate zone could be the reason why Welmac had 2 generations compared to the 2.4 and 2.3 generations of the Arbor and Neuhof respectively. According to Kiritani (2006), an increase of 1 °C in annual mean temperature is sufficient to increase the number of generations over a season (Kiritani 2006). The climate predictions in the Levubu area are projected to increase by 3 °C over the next fifty years. This implies that the number of generations could increase to approximately five generations at Arbor and two generations at Highfield.

The predicted number of generations of the major pest of *Antestiopsis thunbergii* (Leston) (Hemiptera: Pentatomidae) also decreased with increasing elevations from 1081 m a.s.l (3.2 generations) to 1705 m a.s.l (1.9 generations) along the Kilimanjaro transect, Tanzania (Azrag et al. 2018). The difference in the number of generations between these two stink bug species (*B. distincta* and *A. thunbergii*) may be due to their thermal adaptation and the methodology used to predict seasonal number of generations. The minimum temperatures ranged from 17.2 to 13.9 °C and mean maximum temperatures from 28.6 to 25.6 °C from the lowest (1081 m a.s.l) to the highest elevation (1705 m a.s.l) along the Kilimanjaro transect (Azrag et al. 2018). The mean temperatures of the current study ranged from 20 to 17.1 °C.

In addition, Azrag et al. (2018) estimated number of generations using a generation time (*T*), estimated at constant and fluctuating temperatures while the current study used the life stage specific degree-days (DD) estimated at constant temperatures. Under laboratory conditions, *A. thunbergii* completed a generation at 18 °C (Azrag et al. 2017), whereas *B. distincta* developed up to instar 2 (Appendix A) at the same temperature. This means that *A. thunbergii* is adapted to cooler environments than *B. distincta*.

The seasonal number of generations is an important parameter that influences pest abundance (Kiritani 2006). The abundance of *B. distincta* may be expected to be high in the Cwa climate zone. Arbor could be more susceptible to damage because all nymphal stages of *B. distincta* (except instar 1) can inflict damage to matured nuts. However, the damage intensity might be influenced by the size of the orchard because the heteropterans in macadamias have been shown to be low-density feeders with a current economic threshold of four stink bugs on 10 trees, representing 125 stink bugs that can cause damage at the phenological stage of the macadamia nut per hectare (Schoeman 2020). In relation to the Arbor's mean temperature (20 °C), *B. distincta* produced an average of 50 eggs per female at 21 °C (Chapter 2), suggesting fewer eggs at this temperature. Despite the limitations of the laboratory trials under constant temperatures, nut damage might decrease from Arbor to Highfield, depending on the management strategies and temperature in their respective climate zones.

Crop damage is expected to increase due to climate change (Deutsch et al. 2018), and this might be true for *B. distincta* because it's not stressed by the current heat and cold extremes across all climatic zones. In addition, the thermal biology of this pest shows that it can harden and acclimate in response to extreme temperatures (Muluvhahothe et al. 2023), develop and reproduce at temperatures ranging from 19 to 30 °C (Appendix A, chapter 2). Extreme weather events such as sudden heat waves and cold spells (Meehl and Tebaldi 2004; Palmer 2014) might positively influence *B. distincta* population because of its plastic traits. However, extremes' intensity and frequency affect insect growth (Roitberg and Mangel 2016), implying that the duration of extremes above the thresholds (five hours) might affect *B. distincta* life stages. In contrast, a global analysis of the seasonal abundance of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) showed that extreme temperatures determine the limits of development and survival and are more influential than mean temperatures (Ørsted et al. 2021). The latter study concluded that the abundance of *D. suzukii* can be reduced by temperatures above 25 °C.

Seasonal temperature variations shape the populations of *B.distincta* in the macadamia orchards*,* with low abundance in winter associated with food scarcity and high abundance in summer when nuts are developing (Schoeman 2018). Saulich and Musolin (2012) suggested that winter temperatures induce true diapause in stink bugs despite the food scarcity. The fact that orchards' extreme temperatures did not affect all *B. distincta* life stages suggest that it does not experience diapause but survives low temperatures and experience reproductive diapause (Schoeman 2013). This corroborates with the high number of instars recorded during the winter period in macadamia orchards in Nelspruit, South Africa (Schoeman 2013). Schoeman (2013) further highlighted that caged adults did not lay eggs between June and July, suggesting a possible reproductive diapause when food is scarce. The adults of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) have been reported to undergo reproductive diapause in temperate locations during the fall season in the United States (Nielsen et al. 2017).

Temperature is a primary constraint of seasonal abundance, with harsh winter extremes expected to slow the summer population build-up and hinder the population's maximum abundance of the next season (Panel et al. 2018). The latter is determined by the number of adults surviving the winter period (Dalton et al. 2011). The winter survival of *H. haly*s and *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) stink bug species is expected to increase by 15% for each 1 °C increase in Japan (Kiritani 2006). The thermal tolerance of *B. distincta* adults exceeded the minimum and maximum temperatures observed in each climatic zone, while females acclimated at lower temperatures (Muluvhahothe et al. 2023). *B. distincta* adults can therefore survive winter temperatures. The results reported by Schoeman (2013) for the seasonal *B. distincta* abundance are important in terms of instar winter survival. However, they are unreliable for adult survival because they have alternative food sources during winter (Fourie et al. 2022). Thus, the abundance of *B. distincta* adults in macadamia orchards during winter may depend on the diverse agricultural landscapes surrounding the orchards because complex landscapes reduce pest hotspots (Paredes et al. 2021).

High TSM of *B. distincta* might enable this pest to expand its geographical range because it is not living close to its optimal temperature (T_{opt}) and should experience increased fitness in response to warming. Deutsch et al. (2008) observed that the fitness of species living closer to their optimal performance temperatures (T_{opt}) will decrease with little warming and contrast with the improved fitness of species occupying habitats that are far from their optimal performance temperatures such as those at high latitudes or altitudes.

Overall, fitness in all orchards followed the same trend peaking from August to December 2021 and again from May to August 2022. The latter implies that *B. distincta* populations were fit during the whole season but fitter during nut development and post-harvest in all climatic zones. This would be expected as all the life stages are not living closer to their T_{opt}. Decreased fitness in summer is due to high temperatures as the rise closer to the T_{opt}. Similar to this study, the fitness of ectotherms in temperate regions decreased due to the increased frequency of heat stress in response to climate change (Kingsolver et al. 2013). In contrast, the fitness of *E. saccharina* increased in a warmer site in Mpumalanga compared to the colder site in Kwazulu-Natal, South Africa (Kleynhans et al. 2018). The temperature variations experienced by *B. distincta* in Limpopo, South Africa and *E. saccharina* adult moths in Mpumalanga, South Africa are likely to be the reason for the fitness differences. Nevertheless, Kleynhans et al. (2018) also provided strong evidence of population fitness at the cold site in Kwazulu-Natal which corroborates with the fitness of *B. distincta* at cold temperatures. These results imply that *B. distincta* remains active throughout the season with improved performance during the period of food scarcity, which could favour its population persistence.

In conclusion, the climatic zones interact with elevation to shape the seasonal population dynamics of *B. distincta* in the Levubu area. Macadamia orchards in the Cwa climate zone and those situated at lower elevations may be prone to damage as the number of generations increased with decreasing elevation. Global warming could increase the number of generations and abundance of this pest while negatively influencing fitness during summer as temperatures often exceed their upper thermal limits. Orchards in and near the Bsh climate zone such as the Neuhof might be the most affected as warming is expanding the hot-dry zones of agricultural landscapes along elevational gradients (Wang et al. 2022). At a large scale, the physiological traits of *B. distincta* life stage can be used to model the future suitable habitats and distribution across all macadamia growing regions in South Africa using climatic data under future climate scenarios. This will help identify the geographical limits of *B. distincta* and regions that may be at risk of outbreaks as the climate changes.

Appendices

Appendix A: Mean developmental duration (days, mean ± SE) of *B. distincta* at seven constant temperatures. Different letters indicate columns indicate significant differences between temperatures (Kruskal-Wallis, P > 0.05).

	Developmental stage						
Temp (°C)	**Egg**	**Instar 1**	**Instar 2**	**Instar 3**	**Instar 4**	**Instar 5**	**Egg to adult**
15	-	-	-	-	-	-	-
18	15.5	-	-	-	-	-	-
19	9.56 ± 0.20^{a}	8.93 ± 0.56^{a}	15.9 ± 0.88^{a}	10.9 ± 0.85^{a}	11.0 ± 0.80^{a}	18.0 ± 1.99^{a}	74
25	6.20 ± 0.16^{b}	5.30 ± 0.15^{ab}	7.60 ± 0.36^{a}	6.10 ± 0.32^{a}	6.80 ± 0.11^{a}	10.5 ± 0.26^{a}	42
30	4.00 ±0.00^{bc}	3.72 ± 0.09^{b}	7.53 ± 0.38^{a}	8.59 ± 1.22^{a}	8.90 ± 1.76^{a}	11.7 ± 1.42^{a}	39
35	4.00 ± 0.00^{c}	3.7	-	-	-	-	-
40	-	-	-	-	-	-	-

Appendix B: The minimum, mean (mean ± SE), maximum temperatures (°C), and relative humidity (%RH) for a period of 5 years (October 2018 to February 2023) in the selected macadamia orchards obtained from the iLeaf weather stations.

Climatic zones (Orchard)	Minimum temp (°C)	Mean temp (°C)	Maximum temp (°C)	Mean Relative Humidity (%)
Cwa (Arbor)	1.7.	20.7 ± 0.03	42.7	77.2 ± 0.11
Cwa/Bsh (Welmac)	-0.3	20.0 ± 0.03	42.0	65.2 ± 0.11
Cwa/Cwb/Bsh (Neuhof)	1.1	20.4 ± 0.03	42.0	65.9 ± 0.11
Cwb (Highfield)	2.8	17.3 ± 0.05	41.5	64.9 ± 0.22

CHAPTER 5

Conclusions and recommendations

Here, for the first time, the effect of temperature on the survival and development of the two-spotted stink bug, *Bathycoelia distincta,* a major pest of macadamia in South Africa, was determined, using laboratory-based assays. The development of integrated pest management (IPM) requires an understanding of the thermal biology of this species. Specifically, this work investigated three interconnected topics: the thermal biology of *B. distincta* (Chapter 2): thermal developmental temperatures that allowed it to complete its development, mate, and reproduce. Phenotypic plasticity (Chapter 3): the ability to harden and acclimate in response to extreme temperatures; and climate-related population dynamics (Chapter 4): explored the implication of its thermal biology for population turnover and fitness across climate zones. The work developed a mechanistic understanding of the role of temperature in the population dynamics of *B. distincta* and applied it within a predictive framework that could inform integrated pest management.

The results showed a monotonic increase for intrinsic capacity of increase (population growth) from 19 to 29 °C with a peak of the net reproductive rate at 25 °C. Five-year annual mean temperatures of the macadamia orchards ranged from 17 (Highfield) to 21 °C (Arbor) in the Levubu area. Temperature in this area is projected to increase by 3 °C over the next fifty years. It is possible that the populations of *B. distincta* will increase in response to climate change and thereby increasing nut damage. A peak of the net reproductive rate at 25 °C would result in outbreaks because females will produce more offspring despite the population growth rate. The population increase and abundance of *B. distincta* will also be enhanced by its life-stage specific high survival rates (51 - 100 %) across different temperatures. However, this may be affected by predators, diseases (Daane et al. 2022) and heavy rainfalls in natural environments. In addition, the population growth and survival rates were estimated at constant temperatures, which do not entirely reflect the fluctuation nature of temperature in natural habitats. Population growth rates of stink bug species have been largely estimated using constant temperatures (Nielsen et al. 2008; Yazdanpanah et al. 2019; Govindan and Hutchison 2020), but new findings on the importance of extreme temperatures in determining species abundance (Ørsted et al. 2021) suggest a need for considering fluctuating temperatures (Khelifa et al. 2019).

Daily extreme temperatures influence the activity of insects because the ambient temperature dictates their metabolic rate. In the case of pests, these temperatures can result in increased crop damage due to high consumption rates (Skendžić et al. 2021). *B. distincta* life stages were thermally plastic in response to extreme temperatures over short time periods and gradual increase/decrease of temperatures. Extremes temperatures above thermal limits may pose serious threats to insects' survival and performance, but *B. distincta* appeared not to be thermal stressed at Arbor, Welmac, Neuhof and Highfield across all climatic zones. A thermally stressed pest may not be able to cause as much damage as one that is not stressed. Thus, the current heat waves and cold snaps associated with climate change may instead increase nut damage through the high consumption rate of *B. distincta* life stages. The findings of this study also suggest that *B. distincta* may be adapted to warmer environments, although they are not living closer to their thermal optimum temperatures. However, the mean average temperature of all the farms investigated was less than 22 °C. The current thermal conditions of *B. distincta* explain the egg's lower developmental threshold of 14 °C. However, eggs did develop at 15 °C in the laboratory, which could be attributed to a low relative humidity (26 %) compared to the actual relative humidity they experienced in the orchards (Welmac: 65 % to Highfield: 72 %).

The plastic response of *B. distincta* life stages seemed to resemble their thermoregulatory behaviour in natural environments. (i) Instar 1 was the most temperature-sensitive life stage. When the egg hatches, this stage aggregate around the empty eggshells and do not feed. This behaviour has been shown to improve their survival (McPherson and McPherson 2000) although immobile to less mobile life stages are thought to be more thermally plastic because they have limited ways of regulating their body temperatures behaviourally. As this might not be true for *B. distincta* instar 1, placing each of them individually in vials during the thermal tolerance experiments could have negatively impacted their survival. This suggest that thermal tolerance experiments of instar 1 must not be investigated individually. (ii) Instar 2 was the most thermally plastic life stage. *B. distincta* starts feeding at this stage, meaning it might require thermal energy to search for macadamia nuts around the tree. This explains could explain why it is the most darkly pigmented life stage because dark color absorbs more heat. (iii) Female adults showed plastic response especially to low acclimation temperatures. It was hypothesized that females return to macadamia orchards first before males searching for oviposition sites after winter.

As mentioned previously that most eggs are found in more denser trees, this plastic trait of females might be useful when they search for oviposition sites because they have been reported to start laying eggs in the first week of August (Schoeman 2013) when temperatures are still low. Plastic traits of the latter stages (especially instar 2 and females) may aid to *B. distincta* abundance in response to climate change because feeding and reproducing are key factors of species persistence. Instar 2 and instar 4 were the only life stages that developed faster on the sweetcorn diet compared to its host plant. This means that *B. distincta* can potential be a pest of concern in other crops with fast development facilitated by the quick response of instar 2 to alternative diets and extreme temperature.

B. distincta is not living closer to its thermal optimum temperatures (Chapter 2) and its genetic diversity is not restricted to macadamia orchards only (Pal et al. 2022) and its currently living far from its thermal optimum temperatures. This pest may be able to expand its geographical range with the expansion of macadamia plantation in South Africa. The ability of *B. distincta* life stages to withstand an extremely high temperature of 41 °C for 2 hours (Muluvhahothe et al. 2023) can enable it to survive at different thermal habitats and enhance geographical expansion in response to climate change. In addition, climate change is projected to expand the certain climate zones, such as the Bsh zone (Wang et al. 2022). Chapter 4 revealed that macadamia orchards in the Cwa climate zone (Monsoon-influenced humid subtropical climate) at lower elevations will have increased number of generations compared to those at higher elevations. Due to climate change, warmer climate zones may become hotter while cooler zones become warmer. This will also cause outbreaks in the climate zones such as the Cwa and pose management challenges in the absence of effective IPM, such as cover crops. In addition, the priority of IPM should focus on the Cwa and Bsh climate zones because increased generations will require continuous chemical application, ultimately reducing productivity through poor soil quality and declining beneficial insect populations.

Modelling populations dynamics in relation to their on thermal physiological traits could be an important step towards effective management strategies of *B. distincta*. First, the degree-day (DD) models are important in forecasting phenological events such as oviposition time, adult emergence, and number of generations that may occur during the seasons. Scouting prior chemical application is an adapted strategy to identify the pest status in the orchards. This process is timing consuming and may not be effective in big macadamia orchards. Thus, the DD model can be used to confirm scouting data when the adults return to the orchards and apply chemicals based on its estimations throughout the growing season.

This will be especially important in timing chemical application in the face of climate change because *B. distincta* develop faster with shorter generation lengths at higher temperatures. Estimating DD requires lower and upper developmental threshold temperatures, which were quantified in the current study. Macadamia growers can use the DD model based on their climatic conditions to accurately predict the phenology of *B. distincta* for chemical applications as temperature can differ at short distances.

Future studies should focus on (i) modelling the life-stage specific thermal physiological traits across all macadamia growing regions in South Africa for current and potential distribution limits of *B. distincta*, (ii) mechanism underlying the plastic traits of instar 2, and (iii) developmental rates at fluctuating temperatures.

References

Ahlmann-Eltze, C. and Patil, I., 2021. ggsignif: R package for displaying significance brackets for 'ggplot2'.

Alford, L., Andrade, T.O., Georges, R., Burel, F. and Van Baaren, J., 2014. Could behaviour and not physiological thermal tolerance determine winter survival of aphids in cereal fields?. *Plos One*, 9(12), 114982.

Azrag, A.G. A., Murungi, L.K., Tonnang, H.E.Z., Mwenda, D., Rabin, R., 2017. Temperature-dependent models of development and survival of an insect pest of African tropical highlands, the Coffee Antestia Bug *Antestiopsis Thunbergii* (Hemiptera: Pentatomidae). *Journal of Thermal Biology* 70: 27-36.

Azrag, A.G., Pirk, C.W., Yusuf, A.A., Pinard, F., Niassy, S., Mosomtai, G. and Babin, R., 2018. Prediction of insect pest distribution as influenced by elevation: Combining field observations and temperature-dependent development models for the coffee stink bug, *Antestiopsis thunbergii* (Gmelin). *Plos One*, 13(6), 0199569.

Bale, J.S., 2002. Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1423), 849-862.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., and Good, J.E., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores: rising temperature and insect herbivores. *Global Change Biology* 8(1): 1-16.

Baudier, K., Mudd, A., Erickson, S., and ODonnell, S., 2015. Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). *Journal of Animal Ecology*, 84(5), 1322-1330.

Barton, M.G., and Terblanche, J.S., 2014. Predicting performance and survival across topographically heterogeneous landscapes: the global pest insect *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: N octuidae). *Austral Entomology*, 53(3), 249-258.

Battisti, A., and Larsson, S., 2015. Climate change and insect pest distribution range. In Climate change and insect pests. Wallingford UK: *CABI*, 1-15.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., and Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data*, *5*(1), 1-12.

Birch, L.C., 1948. The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology* 17(1): 15-26.

Bouma, E., 2003. GEWIS, a weather-based decision support system for timing the application of plant protection products. *EPPO Bulletin* 33(3): 483–87.

Bowler, K., and Terblanche, J., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence?. *Biological Reviews*, 83(3), 339-355.

Braschler, B., Chown, S., and Duffy, G., 2021. Sub-critical limits are viable alternatives to critical thermal limits. *Journal of Thermal Biology*, 101, p.103106.

Bruwer, I.J., Giliomee, J.H. and Pringle, K.L., 2021. The relationship between proboscis length and the ability of certain Heteroptera to damage macadamia kernels. *African Entomology*, *29*(1), 112-124.

Campbell, A., Frazer, B.D., Gilbert, N.G.A.P., Gutierrez, A.P. and Mackauer, M., 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* 11(2): 431-38.

Carey, J.R., 2001. Insect biodemography. *Annual Review of Entomology* 46(1): 79-110.

Carrillo, W., Carpio, C., Morales, D., Vilcacundo, E. and Alvarez, M., 2017. Fatty acids composition in macadamia seed oil (Macadamia integrifolia) from Ecuador. *Asian Journal of Pharmaceutical and Clinical Research*, 10, 303-306.

CFIA., 2014. RMD-14-12: Revision of the geographic boundaries of the regulated areas for the blueberry maggot Rhagoletis mendax Curran in the province of Ontario. http://www.inspection.gc.ca/plants/plantpests-invasive-species/directives/risk-management/rmd-14-02/eng/1406731989341/1406732109440 [accessed on 15 February 2023 2023]

Chanthy, P., Martin, R., Gunning, R. and Andrew, N., 2012. The effects of thermal acclimation on lethal temperatures and critical thermal limits in the green vegetable bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). *Frontiers in Physiology*, 3.

Chanthy, P., Robert, M., Robin G., and Nigel A., 2015. influence of temperature and humidity regimes on the developmental stages of green vegetable bug, *Nezara Viridula* (L.) (Hemiptera: Pentatomidae) from inland and coastal populations in Australia. *General and Applied Entomology* 43, 37-55.

Chen, D., and Chen, H.W., 2013. Using the Köppen classification to quantify climate variation and change: an example for 1901–2010. *Environmental Development,* 6, 69-79.

Chidawanyika, F. and Terblanche, J.S., 2011. Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evolutionary Applications*, 4(4), 534-544.

Chidawanyika, F., Nyamukondiwa, C., Strathie, L. and Fischer, K., 2017. Effects of thermal regimes, starvation and age on heat tolerance of the parthenium beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) following Dynamic and Static Protocols. *Plos One*, 12(1), p.e0169371.

Chown, S. L., Sørensen, J. G., and Terblanche, J. S., 2011. Water loss in insects: an environmental change perspective. *Journal of Insect Physiology*, 57(8), 1070-1084.

Chown, S.L. and Terblanche, J.S., 2006. Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology*, 33, 50-152.

Chown, S.L., and Nicolson, S., 2004. *Insect physiological ecology: mechanisms and patterns*. Oxford University Press.

Chown, S.L., Jumbam, K.R., Sørensen, J.G. and Terblanche, J.S., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology*, 23(1), 133-140.

Chown, S.L., Sørensen, J.G., and Terblanche, J.S., 2011. Water loss in insects: an environmental change perspective. *Journal of insect physiology*, 57(8), 1070-1084.

Chown, S.L., and Nicolson, S., 2004. *Insect physiological ecology: mechanisms and patterns*. Oxford University Press.

Cira, T.M., Koch, R.L., Burkness, E.C., Hutchison, W.D., and Venette, R.C., 2018. Effects of diapause on *Halyomorpha halys* (Hemiptera: Pentatomidae) cold tolerance. *Environmental Entomology*, 47(4), 997-1004.

Cook, R.D., 1977. Detection of influential observation in linear regression. *Techno metrics*, 19(1), 15-18.

Coulin, C., de la Vega, G.J., Chifflet, L., Calcaterra, L.A., and Schilman, P.E., 2019. Linking thermo-tolerances of the highly invasive ant, Wasmannia Auropunctata, to its current and potential distribution. *Biological Invasions*, 21, 3491-3504.

Cullen, E.M. and Zalom, F.G., 2000. Phenology-based field monitoring for consperse stink bug (Hemiptera: Pentatomidae) in processing tomatoes. *Environmental Entomology*, 29(3), 560-567.

Czajka, M.C., and Lee, R.E., 1990. A rapid cold-hardening response protecting against cold shock injury in Drosophila melanogaster. *Journal of Experimental Biology*, 148(1), 245-254.

Da Silva, P.G., and Daane, K.M., 2014. Life history parameters of *Chinavia Hilaris* (Hemiptera: Pentatomidae), a stink bug injurious to pistachios in California. *Journal of Economic Entomology*, 107(1): 166-73.

Daane, K.M., da Silva, P.G., Stahl, J.M., Scaccini, D., and Wang, X., 2022. Comparative life history parameters of three stink bug pest species ed. Silvia Rondon. *Environmental Entomology*,51(2): 430-39.

Dalton, D.T., Walton, V.M., Shearer, P.W., Walsh, D.B., Caprile, J. and Isaacs, R., 2011. Laboratory survival of Drosophila suzukii under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. *Pest Management Science*, 67(11), 1368-1374.

Damos, P.T., and Savopoulou-Soultani, M., 2008. Temperature-dependent bionomics and modeling of *Anarsia Lineatella* (Lepidoptera: Gelechiidae) in the Laboratory. *Journal of Economic Entomology*, 101(5): 1557-67.

Dara, S.K., 2019. The new integrated pest management paradigm for the modern age. *Journal of Integrated Pest Management*, 10(1), 12.

de Matos, S.T.S., dos Santos-Cividanes, T.M., Cividanes, F.J., dos Santos, L.D.C., Suguino, E., and Ribeiro, A.A., 2019. First record of lepidopteran borers in macadamia nuts in Brazil. *Florida Entomologist*, 102(3), 649-650.

de Sá Júnior, A., de Carvalho, L.G., Da Silva, F.F. and de Carvalho Alves, M., 2012. Application of the Köppen classification for climatic zoning in the state of Minas Gerais, Brazil. *Theoretical and Applied Climatology*, 108, 1-7.

Deere, J.A. and Chown, S.L., 2006. Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *The American Naturalist*, 168(5), 630-644.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. and Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across Latitude. *Proceedings of the National Academy of Sciences,* 105(18): 6668-72.

Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., and Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate. *Science*, 361(6405): 916-19.

DeWitt, T.J., and Scheiner, S.M. Eds., 2004. Phenotypic plasticity: functional and conceptual approaches. *Oxford University Press.*

Dhaliwal, G.S., Jindal, V. and Dhawan, A.K., 2010. Insect pest problems and crop losses: changing trends. *Indian Journal of Ecology*, 37(1), 1-7.

Dhaliwal, G.S., Arora, R., and Dhawan, A.K., 2004. Crop losses due to insect pests in Indian agriculture: an update. *Indian Journal of Ecology*, 31(1), 1-7.

Dhillon, M.K., and Hasan, F., 2017. Temperature-dependent development of diapausing larvae of *Chilo Partellus* (Swinhoe) (Lepidoptera: Crambidae). *Journal of Thermal Biology,* 69: 213-20.

Diffenbaugh, N.S., Krupke, C.H., White, M.A., and Alexander, C.E., 2008. Global warming presents new challenges for maize pest management. *Environmental Research Letters*, 3(4), p.044007.

Diffenbaugh, N.S., Pal, J.S., Trapp, R.J., and Giorgi, F., 2005. Fine-scale processes regulate the response of extreme events to global climate change. *Proceedings of the National Academy of Sciences*, 102(44), 15774-15778.

Dingha, B.N., and Jackai, L.E., 2017. Laboratory rearing of the brown marmorated stink bug (Hemiptera: Pentatomidae) and the impact of single and combination of food substrates on development and survival. *The Canadian Entomologist*, 149(1), 104-117.

Escribano-Álvarez, P., Pertierra, L.R., Martínez, B., Chown, S.L. and Olalla-Tárraga, M.Á., 2022. Half a century of thermal tolerance studies in springtails (Collembola): A review of metrics, spatial and temporal trends. *Current Research in Insect Science*, *2*, p.100023.

Fand, B.B., Kamble, A.L., and Kumar, M., 2012. Will climate change pose serious threat to crop pest management: A critical review. *International journal of scientific and Research publications*, *2*(11), 1-14.

Farahani, S., Bandani, A.R., Alizadeh, H., Goldansaz, S.H., and Whyard, S., 2020. Differential expression of heat shock proteins and antioxidant enzymes in response to temperature, starvation, and parasitism in the Carob moth larvae, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). *Plos One*, *15*(1), p.e0228104.

Fasolo, A. G., and Krebs, R. A., 2004. A comparison of behavioural change in Drosophila during exposure to thermal stress. *Biological Journal of the Linnean Society*, 83(2), 197-205.

Fernandez, D., Millán, A., Rizzo, V., Comas, J., Lleopard, E., Pastor, J., Pallarés, S., Abellán, P., Spada, M., Bilton, D. and Ribera, I., 2018, February. The CAVEheAT project: climate change, thermal niche and conservation of subterranean biodiversity. In *ARPHA Conference Abstracts* (Vol. 1, p. e30105). Pensoft Publishers.

Fisher, J.J., Rijal, J.P., and Zalom, F.G., 2021. Temperature and humidity interact to influence brown marmorated stink bug (Hemiptera: Pentatomidae), survival. *Environmental Entomology*, 50(2), 390-398.

Fourie, A., Venter, S.N., Slippers, B., and Fourie, G., 2022. A detection assay to identify alternative food sources of the two-spotted stink bug, *Bathycoelia distincta* (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, 115(2), 519-525.

Funayama, K., 2006. A new rearing method using carrots as food for the brown-marmorated stink bug, *Halyomorpha Halys* (Stål) (Heteroptera: Pentatomidae). *Applied Entomology and Zoology*, 41(3): 415-18.

Gabre, R.M., Adham, F.K., and Chi, H., 2005. Life table of *Chrysomya Megacephala* (Fabricius) (Diptera: Calliphoridae). *Acta Oecologica* 27(3): 179-83.

Geerinck, M.W., Van Hee, S., Gloder, G., Crauwels, S., Colazza, S., Jacquemyn, H., Cusumano, A., and Lievens, B., 2022. Diversity and composition of the microbiome associated with eggs of the southern green stinkbug, *Nezara Viridula* (Hemiptera: Pentatomidae). *Microbiology Open*, 11(6): e1337.

Geng, S., and Jung, C., 2018. Temperature-dependent development of immature *Phyllonorycter Ringoniella* (Lepidoptera: Gracillariidae) and its stage transition models. *Journal of Economic Entomology*, 111(4): 1813-23.

Golden, M., Follett, P.A. and Wright, M.G., 2006. Assessing *Nezara viridula* (Hemiptera: Pentatomidae) feeding damage in macadamia nuts by using a biological stain. *Journal of economic entomology*, 99(3), 822-827.

Govindan, B.N., and Hutchison, W.D., 2020. Influence of temperature on age-stage, two-sex life tables for a minnesota-acclimated population of the brown marmorated stink bug (*Halyomorpha Halys). Insects*, 11(2): 108.

Grant, B. W., and Porter, W. P., 1992. Modeling global macroclimatic constraints on ectotherm energy budgets. *American Zoologist*, 32(2), 154-178.

Gray, E.M., 2013. Thermal acclimation in a complex life cycle: the effects of larval and adult thermal conditions on metabolic rate and heat resistance in *Culex pipiens* (Diptera: Culicidae). *Journal of Insect Physiology*, 59(10), 1001-1007.

Grigaltchik, V.S., Ward, A.J., and Seebacher, F., 2012. Thermal acclimation of interactions: differential responses to temperature change alter predator–prey relationship. *Proceedings of the Royal Society B: Biological Sciences*, 279(1744), 4058-4064.

Hagstrum, D.W., and Milliken, G.A., 1988. Quantitative analysis of temperature, moisture, and diet factors affecting insect development. *Annals of the Entomological Society of America*, 81(4): 539–46.

Hallman, G.J., and Denlinger, D.L., 2019. *Temperature sensitivity in insects and application in integrated pest management*. CRC Press.

Harcourt, D.G., and Yee, J.M., 1982. Polynomial algorithm for predicting the duration of insect life stages 1. *Environmental Entomology*, 11(3): 581-84.

Harvey, J.A., Heinen, R., Gols, R., and Thakur, M.P., 2020. Climate change-mediated temperature extremes and insects: From outbreaks to breakdowns. *Global change biology*, 26(12), 6685-6701.

Herms, D.A., 2004. Using degree-days and plant phenology to predict pest activity. In *IPM (integrated pest management) of midwest landscapes* (Vol. 58, 49-59). St. Paul, MN: Minnesota Agricultural *Experiment Station Publication*

Herrera, A.M., Dahlsten, D.D., Tomic-Carruthers, N., and Carruthers, R.I., 2005. Estimating temperature-dependent developmental rates of *Diorhabda Elongata* (Coleoptera: Chrysomelidae), a biological control *Agent of Saltcedar* (Tamarix Spp.). *Environmental Entomology,* 34(4): 775-84.

Hoffmann, A. A., Hallas, R., Sinclair, C., and Mitrovski, P. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution*, 55(8), 1621-1630.

Hoffmann, A.A., Sørensen, J.G. and Loeschcke, V., 2003. Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. *Journal of thermal Biology*, 28(3), 175-216.

Hofmann, G.E., and Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Annual review of physiology*, 72, 127-145.

Hope, R.M., 2022. _Rmisc: Ryan Miscellaneous_. R package version 1.5.1, https://CRAN.R-project.org/package=Rmisc.

Horne, C.R., Hirst, A.G., Atkinson, D., Almeda, R., and Kiørboe, T., 2019. Rapid shifts in the thermal sensitivity of growth but not development rate causes temperature–size response variability during ontogeny in arthropods. *Oikos*, 128(6), 823-835.

Hu, W., Fitzgerald, M., Topp, B., Alam, M., and OHare, T.J., 2019. A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. *Journal of Functional Foods*, 62, p.103520.

Huey, R.B., and Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *The Quarterly review of biology*, 51(3), 363-384.

Huey, R.B., and Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*, 19(1), 357-366.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F.E., Poehling, H.M. and Borgemeister, C., 2009. Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *Plos One*, 4(8), p.e6487.

Jarošík, V., Honěk, A., and Dixon, A.F.G., 2002. Developmental Rate Isomorphy in Insects and Mites. *The American Naturalist*, 160(4): 497-510.

Jaworski, T., and Hilszczański, J., 2013. The effect of temperature and humidity changes on insects development their impact on forest ecosystems in the context of expected climate change.

Johnson, D.J., and Stahlschmidt, Z.R., 2020. City limits: Heat tolerance is influenced by body size and hydration state in an urban ant community. *Ecology and Evolution*, 10(11), 4944-4955.

Jones, V.P., 2002. Macadamia integrated pest management: IPM of insects and mites attacking macadamia nuts in Hawaii. University of Hawaii.

Jumbam, K.R., Jackson, S., Terblanche, J.S., McGeoch, M.A. and Chown, S.L., 2008. Acclimation effects on critical and lethal thermal limits of workers of the Argentine ant, *Linepithema humile*. *Journal of Insect Physiology*, 54(6), 1008-1014.

Käfer, H., Kovac, H., Simov, N., Battisti, A., Erregger, B., Schmidt, A.K., and Stabentheiner, A., 2020. Temperature tolerance and thermal environment of European seed bugs. *Insects*, 11(3), p.197.

Karuppuchamy, P. and Venugopal, S., 2016. Integrated pest management. In Ecofriendly pest management for food security. *Academic Press,* 651-684.

Kaspari, M., Alonso, L. and ODonnellkwd, S., 2000. Three energy variables predict ant abundance at a geographical scale. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1442), 485-489.

Kawate, M.K. and Tarutani, C.M., 2004, Pest Management Strategic Plan for Macadamia Nut Production in Hawaii. In *Workshop Summary*.

Kerr, N.Z., Wepprich, T., Grevstad, F.S., Dopman, E.B., Chew, F.S., and Crone, E.E., 2020. Developmental trap or demographic bonanza? Opposing consequences of earlier phenology in a changing climate for a multivoltine butterfly. *Global Change Biology*, 26(4), 2014-2027.

Khelifa, R., Blanckenhorn, W.U., Roy, J., Rohner, P.T., and Mahdjoub, H., 2019. Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. *Journal of Animal Ecology*, 88(12), 1901-1912.

Khun, K.K., Ash, G.J., Stevens, M.M., Huwer, R.K., and Wilson, B.A., 2021. Transmission of *Metarhizium anisopliae* and *Beauveria bassiana* to adults of *Kuschelorhynchus macadamiae* (Coleoptera: Curculionidae) from infected adults and conidiated cadavers. *Scientific Reports*, 11(1), 2188.

Kingsolver, J.G., and Huey, R., 2008. Size, temperature, and fitness: three rules. *Evolutionary ecology research*, 10(2), 251-268.

Kingsolver, J.G., and Buckley, L.B., 2020. Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Current opinion in insect science*, 41, 17-24.

Kingsolver, J.G., Diamond, S.E., and Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, 27(6), 1415-1423.

Kiritani, K., 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Population Ecology*, 48(1), 5-12.

Kleynhans, E., Barton, M.G., Conlong, D.E., and Terblanche, J.S., 2018. Population dynamics of *Eldana saccharina* Walker (Lepidoptera: Pyralidae): application of a biophysical model to understand phenological variation in an agricultural pest. *Bulletin of Entomological Research*, 108(3), 283-294.

Kleynhans, E., Conlong, D.E., and Terblanche, J.S., 2014. Host plant-related variation in thermal tolerance of *Eldana saccharina*. *Entomologia Experimentalis et Applicata*, 150(2), 113-122.

Klockmann, M., Günter, F., and Fischer, K., 2017. Heat resistance throughout ontogeny: body size constrains thermal tolerance. *Global Change Biology*, 23(2), 686-696.

Klok, C.J., and Chown, S.L., 1997. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). *Journal of Insect Physiology*, 43(7), 685-694.

Klok, C.J., and Chown, S.L., 2001. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology*, 47(1), 95-109.

Kontodimas, D.C., Eliopoulos, P.A., Stathas, G.J., and Economou, L.P., 2004. Comparative Temperature-dependent development of *Nephus Includens* (Kirsch) and *Nephus Bisignatus* (Boheman) (Coleoptera: Coccinellidae) preying on *Planococcus Citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. *Environmental Entomology,* 33(1): 1-11.

Lara, D., Vilcacundo, E., Carrillo, C., Carpio, C., Silva, M., Alvarez, M., and Carrillo, W., 2017. Obtention of protein concentrate and polyphenols from macadamia (*Macadamia integrifolia*) with aqueous extraction method. *Asian Journal of Pharmaceutical and Clinical Research,* 10(2), 138-142.

Le Lann, C., Roux, O., Serain, N., Van Alphen, J.J., Vernon, P. and Van Baaren, J., 2011. Thermal tolerance of sympatric hymenopteran parasitoid species: does it match seasonal activity?. *Physiological Entomology*, 36(1), 21-28.

Lee Jr, R.E., 2010. Rapid cold-hardening: ecological significance and underpinning mechanisms. *Low Temperature Biology of Insects*, 35-58.

Lee Jr, R.E., Elnitsky, M.A., Rinehart, J.P., Hayward, S.A., Sandro, L.H., and Denlinger, D.L., 2006. Rapid cold-hardening increases the freezing tolerance of the Antarctic midge Belgica antarctica. *Journal of Experimental Biology*, 209(3), 399-406.

Leroi, A. M., Bennett, A. F., and Lenski, R. E., 1994. Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption*. Proceedings of the National Academy of Sciences*, 91(5), 1917-1921.

Li, H.B., Shi, L., Lu, M.X., Wang, J.J., and Du, Y.Z., 2011. Thermal tolerance of *Frankliniella occidentalis*: effects of temperature, exposure time, and gender. *Journal of Thermal Biology*, 36(7), 437-442.

Lin, T.K., Zhong, L., and Santiago, J.L., 2017. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International Journal of Molecular Sciences*, 19(1), 70.

Loeschcke, V., and A. A. Hoffmann., 2002. The detrimental acclimation hypothesis. *Trends in Ecology and Evolution* 17:407-408.

Lockwood, J.A. and Story, R.N., 1986. Adaptive functions of nymphal aggregation in the southern green stink bug, Nezara viridula (L.) (Hemiptera: Pentatomidae). *Environmental Entomology*, 15(3), 739-749.

Manzoor, U., Haseeb, M., Chander, S., and Moraeit, M., 2020. Determination of thermal constant and development threshold of red cotton bug, *Dysdercus Cingulatus. National Academy Science Letters* 43(4): 307-10.

Marais, E., and Chown, S. L., 2008. Beneficial acclimation and the Bogert effect. *Ecology Letters*, 11(10), 1027-1036.

Marais, E., Terblanche, J.S., and Chown, S.L., 2009. Life stage-related differences in hardening and acclimation of thermal tolerance traits in the kelp fly, *Paractora dreuxi* (Diptera, Helcomyzidae). *Journal of Insect Physiology*, 55(4), 336-343.

Mason, R.L., 2000., Macadamia nut quality research: the processing challenge. *Food Australia*, 52(9), 416-419.

Mast, A.R., Willis, C.L., Jones, E.H., Downs, K.M., and Weston, P.H., 2008. A smaller Macadamia from a more vagile tribe: inference of phylogenetic relationships, divergence times, and diaspore evolution in Macadamia and relatives (tribe Macadamieae; Proteaceae). *American Journal of Botany*, 95(7), 843-870.

McPherson, J. E., and McPherson, R.M., 2000. Stink bugs of economic importance in America North of Mexico. *Boca Raton*: CRC Press.

Mech, A. M., Tobin, P. C., Teskey, R. O., Rhea, J. R., and Gandhi, K. J., 2018. Increases in summer temperatures decrease the survival of an invasive forest insect. *Biological Invasions*, 20(2), 365-374.

Medeiros, R.S., Ramalho, F.S., Serrão, J.E., and Zanuncio, J.C., 2003. Temperature influence on the reproduction of *Podisus Nigrispinus*, a predator of the noctuid larva *Alabama Argillacea*. *Biological Control*, 48(6): 695-704.

Meehl, G.A., and Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305(5686), 994-997.

Mellanby, K., 1939. Low temperature and insect activity. *Proceedings of the Royal Society of London. Series B-Biological Sciences,* 127(849), 473-487.

Mendesil, E., Jembere, B., and Seyoum, E., 2003. Ocurrence of coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) on Coffeea arabica L. in Ethiopia. *Ethiopian Journal of Biological Sciences.*, *2*, 61-72.

Mereles Ceuppens, L.G., Ferro, E.A., Alvarenga, N.L., Caballero, S.B., Wiszovaty, L., Piris Jara, P.A., and Michajluk, B.J., 2017. Chemical composition of *Macadamia integrifolia* (Maiden and Betche) nuts from Paraguay. *International Food Research Journal*, 24(6).

Mitchell, A. and Maddox, C., 2010. Bark beetles (Coleoptera: Curculionidae: Scolytinae) of importance to the Australian macadamia industry: an integrative taxonomic approach to species diagnostics. *Australian Journal of Entomology*, 49(2), 104-113.

Moghadam, N.N., Ketola, T., Pertoldi, C., Bahrndorff, S., and Kristensen, T.N., 2019. Heat hardening capacity in *Drosophila melanogaster* is life stage-specific and juveniles show the highest plasticity. *Biology Letters*, *15*(2), p.20180628.

Motswagole, R., Gotcha, N. and Nyamukondiwa, C., 2019. Thermal biology and seasonal population abundance of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae): implications on pest management. *International Journal of Insect Science*, 11, p.1179543319863417.

Muluvhahothe, M.M., Joubert, E., and Foord, S.H., 2023. Thermal tolerance responses of the two-spotted stink bug, *Bathycoelia Distincta* (Hemiptera: Pentatomidae), vary with life stage and the sex of adults. *Journal of Thermal Biology*, 111: 103395.

Musser, F.R., and Shelton, A.M., 2005. The influence of post-exposure temperature on the toxicity of insecticides to *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Pest Management Science: formerly Pesticide Science*, 61(5), 508-510.

Mutamiswa, R., Machekano, H., Chidawanyika, F., and Nyamukondiwa, C., 2019. Life-stage related responses to combined effects of acclimation temperature and humidity on the thermal tolerance of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *Journal of Thermal Biology*, 79, 85-94.

Navarro, S.L., and Rodrigues, C.E., 2016. Macadamia oil extraction methods and uses for the defatted meal by product. *Trends in Food Science and Technology*, 54, 148-154.

Nielsen, A.L., Hamilton, G.C., and Matadha, D., 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environmental Entomology*, 37(2), 348-355.

Nielsen, A.L., Fleischer, S., Hamilton, G.C., Hancock, T., Krawczyk, G., Lee, J.C., Ogburn, E., Pote, J.M., Raudenbush, A., Rucker, A. and Saunders, M., 2017. Phenology of brown marmorated stink bug described using female reproductive development. *Ecology and Evolution*, 7(17), 6680-6690.

Nietschke, B.S., Magarey, R.D., Borchert, D.M., Calvin, D.D., and Jones, E., 2007. A Developmental Database to Support Insect Phenology Models. *Crop Protection* 26(9): 1444-48.

Nyamukondiwa, C., and Terblanche, J.S., 2010. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies Ceratitis capitata and Ceratitis rosa: thermal history affects short-term responses to temperature. *Physiological Entomology*, 35(3), 255-264.

Nyamukondiwa, C., Chidawanyika, F., Machekano, H., Mutamiswa, R., Sands, B., Mgidiswa, N., and Wall, R., 2018. Climate variability differentially impacts thermal fitness traits in three coprophagic beetle species. *Plos One*, 13(6), p.e0198610.

Nyamukondiwa, C., Kleynhans, E., and Terblanche, J.S., 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology*, 35(5), 565-575.

Nyamukondiwa, C., Weldon, C.W., Chown, S.L., le Roux, P.C., and Terblanche, J.S., 2013. Thermal biology, population fluctuations and implications of temperature extremes for the management of two globally significant insect pests. *Journal of Insect Physiology*, 59(12), 1199-1211.

Oyen K.J., and Dillon, M.E., 2018. Critical thermal limits of bumblebees (*Bombus impatiens*) are marked by stereotypical behaviours and are unchanged by acclimation, age or feeding status. *Journal of Experimental Biology*, 221(8), p.165589.

Ørsted, M., Lye, J., Umina, P.A. and Maino, J.L., 2021. Global analysis of the seasonal abundance of the invasive pest Drosophila suzukii reveal temperature extremes determine population activity potential. *Pest Management Science*, 77(10), 4555-4563.

Pal, E., Allison, J.D., Hurley, B.P., Slippers, B. and Fourie, G., 2022. Genetic diversity of the two-spotted stink bug *Bathycoelia distincta* (Pentatomidae) associated with macadamia orchards in South Africa. *Plos One*, 17(6), p.e0269373.

Palmer, T., 2014. Record-breaking winters and global climate change. *Science*, 344(6186), 803-804.

Panel, A.D., Zeeman, L., Van der Sluis, B.J., Van Elk, P., Pannebakker, B.A., Wertheim, B. and Helsen, H.H., 2018. Overwintered Drosophila suzukii are the main source for infestations of the first fruit crops of the season. *Insects*, 9(4), 145.

Paredes, D., Rosenheim, J.A., Chaplin-Kramer, R., Winter, S., and Karp, D.S., 2021. Landscape simplification increases vineyard pest outbreaks and insecticide use. *Ecology Letters*, 24(1), 73-83.

Patil, I., 2021. Visualizations with statistical details: The ggstatsplot approach. *Journal of Open Source Software*, 6(61), 3167, doi:10.21105/joss.03167.6(61), 3167, doi:10.21105/joss.03167.

Pieterse, W., Terblanche, J.S. and Addison, P., 2017. Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)?. *Journal of Insect Physiology*, 98, 1-6.

Pigliucci, M., Murren, C.J., and Schlichting, C.D., 2006. Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, 209(12), 2362-2367.

Piyaphongkul, J., Pritchard, J., and Bale, J., 2012. Can tropical insects stand the heat? A case study with the brown planthopper *Nilaparvata lugens* (Stål). *Plos One*, 7(1), p.e29409.

Piyaphongkul, J., Suraksakul, P., Tangchitsomkid, N., and Sahaya, S., 2018. Thermal acclimation capacity of Jack Beardsley mealybug (*Pseudococcus jackbeardsleyi)* to survive in a warming world. *Journal of Asia-Pacific Entomology*, 21(2), 737-742.

Piza, P.L.B.D.T., and Moriya, L.M., 2014. Cultivo da macadâmia no Brasil. *Revista Brasileira de Fruticultura*, 36, 39-45.

Prasannath, K., Galea, V.J., and Akinsanmi, O.A., 2022. Influence of climatic factors on dry flower, grey and green mould diseases of macadamia flowers in Australia. *Journal of Applied Microbiology*, 132(2), 1291-1306.

Price, T.D., Qvarnström, A., and Irwin, D.E., 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1523), 1433-1440.

University of New England., 2023. National map of Macadamia orchards in South Africa: www.une.edu.au/webapps. Accessed January 2023.

Quiroz, D., Kuepper, B., Wachira, J., and Emmott, A., 2019. Value chain analysis of macadamia nuts in Kenya. *Centre for the Promotion of Imports from Developing Countries (CBI): Amsterdam, The Netherlands*.

R Core Team., 2022. R: A language and environment for statistical computing. *R Foundation for Statistical Computing,* Vienna, Austria.URL https://www.R-project.org/

Radchuk, V., Turlure, C., and Schtickzelle, N., 2013. Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *Journal of Animal Ecology*, 82(1), 275-285.

Rafia, B., 2013. Phytosterols in human nutrition. *International Journal of Scientific Research. Rev*, *2*, 1-10.

Rahman, M.M., Rasul, M., Hassan, N.M.S. and Hyde, J., 2016. Prospects of biodiesel production from macadamia oil as an alternative fuel for diesel engines. *Energies*, 9(6), p.403.

Ramniwas, S., Kumar, G., and Singh, D., 2020. Rejection of the beneficial acclimation hypothesis (BAH) for short term heat acclimation in *Drosophila nepalensis*. *Genetica*, 148(3), 173-182.

Rebaudo, F., Struelens, Q., and Dangles, O., 2018. Modelling Temperature-dependent development rate and phenology in arthropods: The DEVRATE Package for R ed. Samantha Price. *Methods in Ecology and Evolution*, 9(4): 1144-50.

Reed, D.A., Ganjisaffar, F., Palumbo, J.C., and Perring, T.M., 2017. Effects of temperatures on immature development and survival of the invasive stink bug *Bagrada hilaris* (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, 110(6), 2497-2503.

Renault, D., Hance, T., Vannier, G., and Vernon, P., 2003. Is body size an influential parameter in determining the duration of survival at low temperatures in *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)? *Journal of Zoology* 259(4): 381-88.

Rinehart, J.P., Hayward, S.A., Elnitsky, M.A., Sandro, L.H., Lee, R.E., and Denlinger, D.L., 2006. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. *Proceedings of the National Academy of Sciences*, 103(38), 14223-14227.

Rivera, K.L., and Mitchell, P.L., 2020. Feeding behaviour of *Nezara viridula* first instars: EPG analysis and effect of food availability on subsequent development. *Journal of Agricultural and Urban Entomology*, 36(1), 47-63.

Rodrigues, Y.K., and Beldade, P., 2020. Thermal plasticity in insects response to climate change and to multifactorial environments. *Frontiers in Ecology and Evolution*, p.271.

Rogers, D.J., Terblanche, J.S., and Owen, C.A., 2021. Low-temperature physiology of climatically distinct south African populations of the biological control agent *Neochetina eichhorniae*. *Ecological Entomology*, 46(1), 138-141.

Rohr, J.R., Civitello, D.J., Cohen, J.M., Roznik, E.A., Sinervo, B., and Dell, A.I., 2018. The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21(9), 1425-1439.

Roitberg, B.D., and Mangel, M., 2016. Cold snaps, heatwaves, and arthropod growth. *Ecological Entomology*, 41(6), 653-659.

Roltsch, W.J., Zalom, F.G., Strawn, A.J., Strand, J.F., and Pitcairn, M.J., 1999. Evaluation of several degree-day estimation methods in California climates. *International Journal of Biometeorology*, *42*, 169-176.

Ruthsatz, K., Dausmann, K.H., Peck, M.A., and Glos, J., 2022. Thermal tolerance and acclimation capacity in the European common frog (*Rana temporaria*) change throughout ontogeny. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*.

SAMAC., 2021. Industry overview. https://samac.org.za/industry-overview-3/. Accessed May 2021

Santos, M., Castañeda, L. E., and Rezende, E.L., 2011. Making sense of heat tolerance estimates in ectotherms: lessons from *Drosophila*: modelling survival probability in heat resistance assays. *Functional Ecology* 25(6): 1169-80.

Santos, P.A.B.D., Monti, C.A.U., Carvalho, L.G.D., Lacerda, W.S. and Schwerz, F., 2021. Air temperature estimation techniques in Minas Gerais state, Brazil, Cwa and Cwb climate regions according to the Köppen-Geiger climate classification system. *Ciência e Agrotecnologia*, *45*.

Saulich, A.K., and Musolin, D.L., 2012. Diapause in the seasonal cycle of stink bugs (Heteroptera, Pentatomidae) from the temperate zone. *Entomological Review*, 92(1), 1-26.

Savaris, M., Lampert, S., Hickmann, F., Aurélio, M.S., Schwertner, C.F., and Pereira, P.R., 2022. Rearing the green-belly stink bug, diceraeus furcatus (Fabricius, 1775) (Hemiptera: Pentatomidae) in laboratory. *Entomological Communications* 4: ec04022-ec04022.

Scherrer, S., Lepesqueur, C., Vieira, M.C., Almeida-Neto, M., Dyer, L., Forister, M. and Diniz, I.R., 2016. Seasonal variation in diet breadth of folivorous Lepidoptera in the Brazilian cerrado. *Biotropica*, *48*(4), 491-498.

Schlichting, C.D., 2004. The role of phenotypic plasticity in diversification. *Phenotypic plasticity: functional and conceptual approaches*, 191-200.

Schoeman, P.S., and Mohlala, R., 2012. Prediction of ideal spraying dates for the stink bug and nut borer complexes. *South African Macadamia Association Yearbook*, p. 20.

Schoeman, P.S., 2009. Key biotic components of the indigenous tortricidae and heteroptera complexes occurring on macadamia in South Africa, Doctoral dissertation, *North-West University, Potchefstroom Campus*.

Schoeman, P.S., 2011. Integrated management of macadamia pests with emphasis on alternative pesticides and more effective monitoring. *Agricultural Research Council*

Schoeman, P.S., 2013. Phytophagous Stink Bugs (Hemiptera: Pentatomidae; Coreidae) Associated with Macadamia in South Africa. *Open Journal of Animal Sciences* 03(03): 179-83.

Schoeman, P.S., 2018. Relative seasonal occurrence of economically significant heteropterans (Pentatomidae and Coreidae) on macadamias in South Africa: implications for management. *African Entomology*, *26*(2), 543-549.

Schoeman, P.S., 2020. Damage potential of indigenous Heteroptera species occurring on Macadamia nuts (*Macadamia integrifolia* Maiden and Betche and *Macadamia tetraphylla* L. Johnson) in South Africa during the early and late season. *International Journal of Tropical Insect Science*, 40(1), 217-219.

Schoeman, P.S., and De Villiers, E.A, 2015. Subtropical fruit and nut trees, coffee and tea. In: *Insects of Cultivated Plants and Natural Pastures in Southern Africa*. Eds, Prinsloo, G.L. and Uys, V.M. *Entomological Society of Southern Africa*, 560-565.

Schumann, F.W., and Todd, J.W., 1982. Population dynamics of the southern green stink bug (Heteroptera: Pentatomidae) in relation to soybean phenology. *Journal of Economic Entomology*, 75(4), pp.748-753.

Sentis, A., Veselý, L., Let, M., Musil, M., Malinovska, V., and Kouba, A., 2022. Short-term thermal acclimation modulates predator functional response. *Ecology and Evolution*, 12(2), p.e8631.

Sharma, H.C., 2014. Climate change effects on insects: implications for crop protection and food security. *Journal of Crop Improvement*, 28(2): 229-59.

Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., and Chiu, J.C., 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. *BMC Ecology*, 16(1), 1-18.

Shrestha, S., 2019. Effects of climate change in agricultural insect pest. *Acta Scientific Agriculture*, *3*(12), 74-80.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D., Marshall, D.J., Helmuth, B.S., and Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?. *Ecology Letters*, 19(11), 1372-1385.

Sinclair, B.J., Vernon, P., Klok, C.J., and Chown, S.L., 2003. Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution*, 18(5), 257-262.

Skendžić, S., Zovko, M., Živković, I.P., Lešić, V., and Lemić, D., 2021. The impact of climate change on agricultural insect pests. *Insects*, 12(5), p.440.

Skillman, V.P., and Lee, J.C., 2017. Nutrient content of brown marmorated stink bug eggs and comparisons between experimental uses. *Journal of Insect Science* 17(6): 120.

Sommer, R.J., 2020. Phenotypic plasticity: from theory and genetics to current and future challenges. *Genetics*, 215(1), 1-13.

Sonnekus, B., Slippers, B., Hurley, B.P., Joubert, E., Stiller, M., and Fourie, G., 2022. Diversity and molecular barcoding of stink bugs (Hemiptera: Pentatomidae) associated with macadamia in South Africa. *Insects*, 13(7), p.601.

Sørensen, M.H., Kristensen, T.N., Lauritzen, J.M.S., Noer, N.K., Høye, T.T., and Bahrndorff, S., 2019. Rapid induction of the heat hardening response in an Arctic insect. *Biology Letters*, 15(10), p.20190613.

Southwood, T.R.E., and Henderson, P.A., 2009. Ecological methods. *John Wiley and Sons*.

Stevenson, R.D., 1985. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *The American Naturalist*, 126(3), 362-386.

Stuart-Fox, D., Newton, E., and Clusella-Trullas, S., 2017. Thermal consequences of colour and near-infrared reflectance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1724), p.20160345.

Tarusikirwa, V.L., Mutamiswa, R., English, S., Chidawanyika, F., and Nyamukondiwa, C., 2020. Thermal plasticity in the invasive south American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Thermal Biology*, 90, p.102598.

Taylor, C.M., Coffey, P.L., DeLay, B.D., and Dively, G.P., 2014. The Importance of Gut Symbionts in the Development of the Brown Marmorated Stink Bug, *Halyomorpha Halys* (Stål). *Plos One* 9(3): e90312.

Taylor, F., 1981. Ecology and evolution of physiological time in insects*.* *The American Naturalist*, 117(1): 1–23.

Taylor, P.J., Grass, I., Alberts, A.J., Joubert, E., and Tscharntke, T., 2018. Economic value of bat predation services–A review and new estimates from macadamia orchards. *Ecosystem Services*, 30, 372-381.

Teets, N.M., and Denlinger, D.L., 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiological Entomology*, 38(2), 105-116.

Teets, N.M., Gantz, J.D., and Kawarasaki, Y., 2020. Rapid cold hardening: ecological relevance, physiological mechanisms and new perspectives. *Journal of Experimental Biology*, 223(3), p.203448.

Teets, N.M., Kawarasaki, Y., Potts, L.J., Philip, B.N., Gantz, J.D., Denlinger, D.L., and Lee Jr, R.E., 2019. Rapid cold hardening protects against sublethal freezing injury in an Antarctic insect. *Journal of Experimental Biology*, 222(15), p.206011.

Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., and Chown, S.L., 2008. Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. *Journal of Insect Physiology*, 54(1), 114-127.

Terblanche, J.S., Klok, C.J., Krafsur, E.S., and Chown, S.L., 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. *The American Journal of Tropical Medicine and Hygiene*, 74(5), 786-794.

Terblanche, J.S., Sinclair, B. J., Klok, C.J., McFarlane, M.L., and Chown, S.L., 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology*, 51(9), 1013-1023.

Terblanche, J.S., and Hoffmann, A.A., 2020. Validating measurements of acclimation for climate change adaptation. *Current opinion in insect science*, *41*, 7-16.

Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., and Chown, S.L., 2008. Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. *Journal of Insect Physiology*, 54(1), 114-127.

Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., and Chown, S.L., 2007. Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B: Biological Sciences*, 274(1628), 2935-2943.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., and Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology*, 214(22), 3713-3725.

Terblanche, J.S., Klok, C.J., Krafsur, E.S., and Chown, S.L., 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. *The American Journal of Tropical Medicine and Hygiene*, 74(5), p.786.

Terblanche, J.S., Mitchell, K.A., Uys, W., Short, C. and Boardman, L., 2017. Thermal limits to survival and activity in two life stages of false codling moth *Thaumatotibia leucotreta* (Lepidoptera, Tortricidae). *Physiological Entomology*, 42(4), 379-388.

Thomson, L.J., Robinson, M., and Hoffmann, A.A., 2001. Field and laboratory evidence for acclimation without costs in an egg parasitoid. *Functional Ecology*, 15(2), 217-221.

Tillman, P. G., 2011. Influence of corn on stink bugs (Heteroptera: Pentatomidae) in subsequent crops. *Environmental Entomology* 40(5): 1159-76.

Tochen, S., Woltz, J.M., Dalton, D.T., Lee, J.C., Wiman, N.G., and Walton, V.M., 2016. Humidity affects populations of *Drosophila Suzukii* (Diptera: Drosophilidae) in blueberry. *Journal of Applied Entomology* 140(1-2): 47-57.

Tonnang, H.E.Z., Sokame, B.M., Abdel-Rahman, E.M., and Dubois, T., 2022. Measuring and modelling crop yield losses due to invasive insect pests under climate change. *Current Opinion in Insect Science* 50: 100873.

Uelmen Jr, J.A., Lindroth, R.L., Tobin, P.C., Reich, P.B., Schwartzberg, E.G. and Raffa, K.F., 2016. Effects of winter temperatures, spring degree-day accumulation, and insect population source on phenological synchrony between forest tent caterpillar and host trees. *Forest Ecology and Management*, 362, 241-250.

Van Dyck, H., Bonte, D., Puls, R., Gotthard, K., and Maes, D., 2015. The lost generation hypothesis: could climate change drive ectotherms into a developmental trap?. *Oikos*, 124(1), 54-61.

Wang, W., Pijl, A., and Tarolli, P., 2022. Future climate-zone shifts are threatening steep-slope agriculture. *Nature Food*, 3(3), 193-196.

Weaving, H., Terblanche, J.S., Pottier, P., and English, S., 2022. Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nature Communications*, 13(1), p.5292.

Weier, S.M., Moodley, Y., Fraser, M.F., Linden, V.M., Grass, I., Tscharntke, T., and Taylor, P.J., 2019. Insect pest consumption by bats in macadamia orchards established by molecular diet analyses. *Global Ecology and Conservation*, *18*, p.e00626.

Whitman, D.W., and Agrawal, A.A., 2009. What is phenotypic plasticity and why is it important? *Phenotypic plasticity of insects: Mechanisms and consequences*, 1-63.

Wilson, C., and Tisdell, C., 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics*, 39(3), 449-462.

Wilson, R.S., and Franklin, C.E., 2002. The detrimental acclimation hypothesis. *Trends in Ecology and Evolution*, *17*(9), p.408.

Worland, M.R., 2005. Factors that influence freezing in the sub-Antarctic springtail Tullbergia antarctica. *Journal of Insect Physiology*, *51*(8), 881-894.

Wu, H., Hu, X.P., and Appel, A.G., 2017. Temperature-dependent development and thermal sensitivity of *Blaptica dubia* (Blattodea: Blaberidae). *Journal of Economic Entomology*, 110(2), 546-551.

Yamahira, K., Kawajiri, M., Takeshi, K., and Irie, T., 2007. Inter-and intrapopulation variation in thermal reaction norms for growth rate: evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution*, 61(7), 1577-1589.

Yazdanpanah, A., Nouri-Ganbalani, G., Razmjou, J., Basirat, M., and Fathi, S.A.A., 2019. Effects of different temperatures on the demography of pistachio green stink-bug, *Brachynema Germari* Kolenati (Hemiptera: Pentatomidae), under laboratory conditions. *Revista de la Sociedad Entomológica Argentina* 78(4): 29-36.

Yi, S.X., Moore, C.W., and Lee, R.E., 2007. Rapid cold-hardening protects *Drosophila melanogaster* from cold-induced apoptosis. *Apoptosis*, 12(7), 1183-1193.

Zhang, W.Y., Storey, K.B., and Dong, Y.W., 2021. Synchronization of seasonal acclimatization and short-term heat hardening improves physiological resilience in a changing climate. *Functional Ecology*, 35(3), 686-695.

Zhao, F., Xing, K., Hoffmann, A.A., and Ma, C.S., 2019. The importance of timing of heat events for predicting the dynamics of aphid pest populations. *Pest Management Science*, 75(7), 1866-1874.

Zhu, G., Zhao, H., Xue, M., Qu, C., and Liu, S., 2022. Effects of heat hardening on life parameters and thermostability of *Bradysia odoriphaga* larva and adults. *Journal of Applied Entomology*, 146(1-2), 185-195.