Evolutionary, physiological and phenological responses of *Spodoptera frugiperda* to climate change

Abongile Mbande

Thesis submitted in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY IN ENTOMOLOGY (Ph.D.)

To the Department of Zoology and Entomology

Faculty of Natural and Agricultural Sciences

University of the Free State

Bloemfontein

Republic of South Africa

2023

List of Supervisors

Frank Chidawanyika (PhD): Associate Researcher, University of the Free State, Bloemfontein 9300, South Africa & Scientist, International Center for Insect Physiology and Ecology (Icipe), Nairobi, Kenya.

Reyard Mutamiswa (PhD): Post-Doctoral Fellow, University of the Free State.

Student Declaration

I Abongile Mbande declare that the Doctorate research thesis that I herewith submit at the University of the Free State, is my independent work and that I have not previously submitted it for qualification at another institution of higher education.

May 2023

Supervisor Declaration

International Centre of Insect Physiology and Ecology
P. O. Box 30772-00100 Nairobi, Kenya
Tel: +254 20 8632000; Fax: +254 20 8632001
icipe@icipe.org — www.icipe.org

18 September 2023

To whom it may concern,

I, Frank Chidawanyika, in my capacity as the supervisor for Abongile Mbande (Student number: 2018417267) hereby approve the submission of her PhD thesis titled ***Evolutionary, physiological and phenological responses of Spodoptera frugiperda to climate change***. I confirm that the work has not been previously, either in part or its entirety, submitted elsewhere for the sake of degree purposes.

Yours faithfully,

Dr Frank Chidawanyika

Associate Researcher, External Supervisor
Department of Zoology and Entomology, UFS

Dedication

This thesis is dedicated to my missing mother Nobandla, my late father Sikhulu Elijas Mbande and my entire family.

Abstract

The threat of biological invasions due to global climate change and anthropogenic activities is growing globally. Environmental changes, such as increasing mean temperatures due to global climate warming, may exacerbate the spread of invasive insect species by improving their voltinism, survival, reproductive success and geographic range expansion. The fall armyworm (FAW) *Spodoptera frugiperda* is one of the most economically important invasive insect pests attacking sub-Saharan Africa (SSA) staple crops, such as maize and sorghum. First reported in West Africa in 2016, FAW has spread to the rest of SSA. Key questions arising from this rapid geographic expansion is how climate and its physiological attributes aid its invasion potential. This is particularly important in this era of global climate change where phenological mismatches are known to also influence their host utilization and survival across different life-stages and physiological status such as mating or feeding. Evolutionary responses at individual and population also play a pivotal role in survival and population persistence across various temporal scales. This study explored the evolutionary, physiological and phenological responses of *S. frugiperda* to climate change. First, I measured the effects of short-term exposure to heat shock on physiological responses *vis* critical thermal limits (critical thermal minima [CT_{min}] and critical thermal maxima [CT_{max}]), and ecological traits *vis* reproductive success (fecundity [number of eggs per female] and hatching success) and longevity using virgin adults following heat shock at 32, 35, and 38 °C for 2 hours. Second, I assessed the effects of repeatable exposure to cold (CT_{min}) and chill coma recovery time (CCRT) for 0; 24; 48 and 72 hours and the body lipid content (BLC) and body water content (BWC) on adult fall armyworm (24 h old). Last, I investigated the thermal tolerance of *S. frugiperda* at 3, 6 and 9 days old adults heat shocked at adult, pupal and larval stages, at 40℃ for 2 hours. Heat shock improved tolerance to heat (measured as CT_{max}) but negatively affected cold tolerance (measured as CT_{min}). Males were more vulnerable to heat stress than females. Interestingly,

heat shock also reduced fecundity, hatching success and adult longevity. Cold tolerance measured as CT_{min} improved with repeated exposure in 5^{th} instar larvae, virgin males, and females while CCRT improved in 4^{th}, 5^{th} and 6^{th} instar larvae following repeated cold exposure. Heat tolerance in virgin females showed highest CTmax in 6 day olds while it fluctuated in males. Mated males had higher CT_{max} than mated females across all heat shocked developmental stages. This study shows that, under heat stress, reproductive fitness in FAW is largely compromised due to low fecundity and stress resistance that is dependent on mating status, age and life-stage. Taken together, the results show that thermal variation has fitness consequences for FAW at both individual and population level. These results provide valuable data that inform mechanistic models for the geographic distribution of FAW under variable climate scenarios. This will especially be critical in developing early warning systems and area-wide strategies for FAW control.

Keywords: Environmental stress, invasive species, longevity, population persistence, reproductive fitness, trade-off, thermal tolerance.

Acknowledgements

First and foremost, praises and thanks to God for his never-ending blessings throughout my research. I would like to express my deep and sincere gratitude to the University of the Free State, department of Zoology and Entomology head of department (HOD), Professor Liesel Van As for her support, compassion and understanding especially after she learned of my mother's disappearance. I would also like to thank my supervisor Dr. Frank Chidawanyika. I appreciate his guidance, support and patience throughout this project and for pushing me into being a better researcher. It was a great privilege and honor to study and work with him, I am grateful for everything he offered me. I would also like to thank my Co-supervisor Dr. Reyard Mutamiswa, your understanding and support kept me sane, when I wanted to quit, you were a voice of reason, you believed in me more than I believed in myself, if it wasn't for you I would not have completed this degree. I would also like to acknowledge Professor Hannalene Du Plessis from the North West University, for supplying me with some of the insects I used to boost my laboratory culture. I would also like to acknowledge Dr Gerald Chikowore for microclimatic data. I would also like to thank Lugisani Mulaudzi for assisting me with mass rearing and her friendship, I will always hold the moments we shared close to my heart. I would also like to thank the National Research Foundation (NRF) Freestanding, Innovation and Scarce Skills for the financial support between 2018 and 2019 for their financial support. A big thank you goes to the SACTA NPC trust bursary that took me when I thought my dream of being a Doctor of Philosophy in entomology was over. Kind support from the University of the Free State for funding. Lastly, a big thanks goes to my family especially my sister Sekiwe who took the role of both my parents without expecting anything in return, my partner Zane for both emotional and financial support throughout my research, my friends for their support and my children, Iminathi and Somila for motivating me during tough times.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

1.1 Effects of climate change on insects

The magnitude and frequency of extreme weather events such as flooding and drought are gradually increasing due to global climate change (IPCC, 2013) with subsequent effects on biodiversity and ecosystem functioning (Walther et al., 2009; Ma & Ma, 2022). This is because climate change may reduce or eliminate abiotic limitations and /or indirectly alter biotic interactions, which may benefit invasive species (Hulme, 2017; Ma & Ma, 2022). This is a concern as it may have an impact on biodiversity, invasion of both plants and animals. For agricultural crop production this has implications directly or indirectly due to diseases and insect pest outbreaks (Parmesan et al., 1999; Qi et al., 2019), whether native or alien (Bale et al, 2002; Parmesan & Yohe, 2003; Chidawanyika et al., 2019; Phophi et al., 2020).

Climate change may directly influence the spread of alien species as they have to track favourable climates (Fig. 1.1) (Huang et al., 2011). Insects are the most destructive and costly invasive taxa, with most exerting devastating impact on agricultural productivity (Paini et al., 2016). Due to their ectothermic nature, the growth, metabolism, survival, fecundity, longevity and ultimately geographic distribution of insects is chiefly influenced by temperature (Chown & Nicolson, 2004, Calosi et al., 2008; Chidawanyika et al., 2012). It is therefore important to understand insect-temperature relationships as they inform both conservation or control options, depending whether the insect species is beneficial or deleterious, respectively (Tobin et al., 2003; Du Plessis et al., 2020).

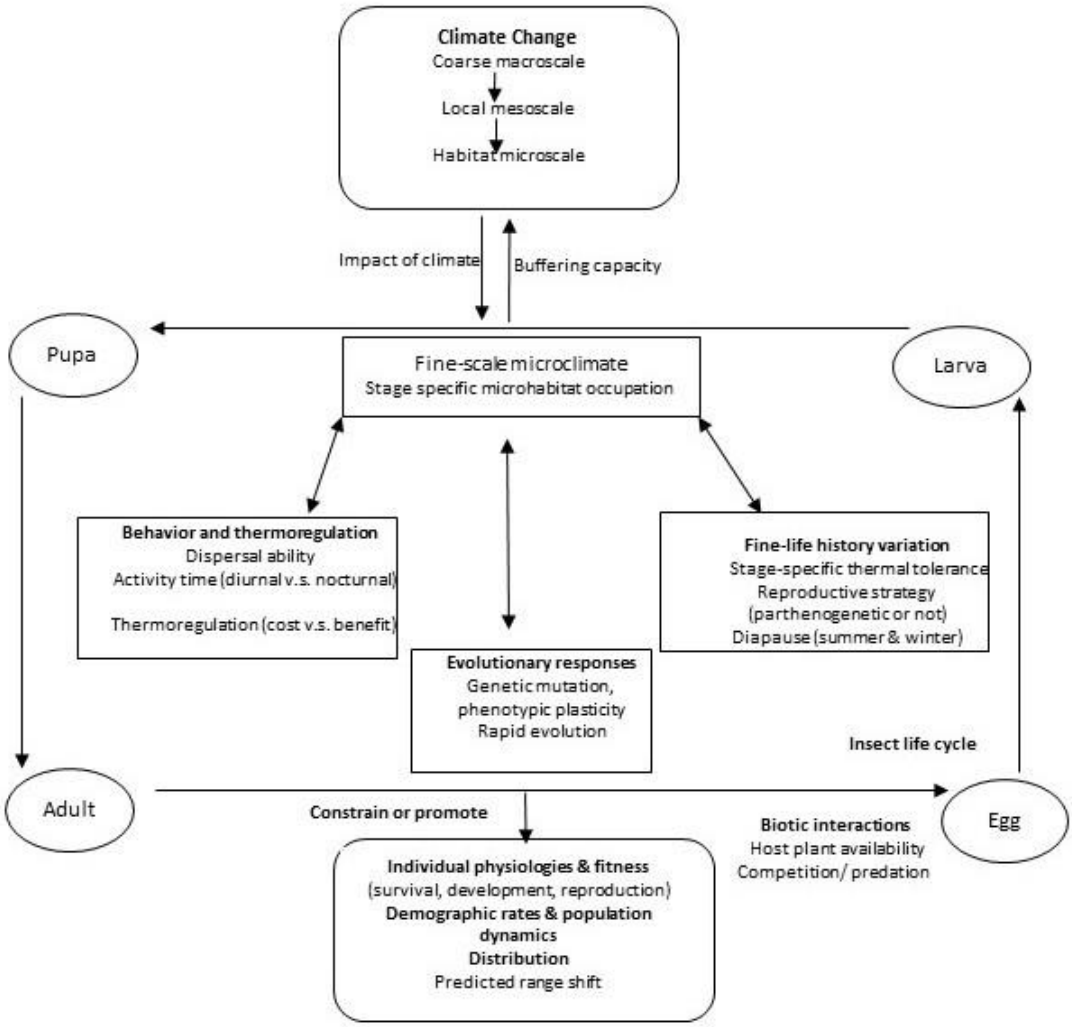

Figure 1. 1 A conceptual framework incorporating the capacity of holometabolous insects buffering climate change. Redrawn from (Ma &Ma, 2022).

Insect responses to thermal variation are diverse and can be behavioural or physiological across both acute and chronic time-scales. For example, behavioral thermoregulation can benefit insects through avoidance of shaded cooler environments and moving to sunny spots (Yin et al., 2018). Similarly, larvae can feed on the underside of the leaf whilst avoiding the upper surface exposed to direct heat from the sun (Yin et al., 2018; Guillen et al., 2022). This is in addition to other behavioral responses such as changing time of diurnal and seasonal activities (Caspi et al., 2022; Ma & Ma, 2022). Other insects employ polyphenism where changes in color depending on environmental temperature assist in body thermoregulation (Markl et al., 2022). Other examples of plastic responses to thermal variation include physiological adjustments such as the expression of

heat shock proteins, which act as molecular chaperones against protein denaturation, to withstand extreme heat stress. These heat shock proteins are usually induced following sub-lethal thermal exposure before otherwise lethal environments (Chown & Nicolson, 2004; Angilletta & Angilletta, 2009). Interestingly these heat shock proteins can also confer resistance to cold through cross-tolerance mechanisms (Chidawanyika & Terblanche, 2011). Insects have also evolved mechanisms to withstand cold environments by use of polyols and lipids, which act as anti-freezing agents to preserve body organs under sub-zero temperatures (Chown & Nicolson, 2004), thereby prolonging survival in extreme conditions. Thus, insect responses to thermal variability is highly dynamic and dependent on several factors at both individual and population level through phenotypic plasticity and heritable responses, respectively (Angilletta, 2009). These phenotypically plastic responses are usually referred to as hardening (short term prior-exposure) and acclimation (long-term exposure days to weeks etc) and can be dependent on life stage (Fig. 1.1).

1.2 Measures of insect thermal tolerance

Insect thermal responses can be determined through their developmental rates, lethal limits (high or low) and critical thermal limits (CT_{min} and CT_{max}). Critical thermal limits are temperatures at which a crucial ecological function is lost such as locomotion function, these may be reversible (Mutamiswa et al., 2018). Other variation in both upper and lower thermal limits to survival and activity is closely related to features of the organisms and its environment they inhabit (Hoffmann et al., 2013; Soravia et al., 2021). These may include phylogenetic signal in thermal tolerance (Kellerman et al., 2012), differences in tolerance and behavioural regulation between life stages and the predictability in environmental variation (Chown & Terblanche, 2006). Upper and lower thermal tolerance of terrestrial organisms indicates the rate of decline in upper thermal limits, which is due to magnitude slower than that of lower limits (Sunday et al., 2011; Hoffmann & Chown., 2013; Turriago et al., 2022). Insect upper limits may vary much less with

acclimation than in lower limits (Stockton et al., 2018). Ectotherms in low latitudes may have narrow thermal safety margins (Deutsch et al., 2008; Hoffmann et al., 2013), and this increases chances of extinction. It is estimated that temperatures will increase by 4 ℃ on average by the end of the century (Georgoulias et al., 2022). This could increase the risk of population extinction, but if there is room for adaptation through evolution, plasticity, and cross-generational epigenetic mechanisms, that could release genetic variation, increase adaptation to climate change, and reduce the risk of extinction (Hoffmann et al., 2013; Sgro et al., 2016).

1.3 Plasticity to thermal tolerance

Organisms have evolved adaptive mechanisms to buffer heat stress (Hoffmann et al., 2013). There are three (non-exclusive) methods through, which organisms can adapt to a changing environment. (1) Over a period of time and depending on an organism's life history, a species' distribution may shift to habitats that are more suitable to their physiology and ecology. (2) Adaptation to the current environmental conditions, which involves evolutionary (genetic) change in response to the new selective pressures. (3) In the short term, phenotypic plasticity where the same genes result in different phenotypes, depending on the environment (Chown & Nicolson, 2004). Thus, phenotypic plasticity can be used, particularly by ectotherms, to cope with harsh transient environmental conditions (Terblanche et al., 2010; Chidawanyika & Terblanche, 2011). Plasticity is therefore a key trait that contributes to the success of pest species especially when encountering novel environments (Angilletta, 2009; Harris et al., 2013; Sgro et al., 2016; Xue &Ma, 2020). For example, plasticity in thermal tolerance has been shown to improve fitness of invasive species such as Mediterranean fruit fly, *Ceratitis capitata* (Nyamukondiwa et al., 2010) where rapid cold hardening allowed *C. capatita* to track its diurnal change in environmental temperatures which may optimize both feeding and mating during unfarouble temperature conditions, *Chilo partellus* (Mutamiswa et al., 2019) where

thermal tolerance has been shown to be life-stage dependent with adults that were developmentally acclimated and adult acclimated across temperature and relative humidity showing different thermal fitness, and *Drosophila suzukii* showed that mating status may affect cold tolerance, mated females had lower cold tolerance and mating may lower plasticity of heat tolerance such that acclimation capacity of mated adults decreases (Xue & Ma, 2020) . This therefore suggests that thermal plasticity is a key trait aiding invasion potential (Chidawanyika et al., 2019; Nyamukondiwa et al., 2022).

There are several ways in which thermal plasticity can be acquired. "Acclimation" is when reversible physiological plasticity in thermal tolerance used by organisms to survive in extreme conditions (Weldon et al., 2011; Gunderson & Stillman, 2015) is acquired over a relatively longer period. On the other hand, "hardening" involves reversible induction of physiological responses over short term pre-exposure to extreme heat (sub-lethal) or cold temperatures (Chidawanyika et al., 2011a, b; Foucault et al., 2014). In both hardening and acclimation, physiological changes such as upregulation of heat shock proteins or production of polyols to withstand heat or cold, respectively, may be involved. There are also cases of cross-tolerance where pre-exposure to the opposite thermal extreme can lead to improved tolerance of the opposite (Chidawanyika & Terblanche, 2011; Ge et al., 2013).

Thermal plasticity is not a static trait, but rather a function of both environmental and organismal factors especially for organisms with complex life cycles such as insects that are characterized by different ontogenetic life stages. These life stages may occur in different microhabitats which may vary in temperature means and extremes (Krebs & Loeschcke, 1995), thus the evolution of different plasticity levels. This may consequently limit the achievement of different stage-specific temperature optima within each of these environments since tolerance at different developmental stages is likely to be linked and may be constrained by physiology or trade off (Huey & Kingsolver, 1993; Angilletta, 2009; Blackenhorn et al., 2014). The magnitude of temperature and duration of exposure affects thermal tolerance of an organism (Chown & Nicolson, 2004). For example,

Chidawanyika & Terblanche (2011a) found an increase in mortality in codling moth *Cydia pomonella* with either an increase in temperature or an increase in the exposure at any given temperature. Similar factors such as sex, age, mating and metabolic status affect thermal tolerance (Colinet & Renault, 2014; Rusch et al., 2019; Xue & Ma, 2020). Scharf et al. (2016) found that the upper thermal tolerance (CT_{max}) reduced with age in *Drosophila melanogaster* and Nyamukondiwa & Terblanche (2009) found that fed fruit flies (*Ceratitis capatata* and *Ceratitis rosa*) had increased thermal tolerance. CT_{max} differed by sex in screwworm (*Cochlioyia macellaria*), with females showing an increased thermal tolerance than males regardless of nutrient availability (Rusch et al., 2019). The extent to, which species can cope with extreme temperatures in different life stages and how exposure to such conditions at a juvenile (ontogeny) affects adult survival, reproduction and life histories later in life are of interest in order to understand evolutionary and physiological constraints of thermal tolerance. This is because different physiological pathways may be used during ontogeny, for example using *Drosophila,* studies revealed that short-term exposure to extreme temperatures at larval and pupal stages influenced its development and hatchability and tolerance may be uncoupled across developmental stages (Krebs & Loeschcke, 1994).

Other studies on age -and-stage dependent resistance to high temperatures that showed a decline in thermal tolerance with stage or age include, Nyamukondiwa & Terblanche (2009) for fruit flies, Sales et al. (2021) for flour beetle, *Tribolium castaneum* and cold temperature tolerance in *Chilo partellus* (Mutamiswa et al., 2019). Several studies also showed that sex also differ in many ways to temperature stress, with females showing less sensitivity than males. Heat stress may delay mating and may reduce the frequency of mating (Sales et al., 2018). Sperm and eggs production are affected by temperatures and differential effects of high temperatures on spermatogenesis and oogenesis has been reported in the parasitoid wasp *Anisopteromalus calandrae* (Nguyen et al., 2013). Such sex-specific differences in heat sensitivity are of importance since mating and consequently reproductive success are an

interaction because infertility of one mating partner is enough to result in zero fitness of the other (Blackenhorn et al., 2014; Sales et al., 2018). Hardening (heat or cold) and /or acclimation are important concepts linking stage-and sex-specific thermal tolerance. This is achieved by individuals who become more tolerant to extreme temperatures at adult stage after being exposed to them at juvenile stage (egg, larvae or pupae) (Hoffmann et al., 2003, Blackenhorn et al., 2014; Sales et al., 2021). These carry-over effects may increase survival and reproductive output and fitness at adult stage for both males and females (discussed in Hoffmann et al., 2003; Chown & Nicolson, 2004; Bowler & Terblanche, 2008).

1.4 Economic losses due to invasive insect pests

Invasive species are a major threat to biodiversity and ecosystem service (Amusan & Olawuyi, 2018; Machekano et al., 2018), food production and can act as disease vectors, which may have direct and indirect effects on humans (Haneley & Roberts, 2019). Biological invasions have major economic consequences and can reduce the flow of ecosystem services by reducing biodiversity, competing with native species, altering habitats and may even lead to the extinction of native species and human wellbeing such as access to clean water, human health and climate stabilization (Shackleton et al., 2019; van Wilgen et al., 2020), . Agriculture is very important in southern Africa for security and livelihoods, yet is vulnerable to climate change (Mutamiswa et al., 2017). Crop losses due to insect pests, mites, weeds and pathogens have been a major threat to income of rural families and to food security (Mutamiswa et al., 2017; Savary et al., 2019; Sileshi et al., 2019). Insect pests' geographic ranges are increasing with rising temperatures (Parmesan et al., 1999). This will lead to alien species including invasive pests expanding their host ranges. According to a recent global analysis of 1297 invasive species, Africa was found to be one of the continents with countries that were mostly vulnerable to invasive alien species worldwide (Sileshi et al., 2019). Many AISs affect food crops including crops native to Africa such as sorghum (*Sorghum bicolor*), hungry rice (*Digitaria exilis*), maize (*Zea mays*) or cassava (*Manihot esculenta*), which are

staple food for a large number of low-income consumers (Silesh et al., 2019). Maize is a staple food in Africa and is grown across different agro-ecological zones where many people depend on it for food security (Kumela et al., 2019). Yield losses for 137 pest species on wheat rice, maize and potatoes were estimated at different hotspots world wide including southern Africa (Savary et al., 2019). Their survey showed that in Southern Africa average loss and the range of losses for different crops were wheat 21.5% (10.1–28.1%), rice 30.0% (24.6–40.9%), maize 22.5% (19.541.1%), potato 17.2% (8.1–21.0%) and soybean 21.4% (11.0–32.4%). Southern Africa has been described as climate change hotspot and the region is expected to experience increases in aridity with low adaptive capacity (Nhamo et al., 2019). South Africa has the largest economy in terms of agricultural production and trade in the region (Ransom et al., 2019).

With warming climates due to climate change, the scourge of such pests is projected to increase. This leads to increased costs of crop production due to investment in pest management strategies and outright food insecurity in the cases where crop damage goes unabated. In South Africa, the exact amount of money spent on the control of invasive species is unknown but it could potentially reach a minimum of US$242 million per year (van Wilgen et al., 2020). Developing countries are more vulnerable to threats posed by invasive species because their economies rely mostly on agriculture, fishing, and forestry (Pimentel et al., 2001). About 60% of employment in Africa comes from agriculture and generates more than 40% of the continents foreign exchange and about 80% of rural populations depend on agriculture (Kherallah et al., 2002). Maize is the staple food of about 50% of the population of sub-Saharan Africa (SSA) and yet the region has the lowest maize yields due to invasive species (Kavhiza et al., 2022). For example, *Chilo partelus* (spotted stem borer) is a serious pest of maize and sorghum (Chidawanyika et al., 2012; Mutamiswa et al., 2018). Its economic impact is estimated at US$61-73 million in Ethiopia, US$43-51 million in Kenya annually (Pratt et al., 2017). *Prostephanus truncatus* (larger grain borer) occurs in 17 African countries and is a destructive pest of stored maize and dried cassava. The economic impact

of this pest is estimated at US $15 million cording at 1995 values (Boxall, 2002). The pest also damages cereals, legumes, dried roots, cocoa, coffee beans, wood and tubers. The Oriental fruit fly (*Bactrocera dorsalis*) have been confirmed in 41 countries across Africa. There are no reliable yield losses as estimates are lacking but fruit losses can be 100 % without protection (Cugala et al., 2003). *Tuta absoluta* (tomato leaf miner) is a tomato pest and also attacks crops of the Solanaceae family and has been confirmed in 19 African countries including South Africa with 100% crop loss in countries invaded (Pratt et al., 2017; Naganna et al., 2020). Its range is predicted to expand in tropical Africa and the number of generations per year area also predicted to increase under climate change (Silesh et al., 2019). *Spodoptera frugiperda* annual economic losses are estimated at US$2.5-6.3 billion in maize producing African countries (Day et al., 2017). The *S. frugiperda* has host range of over 100 plant species in 27 families globally (CABI, 2018), but in Africa its main hosts include maize, sugarcane, sorghum and 23 horticultural crops like tomato, potato, soybean, peanut and millet (Makgoba et al., 2021). It threatens the food and nutritional security of millions of farming families in Africa (Nagoshi et al., 2019; Naganna et al., 2020).

1.5 Problem statement and objectives

Invasive species are a major threat both to natural and agricultural ecosystems (Amusan & Olawuyi, 2018; Machekano et al., 2018) leading to food insecurity and poor human well-being. In recent years, Africa has witnessed severe FAW invasion leading to devastating yield and economic losses (Overton et al., 2021; Tambo et al., 2021; . This polyphagous insect is native to the tropics and subtropical regions of America (Rukundo et al., 2020). In Africa alone, if left uncontrolled has a potential to cause maize yield losses estimated between US$2.5 to US$6.2 billion per annum (CABI, 2018). The spread of the FAW throughout several central and southern African countries now includes Togo, Zambia, Zimbabwe, South Africa, Mozambique, Malawi, Namibia, Kenya, Rwanda, Tanzania and Ghana (Goergen et al., 2016). In South Africa, *S. frugiperda* has been reported in several provinces, including Limpopo, North West, Free State, Gauteng, Mpumalanga, KwaZulu-Natal, Western Cape, Northern Cape and Eastern Cape (Rukundo et al., 2020). Some

information that is urgently needed for this species is how climate influences the physiology and population dynamics of this pest. Previous reports have suggested that invasive species are more tolerant to high temperatures than natives (Zerebecki & Sorte, 2001) i.e. invasive species may have enhanced eurythermality, the ability to live in diverse environments, compared to native species occupying the same environment (Zerebecki & Sorte, 2011). This implies that mechanisms such as phenotypic plasticity provide invasive species with a wider physiological tolerance, enabling them to establish successfully under various potential abiotic stressors, such as temperature and desiccation, by adapting through physiological changes (Kelly, 2014). These abiotic factors serve as key 'ecological filters' to invasion. For ectotherms, understanding how an invasive organism may respond to environmental variation may bring about insights regarding patterns of invasion. From the foregoing, the overall goal of this study was to investigate the evolutionary, physiological and phenological responses of *S. frugiperda* to climate change. Specifically, the objectives of this study are.

1. To evaluate the impact of short exposure to high temperatures on survival and fecundity of *S. frugiperda.* It was hypothesized that short term exposure to high temperatures will impact survival and female fecundity.

2. To evaluate basal and plastic responses to thermal variability in *S. frugiperda*. It was hypothesized that adult heat shock stress may have an impact on the physiological and ecological functioning of fall armyworm adults.

3. To investigate the role of life-stage in the thermal tolerance of *S. frugiperda.* It was hypothesized that heat tolerance will decreased with age.

4. To evaluate the effects of sex, age and mating status on thermal tolerance of *S. frugiperda* after heat shock. It was hypothesised that thermal tolerance of *S. frugiperda* varies across age, sex and mating status following heat shock.

5. To investigate Repeatability of critical thermal minima (CTmin) and chill coma recovery time (CCRT), body water content (BWC) and body lipid content (BLC) in *S. frugiperda.* It was also hypothesized that CTmin and CCRT are repeatable traits and may change over time because of cold hardening and body water content and body lipid content will influence cold hardening.

1.6 Study organism

Spodoptera frugiperda (J.E. Smith), commonly known as the fall armyworm (FAW) is a significant economic pest of members of Poacecae plants, feeding on over 80 crops including strategic crops for food security such as maize, sorghum and rice. This polyphagous insect is native to the tropics and subtropical regions of the Americas (Rukundo et al., 2020). It is incapable of diapause with limited capacity to survive freezing winter conditions (Kumela et al., 2019; Nagoshi et al., 2019). *Spodoptera frugiperda* has high dispersal capacity, which contributes to the quick long range spread (Nagoshi et al., 2019). The adult female FAW has brownish-grey forewings that are uniform in color (Fig.1.2.1) while males have shaded grey and brown forewings with triangular white spots on the tip of the wing (Fig. 1.2.2).

Figure 1. 2.1 Adult female *Spodoptera frugiperda*

Figure 1. 3.3 Adult male *Spodoptera Frugiperda*

Females oviposit as early as the same night of mating in batches of 50 to 200 eggs (Fig. 1.3). The females may lay between 1500 to 2000 eggs in total. It usually has 6 instars, The life cycle usually includes six instars. The first and second instars feed on one side of the leaf, whilst the final instars feed on most plant parts, damaging them in the process (Kumela et al., 2019). The larval stage takes about 14 days in summer and 30 days in cool temperatures (Nagoshi et al., 2019). The last instar drops to the ground and pupates in the soil. It takes about 8 to 9 days in summer and 20-30 days in winter for adult eclosion (Kumela et al., 2019; Du Plessis et al., 2020).

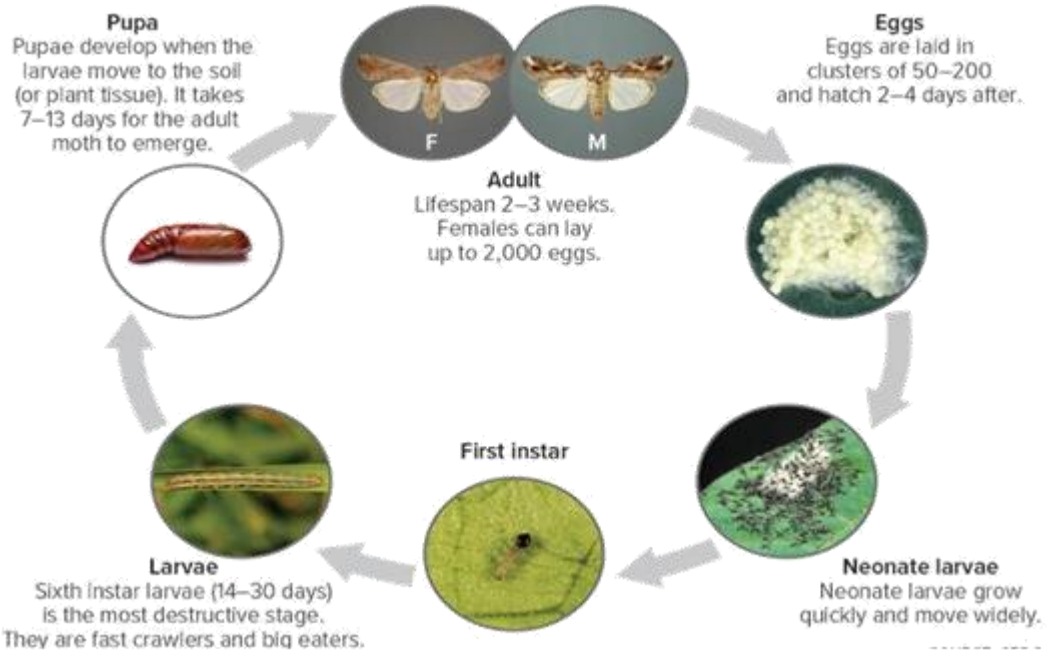

Figure 1. 4 Life cycle of *Spodoptera frugiperda*

The adult females oviposit eggs in large numbers on the foliage, which are then dispersed throughout the crop. In summer, the eggs hatch within 3 days and as soon as they hatch, the larvae begin feeding on the tissues (Cruz et al., 2014). The first and second instar larvae usually eat the green tissue from the side of the leaf leading to the loss of photosynthetic parts of the plant (Chimweta et al., 2020; Suby et al., 2020). The first instar larvae usually start with the most delicate plant parts (Cruz et al., 2010) (Fig. 1.4). From the third instar on, the larvae congregate in the whorl and begin to feed, leaving a pattern of holes in the unfolding leaves. As they grow, their feeding

rate and damage increases (Chuang et al., 2014; Cruz et al., 2010).

Figure 1. 5 *Spodoptera frugiperda* damage to maize (Visser, 2017).

1.7 References

Amusan, L. & Olelekan, S. 2018. The menace of the fall armyworm. New Zealand International Review. 43(2), 20.

Andreadis, S.S., Spanoudis, C.G., Zakka, G., Aslanidou, B., Noukari, S. & Savopoulou-Soultani, M. 2017. Effect of temperature on rate of development, survival and adult longevity of *Phthorimaea operculella* (Lepidoptera: Gelechiidae). European Journal of Entomology, 114, 35.

Angilletta Jr, M.J., Niewiarowski, P.H. & Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology, 27(4), 249-268.

Angilletta, M.J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, USA.

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.& Good, J.E. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology, 8(1), 1-16.

Blanckenhorn, W.U., Gautier, R., Nick, M., Puniamoorthy, N. & Schäfer, M.A. 2014. Stage and sex-specific heat tolerance in the yellow dung fly *Scathophaga stercoraria.* Journal of Thermal Biology, 46, 1-9.

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

Boxall, R.A. 2002. Damage and loss caused by the larger grain borer *Prostephanus truncatus.* Integrated Pest Management Reviews, 7(2), 105-121.

Bryant, S.R., Thomas, C.D. & Bale, J.S. 1997. Nettle-feeding nymphalid butterflies: temperature, development and distribution. Ecological Entomology, 22(4), 390-398.

Buckley, L.B. 2008. Linking traits to energetics and population dynamics to predict lizard ranges in changing environments. American Naturalist, 171, E1–E19.

Calosi, P., Bilton, D. T. & Spicer, J. I. 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biology Letters, 4(1), 99-102

Caspi, T., Johnson, J.R., Lambert, M.R., Schell, C.J. & Sih, A., 2022. Behavioral plasticity can facilitate evolution in urban environments. Trends in Ecology & Evolution.

Chidawanyika, F. & 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. & Terblanche, J.S. 2011a. Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). Journal of Insect Physiology, 57(1), 108-117.

Chidawanyika, F. & Terblanche, J.S. 2011b. 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., Mudavanhu, P. & Nyamukondiwa, C. 2012. Biologically based methods for pest management in agriculture under changing climates: challenges and future directions. Insects, 3(4), 171-1189.

Chidawanyika, F., Nyamukondiwa, C., Strathie, L. & 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), e0169371.

Chimweta, M., Nyakudya, I.W., Jimu, L. & Bray Mashingaidze, A. 2020. Fall armyworm [*Spodoptera frugiperda (*JE Smith)] damage in maize: management options for flood-recession cropping smallholder farmers. International Journal of Pest Management, 66(2), 142-154.

Chown, S.L. & Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, U.K.

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

Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. Journal of Insect Physiology, 47(7), 649-660.

Chuang, W.P., Ray, S., Acevedo, F.E., Peiffer, M., Felton, G.W. & Luthe, D.S. 2014. Herbivore cues from the fall armyworm (*Spodoptera frugiperda*) larvae trigger direct defenses in maize. Molecular Plant-Microbe Interactions, 27(5), 461-470.

Colinet, H. & Renault, D. 2014. Dietary live yeast alters metabolic profiles, protein biosynthesis and thermal stress tolerance of *Drosophila melanogaster*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 170, 6-14.

Colinet, H., Sinclair, B.J., Vernon, P. & Renault, D. 2015. Insects in fluctuating thermal environments. Annual Review of Entomology, 60, 123-140.

Cornelissen, T. 2011. Climate change and its effects on terrestrial insects and herbivory patterns. Neotropical Entomology, 40(2), 155-163.

Day, R. 2018. Invasive species - the hidden threat to sustainable development. Devex. Retrieved from https://www.devex.com/news/sponsored/opinion-invasive-species-the-hidden-threat-to-sustainable-development-91747

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J. & Gomez, J. 2017. Fall armyworm: impacts and implications for Africa. Outlooks on Pest Management, 28(5), 196-201.

Doherty, T., Glen, A., Nimmo, D., Ritchie, E., & Dickman, C. 2016. Invasive species and global biodiversity loss. PNAS, 113(40), 11261-112.

Du Plessis, H., Schlemmer, M.L. & Van den Berg, J. 2020. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects, 11(4), 228. fly. Journal of Evolutionary Biology, 31(1), 98-110.

Foucault, Q., Wieser, A., Waldvogel, A.M., Feldmeyer, B. & Pfenninger, M. 2018. Rapid adaptation to high temperatures in *Chironomus riparius*. Ecology and Evolution, 8(24), 12780-12789.

Ge, Lin-Quan, Liu-Juan Huang, Guo-Qin Yang, Qi-Sheng Song, David Stanley, G. M. Gurr, and Jin-Cai Wu. "Molecular basis for insecticide-enhanced thermotolerance in the brown planthopper N ilaparvata lugens S tål (H emiptera: D elphacidae)." *Molecular ecology* 22, no. 22 (2013): 5624-5634.

Georgoulias, A.K., Akritidis, D., Kalisoras, A., Kapsomenakis, J., Melas, D., Zerefos, C.S. & Zanis, P. 2022. Climate change projections for Greece in the 21st century from high-resolution EURO-CORDEX RCM simulations. Atmospheric Research, 271, p.106049.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PloS One, 11(10), p.e0165632.

Gotcha, N., Terblanche, J.S. & Nyamukondiwa, C. 2018. Plasticity and cross-tolerance to heterogeneous environments: divergent stress responses co-evolved in an African fruit fly. Journal of Evolutionary Biology, 31(1), 98-110.

Grigaltchik, V.S., Ward, A.J. & 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.

Guillén, L., Monribot-Villanueva, J.L., Guerrero-Analco, J.A., Ortega, R., Altúzar-Molina, A., Mena, V., Ruiz-May, E. 7 Aluja, M. 2022. Influence of sunlight incidence and fruit

chemical features on oviposition site selection in mango by Anastrepha obliqua: Implications for management. Insects, 13(2), p.141.

Gunderson, A. R., & Stillman, J. H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceedings of the Royal Society B: Biological Sciences, 282(1808), 20150401.

Hanley, N. & Roberts, M. 2019. The economic benefits of invasive species management. People and Nature, 1(2), 124-137.

Harris, R.M., McQuillan, P. & Hughes, L. 2013. Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). PLoS One, 8(11), p.e80243.

Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Functional Ecology, 27(4), 934-949.

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

Huang, D., Haack, R. A., & Zhang, R. 2011. Does global warming increase establishment rates of invasive alien species? A centurial time series analysis. PloS One, 6(9), e24733.

Huey, R.B. and Kingsolver, J.G., 1993. Evolution of resistance to high temperature in ectotherms. The American Naturalist, 142, S21-S46.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M. & Williams, S.E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philosophical Transactions of the Royal Society B: Biological Sciences, 367(1596), 1665-1679.

Hulme, P.E. 2017. Climate change and biological invasions: evidence, expectations, and response options. Biological Reviews, 92(3), 1297-1313.

IPCC. 2013. Summary for Policymakers in Climate Change: The Physical Science Basic Contribution of Working Group It to the Fifth Assessment Report of the

Intergovermental Panel on Climate Change.

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

Kavhiza, N.J., Zargar, M., Prikhodko, S.I., Pakina, E.N., Murtazova, K.M.S. & Nakhaev, M.R. 2022. Improving crop productivity and ensuring food security through the adoption of genetically modified crops in sub-Saharan Africa. Agronomy, 12(2), 439.

Kearney, M., Shine, R. & Porter, W.P. 2009. The potential for behavioural thermoregulation to buffer "cold-blooded" animals against climate warming. Proceedings of the National Academy of Sciences, 106 (10), 3835-3840.

Kellermann, V., Loeschcke, V., Hoffmann, A.A., Kristensen, T.N., Fløjgaard, C., David, J.R., Svenning, J.C. & Overgaard, J. 2012. Phylogenetic constraints in key functional traits behind species'climate niches: patterns of desiccation and cold resistance across 95 drosophila species. Evolution: International Journal of Organic Evolution, 66 (11), 3377-3389.

Kelley, A.L. 2014. The role thermal physiology plays in species invasion. Conservation Physiology, 2(1), p.cou045.

Kherallah, M., Delgado, C.L., Gabre-Madhin, E.Z., Minot, N. & Johnson, M. 2002. *Reforming agricultural markets in Africa: Achievements and challenges.* The international food policy research institute, The John Hopkins University Press, Baltimore and London.

Klok, C.J. & 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.

Krebs, R.A. & Loeschcke, V. 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Functional Ecology, 8(6), 730-737.

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L. & Tefera, T. 2019. Farmers' knowledge, perceptions, and management practices of the new invasive pest,

fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. International Journal of Pest Management, 65(1), 1-9.

Ma, G. & Ma, C.S. 2022. Potential distribution of invasive crop pests under climate change: incorporating mitigation responses of insects into prediction models. Current Opinion in Insect Science, 49, 15-21.

Machekano, H., Mutamiswa, R. and Nyamukondiwa, C., 2018. Evidence of rapid spread and establishment of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in semi-arid Botswana. Agriculture & Food Security, 7(1), 1-12.

Makgoba, M.C., Tshikhudo, P.P., Nnzeru, L.R. & Makhado, R.A. 2021. Impact of fall armyworm (Spodoptera frugiperda)(JE Smith) on small-scale maize farmers and its control strategies in the Limpopo province, South Africa. *Jàmbá: Journal of Disaster Risk Studies*, *13*(1), 1016.

Markl, G., Ottmann, S., Haasis, T., Budach, D., Krais, S. & Köhler, H.R. 2022. Thermobiological effects of temperature-induced color variations in *Aglais urticae* (Lepidoptera, Nymphalidae). Ecology and Evolution, 12(6), p.e8992.

Mitchell, K.A., Boardman, L., Clusella-Trullas, S. & Terblanche, J.S. 2017. Effects of nutrient and water restriction on thermal tolerance: a test of mechanisms and hypotheses. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 212, 15-23.

Montejo-Kovacevich, G., Martin, S.H., Meier, J.I., Bacquet, C.N., Monllor, M., Jiggins, C.D. & Nadeau, N.J. 2020. Microclimate buffering and thermal tolerance across elevations in a tropical butterfly. Journal of Experimental Biology, 223(8), p.jeb220426.

Motswagole, R., Gotcha, N. & 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, 1179543319863417.

Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2017. Dominance of spotted

stemborer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) over indigenous stemborer species in Africa's changing climates: ecological and thermal biology perspectives. Agricultural and Forest Entomology, 19(4), 344-356.

Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2018. Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. Physiological Entomology, 43(2), 108-119.

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.

Nboyine, J.A., Kusi, F., Abudulai, M., Badii, B.K., Zakaria, M., Adu, G.B., Haruna, A., Seidu, A., Osei, V., Alhassan, S. & Yahaya, A. 2020. A new pest, *Spodoptera frugiperda* (JE Smith), in tropical Africa: Its seasonal dynamics and damage in maize fields in northern Ghana. Crop Protection, 127, 104960.

Nguyen, T.M., Bressac, C. & Chevrier, C. 2013. Heat stress affects male reproduction in a parasitoid wasp. Journal of Insect Physiology, 59(3), 248-254.

Nhamo, L., Matchaya, G., Mabhaudhi, T., Nhlengethwa, S., Nhemachena, C. & Mpandeli, S. 2019. Cereal production trends under climate change: Impacts and adaptation strategies in southern Africa. Agriculture, 9(2), 30.

Noer, N.K., Ørsted, M., Schiffer, M., Hoffmann, A.A., Bahrndorff, S. & Kristensen, T.N. 2022. Into the wild—a field study on the evolutionary and ecological importance of thermal plasticity in ectotherms across temperate and tropical regions. Philosophical Transactions of the Royal Society B, 377(1846), 20210004.

Noh, S., Everman, E.R., Berger, C.M. &Morgan, T.J. 2017. Seasonal variation in basal and plastic cold tolerance: Adaptation is influenced by both long-and short-term phenotypic plasticity. Ecology and Evolution, 7(14), 5248-5257.

Nyamukondiwa, C. & Terblanche, J.S. 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, sex and feeding status. Journal of Thermal Biology, 34(8), 406-414.

Nyamukondiwa, C., Kleynhans, E. & 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.

Overton, K., Maino, J.L., Day, R., Umina, P.A., Bett, B., Carnovale, D., Ekesi, S., Meagher, R. & Reynolds, O.L. 2021. Global crop impacts, yield losses and action thresholds for fall armyworm (Spodoptera frugiperda): A review. *Crop Protection*, *145*, p.105641.

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P. & Thomas, M.B. 2016. Global threat to agriculture from invasive species. Proceedings of the National Academy of Sciences, 113(27), 7575-7579.

Parmesan, C. & Yohe, G. A. 2003. Globally coherent fingerprint of climate change impacts across natural systems. Nature, 421, 37

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics, 37: 637–669

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T.& Tennent, W.J. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature, 399(6736), 579

Peacock, L. & Worner, S.P. 2008. Biological and ecological traits that assist establishment of alien invasive insects. New Zealand Plant Protection, 61, 1-7.

Phophi, M.M., Mafongoya, P. & Lottering, S. 2020. Perceptions of climate change and drivers of insect pest outbreaks in vegetable crops in Limpopo province of South Africa. Climate. 8(2), 27.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., Wong, E.,

Russel, L., Zern, J., Aquino, T. & Tsomondo, T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, ecosystems and environment, 84(1), 1-20.

Prasad, T.V., Gopal, V.N. & Gedia, V.N. 2008. Seasonal abundance of Sesbania thrips *Caliothrips indicus* Bagnall in groundnut. Journal of Agrometeorology, 1, 211–214.

Pratt, C.F., Constantine, K.L. &Murphy, S.T. 2017. Economic impacts of invasive alien species on African smallholder livelihoods. Global Food Security, 14, 31-37.

Qi, X., Majeed, M.Z. & Zhang, W. 2019. Adaptation of Drosophila species to climate change—A literature review since 2003. Journal of Integrative Agriculture, 18(4), 805-814.

Qu, Y., Li, H., Gao, J., Xu, X. & Ji, X. 2011. Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two *Phrynocephalus lizards* (Agamidae), with a review of species studied. Current Zoology, 57(6), 684-700.

Ransom, E., Cockerill, J. & Weatherly, E.R. 2019. The strategic role of agriculture in the economic space of the Southern Africa region. Transforming Agriculture in Southern Africa, 7.

Rukundo, P., Karangwa, P., Uzayisenga, B., Ingabire, J.P., Waweru, B.W., Kajuga, J. & Bizimana, J.P. 2020. Outbreak of fall armyworm (*Spodoptera frugiperda*) and its impact in Rwanda agriculture production. Sustainable Management of Invasive Pests in Africa, (supp.14) 139-157.

Rusch, T.W., Adutwumwaah, A., Beebe, L.E., Tomberlin, J.K. & Tarone, A.M., 2019. The upper thermal tolerance of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae). Journal of Thermal Biology, 85, 102405.

Sales, K., Vasudeva, R. & Gage, M.J. 2021. Fertility and mortality impact of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. Royal Society Open Science, 8(3), 201717.

Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł.,

Hebberecht, L., Thomas, P., Franco, A. & Gage, M.J. 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nature Communications, 9(1), 4771.

Savary, S., Teng, P.S., Willocquet, L. & Nutter Jr, F.W. 2006. Quantification and modeling of crop losses: a review of purposes. Annual Reviews of Phytopathology, 44, 89-112.

Scharf, I., Galkin, N. & Halle, S. 2015. Disentangling the consequences of growth temperature and adult acclimation temperature on starvation and thermal tolerance in the red flour beetle. Evolutionary Biology, 42, 54-62.

Scharf, I., Wexler, Y., MacMillan, H.A., Presman, S., Simson, E. & Rosenstein, S., 2016. The negative effect of starvation and the positive effect of mild thermal stress on thermal tolerance of the red flour beetle, *Tribolium castaneum.* The Science of Nature, 103, 1-10.

Sgro, C.M., Terblanche, J.S. & Hoffmann, A.A. 2016. What can plasticity contribute to insect responses to climate change? Annual review of Entomology, 61,433-451.

Shackleton, C.M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S.E. & Fabricius, C. 2007. Assessing the effects of invasive alien species on rural livelihoods: case examples and a framework from South Africa. Human Ecology, 35, 113–127.

Shackleton, R.T., Le Maitre, D.C. & Richardson, D.M. 2015. Stakeholder perceptions and practices regarding *Prosopis* (mesquite) invasions and management in South Africa. Ambio, 44, 569– 581.

Shackleton, R.T., Shackleton, C.M. & Kull, C.A. 2019. The role of invasive alien species in shaping local livelihoods and human well-being: A review. Journal of Environmental Management, 229, 145-157

Sileshi, G.W., Gebeyehu, S. & Mafongoya, P.L. 2019. The threat of alien invasive insect and mite species to food security in Africa and the need for a continent-wide response. Food Security, 11(4), 763-775.

Sinclair, B.J., Williams, C.M. & Terblanche, J.S. 2012. Variation in thermal performance

among insect populations. Physiological and Biochemical Zoology, 85(6), 594-606.

Soravia, C., Ashton, B.J., Thornton, A. & Ridley, A.R. 2021. The impacts of heat stress on animal cognition: Implications for adaptation to a changing climate. Wiley Interdisciplinary Reviews: Climate Change, 12(4), p.e713.

Stockton, D.G., Wallingford, A.K. & Loeb, G.M. 2018. Phenotypic plasticity promotes overwintering survival in a globally invasive crop pest, *Drosophila suzukii*. Insects, 9(3), 105.

Suby, S.B., Soujanya, P.L., Yadava, P., Patil, J., Subaharan, K., Prasad, G.S., Babu, K.S., Jat, S.L., Yathish, K.R., Vadassery, J. & Kalia, V.K. 2020. Invasion of fall armyworm (*Spodoptera frugiperda*) in India: nature, distribution, management and potential impact. Current Science, 119(1), 44-51.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proceedings of the Royal Society B: Biological Sciences, 278(1713), 1823-183

Synthesis. Oxford, UK: Oxford University Press.

Tambo, J.A., Kansiime, M.K., Rwomushana, I., Mugambi, I., Nunda, W., Mloza Banda, C., Nyamutukwa, S., Makale, F. & Day, R. 2021. Impact of fall armyworm invasion on household income and food security in Zimbabwe. Food and energy security, 10(2), 299-312.

Terblanche, J.S., Nyamukondiwa, C. & Kleynhans, E. 2010. Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). Entomologia Experimentalis et Applicata, 137(3), 304-315.

Tobin, P.C., Nagarkatti, S. & Saunders, M.C. 2003. Phenology of Grape berry moth (Lepidoptera: Tortricidae) in cultivated grape at selected geographic locations. Environmental Entomology, 32, 340–346.

Turriago, J.L., Tejedo, M., Hoyos, J.M. & Bernal, M.H. 2022. The effect of thermal

microenvironment in upper thermal tolerance plasticity in tropical tadpoles. Implications for vulnerability to climate warming. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 337(7), 746-759.

van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R. & Zengeya, T.A. 2020. Biological invasions in South Africa: an overview. In Biological Invasions in South Africa. Springer, Cham, 3-31.

Visser, D. 2017. Fall armyworm: an identification guide in relation to other common caterpillars, a South African perspective, 26.

Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukat, Z., Bugmann, H. & Czucz, B. 2009. Alien species in a warmer world: risks and opportunities. Trends in Ecology & Evolution, 24(12), 686-693.

Weldon, C.W., Terblanche, J.S. & Chown, S.L. 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. Journal of Thermal Biology, 36(8), 479-485.

Xue, Q. & Ma, C.S. 2020. Aged virgin adults respond to extreme heat events with phenotypic plasticity in an invasive species, *Drosophila suzukii*. Journal of Insect Physiology, 121, 104016.

Xue, Q., Majeed, M.Z., Zhang, W. & MA, C.S. 2019. Adaptation of Drosophila species to climate change-A literature review since 2003. Journal of Integrative Agriculture, 18(4), 805-814.Zerebecki, R.A. & Sorte, C.J. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. PLOS One, 6(4), p.e14806.

Yin, W.D., Hoffmann, A.A., Gu, X.B. and Ma, C.S., 2018. Behavioral thermoregulation in a small herbivore avoids direct UVB damage. *Journal of insect physiology*, *107*, pp.276-283.

Chapter 2

Contrasting effects of acute heat shock on physiological and ecological performance of the fall armyworm*

*Published as: **Mbande, A**., Mutamiswa, R., Nyamukondiwa, C. & Chidawanyika, F. (2023).

Contrasting effects of acute heat shock on physiological and ecological performance of the fall armyworm. *Entomologia Experimentalis et Applicata.* **171**: 525-534.

2.1. Introduction

Ectotherms' body temperatures are always closely related to prevailing ambient conditions (Angiletta, 2009). Given the role of temperature in organismal activity, function, and biochemical processes (Chown & Nicolson, 2004), it thus plays a key role in determining overall animal fitness (Bale et al., 2002; Mutamiswa, 2017). Insects are highly responsive to temperature because they are ectotherms. Consequently, climate change-associated heat waves are expected to affect their performance. For example, climate stress may negatively affect native species and benefit invasive alien species (Nyamukondiwa et al., 2022), and coupled with failed natural pest suppression under climate change (Mironidis & Savopoulou-Soultani, 2010; Chidawanyika et al., 2019), continuous bouts of heat waves may thus favor invasive pest proliferation. However, species' responses to thermal variation at both organismal and population level are dynamic and may be mediated by several basal, plastic, and genetically determined factors that are environmentally dependent (Angilletta, 2009; Sgro et al., 2016).

Organisms have evolved various adaptive mechanisms to buffer against stressful environments (Hoffmann et al., 2013). This adaptation can be in two forms: (1) phenotypic plasticity (occurring at individual level or within generation), and (2) genetic changes through natural selection (evolution of stress traits that may favor the fittest) (Angilletta, 2009). Phenotypic plasticity involves changes in an organism's behavior, morphology, and/or physiology in response to changes in environmental conditions (Chown & Nicolson, 2004). Phenotypic plasticity takes two forms, i.e., hardening in the short term and acclimation on longer timescales (Sgro et al., 2016). Both forms allow individuals to respond rapidly to stress outside their thermal limits (Chidawanyika & Terblanche, 2011; Sgro et al., 2016). Phenotypic plasticity of a genotype plays a critical role in many evolutionary processes such as selection within and among species (Salamin et al., 2010) and the establishment of barriers of reproduction between and within populations. Although the phenomenon is nearly ubiquitous

in insects and is mainly adaptive (Chown & Nicolson, 2004; Angiletta 2009), phenotypic plasticity can also be maladaptive. Maladaptive plasticity occurs when a population encounters an environment that induces the production of phenotypes away from the local optimum, resulting in a negative relationship between the direction of plasticity and that of adaptive evolution (Ghalambor et al., 2015). For example, Svensson et al. (2020) showed that phenotypic plasticity is maladaptive to both *Calopteryx splendens* (Harris) and *Calopteryx virgo* (L.).

Heat stress may also have fitness costs and benefits for insects (Segaiso et al., 2022). However, these costs and benefits are dependent on the level of stress. Although many studies have suggested that a single heat wave may negatively affect insect life-history traits – e.g., through reduced fecundity, egg hatchability, and prolonged development (Liang et al., 2014; Zheng et al., 2017) – considerable studies have also shown the contrary, that sub-lethal acute heat stress may increase fitness, e.g., through increased longevity, reproduction, and heat tolerance (Roux et al., 2010; Sarup et al., 2016). Thus, taxonomic differences in the costs and benefits of heat stress on life-history traits warrant a more thorough investigation. At organismal level, sustained exposure to heat stress has fitness consequences due to injuries at cellular level mediated by protein denaturation (Chown & Nicolson, 2004). Injuries sustained can manifest acutely within the same organisms or, in some cases, may appear in subsequent developmental stages and/or generations (Lu et al., 2014; Su et al., 2021). For example, exposure of larval *Drosophila melanogaster* Meigen to sub-lethal heat stress resulted in both mutations and pupation failure (Cui et al., 2008). Furthermore, parental sub-lethal heat shock decreased egg hatchability in *Mononychellus mcgregori* (Flechtmann & Baker) (Lu et al., 2014) and *Plutella xylostella* (L.) (Zhang et al., 2013). Similarly, in *Ephestia cautella* (Walker), sub-lethal heat shock reduced overall fecundity (Silbermann & Tatar, 2000), consistent with results on *Helicoverpa armigera* (Hübner) (Mironidis & Savopoulou-Soultani, 2010) and *Tribolium castaneum* (Herbst) (Sales et al., 2021). Such reduction in fecundity following heat shock stress has been associated with injury to the oocytes and ovaries (Mironidis & Savopoulou-Soultani, 2010). Similarly, in males, heat stress may reduce

fertility through direct injury to the testes and sperm (Rinehart et al., 2000; Sales et al., 2021) and may reduce mating success in other insect species (Jerbi-Elayed et al., 2015). Heat shock may also reduce female longevity among adults (Chen et al., 2015; Zhang et al., 2016) thereby minimizing their full reproduction potential. Subjection to heat stress is known to induce the production of heat shock proteins that prevent protein denaturation and repair damaged proteins (Nyamukondiwa et al., 2010; Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2017). Thus, heat stress may be a significant determinant of survival and potentially mediate insect population dynamics in nature. As such, an understanding of its effects on ecological and physiological responses is critical in elucidating invasive insect population dynamics, establishment, and spread under changing environments (Chen et al., 2019; Tarusikirwa et al., 2022).

The fall armyworm (FAW), *Spodoptera frugiperda* **(**J.E. Smith) (Lepidoptera: Noctuidae), native to South America, is a highly invasive insect pest that threatens food security (Kenis et al., 2023). Given that FAW adults may occupy thermally divergent habitats, coupled with increasing acute temperature stress, no studies have investigated the effects of previous thermally stressful environments on the eco-physiology of FAW. As stated in previously, under optimum temperature conditions of 28 °C, an adult female usually deposits eggs for 4–5 days, in batches of 0– 200 each day – so, total fecundity is up to 1000 eggs (Kumela et al., 2019).

Fall armyworm larvae have 5–10 instars (six optimally) (Ali et al., 1990; reviewed in Kenis et al., 2023). First and second instars feed on one side of the leaf skeletonizing it in the process, whereas the later instars feed on most plant parts damaging them in the process (Kumela et al., 2019). The larval stage may last up to 14 days in summer and up to 30 days in winter, highly suggestive of temperature-dependent development (Nagoshi et al., 2019). Cognizant of its economic importance and the adult migratory nature, the effects of thermal environments on FAW fitness thus warrant investigation. I assessed the effects of sub-lethal heat shock on eco-physiological performance of this invasive insect pest. Specifically, I determined whether sub-lethal heat shock may influence (1) cold tolerance (critical thermal minima, CT_{min}) and heat tolerance (critical thermal maxima, CT_{max}), (2) reproductive capacity

(fecundity and hatching success), and (3) longevity. Phenology, which refers to the timing of seasonal activities such as development, since phenological responses may be altered by temperature regimes (Gill et al., 2015) and the ecological impact of climate change such as extreme weather events and drought, which are associated with insect outbreaks. I hypothesized that adult heat shock stress may influence physiological and ecological performance of FAW adults.

2.2 Materials and methods

2.2.1 Plant and insect culture

Maize plants, *Zea mays* L. (Poaceae), were grown individually in plastic pots (20 cm diameter) with loamy soil mixed with potting mix (Gromor, Cato Ridge, KwaZulu-Natal province, South Africa) as it is the preferred host of *S. frugiperda* (Assefa, 2018). The plants were watered daily and fertilized weekly with 30 g of fertilizer to maintain the same levels of fertilizer (Lawn & Leaf 7:1:3; Wonder, Isando, South Africa) for 4 weeks before FAW inoculation.

The initial *Spodoptera frugiperda* larval culture (maize strain) was obtained from the Agricultural Research Council

– Plant Health Protection (ARC-PHP) in Pretoria, South Africa, and maintained in the insectary under optimum conditions at 28 ± 2 °C (Ali et al., 1990; Du Plessis et al., 2020), 65 ± 5% relative humidity (RH) and L12:D12 photoperiod. They were allowed to grow into adults and newly laid (F_1 generation) eggs were incubated until larval emergence in cages with 2-week-old fresh maize plants. Thereafter, third larvae were individually transferred into vials containing artificial diet to prevent cannibalism until they pupated. Pupae were then sexed using differences in genitalia, where the genital slit in females is located on the anterior edge of the fourth abdominal segment posterior to the wing covers and is located on the posterior edge of the same segment in males (Tuncer & Aker, 2018).These were transferred into 1-L plastic tubs containing moist soil until adult eclosion.

2.2.2 Adult sub-lethal heat shock treatments

Newly emerged adults of *S. frugiperda* (24- to 48 -h old) were individually heat-shocked in propylene vials at 32, 35, or 38 °C for 2 h in a programmable water bath (model Tx150; Grant Instruments, Shepreth, UK) using the plunge protocol (e.g., Chidawanyika & Terblanche, 2011). These non-lethal high temperature–time combinations for heat shock were chosen based on preliminary trials in which survival was assessed under similar static thermal regimes. In addition, microclimatic conditions experienced in one of the maize-growing areas in South Africa range from –13.7 to 45.6 °C (see Results). A piece of moist cotton wool was placed at the bottom of each vial to prevent desiccation-related mortality. Controls were maintained under optimum conditions (28 ± 2 °C, 65 ± 5% R.H., L12:D12) in cages in the insectarium before measuring life-history traits. All metrics were measured just after heat shock, i.e., 0 h post heat shock treatment and using 24- to 48-h-old moths.

2.2.3 Effects of heat shock on low and high temperature tolerance

Critical thermal limits (CTLs; CT_{min} and CT_{max}) were assessed following Nyamukondiwa & Terblanche (2010). Ten adult FAW from heat shock treatment and controls were individually placed into a double jacketed 'organ pipe' chamber comprising 11 separate 200-mm tubes, connected to a programmable Grant Tx150 water bath filled with a 1:1 mixture of water and propylene glycol and subjected to constant heating and/or cooling. In the 'organ pipe', insects were given 10 min first to equilibrate at 28 °C (optimum developmental temperature) before ramping temperature up (CT_{max}) or down (CT_{min}) at a rate of 0.25 °C per min. This was repeated to yield n = 20 per treatment (20 replications). A thermocouple (type K 36 SWG) (Fluke Corporation, Everett, WA, USA) connected to a digital thermometer (53/54IIB; Fluke Corporation, Everett, WA, USA) was inserted into the control chamber to monitor the chamber temperatures. Critical thermal minima (CT_{min}) and maxima (CT_{max}) were defined as the lower and upper temperatures, respectively, at which the adults lost muscle coordination, which was regarded as a lack of response to mild prodding (e.g., Nyamukondiwa & Terblanche, 2010).

2.2.4 Effects of heat shock on female fecundity and hatching success

Following heat shock, virgin individual adult females and males were paired as follows: treated female × untreated male, treated female × treated male, and untreated female × treated male (20 pairs each). Each pair was transferred into a rearing cage with potted fresh 4-week-old maize plants and a cotton wad soaked in 25% sugar water for oviposition and food provision, respectively. Untreated adults (male and female) were maintained at 28 °C as controls. The plants were monitored daily and leaves with newly deposited eggs were cut from the plant and transferred into Petri dishes and incubated under optimum conditions. Eggs were counted under a Nikon SMZ 645 dissecting microscope in order to determine fecundity. The process was repeated until no newly deposited eggs were observed (up to day 15). Fecundity was defined as the total number of eggs per pair during 15 days.

Hatchlings were counted and removed daily until no more larvae were observed. Hatching success was defined as the total number of larvae that hatched vs. the total number of eggs from each pair.

2.2.5 Effects of heat shock on longevity

Twenty replicates of heat-shocked virgin male and female adults were placed individually in propylene vials with screw cap lids (20 males and 20 females). A moist cotton wick dipped in 10% sugar solution was placed in each vial for provision of food and water. Thereafter, the vials were maintained in the insectary under optimum conditions (28 °C, 65 ± 5% r.h., L12:D12). Controls (virgin males and females) were maintained individually in cages as treatments under optimum conditions before measuring longevity. Mortality was recorded once per day until all the adults were dead.

2.2.6 Microclimate data recording

Microclimatic data were recorded using model DS1920 Thermocron iButtons (five iButtons were used) (0.5 °C accuracy, 0.5 h sampling frequency; Dallas Semiconductors, Dallas, TX, USA) in Clifton, Bloemfontein, Free State province, South Africa (29.1128°S, 26.2312°E) during the period

March 2019 to March 2020 to determine the environmental conditions experienced by *S. frugiperda* in the field. Most *S. frugiperda* host plants are grown in this area at subsistence and commercial level. The iButtons were placed under a tree canopy (shaded environment), 1 m above the ground.

2.3 Data analysis

Data were analyzed in STATISTICA v.13.5.0 (TIBCO, Palo Alto, CA, USA) and R v.4.1.2 (R Core Team, 2021). Data were first checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests, respectively. For CTLs, linear model assumptions of constant variance and normal errors were met, whereas longevity, fecundity, and hatching success data did not conform to assumptions of ANOVA. Therefore, CTLs results were analyzed using full-factorial ANOVA with sex and treatment as categorical factors, and CT_{min} and CT_{max} as dependent variables. Longevity, fecundity, and hatching success data were considered as count data and were analyzed using a generalized linear model (GLM) assuming a Poisson distribution and a log link function in R. A quadratic term was added to the temperature term, to improve model fit based on the Akaike Information Criterion. Tukey-Kramer post-hoc tests were used to separate means.

2.4 Results

2.4.1 Effects of sub-lethal heat shock stress on low and high temperature tolerance

Sub-lethal heat shock significantly influenced low-temperature tolerance (CT_{min}) in both adult females and males (Table 2.1). The interaction effects between sex and treatment were highly significant for CT_{min} (Table 2.1). Sub-lethal heat shock stress at the highest treatment temperature (38 °C) significantly increased CT_{min} (reduced cold tolerance) for both males (6.43 ± 0.09 °C) and females (7.0 ± 2 ± 0.06 °C) (Figure 2.1A). Control treatment showed a significant difference in low-temperature tolerance between females and males with females recording higher CT_{min} than males (Figure 2.1A).

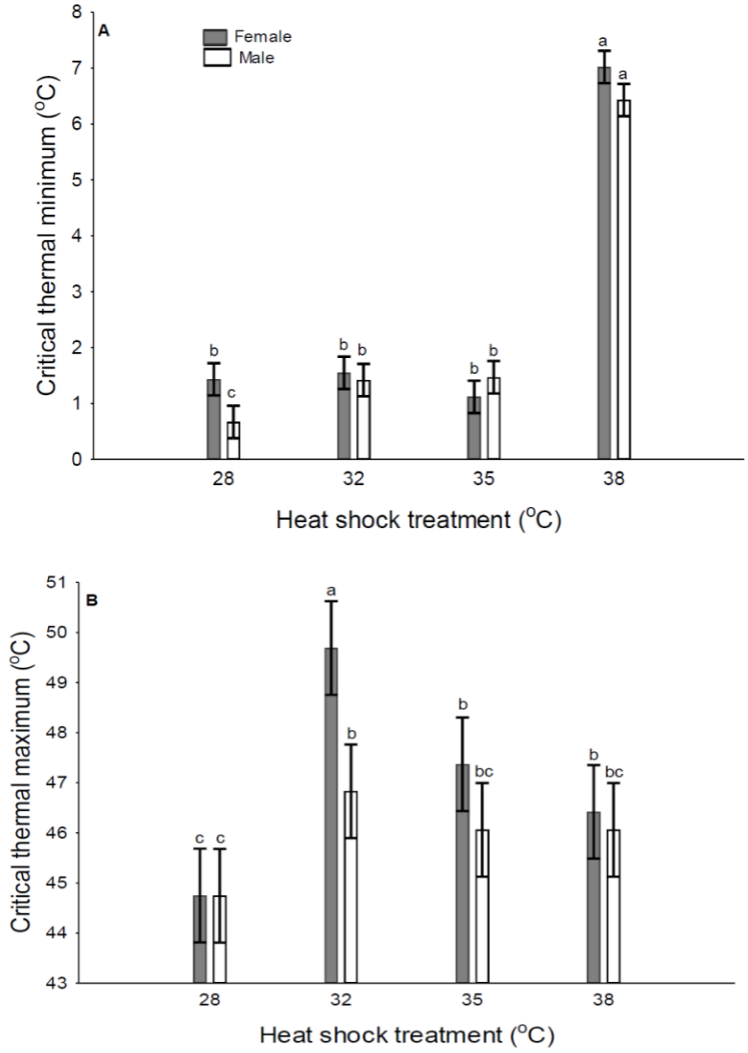

Figure 2. 1 : Effects of prior sub-lethal heat shock treatment (2h) and sex on critical thermal minimum (A) and critical thermal maximum (B) for Spodoptera frugiperda. Error bars represent 95% confidence limits (N = 20) and means with the same letter are not significantly different from each other.

Table 2. 1: Effects of prior sub-lethal heat shock treatment (2h) on *Spodoptera frugiperda* critical thermal limits (CTmin, CTmax). Full factorial ANOVA of the effects of sub-lethal 2-h heat shock treatment at four temperatures on critical thermal minimum (CTmin) and maximum (CTmax) of *Spodoptera frugiperda* males and females

Trait	**Effect**	**SS**	**d.f.**	**MS**	**F**	**P**
CT_{min}	Intercept	1114.61	1	1114.61	2590.63	<0.001
	Sex	3.22	1	3.22	7.49	<0.01
	Temperature	894.02	3	298.01	692.64	<0.001
	Sex*temperature	7.51	3	2.50	5.82	<0.001
	Error	65.40	152	0.43		
CT_{max}	Intercept	345820.5	1	345820.5	77318.17	<0.001
	Sex	51.4	1	51.4	11.50	<0.001
	Temperature	251.3	3	83.8	18.73	<0.001
	Sex*tTemperature	48.8	3	16.3	3.63	0.014
	Error	679.8	152	4.5		

Similarly, heat shock significantly influenced high-temperature tolerance (CT_{max}) in both adult females and males (Table 2.1). It improved CT_{max} in both sexes at 32 °C heat shock treatment with females recording 49.69 ± 0.059 °C and males 46.82 ± 0.191 °C, showing plasticity towards heat stress tolerance (Figure 2.1B). The interaction between treatments was significantly different for high temperature tolerance (Table 2.1). Control treatment had no significant response for CT_{max} for both sexes (Fig. 2.1B).

2.4.2 Effects of heat shock on female fecundity and hatching success

Fecundity and hatching success varied significantly across pairs following heat shock treatments

(Table 2.2, Figure 2.2). There was a significant interaction effect between temperature and pair for both fecundity and hatching success (Table 2.2). Fecundity and hatching success decreased with increase in heat shock temperature across all pairs (Figure 2.2). Following heat shock treatments at 32 to 38 °C, fecundity ranged from 409 to 144 (treated male × untreated female), from 369 to 132 (treated female × untreated male), and from 342 to 81 eggs (treated male × treated female) (Figure 2.2A). Likewise, following the same heat shock treatment conditions, hatching success ranged from 332 to 103 (treated male × untreated

female), from 287 to 99 (treated female × untreated male), and from 301 to 81 eggs (treated male × treated female) (Figure 2.2B). Fecundity and hatching success at 35 °C differed significantly between both sexes treated vs. treated male × untreated female (Figure 2). At 38 °C, a significant difference in fecundity was recorded between the same pairs, but not in hatching success (Figure 2.2). The treated male × treated female pair recorded the lowest fecundity and hatching success at 35 and 38 °C (Figure 2.2).

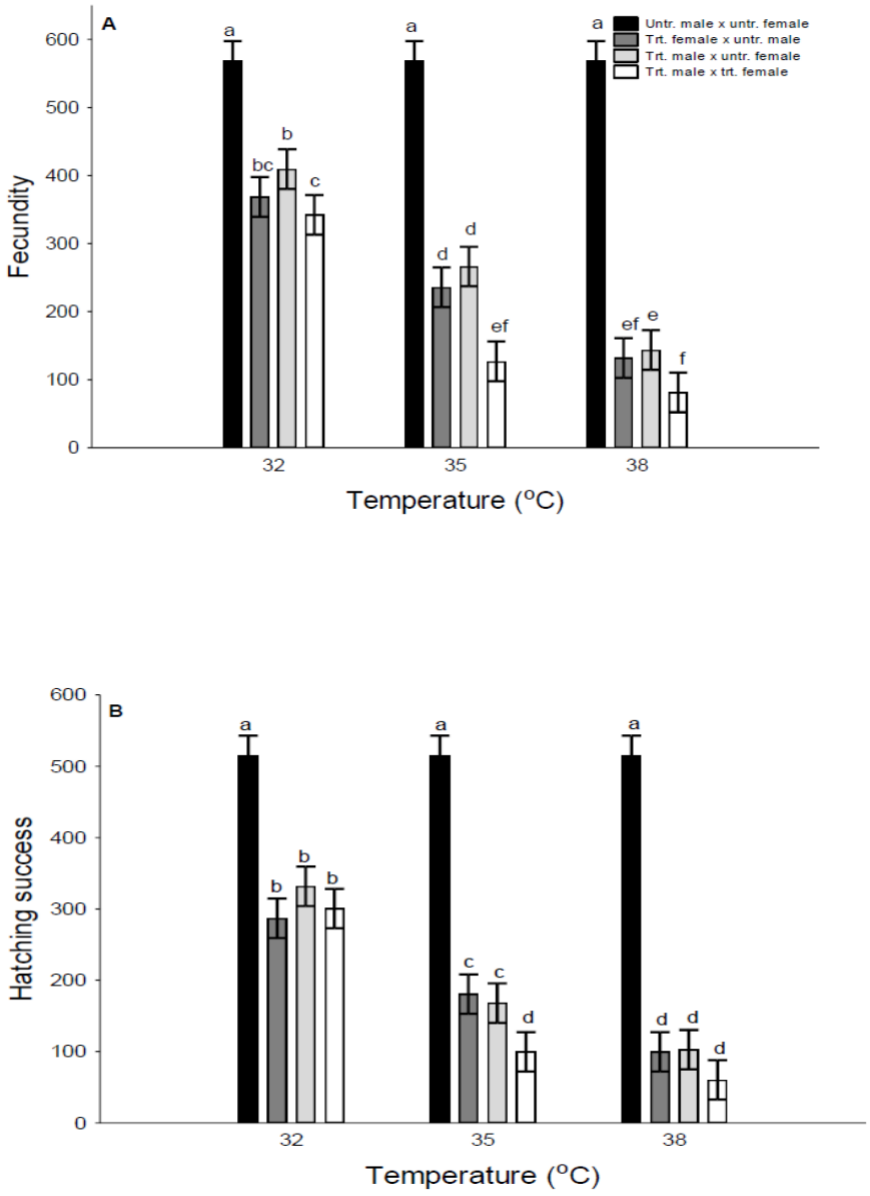

Figure 2. 2 Effects of prior sub-lethal heat shock treatment (2h) and sex on Spodoptera frugiperda fecundity (A) and hatching success (B) (N=20 pairs). Error bars represent 95% confidence limits. Means with the same letter are not significantly different from each other

Fecundity was defined as the number of eggs per pair following adult oviposition for 15 days duration. Hatching success was defined as the number of larvae that hatched versus total number of eggs from each pair after not seeing new hatchlings.

Table 2. 2: Effects of prior sub-lethal heat shock treatment (2h) on *Spodoptera frugiperda* fecundity and hatching success. Factorial analysis using generalized linear model (GLM) of the effects of sub-lethal 2-h heat shock treatment at three temperatures on fecundity and hatching success of *Spodoptera frugiperda* males and females. Pair = male × female.

Trait	Effect	d.f.	χ^2	P
Fecundity	Temperature	2	1496754	<0.001
	Pair	3	5289052	<0.001
	Temperature*pair	6	550537	<0.001
Hatching success	Temperature	2	1144322	<0.001
	Pair	3	5094727	<0.001
	Temperature*pair	6	428048	<0.001

2.4.3 Effects of heat shock on longevity

Overall, longevity was marginally higher in males (z = 2.496, $P = 0.013$) and initially increased and later decreased with increasing heat shock (z = 3.821, $P < 0.001$), as was the case with females (Figure 2.3). However, there were no significant sex*temperature effects across treatments (z = 1.789, $P = 0.074$) (Figure 2.3).

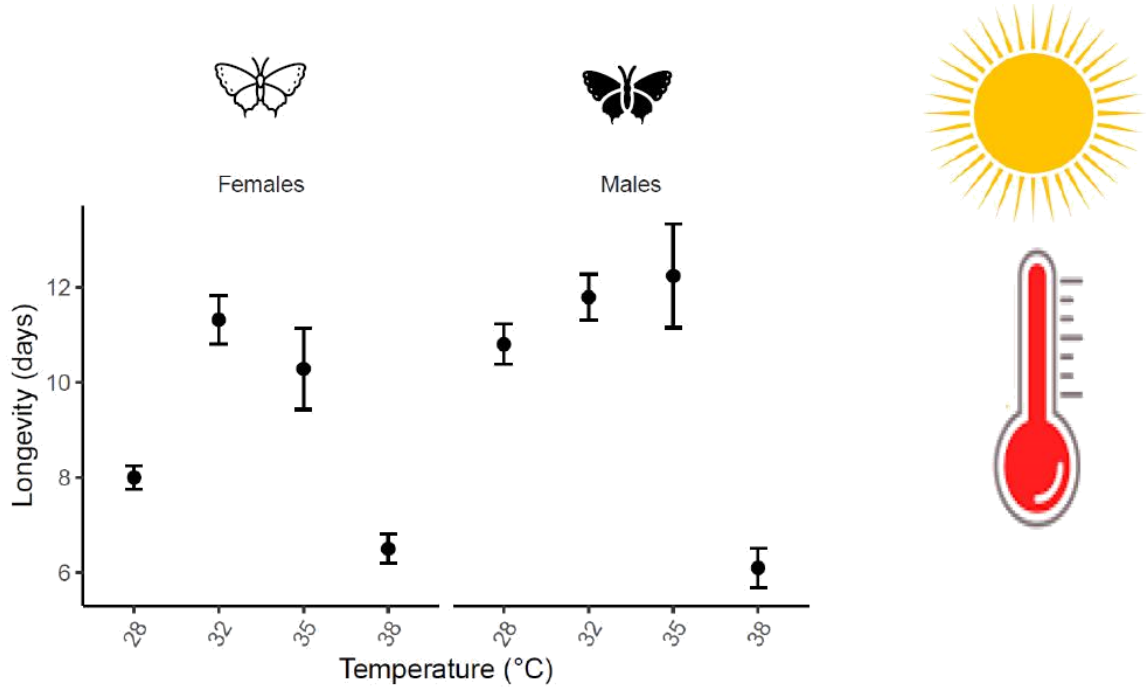

Figure 2. 3 Effects of prior sub-lethal heat shock treatment (2h) and sex on the longevity of *Spodoptera frugiperda* (N=20 pairs). Nodes represent raw data

2.4.4 Microclimate data recordings

Temperature between March 2019 and March 2020 in Clifton, Bloemfontein, South Africa, one of the areas where maize is grown, ranged from –13.7 to 45.6 °C (Figure 2.4A). Analysis of the temperature severity showed that acute heat stress episodes are common under natural conditions (Figure 2.4B). Temperature stress conditions of 30–45 °C were experienced at least 1000×, whereas temperatures of 45–50 °C were experienced 250× in a calendar year (Figure 2.4B).

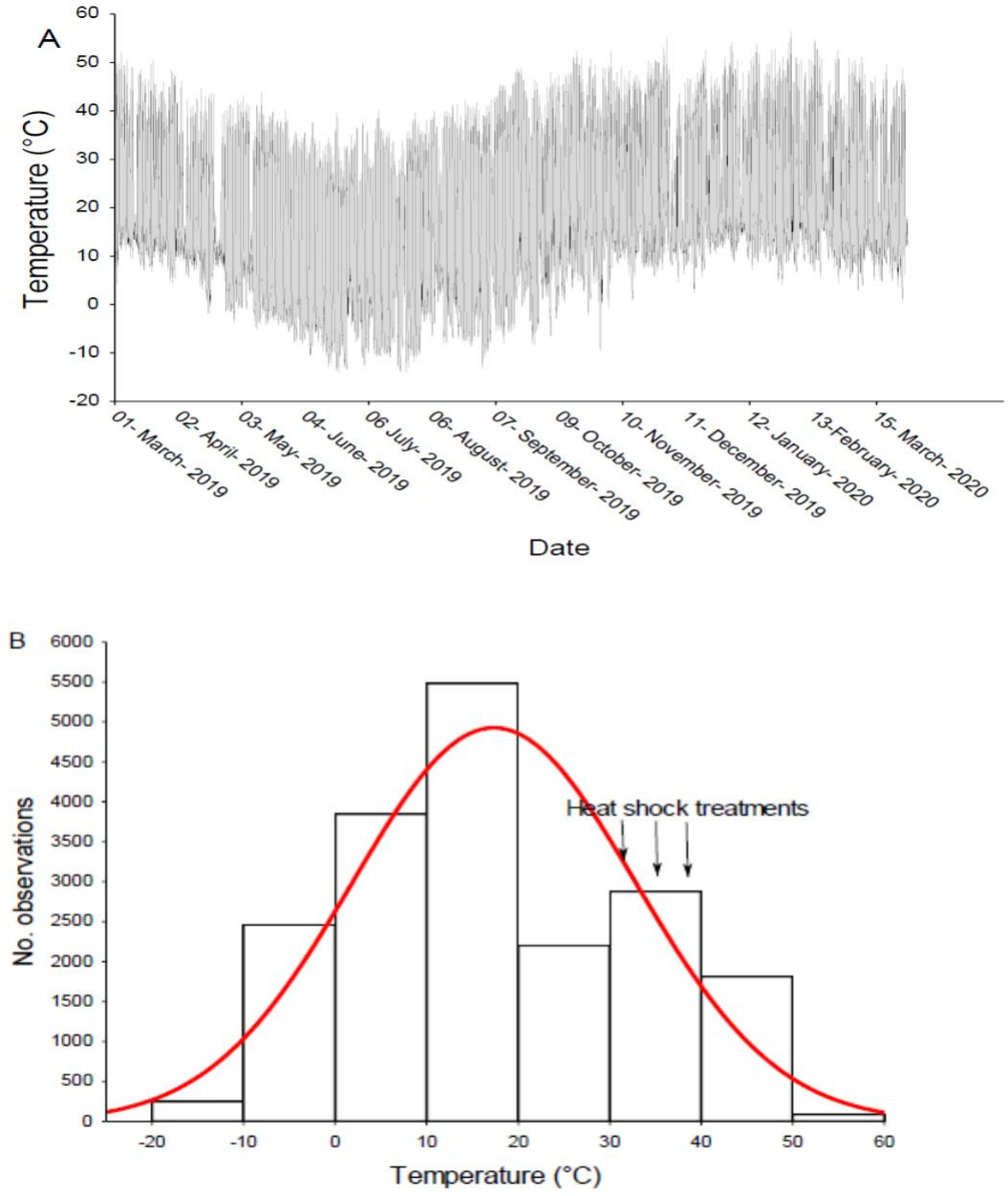

Figure 2. 4 : (A) Microhabitat temperature (denoted by line curve) recorded using Thermocron iButtons at 0.5-h sampling frequency under a tree canopy, 1 m above the ground at Clifton, Free State Province, South Africa, from March 2019 to March 2020. (B) Temperature frequency distribution during the same period. Arrows indicate the heat shock temperatures that were used in laboratory experiments.

2.5 Discussion

In nature, insects experience adverse abiotic stress, and the ability to survive these stressors may determine success (Nyamukondiwa et al., 2022). The current study showed contrasting physiological and ecological responses of *S. frugiperda* following sub-lethal heat shock stress. I found that sub-lethal heat shock treatment compromised cold tolerance (CT_{min}) whereas it improved heat tolerance (CT_{max}) across all treatments. Furthermore, similar to CT_{min} results, I also found that acute sub-lethal heat stress during development may come at the cost of adult survival and fitness, manifested as reduced longevity and reproductive success (lower fecundity and egg hatching success).

Sub-lethal heat stress significantly reduced cold tolerance in this study, showing evidence of cross-susceptibility between heat and cold stress (Tarusikirwa et al., 2022). Although previous studies demonstrated the importance of cross-tolerance in response to heat and cold (Chidawanyika & Terblanche, 2011; Sinclair et al., 2012, 2015), this study showed no evidence for this relationship. This may mean that heat stress can exert cumulative effects leading to poor thermal tolerance, perhaps due to cumulative tissue injuries. These results are also in agreement with Mutamiswa et al. (2018) and Tarusikirwa et al. (2020) who reported reduced cold tolerance (CT_{min}) following heat stress in the lepidopteran species *Busseola fusca* (Fuller) and *Tuta absoluta* (Meyrick).

There was improved heat tolerance (CT_{max}) following sub-lethal heat shock treatments in both sexes indicating the ability to shift CT_{max} phenotypes, otherwise termed phenotypic plasticity. The increase in CT_{max} following heat shock treatment is not surprising as several studies reported significantly higher expression of heat shock proteins (HSP90 and HSP70) in heat shocked relative to untreated individuals, suggesting their role in heat tolerance (reviewed in Feder & Hofmann, 1999; King & McRae, 2015). Upregulation of heat shock proteins, which act as molecular chaperones against protein denaturation following heat stress, may explain the current results (Lopez-Martinez & Denlinger, 2008; Al-Ghzawi et al., 2022; Xing & Zhao, 2022). As heat

shock protein levels were not measured in the current study, this warrants further investigation to fully elucidate mechanisms eliciting these plastic responses. Females had higher heat tolerance (CT_{max}) and plasticity thereof than males, conferring them a survival advantage over males in natural heat stress environments. This is consistent with sex-related differences in heat tolerance reported by Nyamukondiwa & Terblanche (2009). Females often have higher lipid content, owing to higher reproductive investment. Lipids play a key role in thermal tolerance (see Chown & Nicolson, 2004); thus, lipids may explain differences in basal and plastic differences observed here. However, sex-related lipid content differences and their role in the CT_{max} variation should be investigated further to test this hypothesis. For invasive pests in the tropical regions, counter-adaptive physiological adjustments against heat stress sustain dispersal propensity through preservation of key life-history traits such as mating and locomotion (Bujan et al., 2021; Ma & Ma, 2022; Nyamukondiwa et al., 2022). Here, FAW showed capacity for rapid CT_{max} adjustment under heat stress, suggesting plasticity. This plasticity may likely aid its invasiveness, consistent with reports on related species (e.g., Nyamukondiwa et al., 2010; Tarusikirwa et al., 2022).

Fecundity and hatching success decreased with increase in sub-lethal heat shock temperature underlying the cumulative effects of tissue damage (Chown & Nicholson, 2004). Thus, acute heat waves may reduce the severity of FAW invasions and the damage larvae cause to crops in the short term due to poor FAW reproductive success. However, the net invasiveness of FAW will depend on how FAW compensate for heat stress-associated costs of reproduction. The mechanisms behind reduced fecundity and hatching success are not clear. Heat stress is known to elicit dehydration and denaturalization of proteins including enzymes resulting in reduced survival, fecundity, or offspring viability (Somero, 1995). Furthermore, the female reproductive system is known to be sensitive to thermal stress due to disturbance of the

neurohormonal regulation of reproduction (Munoz-Valencia et al., 2013). Studies have reported that heat shock can elicit injury to oocytes and ovarian development leading to a decrease in egg production, whereas in males, heat shock can reduce mating success by directly injuring testes and sperms, resulting in reduced fertility (Krebs & Loeschke, 1994; Rinehart et al., 2000; Cui et al., 2008; Sales et al., 2021). In addition, spermatozoa are generally sensitive to even negligible variation in temperature such that heat-exposed sperm incurs DNA breakage and chromatin decondensation leading to blastocyst and embryo failures (Burfening et al., 1970; Sales et al., 2021). Given that the present study treated both males and females, heat shock may have influenced oogenesis, spermatogenesis, and embryogenesis, which culminated in reduced fecundity and hatching success. As this study did not explore reproductive system responses to heat stress (e.g., sperm and egg counts and viability), nor assess whether the reduction in fecundity was a result of damage to the reproductive system of both sexes or only one sex, this warrants further investigation. Furthermore, given repeated episodes of acute heat stress with climate change (e.g., Stillman, 2019), future studies should also look into the 'chronic' effects of repeated heat stress exposure on the physiology and ecology of insects.

The results showed that the mean longevity increased with increased heat shock treatments for both sexes, but significantly decreased beyond 32 °C treatments, suggesting temperature-related effects on *S. frugiperda* longevity. This result is in agreement with Zhou et al. (2011) who reported a drastic decline in *Ophraella communa* LeSage adult longevity following exposure (for 2 h) to temperatures above 35 °C. Males lived longer than females indicating a higher cost of acute heat stress treatment in females. Such differential sex responses to heat stress may reduce the full reproduction potential of this invasive pest.

Microclimate data revealed that heat shock conditions that were employed in this study are regularly experienced in nature, and that even more severe heat stress conditions are common. Heat stress may thus realistically influence FAW physiological and ecological

success (reproduction and longevity), which may manifest through, e.g., changes in population phenology and dynamics. Further work is necessary to fully test the capabilities of different life-stages to mount plastic responses to diverse stressful environments.

In conclusion, this study shows contrasting effects of acute sub-lethal heat stress on FAW physiological and ecological traits. Cold tolerance was reduced following heat treatment whereas heat tolerance was improved, suggesting cross-susceptibility between heat and cold stress. Sex-related differences in physiological and ecological responses were shown following acute heat stress treatment; for example, females had higher CT_{max} whereas males had higher longevity. This sex-related fitness and survival advantage may partly enhance population persistence under changing environment. I also show that acute heat stress during development may manifest as reduced adult fecundity, hatching success, and longevity. Reduced reproduction and survival following heat stress will have implications for FAW population persistence amidst increased heat waves with climate change. Future investigations are necessary to elucidate the physiological mechanisms that underlie the variations elicited by heat stress on the fitness and life-history traits of *S. frugiperda*. In addition, transgenerational plasticity studies are also recommended, as well as studies of the effects of more chronic and repeated episodes of heat stress. This information is significant in pest risk assessment and in the development of *S. frugiperda* management options for sustainable crop production under changing climate.

2.6 References

Al-Ghzawi, A.A.M.A., Al-Zghoul, M.B., Zaitoun, S., Al-Omary, I.M. & Alahmad, N.A. 2022. Dynamics of heat shock proteins and heat shock factor expression during heat stress in daughter workers in pre-heat-treated (rapid heat hardening) *Apis mellifera* mother queens. Journal of Thermal Biology, 104, 103194.

Ali, A., Luttrell, R.G. & Schneider, J.C. 1990. Effects of temperature and larval diet on development of the fall armyworm (Lepidoptera: Noctuidae). Annals of the Entomological Society of America, 83(4), 725-733.

Angilletta, M.J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, USA.

Assefa, F. & Ayalew, D. 2019. Status and control measures of fall armyworm (*Spodoptera Frugiperda*) infestations in maize fields in Ethiopia: A Review. Cogent Food Agriculture, 5(1), 1641902.

Assefa, F. 2018. Status of fall armyworm (*Spodoptera frugiperda*), biology and control measures on maize crop in Ethiopia: a review. International Journal of Entomological Research, 6(2), 75-85.

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. & Good, J.E. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology, 8(1), 1-16.

Bujan, J., Charavel, E., Bates, O.K., Gippet, J.M., Darras, H., Lebas, C. & Bertelsmeier, C. 2021. Increased acclimation ability accompanies a thermal niche shift of a recent invasion. Journal of Animal Ecology, 90(2), 483-491.

Burfening, P.J., Elliott, D.S., Eisen, E.J. & Ulberg, L.C. 1970. Survival of embryos resulting from spermatozoa produced by mice exposed to elevated ambient temperature. Journal of Animal Science, 30(4), 578-582.

Chen, H., Solangi, G.S., Zhao, C., Yang, L., Guo, J. , Wan, F. & Zhou, Z. 2019. Physiological metabolic responses of *Ophraella communa* to high temperature stress. Frontiers in Physiology, 10, 1053.

Chen, W., Li, D., Zhang, M., Zhao, Y., Wu, W. & Zhang, G. 2015. Cloning and differential expression of five heat shock protein genes associated with thermal stress and development in the polyphagous predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae). Experimental and Applied Acarology, 67, 65-85.

Chidawanyika, F. & Terblanche, J.S. 2011. Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). Journal of Insect Physiology, 57(1), 108-117.

Chidawanyika, F., Mudavanhu, P. & Nyamukondiwa, C. 2019. Global climate change as a driver of bottom-up and top-down factors in agricultural landscapes and the fate of host-parasitoid interactions. Frontiers in Ecology and Evolution, 7, p.80.

Chown, S.L. & Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, U.K.

Cui, X., Wan, F., Xie, M. & Liu, T. 2008. Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. Journal of Insect Science, 8(1), p.24.

Day, R., Abrahams, P. & Bateman, M., et al. 2017. Fall armyworm: impacts and implications for Africa. Outlooks on Pest Management, 28(5), 196-201.

Du Plessis, H., Schlemmer, M.L. & Van den Berg, J., 2020. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects, 11(4), 228.

Feder, M.E. & Hofmann, G.E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and Ecological Physiology. Annual Review of Physiology, 61(1), 243-282.

Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N. & Hughes, K.A. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression

in nature. Nature, 525(7569), 372-375.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PloS One, 11(10), e0165632.

Griffith, T.C., Paterson, I.D., Owen, C.A. & Coetzee, J.A. 2019. Thermal plasticity and microevolution enhance establishment success and persistence of a water hyacinth biological control agent. Entomologia Experimentalis et Applicata, 167(7), 616-625.

Hodgins, K.A., Bock, D.G. & Rieseberg, L.H. 2018. Trait evolution in invasive species. Annual Plant Reviews Online, 459-496.

Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Functional Ecology, 27(4), .934-949.

Hulme, P.E. 2017. Climate change and biological invasions: evidence, expectations, and response options. Biological Reviews, 92(3), 1297-1313.

Jerbi-Elayed, M., Lebdi-Grissa, K., Foray, V., Muratori, F. & Hance, T. 2015. Using multiple traits to estimate the effects of heat shock on the fitness of *Aphidius colemani*. Entomologia Experimentalis et Applicata, 155(1), 18-27.

Jørgensen, L.B., Malte, H., Ørsted, M., Klahn, N.A. & Overgaard, J. 2021. A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. Scientific Reports, 11(1), 1-14.

Kasoma, C., Shimelis, H. & Laing, M.D. 2021. Fall armyworm invasion in Africa: implications for maize production and breeding. Journal of Crop Improvement, 35(1), 111-146.

Kenis, G. Benelli, A. & Biondi, P.A., et al. 2022 Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. Entomologia Generalis.doi: https://10.1127/entomologia/2022/1659.

King, A.M. & McRae, T.H. 2015. Insect heat shock proteins during stress and diapause. Annual Review of Entomology, 60, 59-75.

Krebs, R.A. & Loeschcke, V. 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Functional Ecology, 730-737.

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L. & Tefera, T. 2019. Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. International Journal of Pest Management, 65(1),1-9.

Liang, L.N., Zhang, W., Ma, G., Hoffmann, A.A. & Ma, C.S. 2014. A single hot event stimulates adult performance but reduces egg survival in the oriental fruit moth, *Grapholitha molesta.* Plos One., 9, p.e116339.

Lopez-Martinez, G. & Denlinger, D.L. 2008. Regulation of heat shock proteins in the apple maggot *Rhagoletis pomonella* during hot summer days and overwintering diapause. Physiological Entomology, 33(4), 346-352.

Lu, F., Chen, Q., Chen, Z., Lu, H., Xu, X. & Jing, F. 2014. Effects of heat stress on development, reproduction, and activities of protective enzymes in *Mononychellus mcgregori*. Experimental and Applied Acarology, 63, 267-284.

Ma, G. & Ma, C.S. 2022. Potential distribution of invasive crop pests under climate change: incorporating mitigation responses of insects into prediction models. Current Opinion in Insect Science, 49, 15-21.

Mironidis, G.K. & Savopoulou-Soultani, M. 2010. Effects of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults. Journal of Thermal Biology, 35(2), 59-69.

Montezano, D.G., Sosa-Gómez, D.R., Specht, A., Roque-Specht, V.F., Sousa-Silva, J.C., Paula-Moraes, S.D., Peterson, J.A. & Hunt, T.E. 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. African Entomology, 26(2), 286-300.

Munoz-Valencia, V., Diaz-Gonzalez, F., Manzano-Martínez, M.D.R., Toro-Perea, N. & Cárdenas-Henao, H. 2013. Basal and induced thermotolerance to heat shocks in

Bemisia tabaci biotype B (Hemiptera: Aleyrodidae). Revista Colombiana de Entomología, 39(1), 18-25.

Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2017. Dominance of spotted stemborer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) over indigenous stemborer species in Africa's changing climates: ecological and thermal biology perspectives. Agricultural and Forest Entomology, 19(4), 344-356.

Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2018. Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. Physiological Entomology, 43(2), 108-119.

Nagoshi, R.N., Dhanani, I., Asokan, R., Mahadevaswamy, H.M., Kalleshwaraswamy, C.M.&Meagher, R.L. 2019. Genetic characterization of fall armyworm infesting South Africa and India indicate recent introduction from a common source population. PLoS One., 14, e0217755.

Nyamukondiwa, C. & Terblanche, J.S. 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, sex and feeding status. Journal of Thermal Biology, 34(8), 406-414.

Nyamukondiwa, C. & 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., Kleynhans, E. & 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., Machekano, H., Chidawanyika, F., Mutamiswa, R., Ma, G. & Ma, C.S. 2022. Geographic dispersion of invasive crop pests: the role of basal plastic climate stress tolerance and other complementary traits in the tropics Current Opinion in Insect

Science, 100878.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rinehart, J.P., Yocum, G.D. & Denlinger, D.L. 2000. Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. Physiological Entomology, 25(4), 330-336.

Roux, O., Le Lann, C., Van Alphen, J.J.M. & van Baaren, J. 2010. How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)? Bulletin of Entomological Research, 100(5), 543-549.

Salamin, N., Wüest, R.O., Lavergne, S., Thuiller, W. & Pearman, P.B. 2010. Pearman Assessing rapid evolution in a changing environment. Trends in Ecology & Evolution, 25(12), 692-698.

Sales, K., Vasudeva, R. & Gage, M.J. 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. Royal Society Open Science, 8(3), p.201717.

Sarup, P., Petersen, S.M.M., Nielsen, N.C., Loeschcke, V. & Malmendal, A. 2016. Mild heat treatments induce long-term changes in metabolites associated with energy metabolism in *Drosophila melanogaster*. Biogerontology, 17, 873-882.

Segaiso, B., Machekano, H., Ross, C.C. & Nyamukondiwa, C. 2022. Thermal fitness costs and benefits of developmental acclimation in fall armyworm. Scientific African, 17, p.e01369

Sgrò, C.M., Terblanche, J.S. & Hoffmann, A.A. 2016. What can plasticity contribute to insect responses to climate change? Annual Review of Entomology, 61,433-451.

Silbermann, R. & Tatar, M. 2000. Reproductive costs of heat shock protein in transgenic

Drosophila melanogaster. Evolution, 54(6), 2038-2045.

Sinclair, B.J., Alvarado, L.E.C. & Ferguson, L.V. 2015. An invitation to measure insect cold tolerance: Methods, approaches, and workflow. Journal of Thermal Biology, 53, 180-197.

Sinclair, B.J., Williams, C.M. & Terblanche, J.S. 2012. Variation in thermal performance among insect populations. Physiological and Biochemical Zoology, 85(6), 594-606.

Somero, G. N. 1995. Proteins and temperature. Annual review of physiology, 57(1), 43-68.

Stillman, J.H., 2019. Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems, and human communities. Physiology, 34(2), 86-100.

Su, Y., Li, X., Zhang, W., Zhang, B. & Ma, C.S. 2021. Dynamics of heat shock protein responses to thermal stress changes after metamorphosis in a lepidopteran insect. Archives of Insect Biochemistry and Physiology, 107(2), p.e21791.

Svensson, E.I., Gomez-Llano, M. & Waller, J.T. 2020. Selection on phenotypic plasticity favors thermal canalization. Proceedings of the National Academy of Sciences, 117(47), 29767-29774.

Tarusikirwa, V.L., Cuthbert, R.N., Mutamiswa, R. & Nyamukondiwa, C. 2022. Context-dependent integrated stress resistance promotes a global invasive pest. Insect Science, 29(6), 1790-1804.

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

Xing, K. & Zhao, F. 2022. Acclimation effects of natural daily temperature variation on longevity, fecundity, and thermal tolerance of the diamondback moth (*Plutella xylostella*). Insects, 13(4), 309.

Zhang, G.H., Li, Y.Y., Zhang, K.J., Wang, J.J., Liu, Y.Q. & Liu, H. 2016. Effects of heat stress on copulation, fecundity and longevity of newly-emerged adults of the predatory mite, *Neoseiulus barkeri* (Acari: Phytoseiidae). Systematic and Applied Acarology, 21(3), 295-306.

Zhang, W., Zhao, F., Hoffmann, A.A. & Ma, C.S. 2013. A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. PLoS One., 8 (10), e75923.

Zheng, J., Cheng, X., Hoffmann, A.A., Zhang, B. & Ma, C.S. 2017. Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress? Journal of Insect Physiology, 102, 36-41.

Zhou, Z.S., Guo, J.Y., Min, L. & Wan, F.H. 2011. Effect of short-term high temperature stress on the development and fecundity of *Ophraella communa* (Coleoptera: Chrysomelidae). Biocontrol Science and Technology, 21(7), 809-819.

Chapter 3

Ontogenetic responses of physiological fitness in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in response to repeated cold exposure*

* **Published as: Mbande, A.,** Mutamiswa, R., & Chidawanyika, F. (2023). Ontogenetic responses of physiological fitness in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in response to repeated cold exposure. *Bulletin of Entomological Research* **113**: 449: 455.

3.1. Introduction

Repeatability or reproducibility experiments are profound tools that were originally developed for independent testing of the precision of experimental protocols. In biological research, the repeatability of observational data can be used to track organismal plastic and genetic responses to stress factors at individual or population level at various temporal scales (Avargues-Weber et al., 2015; Niemelä & Dingemanse, 2017; Näslund, 2021). Given the escalated attention on climate change in recent years, repeatability studies (though controversial) can be pivotal in investigating basal thermal tolerance and plasticity thereof (Morgan et al., 2018; O'Donnell et al., 2020; O'Neill et al., 2021) where both environmental and genetic phenotypic variation effects can be used to determine within-individual trait variability (Grinder et al., 2020). In this study repeatability was used as a measure of physiological trait (cold tolerance) to evaluate the ability of *S. frugiperda to* tolerate repeatable exposure to cold temperature conditions. If the thermal tolerance of a tested organism is consistent over time, denoting high repeatability, it indicates that the adaptive potential of the trait is high, whilst the converse is true for low repeatability (Morgan et al., 2018).

For insects, body temperature depends on ambient conditions mediating biochemical and physiological processes therein (Chown & Nicolson, 2004; Sinclair et al., 2015). Subsequently, such organismal responses mediate development and can cascade to population level through factors such as seasonality, geographic distribution and voltinism (Du Plessis et al., 2020; Phophi et al., 2020; Tarusikirwa et al., 2020; Nyamukondiwa et al., 2022). Of interest is how the magnitude and frequency of thermal extremes in the form of heat waves and cold snaps wrought by the changing climates influence pest physiology, survival and key life-history traits (Tollefson, 2014) as it has direct implications on their population dynamics (Chidawanyika et al., 2012, 2020) and ultimately food security (Gregory et al., 2009). Thus, apart from magnitude of thermal exposure, insects experience different levels of thermal fluctuations (e.g. acute vs chronic, rapid vs slow fluctuations and/ or repeated exposures) typical of diel and seasonal changes (Colinet et al., 2015).

Such extremes, and not average temperatures, drive several organismal responses, including evolutionary adaptations within and across generations (Cox et al., 2010; Travis et al., 2014; Buckley et al., 2016) and define geographic ranges *via* various demographic tipping points (Lynch et al., 2014).

Indeed, insects have evolved diverse morphological, physiological, and behavioural adaptations to withstand and colonize otherwise lethal novel environments (Bale et al., 2002; Neal et al., 2021). For example, overwintering insects are known to survive stressful low temperatures through employing cold tolerance strategies such as rapid cold hardening (RCH), freeze tolerance and freeze avoidance (Sinclair et al., 2015; Feng et al., 2018). Freeze tolerant insects survive intracellular ice formation through use of cryoprotectants, removal of ice nucleators and anti-freeze heat shock proteins synthesis (Elnitsky et al., 2008; Storey & Storey, 2012; Toxopeus et al., 2019). On the contrary, freeze intolerant/avoidant insects cannot withstand internal ice formation but survive through keeping their body fluids in a supercooled condition (Sinclair et al., 2015; Andreadis & Athanasiou, 2017). Rapid cold hardening, a form of phenotypic plasticity, confers survival advantages at low lethal temperature after brief pre-treatment to a prior sub-lethal temperature shock (Lee et al., 1987; Teets et al., 2013). Over longer time scales such prior exposure to sub-lethal temperatures also confer advantages to identical future thermal stress in what is referred to as beneficial acclimation (Leroi et al., 1994).

In nature, insects may thus face multiple stressors including repeated cold stress during diel and seasonal thermal fluctuations (Marshall & Sinclair, 2010) where the above-mentioned plastic responses play a role. Mimicking such repeated thermal exposure in manipulative experiments allows investigation of the relationship between repeatability and adaptive responses (Boake, 1989; Morgan et al., 2018; Grinder et al., 2020). In this study, I used common measures of cold tolerance in critical thermal minimum (CT_{min}) and chill coma

recovery time (CCRT) as proxies for cold hardiness (Andersen et al., 2015; Mutamiswa et al., 2018; Izadi et al., 2019).

Critical thermal minimum is an organism's lower thermal tolerance limit where an insect is incapacitated due to compromised neuromuscular activity (Sinclair et al., 2015; Izadi et al., 2019). If low temperature conditions persist, CTmin is followed by chill coma where paralysis due to complete loss of neuromuscular function occurs (Hazell et al., 2011; O'Neill et al., 2021). The time that an insect requires to regain neuromuscular function following chill coma is what is then regarded as CCRT (Sinclair et al., 2015). Given their ubiquitous occurrence in nature and capacity to define limits for organismal activity, these key indices provide valuable ecologically relevant measures of insect cold tolerance. Thus, understanding the evolutionary capacity following repeated exposure provides important information on their adaptive capacity and potential geographic range expansion in invasive insects such as *S. frugiperda*. This will help can inform pest management strategies and contribute to a broader understanding of insect responses to changing environments.

Spodoptera frugiperda is a highly invasive insect pest native to the tropics and sub-tropics of America (Goergen et al., 2016). The larvae of this polyphagous insect cause significant economic losses in several important crops but inflict the most damage in the Poaceae family (Lu & Adang, 1996; Nboyine et al., 2020). *Spodoptera frugiperda* does not diapause, instead it is known to migrate to environments with favorable conditions for survival (Du Plessis et al., 2020; Vetanpast & Park, 2022). It has been reported to survive in Africa all year-round due to conducive biophysical environment (Du Plessis et al., 2018; Early et al., 2018; Keosentse et al., 2021).

The upregulation of glycerol-3-phosphate dehydrogenase (GPDH) and glycerol kinase (GK) genes for increased synthesis of the cryoprotectant glycerol has been attributed as the key physiological response to withstand cold environments in *S. frugiperda* (Vatanparast & Park, 2022). However, survival has been reported to be limited in some cases in Asia where harsh winters decimate seasonal populations while annual reinvasions provide new propagules (Vatanparast & Park, 2022). Nevertheless, little is known about the role of acquired/induced cold tolerance in the fitness of *S. frugiperda* following prior exposure. Yet, induced

cold tolerance can play a key role in preserving and improving key life-history activities at acute temporal scales.

Here, I examined the consequences of repeated cold exposure on low thermal tolerance (CT_{min} and CCRT) of *S. frugiperda* life stages across 72 hours. I hypothesize that the values of CT_{min} and CCRT will remain consistent over repeated cold exposures due to cold hardening. Since body water and lipid content is associated with basal and induced cold tolerance in insects (or lack thereof), I subsequently assessed the two parameters following thermal exposure to draw inferences on the performance of *S. frugiperda* and subsequent management.

3.2. Materials and methods

3.2.1 Insect culture and maintenance

The initial colony of *S. frugiperda* was obtained as larvae from the Agricultural Research Council, Plant Health Protection (ARC-PHP) Pretoria, South Africa. Thereafter, the insects were maintained on artificial diet in the insectary under optimum conditions of 28^{o} C (Ali et al., 1990; Du Plessis et al., 2020), 65±5% relative humidity (RH) and 12L: 12D photoperiod. Since cannibalism is reportedly predominant among late larval instars (Chapman et al., 1999), each third instar larva was individually placed in a separate 100 ml plastic vial with perforated screw-cap lid and soybean wheat germ artificial diet (Southland Products Inc., Lake Village, Arkansas, USA) until pupation to prevent cannibalism. Pupae were maintained in open Petri dishes (30 × 30 × 30 cm) in collapsible rearing cages made of mesh cloth until adult eclosion. Adults were provided with 25% sugar-water from a moistened cotton wool placed in a petri dish. At least two maize plants (3-4 weeks old) were placed in each rearing cage as oviposition substrate for gravid females. After hatching, the 1^{st} instar larvae were transferred to artificial diet for subsequent rearing. For all the experiments F_1 generation of 4^{th}, 5^{th}, 6^{th} instar larvae and 24-48 -h old virgin adults were used.

3.2.2 Critical thermal minimum (CTmin) and repeated cold exposure assays

To evaluate the relationship between CT_{min} and repeated cold exposure, larvae and adults (males and females) of *S. frugiperda* underwent repeated cold tolerance (CT_{min}) assays at 0 (control), 24, 48 and 72 -h intervals. Critical thermal minima were assayed using standardised dynamic and ecologically relevant protocols (Chidawanyika & Terblanche, 2011b). Ten replicate larvae and adults were individually placed randomly in a series of 200 mm glass tubes ('organ pipes') connected to an insulated double-jacketed chamber linked to a programmable water bath (Grant model Tx150; Grant Instruments, UK) filled with 1:1 water: propylene glycol. In the 'organ pipes', insects were allowed to equilibrate for 10 minutes at 28 ˚C (optimum temperature) before decreasing the temperature at a rate of 0.25 ˚C/min until their CT_{min} were recorded. This was repeated twice for each life stage to yield sample sizes of n=20 per treatment. To record chamber temperature, a thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into a control (centre) glass tube of the organ pipes. After each assay, insects were given time to recover before repeating the same assay across 24, 48, and 72 -h intervals using the same batch of insects. Critical thermal minimum was considered as the temperature at which insects did not respond to gentle prodding (e.g., Nyamukondiwa & Terblanche 2010).

3.2.3 Influence of repeated cold exposure on chill coma recovery time (CCRT)

Chill coma recovery time was assessed following Mutamiswa et al., 2018. A total of 10 replicate larvae and adults were placed individually in 7 ml screw-cap glass vials with 1 mm dimeter holes pierced through cap for ventilation. The vials were then placed into a large zip-lock bag, which was subsequently submerged into a water bath (Grant LTC40 model TX150) filled with 1:1 water: propylene glycol mixture and set at 0 °C for 1 hour. After 1 hour at chill-coma temperature, the tubes were removed from the water bath and transferred to a Memmert climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at 28°C, 65% RH for recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer where observations were recorded. This was repeated twice for each life stage to yield sample sizes of n = 20 per

treatment. After each assay, insects were exposed to the same treatment and CCRT measured across 24, 48 and 72 -h intervals using the same batch of insects. Chill coma recovery time was defined as the time (in minutes) required for an adult to stand upright on its legs (Milton & Partridge, 2008).

3.2.4. Determination of body water content

After 72 h interval following CT_{min} and repeated cold exposure assays, body water content of the insects were determined. Larvae (4^{th}, 5^{th} and 6^{th} instar) and adults were individually placed in a pre-weighed 50 ml Eppendorf tubes and the initial mass of each insect before oven drying was measured (to 0.0001g) on a Scout Pro (DHAUS) microbalance (model: Scout Pro SPU 123, USA). Thereafter, insects were placed in a Memmert drying oven (UL50, Memmert, Germany) set at 60 °C for 72 h. Insects were allowed to cool under laboratory temperature conditions of 28°C for 30 minutes thereafter, dry mass was measured (to 0.0001) on a microbalance. To determine BWC, dry mass was subtracted from the initial mass following (Bazinet et al., 2010; Weldon et al., 2018).

3.2.5 Determination of body lipid content

Following BWC assays, the tested insects were further oven dried for another 72 h at 60°C. Thereafter, the insects were individually washed in 1,5ml diethyl ether and then gently agitated at 250 rmp for 24 -h at 37°C using ST 5 CAT orbital shaker (model: Zipperer GmbH, D 79219 Staufen, Germany) following the methods of Mitchell et al. (2017). The diethyl ether was then removed from the tubes and insects were oven dried again at 60 °C for 24 -h, before reweighing. The lipid content for each individual was calculated by subtracting the lipid free dry mass from the initial dry mass. Controls were exposed to the same conditions before measuring their lipid content.

3.2.6 Data analysis

Data analyses were carried out in STATISTICA, 13.5.0 version (Statsoft Inc.2021 and R version 4.1.2 (R development Core Team, 2021). Normality and equality of variances were first checked using the Shapiro-Wilk and Hartley-Bartlett tests respectively. Data for CCRT was linear and met the conditions for normality and equality of variances ($W = 0.83$, $p = 0.12$) and were analyzed using generalized linear models (GLZ) assuming a Gaussian distribution in R. The CTmin data also met the linear model assumptions and were

analyzed using repeated measures (time intervals) ANOVA. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous means. The relationship between CTmin and body water content and body lipid content were examined using linear regression in STATISTICA.

3.3 Results

3.3.1 CTmin and repeated cold exposure assays

Critical thermal minima significantly varied across life stages following repeated cold exposure($F_{16, 282} = 134.59$, $P < 0.001$) (Table 3.1 and Fig. 3.1). In 5th instar and virgin adults, cold tolerance (CT_{min}) improved with repeated cold exposure (Fig. 3.1). However, 6th instar larvae showed compromised cold tolerance with CT_{min} increasing with repeated exposure (Fig 3.1). Virgin females recorded the lowest CT_{min} across all assays relative to other life stages (Fig. 3.1).

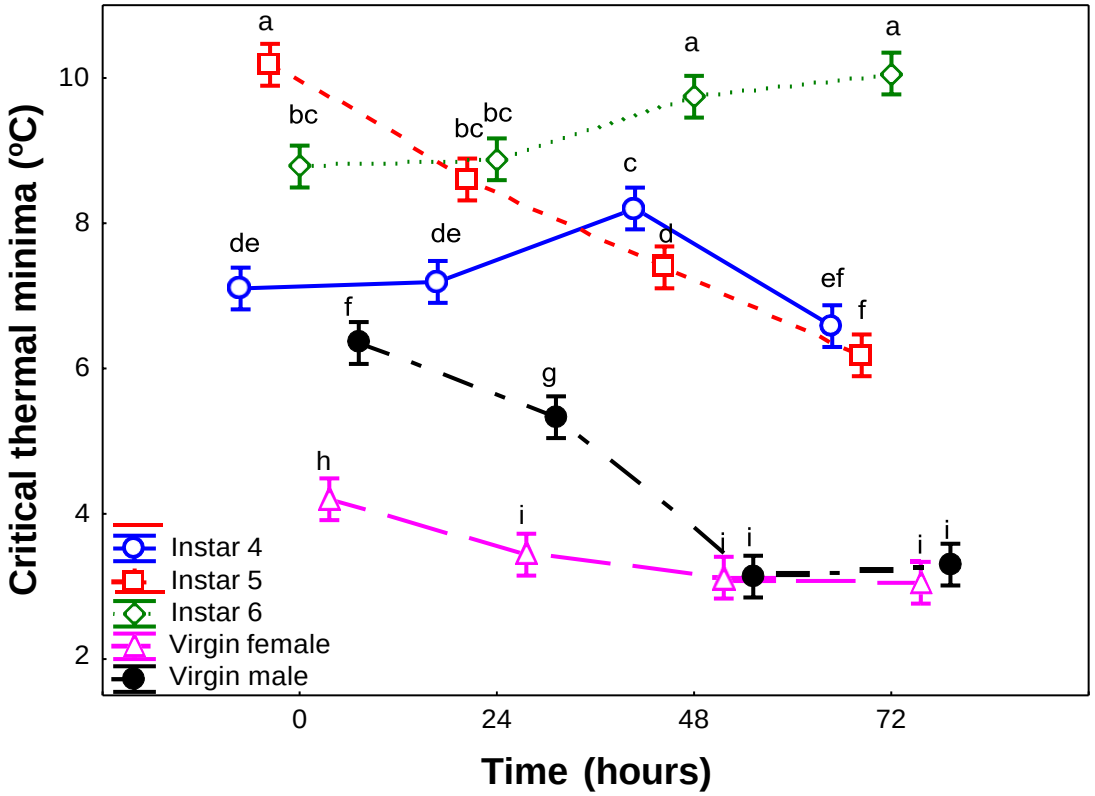

Figure 3. 1 Critical thermal minimum in adult (virgin male and female) and larval stages of *Spodoptera frugiperda* following repeated cold exposure. Data points represent means of n = 20 whilst error bars denote 95% confidence limits for each sex and life-stage. Different letters above error bars denote significant differences.

Table 3. 1: Full factorial ANOVA of the effects of sub-lethal 2-h heat shock treatment at four temperatures on critical thermal minimum (CT_{min}) and maximum (CT_{max}) of *Spodoptera frugiperda* males and females

Trait	**Effect**	**SS**	**d.f.**	**MS**	**F**	**P**
CT_{min}	Intercept	1114.61	1	1114.61	2590.63	<0.001
	Sex	3.22	1	3.22	7.49	<0.01
	Temperature	894.02	3	298.01	692.64	<0.001
	Sex*temperature	7.51	3	2.50	5.82	<0.001
	Error	65.40	152	0.43		
CT_{max}	Intercept	345820.5	1	345820.5	77318.17	<0.001
	Sex	51.4	1	51.4	11.50	<0.001
	Temperature	251.3	3	83.8	18.73	<0.001
	Sex*Temperature	48.8	3	16.3	3.63	0.014
	Error	679.8	152	4.5		

3.3.2 CCRT and repeated cold exposure assays

As in CT_{min} assays, CCRT varied significantly across life stages with repeated cold exposure ($F_{16, 282} = 4.06$, $P < 0.001$) (Fig. 3.2). Chill coma recovery times of tested instars (4^{th} , 5^{th} and 6^{th} instar,) decreased with repeated cold exposure (Fig. 3.2). In adults (virgin males and females), CCRT improved following repeated exposure at 24 h interval and was compromised after 48 and 72 h intervals (Fig. 3.2).

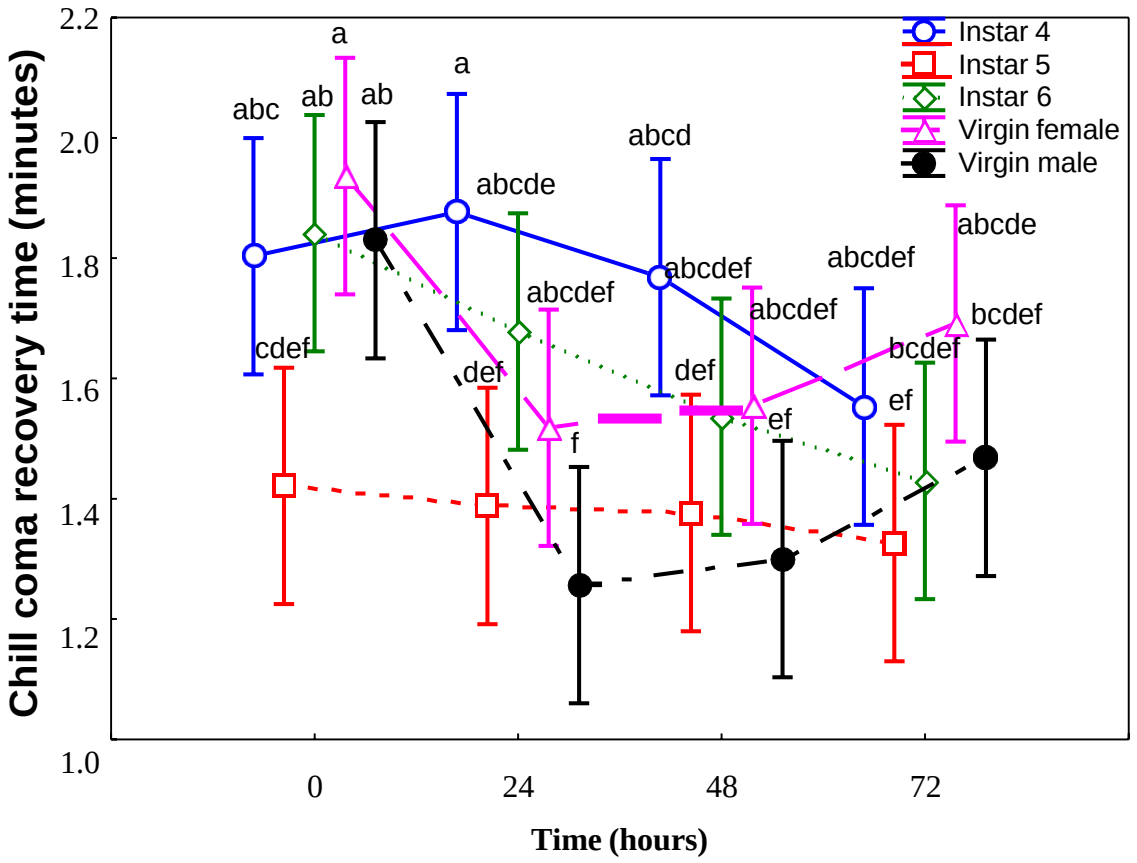

Figure 3. 2 Chill coma recovery time in adult (virgin male and female) and larval stages of *Spodoptera frugiperda* following repeated cold exposure. Data points represent means of n = 20 whilst error bars denote 95% confidence limits for each sex and life-stage. Different letters above error bars denote

3.3.3 Body water and lipid content

Body water content did not vary significantly among life stages ($F_{4,95} = 2.01$, $P = 0.98$) (Fig. 3.3A). There was no significant difference in BWC between all tested life stages (Fig.3.3A). Nevertheless, BWC was not significantly correlated with low temperature tolerance (measured as CT_{min}) (Fig. 3.3B).

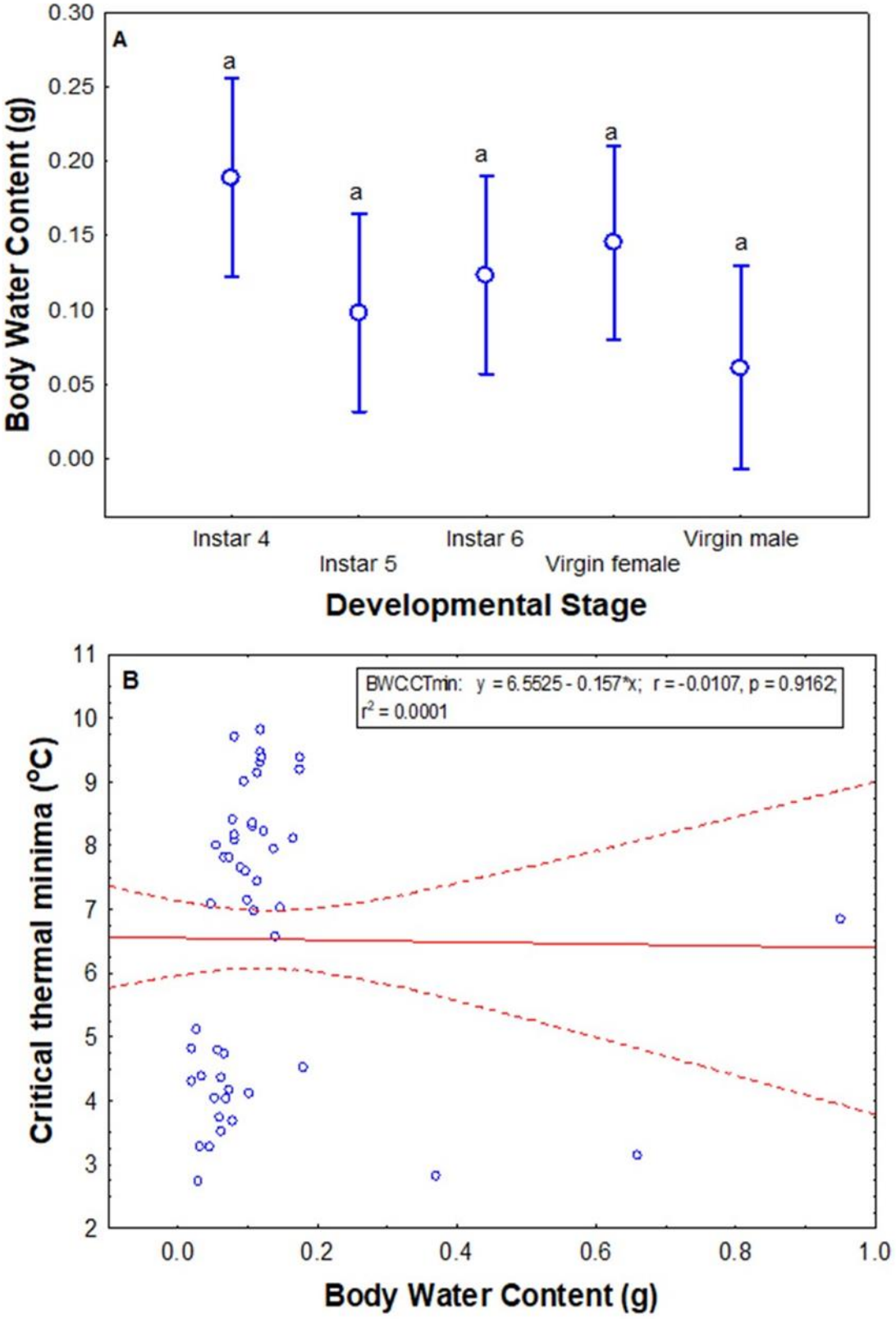

Figure 3. 4 Body water content (g) across different life stages (A) and relationship between body water content and critical thermal minima (CTmin) in *Spodoptera frugiperda* (B).

Similar to BWC, BLC did not significantly vary among life stages ($F_{4,95} = 2.94$, $P = 0.24$) (Fig.3.4A). As in BWC, BLC was not significantly correlated with low temperature tolerance suchthat CT_{min} decreased with BLC (Fig. 3.4B).

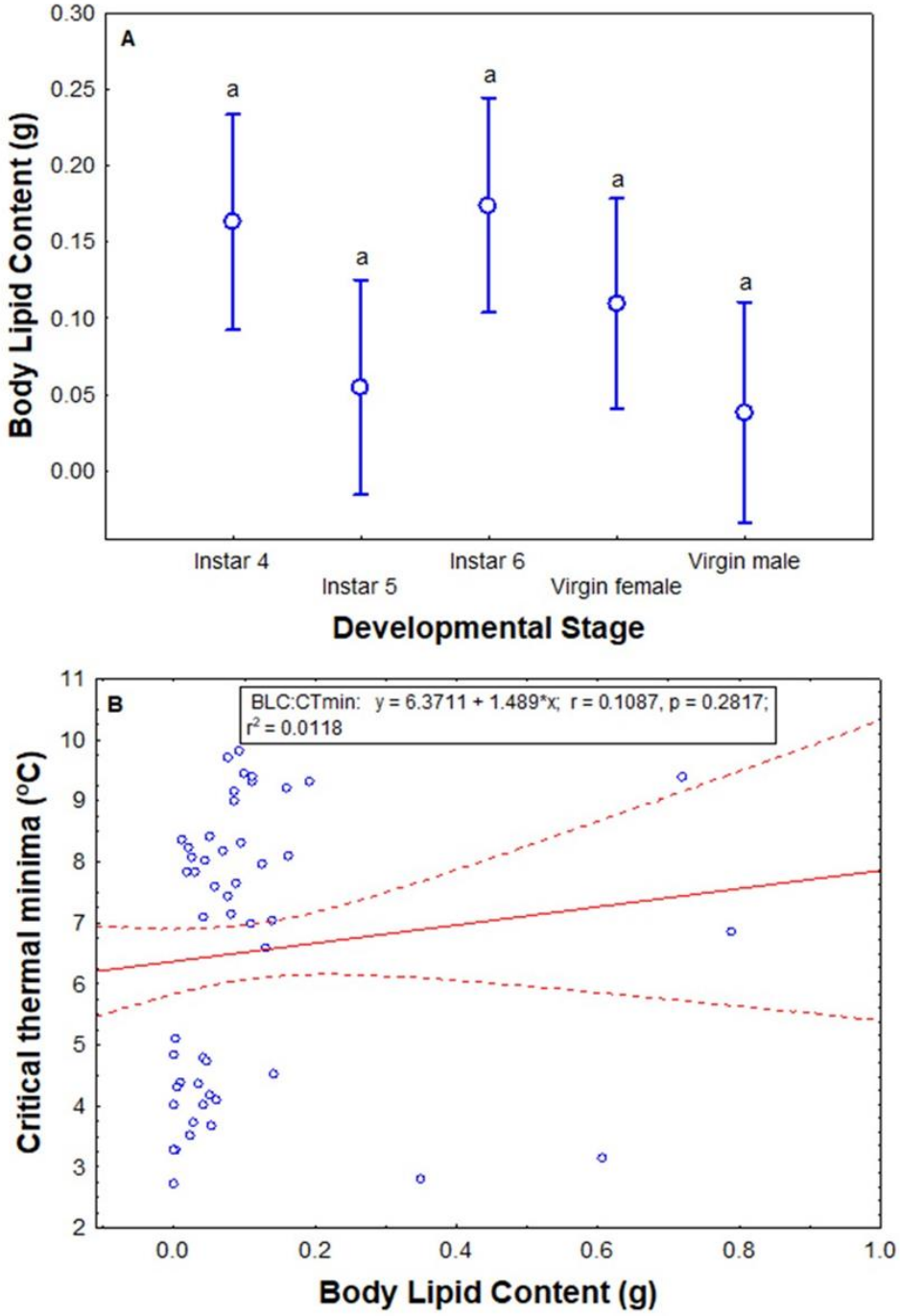

Figure 3. 5 Body lipid content (g) across different life stages (A) and the relationship between body lipid content and critical thermal minima (CTmin) in *Spodoptera frugiperda* (B).

3.4 Discussion

Insect physiological and behavioral adaptations are very important for determining survival and population dynamics in both transient and seasonal cold spells (Chown & Nicolson, 2004; Terblanche et al., 2011; Andrew & Camp, 2016). As expected, our results showed that repeated cold exposure influences the fitness of *S. frugiperda* (determined as CT_{min} and CCRT). While insects may face multiple temperature variabilities in winter season, the repeated cold exposures can trigger responses that may set the insect on a different physiological path relative to a single exposure (Marshall & Sinclair, 2010, 2012). In the current study, CT_{min} improved with repeated exposure in 5^{th} instar larvae, virgin males and females in agreement with Renault et al. (2004) who reported improved survival in beetles that were exposed to repeated cold exposure. A Similar trend was reported in *D. melanogaster*, with low temperature tolerance improving following repeated cold exposure in tested insects (Le Bourg, 2007). However, compromised and fluctuating CT_{min} were recorded in 6^{th} instar and 4^{th} instar larvae respectively. Given this variation across life stages, it therefore indicates that repeated thermal exposure impacts on CT_{min} are life-stage dependent. While 5^{th} instar larvae, virgin males and females showed enhanced CT_{min} across subsequent exposures, virgin females recorded the lowest CT_{min} across treatment intervals indicating that they were the most thermally tolerant. This gives them a fitness and survival advantage when they encounter extreme cold conditions in nature.

In the present study, repeated thermal exposure improved CCRT in 4^{th}, 5^{th} and 6^{th} instar larvae and this in consonance with Anderson et al. (2017) who reported improved chill-comarecovery, cellular survival, and cold tolerance in *Locusta migratoria* following brief cold exposure periods. However, compromised CCRTs were recorded in adults (males and females)in keeping with van Dooremalen et al. (2011) who reported CCRT decrease in *Orchesella cincta* following repeated cold exposure. The variations in the current study underlie that CCRTresponses are life-

stage dependent. Although CCRT and CT_{min} are measures of cold tolerance,surprisingly, 6th instar larvae recorded compromised CT_{min} and enhanced CCRT indicating thatresponses also vary across traits, thus can be trait dependent.

The changes in cold tolerance across consecutive measurements provide insight into potential benefits of short-term acclimation to extreme cold events through cold hardening. This study showed evidence of cold hardening in *S. frugiperda* as indicated by improved cold tolerance in some of the life stages. This suggests significant adaptive potential for cold tolerance in this invasive insect species and that individuals may also respond directly to low temperature extremes through phenotypic plasticity. While *S. frugiperda* has been reported to overwinter and survive all year round in Africa (Prasana et al., 2018; Kebede & Shimalis, 2019; Keosentse et al., 2021), the results indicate its potential to adapt to variable thermal extremes in winter and this may give it fitness and survival advantage in the face of climate change. Insects reportedly enhance their cold tolerance through carbohydrate cryoprotectants accumulation, antifreezes synthesis, lipid membranes reordering and either removal (freeze avoiding) or retaining (freeze tolerant) of ice nucleators (Lee, 2010). Therefore, differential life stage responses shown in this study following repeated exposure assays may be a result of variation in these physiological components of cold hardiness. However, this warrants further investigation to fully elucidate the responses.

Cold tolerance is dependent on the water content remaining unfrozen in many cold hardened insects by allowing basal metabolism to continue at low temperature levels (Collinet et al., 2006; Alfaro-Tapia et al., 2021). Reports have shown that reduction in body water content and subsequent increase in solute concentration may increase cold tolerance in insects (Worland, 1996). In the current study there was no relationship between cold tolerance and body water content. This may be because insects in our assays did not experience repeated cold conditions that trigger any water loss and subsequent solute concentration increase. While Keosentse et al. (2022) reported that BWC increased with larval stage in *S. frugiperda*, these results report

otherwise on CT_{min} following repeated exposure. This may be because this present study measured BWC following plastic responses while Keosentse et al. (2022) measured basal BWC. Given these responses, it indicates that *S. frugiperda* may trade-off basal BWC for plasticity of thermal tolerance.

Lipid content plays a vital role in cold tolerance as they can serve as anti-freezers in the insect haemolymph (Sinclair et al., 2018; Trenti et al., 2022). In winter, most insects do not feed and may face the unreplaced energy consumption, water loss and low temperatures (Sinclair et al., 2013; Williams et al., 2015). Low temperature is one of the stressors which affect neutral lipid fluidity and mobilization and energy drain, since lipids are the primary overwintering source of fuel (Sinclair & Marshall, 2018). As such, most overwintering insects end winter with fewer lipid stores than at the beginning (Sinclair, 2015). For example, in laboratory reared colonies of *D. melanogaster*, glycogen levels decreased following repeated cold exposure (Marshall & Sinclair, 2010). In addition, there was a positive correlation between body lipid content and cold tolerance in *Drosophila* spp. (Hoffmann et al., 2001; Kaczmarek & Bogus, 2021). However, in the current study, these results showed no significant correlation between BLC and cold tolerance in *S. frugiperda*. A recent study attributed glycerol as the key cryoprotectant used by *S. frugiperda* (Vatanparast & Park, 2022). This therefore suggests that the influence of BLC on cold tolerance may be species dependent and glycerol may be more important in this species.

In conclusion, the current study documents life stage-related variation in cold tolerance for *S. frugiperda* following repeated thermal exposure. This study suggests that repeated cold exposure differentially influences the fitness of *S. frugiperda* in nature where vulnerability is life-stage and trait dependent. In addition, the study provides evidence that cold hardening may be an important mechanism for *S. frugiperda* to cope with repeated cold exposure over the short-term. These cold tolerance responses may provide temporal fitness benefits following

repeated cold conditions in nature hence population persistence under changing environments. The results also have direct implications on the geographic distribution of the pest under climate change scenarios where warming winter seasons will lead to even further spatial expansion and multivoltinism due to favourable conditions. For a polyphagous pest such as *S. frugiperda,* this will be critical as alternative hosts will support multiple generations enough to exert pest pressure on the main crop in the subsequent season (Vatanparast & Park, 2022). In such cases, management practices should consider area-wide monitoring of the pest populations even during off-season for early integrated pest management practices. This may include improved phytosanitary measures and reduction of alternative hosts on-farm. The use of parasitoids to suppress the pest populations to reduce the pressure in the main crop in impending season. While the current study investigated repeatability of cold tolerance, Chill Coma Recovery Time and plasticity for laboratory reared S. *frugipreda,* future studies should consider using wild populations for the same traits to determine survival, fecundity and longevity under climate change. Future studies should determine the intensity of parasitoid levels to maintain pest pressure well below economic injury levels.

3.5 References

Alfaro-Tapia, A., Alvarez-Baca, J.K., Tougeron, K., Lavandero, B., Le Lann, C. & Van Baaren, J. 2021. Overwintering strategies and life-history traits of different populations of *Aphidius platensis* along a latitudinal gradient in Chile. Entomologia Generalis, 42(1), 127-145.

Ali, A., Luttrell, R.G. & Schneider, J.C. 1990. Effects of temperature and larval diet on development of the fall armyworm (Lepidoptera: Noctuidae). Annals of the Entomological Society of America, 83(4), 725-733.

Andersen, J.L., Manenti, T., Sørensen, J.G., MacMillan, H.A., Loeschcke, V. & Overgaard, J. 2015. How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. Functional Ecology, 29(1), 55-65.

Andersen, M.K., Folkersen, R., MacMillan, H.A. & Overgaard, J. 2017. Cold acclimation improves chill tolerance in the migratory locust through preservation of ion balance and membrane potential. Journal of Experimental Biology, 220(3), 487-496.

Andreadis, S.S. & Athanassiou, C.G. 2017. A review of insect cold hardiness and its potential in stored product insect control. Crop Protection, 91, 93-99.

Andrew, S.C. & Kemp, D.J. 2016. Stress tolerance in a novel system: genetic and environmental sources of (co) variation for cold tolerance in the butterfly *Eurema smilax*. Austral Ecology, 41(5), 529-537.

Angilletta, M. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford, UK: Oxford University Press.

Assefa, F. & Ayalew, D. 2019. Status and control measures of fall armyworm (*Spodoptera frugiperda*) infestations in maize fields in Ethiopia: A review. Cogent Food & Agriculture, 5(1), 1641902.

Avargues-Weber, A., Lihoreau, M., Isabel, G. & Giurfa, M. 2015. Information transfer beyond the waggle dance: Observational learning in bees and flies. Frontiers in Ecology and Evolution, 3: p.24.

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.

Bell, G. 2013. Evolutionary rescue and the limits of adaptation. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1610**)**, 20120080.

Boake, C.R. 1989. Repeatability: its role in evolutionary studies of mating behavior. Evolutionary Ecology, 3, 173-182.

Buckley, L.B. & Huey, R.B. 2016. How extreme temperatures impact organisms and the evolution of their thermal tolerance. Integrative and Comparative Biology, 56(1), 98-109.

Byers, J.E. 2002. Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos, 97(3), 449-458.

Chapman, J.W., Williams, T., Escribano, A., Caballero, P., Cave, R.D. & Goulson, D. 1999. Age-related cannibalism and horizontal transmission of a nuclear polyhedrosis virus in larval *Spodoptera frugiperda*. Ecological Entomology, 24(3), 268-275.

Chidawanyika, F., Mudavanhu, P. & Nyamukondiwa, C. 2012. Biologically based methods for pest management in agriculture under changing climates: challenges and future directions. Insects, 3(4), 1171-1189.

Chidawanyika, F., Nyamukondiwa, C., Strathie, L. & 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), e0169371.

Chowanski, S., Lubawy, J., Spochacz, M., Ewelina, P., Grzegorz, S., Rosinski, G. & Slocinska, M. 2015. Cold induced changes in lipid, protein and carbohydrate levels in the tropical insect *Gromphadorhina coquereliana*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 183, 57-63.

Chown S.L. & Nicolson, S.W. 2004. Insect physiological ecology: Mechanisms and patterns. Oxford (UK): Oxford University Press.

Chown, S.L. & Terblanche, J.S. 2007. Physiological diversity in insects: ecological and evolutionary contexts. Advances in Insect Physiology, 33, 50–152.

Colinet, H., Nguyen, T.T.A., Cloutier, C., Michaud, D. & Hance, T. 2007. Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. Insect Biochemistry and Molecular Biology, 37(11), 1177-1188.

Cox, J., Schubert, A.M., Travisano, M. & Putonti, C. 2010. Adaptive evolution and inherent tolerance to extreme thermal environments. BMC Evolutionary Biology, 10(1), 1-11.

Crozier, L. 2003. Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*. Oecologia, 135, 648-656.

Cubillos, C., Cáceres, J.C., Villablanca, C., Villarreal, P., Baeza, M., Cabrera, R., Graether, S.P. & Veloso, C. 2018. Cold tolerance mechanisms of two arthropods from the Andean Range of Central Chile: *Agathemera crassa* (Insecta: Agathemeridae) and *Euathlus condorito* (Arachnida: Theraphosidae). Journal of Thermal Biology, 74, 33-139.

Danks, H.V. 2000. Dehydration in dormant insects. Journal of Insect Physiology, 46(6), 837-852.

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J. & Gomez, J. 2017. Fall armyworm: impacts and implications for Africa. Outlooks on Pest Management, 28(5), 196-201.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences, 105(18), 6668-6672.

Diaz, R., Overholt, W.A., Samayoa, A., Sosa, F., Cordeau, D. & Medal, J. 2008. Temperature-dependent development, cold tolerance, and potential distribution of *Gratiana boliviana* (Coleoptera: Chrysomelidae), a biological control agent of tropical soda apple, *Solanum viarum* (Solanaceae). Biocontrol Science and Technology, 18(2), 193-207.

Dohm, M.R. 2002. Repeatability estimates do not always set an upper limit to heritability. Functional Ecology, 16, 273–280.

Du Plessis, H., Schlemmer, M.L. & Van den Berg, J. 2020. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects, 11(4), 228.

Early, R., González-Moreno, P., Murphy, S.T. & Day, R. 2018. Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota, 40, 25-50.

Elnitsky, M.A., Hayward, S.A., Rinehart, J.P., Denlinger, D.L. & Lee Jr, R.E. 2008. Cryoprotective dehydration and the resistance to inoculative freezing in the Antarctic midge, *Belgica antarctica*. Journal of Experimental Biology, 211(4), 524-530.

Feng, Y., Zhang, L., Li, W., Yang, X. & Zong, S. 2018. Cold hardiness of overwintering larvae of *Sphenoptera sp*. (Coleoptera: Buprestidae) in Western China. Journal of Economic Entomology, 111(1), 247-251.

Findsen, A., Andersen, J.L., Calderon, S. & Overgaard, J. 2013. Rapid cold hardening improves recovery of ion homeostasis and chill coma recovery time in the migratory locust, *Locusta migratoria.* Journal of Experimental Biology, 216(9), 1630-1637.

Fischer, K., Dierks A., Franke, K., Geister, T.L., Liska, M. & Winter S et al. 2010. Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus anynana.* PLoS One**,** 5(12), e15284.

Garcia, M.J. & Teets, N.M. 2019. Cold stress results in sustained locomotor and behavioral deficits in *Drosophila melanogaster*. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 331(3), 192-200.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One, 11,e0165632.

Grinder, R.M., Bassar, R.D. & Auer, S.K. 2020. Upper thermal limits are repeatable in *Trinidadian guppies*. Journal of Thermal Biology, 90, 102597.

Gregory, P.J., Johnson, S.N., Newton, A.C., Ingram, J.S. 2009. Integrating pests and pathogens into the climate change/food security debate. Journal of Experimental Botany, 60(10), 2827-38.

Gunderson, A.R. & Stillman, J.H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceedings of the Royal Society B: Biological Sciences, 282(1808), p.20150401.

Hazell, S.P. & Bale, J.S. 2011. Low temperature thresholds: Are chill coma and CTmin synonymous? Journal of Insect Physiology, 57(8), 1085–1089.

Hoffmann A. A. & Sgro C. M. 2011. Climate change and evolutionary adaptation. Nature, 470(7335), 479–85.

Hoffmann, A.A. & Sgro, C.M. 2018. Comparative studies of critical physiological limits and vulnerability to environmental extremes in small ectotherms: How much environmental control is needed? Integrative Zoology, 13(4**)**, 355-371.

Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. 2012. Upper thermal limits in terrestrial ectotherms: how constrained are they? Functional Ecology, 27(4), 934–949.

Hoffmann, A.A., Hallas, R., Sinclair, C. and Partridge, L. 2001. Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. Evolution, 55(2), 436-438.

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

Isobe, K., Takahashi, A. & Tamura, K. 2013. Cold tolerance and metabolic rate increased by cold acclimation in *Drosophila albomicans* from natural populations. Genes and Genetic Systems, 88(5), 289-300.

Izadi, H., Mohammadzadeh, M. & Mehrabian, M. 2019. Cold tolerance of the *Tribolium castaneum* (Coleoptera: Tenebrionidae), under different thermal regimes: impact of cold acclimation. Journal of Economic Entomology, 112(4), 1983-1988.

Kaczmarek, A. & Boguś, M. 2021. The metabolism and role of free fatty acids in key physiological processes in insects of medical, veterinary and forensic importance. Peer J, 9, p.e12563.

Kasoma, C., Shimelis, H. & Laing, M.D. 2021. Fall armyworm invasion in Africa: implications for maize production and breeding. Journal of Crop Improvement, 35(1), 111-146.

Kebede, M. & Shimalis, T. 2018. Out-break, distribution and management of fall armyworm, *Spodoptera frugiperda* JE Smith in Africa: the status and prospects. Academy of Agricultural Journal, 3, 551-568.

Kelley, A.L. 2014. The role thermal physiology plays in species invasion. Conservation Physiology, 2(1), DOI: cou045.

Keosentse, O., Mutamiswa, R., Du Plessis, H. & Nyamukondiwa, C. 2021. Developmental stage variation in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) low temperature tolerance: Implications for overwintering. Austral Entomology, 60(2), 400-410.

Kiritani, K. 2013. Different effects of climate change on the population dynamics of insects. Applied Entomology and Zoology, 48(2), 97–104.

Le Bourg, E. 2007. Hormetic effects of repeated exposures to cold at young age on longevity, aging and resistance to heat or cold shocks in *Drosophila melanogaster*. Biogerontology, 8(4), 431-444.

Lee Jr, R.E., Chen, C.P. & Denlinger, D.L. 1987. A rapid cold-hardening process in insects. Science, 238(4832), 1415-1417.

Lee, R. E., Jr. 2010. *A primer on insect cold-tolerance* 3-34 in *Insect Low Temperature Biology* (ed. D. L. Denlinger and R. E. Lee), New York: Cambridge University Press.

Leroi, A.M., Bennett, A.F. & 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.

Lynch, H.J., Rhainds, M., Calabrese, J.M., Cantrell, S., Cosner, C. & Fagan, W.F. 2014. How climate extremes not means define a species' geographic range boundary via a demographic tipping point. Ecological Monographs, 84(1**)**, 131-149.

Manrique, V., Diaz, R., Montemayor, C., Serrano, D. & Cave, R.D. 2012. Temperature-dependent development and cold tolerance of *Microtheca ochroloma* (Coleoptera: Chrysomelidae), a pest of cruciferous crops in the South Eastern United States. Annals of the Entomological Society of America, 105(6), 859-864.

Marshall, K.E. & Sinclair, B.J. 2010. Repeated stress exposure results in a survival–reproduction trade-off in *Drosophila melanogaster*. Proceedings of the Royal Society B: Biological Sciences, 277(1683**)**, 963-969.

Marshall, K.E. & Sinclair, B.J. 2012. The impacts of repeated cold exposure on insects. Journal of Experimental Biology, 215(10), 1607–1613.

Milton, C.C. & Partridge, L. 2008. Brief carbon dioxide exposure blocks heat hardening but not cold acclimation in *Drosophila melanogaster*. Journal of Insect Physiology, 54(1), 32-40.

Mitchell, K.A., Boardman, L., Clusella-Trullas, S. & Terblanche, J.S. 2017. Effects of nutrient and water restriction on thermal tolerance: a test of mechanisms and hypotheses. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 212, 15-23.

Mohammadzadeh, M. & Izadi, H. 2018. Cooling rate and starvation affect supercooling point and cold tolerance of the Khapra beetle, *Trogoderma granarium* Everts fourth instar larvae (Coleoptera: Dermestidae). Journal of Thermal biology, 71, 24-31.

Morgan, R., Finnøen, M.H. & Jutfelt, F. 2018. CT_{max} is repeatable and doesn't reduce growth in zebrafish. Scientific Reports, 8(1), 1-8.

Musselman, L.P. & Kühnlein, R.P. 2018. Drosophila as a model to study obesity and metabolic disease. Journal of Experimental Biology, 221(suppl_1), p, jeb163881.

Mutamiswa, R., Machekano, H., Chidawanyika, F. & 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.

Mutamiswa, R., Machekano, H., Chidawanyika, F. & Nyamukondiwa, C. 2018. Thermal resilience may shape population abundance of two sympatric congeneric Cotesia species (Hymenoptera: Braconidae). PLoS One, 13(2**)**, p.e0191840.

Näslund, J. 2021. Behavioural repeatability in larval *Limnephilus lunatus* Curtis, 1834 (Trichoptera) in an open-field test, Aquatic Insects, 42(1), 62-77.

Neal, A.S., Diaz, R., Qureshi, J.A. & Cave, R.D. 2021. Adult cold tolerance and potential North American distribution of *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae). Biological Invasions, 23, 3719-3731.

Niemelä, P.T. & Dingemanse, N.J. 2017. Individual versus pseudo-repeatability in behaviour: Lessons from translocation experiments in a wild insect. Journal of Animal Ecology, 86(5), 1033-1043.

Nyamukondiwa, C., Machekano, H., Chidawanyika, F., Mutamiswa, R., Ma, G., & Ma, C. S. 2022. Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance and other complementary traits in the tropics. Current Opinion in Insect Science, 100878.

Nyamukondiwa, C. & Terblanche, J.S. 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa):* effects of age, sex and feeding status. Journal of Thermal Biology, 34(8), 406–414.

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

O'Donnell, M.J., Regish, A.M., McCormick, S.D. & Letcher, B.H. 2020. How repeatable is CTmax within individual brook trout over short-and long-time intervals? Journal of Thermal Biology, 89, 102559.

O'Neill, E., Davis, H.E. & MacMillan, H.A. 2021. A lack of repeatability creates the illusion of a trade-off between basal and plastic cold tolerance. Proceedings of the Royal Society B, 288(1964**)**, 20212121.

Overgaard, J., Sørensen, J.G., Petersen, S.O., Loeschcke, V. & Holmstrup, M. 2005. Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. Journal of Insect Physiology, 51(11), 1173-1182.

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P. & Thomas, M.B. 2016. Global threat to agriculture from invasive species. Proceedings of the National Academy of Sciences USA, 113(27), 7575-7579.

Parkash, R. & Lambhod, C. 2021. Plastic changes in cold and drought tolerance of *Drosophila nepalensis* correlate with sex-specific differences in body melanization, cuticular lipid mass, proline accumulation, and seasonal abundance. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 258, 110985.

Ramniwas, S., Kajla, B. & Parkash, R. 2012. Extreme physiological tolerance leads the wide distribution of *Zaprionus indianus* (Diptera: Drosophilidae) in temperate world. Acta Entomologica Sinica Journal, 55(11), 1295-1305.

Reddy, P.P. 2013. Impact of climate change on insect pests, pathogens and nematodes. Pest Management in Horticultural Ecosystems, 19(2), 225-233.

Renault, D., Nedved, O., Hervant, F. & Vernon, P. 2004. The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. Physiological Entomology, 29(2), 139-145.

Scharf, I., Galkin, N. & Halle, S. 2015. Disentangling the consequences of growth temperature and adult acclimation temperature on starvation and thermal tolerance in the red flour beetle. Evolutionary Biology, 42, 54-62.

Sinclair, B.J. & Marshall, K.E. 2018. The many roles of fats in overwintering insects. Journal of Experimental Biology, 221(Suppl_1), jeb161836.

Sinclair, B.J. 2015. Linking energetics and overwintering in temperate insects. Journal of Thermal Biology, 54, 5-11.

Sinclair, B.J., Alvarado, L.E.C. & Ferguson, L.V. 2015. An invitation to measure insect cold tolerance: methods, approaches, and workflow. Journal of Thermal Biology, 53, 180-197.

Sinclair, B.J., Ferguson, L.V., Salehipour-Shirazi, G. & MacMillan, H.A. 2013. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integrative and Comparative Biology, 53(4), 545-556.

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

Singh, T., Bhat, M.M. & Khan, M.A. 2009. Insect adaptations to changing environments-temperature and humidity. International Journal of Industrial Entomology, 19(1), 155-164.

Sparks, T.H., Dennis, R.L., Croxton, P.J. & Cade, M. 2007. Increased migration of Lepidoptera linked to climate change. European Journal of Entomology, 104(1), 139-143.

Storey, K.B. & Storey, J.M. 2012. Insect cold hardiness: metabolic, gene, and protein adaptation. Canadian Journal of Zoology, 90(4), 456-475.

Tarusikirwa, V.L., Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2020. Cold hardiness of the South American tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae): both larvae and adults are chill-susceptible. Pest Management Science, 77(1), 184-193.

Teets, N.M. & 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. & Kawarasaki, Y. 2020. Rapid cold hardening: ecological relevance, physiological mechanisms and new perspectives. Journal of Experimental Biology, 223(3), jeb203448.

Teets, N.M., Kawarasaki, Y., Potts, L.J., Philip, B.N., Gantz, J.D., Denlinger, D.L. & Lee Jr, R.E. 2019. Rapid cold hardening protects against sublethal freezing injury in an Antarctic insect. Journal of Experimental Biology, 222(15), jeb206011.

Teets, N.M., Peyton, J.T., Colinet, H., Renault, D., Kelley, J.L., Kawarasaki, Y., Lee Jr, R.E. & Denlinger, D.L. 2012. Gene expression changes governing extreme dehydration tolerance in an Antarctic insect. Proceedings of the National Academy of Sciences, 109(50), 20744-20749.

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

Tollefson, J. 2014. The case of the missing heat. Nature, 505(7483), 276.

Toxopeus, J., Koštál, V. & Sinclair, B.J. 2019. Evidence for non-colligative function of small cryoprotectants in a freeze-tolerant insect. Proceedings of the Royal Society B, 286(1899), 20190050.

Travis, W.R. 2014. Weather and climate extremes: Pacemakers of adaptation? Weather and Climate Extremes, 5, 29-39.

Trenti, F., Sandron, T., Guella, G. & Lencioni, V. 2022. Insect cold tolerance and lipidome: Membrane lipid composition of two chironomid species differently adapted to cold. Cryobiology, 106, 84-90.

van Damme, V., Berkvens, N., Moerkens, R., Berckmoes, E., Wittemans, L., De Vis, R., Casteels, H., Tirry, L. & De Clercq, P. 2015. Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. Journal of Pest Science, 88, 533-541.

van Dooremalen, C., Suring, W. & Ellers, J. 2011. Fatty acid composition and extreme temperature tolerance following exposure to fluctuating temperatures in a soil arthropod. Journal of Insect Physiology, 57(9), 1267-1273.

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

Vatanparast, M. & Park, Y. 2022. Cold tolerance strategies of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). Scientific Reports, 12(1), 1-16.

Williams, C.M., Henry, H.A. & Sinclair, B.J. 2015. Cold truths: how winter drives responses of terrestrial organisms to climate change. Biological Reviews, 90(1), 214-235.

Worland, M.R. 1996. The relationship between water content and cold tolerance in the Arctic collembolan *Onychiurus arcticus* (Collembola: Onychiuridae). European Journal of Entomology, 93, 341-348.

Zachariassen, K.E. 1985. Physiology of cold tolerance in insects. Physiological Reviews, 65(4), 799-832.

Zeilstra, I. & Fischer, K. 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. Physiological Entomology, 30(1), 92-95.

Zerebecki, R.A. & Sorte, C.J. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. PLOS one, 6(4), e14806.

Chapter 4

Thermal tolerance in *Spodoptera frugiperda*: influence of age,sex and mating status*

*__Submitted as: Mbande, A.,__ Mutamiswa, R., & Chidawanyika, F. (2023). Thermal tolerancein *Spodoptera frugiperda*: influence of age, sex and mating status. Scientific African.
SCAF-D-23-00920. *Accepted*

4.1 Introduction

Invasive insect pests are a major threat to global agricultural crop production (Machekano et al., 2018). With warming climates due to climate change, the scourge of such pests is projected to increase (LastuVka et al., 2009; Skendzic et al., 2021). This leads to increased costs of crop production due to investment in pest management strategies and outright food insecurity in the cases where crop damage goes unabated (Shrestha, 2019; Skendžić et al., 2021; Tonnang et al., 2022. Extreme weather events associated with climate change are increasing and these may influence not only insect biogeography but individual physiological performance and their population dynamics at various temporal scales (Chidawanyika et al., 2012). For example, heatwaves which are usually acute in nature, may disrupt biological functions of organisms with cascading effects to populations through limited activity and survival (Sales et al., 2021). Such rapid extreme climatic events may even have profound impact on insects than gradual temperature changes (Chidawanyika & Terblanche, 2011a; Soroye et al., 2020; Sales et al., 2021).

The physiological adjustments that are employed by insects to buffer against thermal stress improve fitness through rapid and pliable non-heritable phenotypically plastic responses (Chown & Terblanche, 2006; Sgro et al., 2016). This ability to buffer extreme temperatures is important for invasion success and is reportedly prevalent in pests with high dispersal propensity (Xue & Ma, 2020; Nyamukondiwa et al., 2022). Indeed, plasticity is very beneficial to insects as it increases their thermal safety margins, thereby enabling activity in otherwise unfavorable environments (Angilletta, 2009; Kristensen et al., 2008; Nyamukondiwa et al., 2010; Chidawanyika & Terblanche 2011a, b; Xue & Ma, 2020). Understanding of heat tolerance and its plasticity is of great importance in the prediction of invasion risk and success under climate change.

The extent to which phenotypic plasticity buffer climate change-associated effects is a subject for debate and its effects on the maintenance of ecosystem function is still not clear (Munday et al., 2013; Sorensen et al., 2016) but ontogeny is known as the major factor that influences the magnitude of phenotypic plasticity responses to abiotic stressors (Lockwood et al., 2018; Mutamiswa et al., 2019) where the less mobile life-stages exhibit most plasticity (Chown & Terblanche, 2006). Thermal tolerance varies between populations, among species, individuals and within individuals (Bowler &

Terblanche, 2008; Blanckenhorn et al., 2014). Among other factors thataffect insect biochemical and physiological process, including thermal tolerance are the age, sex, mating status, body size, feeding or nutritious status (Bowler & Terblanche, 2008; Chidawanyika & Terblanche 2011b; Chidawanyika et al., 2017; Li et al., 2022). Ontogeny, age and sex specific variations within individuals received relatively little attention even though they are important in understanding thermal adaptations of organisms (Bowler &Terblanche, 2008). Thermal tolerance varies and is not expected to be constant throughout an individual's lifetime especially for organisms such as insects whose life cycles are complexand different life stages inhabit different microhabitats (Krebs & Loeschcke, 1995; Klock & Chown, 2001; Mutamiswa et al., 2019). The extent to which species cope with extreme temperature conditions at different life stages and how exposure of juvenile stages to such extremes may affect survival and reproduction at a later stage (Bowler & Terblanche, 2008; Blanckenhorn et al., 2014). For example, in Drosophila, short term exposure to extreme temperatures at larval and pupal stages influenced development and hatching success and resistance to extreme temperatures was uncoupled across developmental stages (Krebs & Loeschcke, 1995). This shows that different physiological pathways are used during ontogenyand these may or not be mediated by the production of heat shock proteins (HSPs) (Hoffmann et al., 2003; Bowler & Terblanche 2008). Acclimation at larval stage improved physiological response to thermal tolerance in *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), which were carried over to each stage of development until adult stage (Mutamiswa et al., 2019). This suggests that plastic physiologicalresponses acquired during development may give adult fitness and survival at a later stage (Chidawanyika & Terblanche, 2011b).

The reproductive function of organisms is known to be sensitive to heat (e.g. Zizzari & Ellers,2011; Xue & Ma, 2020) especially that of males (Sales et al. 2018; Malawey et al., 2021). Sexes may differ in various ways in their sensitivity to heat stress, with females being less sensitive than males (Roux et al.,2010; Zhang et al., 2015a). Sperm and egg production are both affected by temperatures. Exposure to extreme conditions (low or high) may not only reduce temperature sensitive performance but may cause irreversible damage to fertility such as deformed sperm and sterility (permanent such as egg or sperm mortality or

temporal) (Sales et al., 2018; 2021). Heat shock may also reduce male reproductive success by reducing sexual attractiveness to females (Krebs & Thompson, 2005). In *Drosophila mojavensis* (Diptera: Drosophilidae), heat stress caused males to be less attractive to females due to change in the epicuticular hydrocarbons production (Markow & Toolson, 1990). From the foregoing, it is apparent that temperature plays a key role in mediating the population dynamicsof ectotherms through life-stage and sex-linked carry over effects.

Spodoptera frugiperda is multivoltine and adults have high dispersal propensity, flying up to 100 km per night (Westbrook et al., 2016; Midega et al., 2018; Fieldmann et al., 2019; Garciaet al., 2019; Du Plessis et al., 2020; Zheng et al., 2022). Apart from these population level predisposing factors, thermal tolerance is likely to play a key role in the invasion ecology of the *S. frugiperda*. It is therefore important to understand the role of climate, and specifically extreme hot conditions on the population dynamics and distribution of *S. frugiperda* given that global temperatures are projected to increase due to global warming (Skendžić et al., 2021). To date studies on thermal tolerance of *S. frugiperda,* investigating CCRT, CTmin and SCP were conducted on field populations (Keosentse et al., 2021; Segaiso et al., 2023). This study aimed to determine the role of heat shock at juvenile and adult stages on the thermal tolerance of *S. frugiperda* of variable age, sex and mating status. Specifically, I investigated the effects of adult and juvenile heat shock on heat tolerance measured as CT_{max} across age, sex and mating status. It was hypothesized that basal thermal tolerance of *S. frugiperda* varies across age, sex and mating status following heat shock. The results are significant in pest risk assessments and informing pest management options under shifting abiotic environments.

4.1.1 Materials and methods

4.1.2 Insect culture

The initial colony of *S. frugiperda* was obtained as larvae and pupae from North West University, Potchefstroom, South Africa. Pupae were maintained in wide mouth plastic tubs (1liter) containing moist soil

until adult eclosion. Larvae were maintained in vials containing artificial diet from Southland product INC, USA. Insects were kept at an optimum temperature of 28 ˚C ((Ali et al., 1990; Du Plessis et al., 2020), 65± 5 relative humidity and 12L: 12D photoperiod (Keosentse et al., 2022) in the insectarium at the University of the Free State, Bloemfontein, South Africa. Adults were sexed at pupal stage following Kalleshwaraswamy et al. (2018). This process involved observing the distance between genital opening and anal slot of pupa under the dissecting microscope (Leica 10445538, MZ8, Leica Corporation, Switzerland), with the females exhibiting longer distance than males. In addition, males have two pairs of rounded tubercles, one pair around the genital opening and the other around the anal opening while females have one pair around the anal opening only. Following eclosion, individual male and female adults were paired, and each pair released into a (40×40×60 cm) cage with potted maize plants (2 weeks old) for oviposition for gravid females. The plants were monitored daily for the presence of eggs. Adult moths were provided with 10% sucrose solution soaked in moistened cotton wool placed in a Petri dish for feeding *ad libitum* (Keosentse et al., 2022).

4.1.3Effects of adult heat shock on age, sex, and mating status

To evaluate the effects of age, sex (female and male) and mating status on basal thermal tolerance of *S. frugiperda*, 3, 6 and 9 day old virgin and mated adults were heat shocked at 40°C for 2 hours in propylene vials with a small piece of moist cotton wool placed on the bottom of each vial to prevent desiccation related mortality (Fig. 4.1). Following heat shock, critical thermal maxima (CT_{max}) was measured following dynamic protocols outlined by Nyamukondiwa & Terblanche, (2009) and Chidawanyika & Terblanche (2011 a). Ten adult *S. frugiperda* from heat shock treatments were individually placed into a double jacketed “organpipe” chamber comprising 11 separate 200 mm tubes, connected to a programmable water bath(Grant model Tx150; Grant Instruments, UK) filled with 1:1 water: propylene glycol and wassubjected to a constant heating. In the ‘organ pipe’, insects were given 10 minutes first to equilibrate at 28 ˚C (which is their optimal developmental temperature) before ramping temperature up (CT_{max}) at a rate of 0.25 ˚C min^{-1}. This was repeated twice to yield n=20 per

treatment (20 replications). A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the control chamber to monitor chamber temperatures. Critical thermal maximum was regarded as the uppertemperature at which the adult lost coordinated movement or ability to self-right after mild proding with a soft hair camel brush.

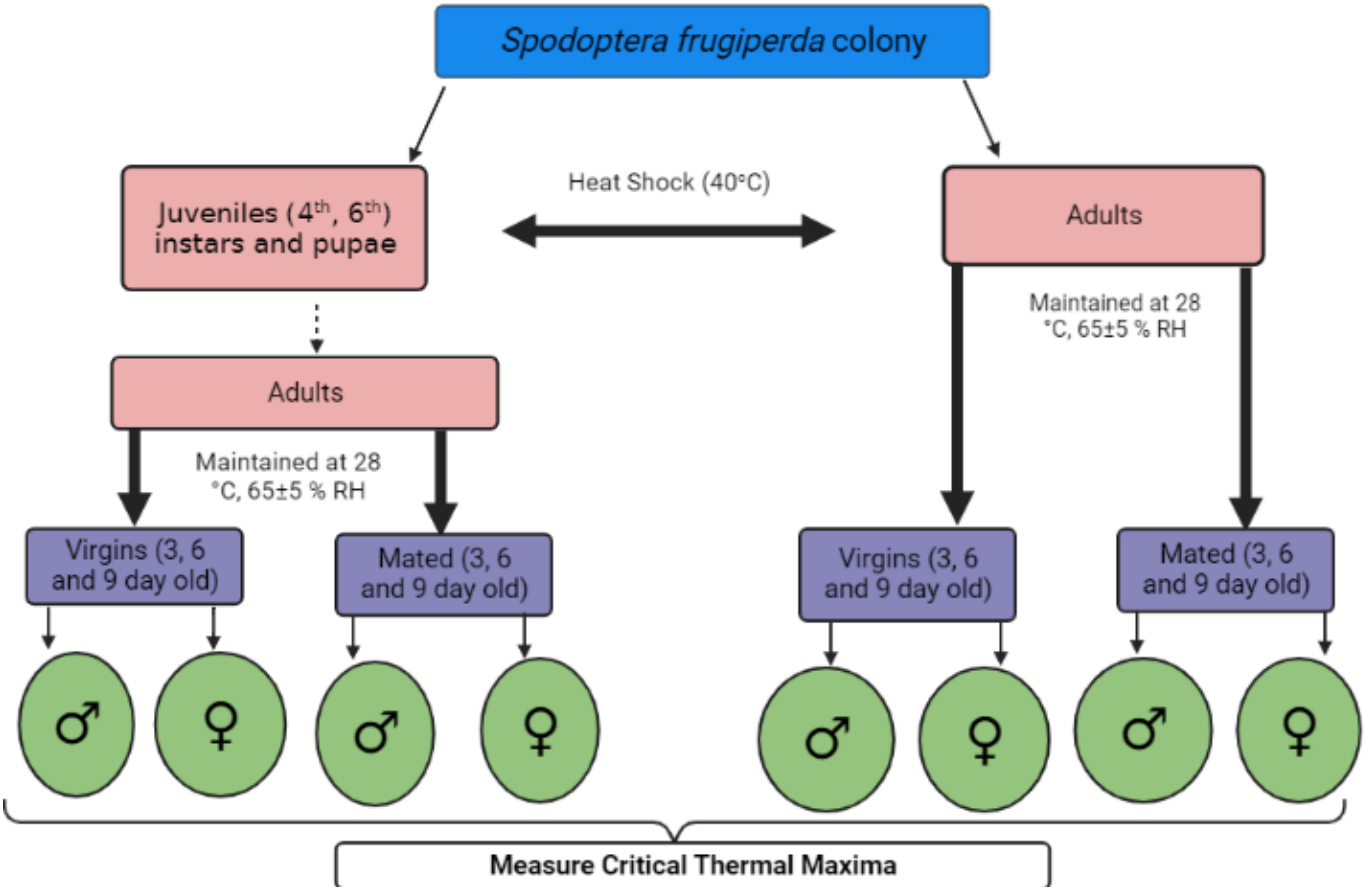

Figure 4. 2 Schematic representation of *Spodoptera frugiperda* heat shock assays.

4.1.4 Effects of juvenile heat shock on adult thermal tolerance

To determine the effects of age and developmental heat shock on *S. frugiperda,* 4th, and 6th instar larvae and 24 - 48 h old pupae were heat shocked at 40 °C for 2 hours (Fig. 4.1). Thereafter, they were returned to theinsectarium where they were kept at 28℃, 65 ± 5 % relative humidity until adult eclosion andwere grouped according to mated or virgin, age (3, 6 and 9 days old) and sex (female and male).Following heat shock, CT_{max} was measured as specified in section 4.2.2. (Fig. 4.1).

4.1.5 Data analysis

Data were first checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests respectively. In all cases, linear model assumptions of analysis of variance (ANOVA) were met (Shapiro–Wilk and Hartley–Bartlett tests, $p > 0.05$). As a result, CT_{max} data were analysed in STATISTICA, version 13.0 (Statsoft Inc., Tulsa,Oklahoma) using full factorial ANOVA with life stage, age, sex and mating status being the categorical factors while CT_{max} was the dependent variable. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups.

4.2 Results

4.2.1 Adult heat shock

Critical thermal maxima significantly varied across mating status, age and sex in tested insects following heat shock ($P < 0.01$) (Table 4.1). There was a significant interaction effect in CT_{max} between mating status and sex, age and sex as well as mating status, age and sex ($P < 0.001$) (Table 4.1). In virgin females, CT_{max} increased with age while fluctuating in males. However, in mated females CT_{max} decreased with age and fluctuated in males (Fig. 4.2). There was a significant difference in CT_{max} between virgin 6 day old females and males with females

recording higher heat tolerance (Mean ±SE) (46.8 ± 0.16℃) than males (Mean ±SE) (44 ±0.66℃) (Fig. 4.2). However, no significant difference in CT_{max} was recorded between virgin males and females aged 9 (Mean ±SE) (46.45± 1.22℃; 47.5± 0.5℃) days as well as 3, (48± 0.5℃; 47.5± 0.33℃) 6 (45± 1.5℃; 46.5± 0.28℃) and 9 (46.5± 0.5℃; 46.7± 0.33℃) day mated males and females (Fig. 2). The 3 day old mated females recorded the highest heat tolerance (Mean ±SE) (46.5 ± 0.5℃) while the 6 day old virgin males recorded the lowest heat tolerance (Mean ±SE) (44 ±0.66℃) (Fig. 4.2). Nevertheless, the interaction between mating status and sex was not significant (P = 0.69) (Table 4.1).

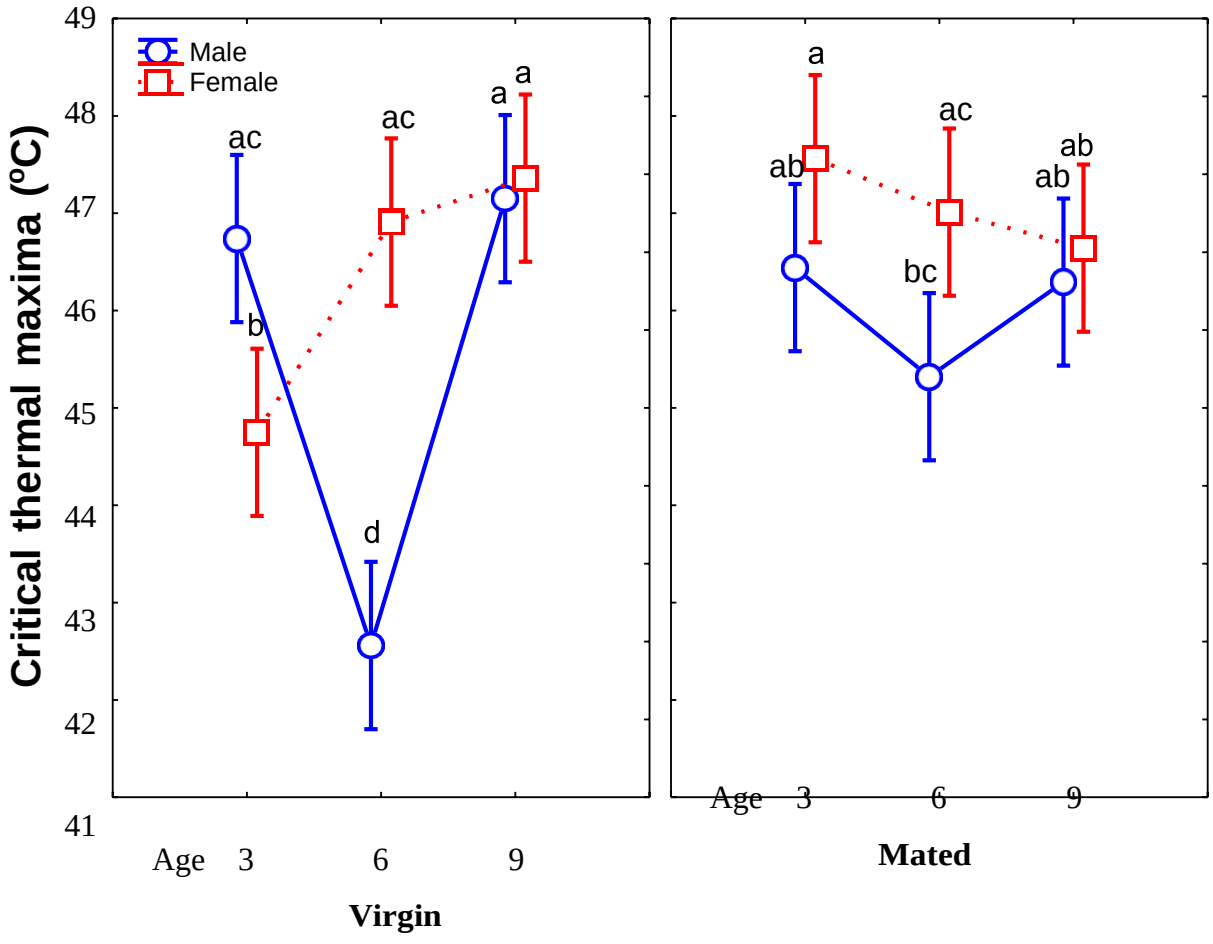

Figure 4. 3 Effects of heat shock on adult thermal tolerance of virgin and mated of *Spodoptera frugiperda.*

Table 4. 1: Effects of adult heat shock on thermal tolerance of virgin and mated adult Spodoptera frugiperda. Summary statistical results from full factorial analysis of variance (ANOVA) showing effects of adult heat shock on thermal tolerance of virgin and mated adult *Spodoptera frugiperda.*

Effect	**SS**	**DF**	**MS**	**F**	***P***
Intercept	256106.9	1	256106.9	136395.7	<0.001
Mating status	12	1	12	6.4	<0.01
Age	41	2	20.5	10.9	<0.001
Sex	27.3	1	27.3	14.6	<0.001
Mating status x Age	30.4	2	15.2	8.1	<0.001
Mating status x Sex	0.3	1	0.3	0.2	0.69
Age x Sex	66.5	2	33.3	17.7	<0.001
Mating status x Age x Sex	41.6	2	20.8	11.1	<0.001
Error	202.8	108	1.9		

4.2.2 Developmental acclimated adults

Critical thermal maxima significantly varied across life stages, sex and age of tested virgin insects (P<0.001) (Table 4.2; Fig. 4.3). The two-way interaction between life stage and age of virgin adults had a significant difference ($P < 0.001$). However, no significant differences were recorded in the two-way interaction between life stage and sex ($P = 0.59$), age and sex ($P = 0.11$) as well as three way interaction between life stage, age and sex of developmentally acclimated virgin adults ($P = 0.12$) (Table 4.2) showing no variation in thermal sensitivity between life stages. Virgin females recorded higher CT_{max} than males across all heat shocked developmental stages (Fig. 4.3). Critical thermal maxima of adult males and females decreased with age following developmental heat shock at 6^{th} instar stage (Fig. 4.3). However, fluctuations in CT_{max} in both adult males and females were recorded following developmental shock at 4^{th} instar and pupal stages.

Table 4. 2: Effects of juvenile heat shock on thermal tolerance of virgin Spodoptera frugiperda adults. Summary statistical results from full factorial analysis of variance (ANOVA) of juvenile heat shock on thermal tolerance of virgin *Spodoptera frugiperda* adults.

Effect	**SS**	**DF**	**MS**	**F**	***P***
Intercept	378051.7	1	378051.7	257113.2	<0.001
Life stage	154.9	2	77.5	52.7	<0.001
Age	95.8	2	47.9	32.6	<0.001
Sex	33.	1	33.8	23.0	<0.001
Life stage x Age	125.6	4	31.4	21.4	<0.001
Life stage x Sex	1.6	2	0.8	0.5	0.59
Age x Sex	6.7	2	3.3	2.3	0.11
Life stage x Age x Sex	10.9	4	2.7	1.9	0.12
Error	238.2	162	1.5		

Table 4. 3: Effects of juvenile heat shock on thermal tolerance of mated adult Spodoptera frugiperda. Summary statistical results from full factorial analysis of variance (ANOVA) of juvenile heat shock on thermal tolerance of mated adult *Spodoptera frugiperda.*

Effect	**SS**	**DF**	**MS**	**F**	***P***
Life stage	1.6	2	0.8	0.44	0.64
Age	312.6	2	156.3	88.02	<0.001
Sex	47.2	1	47.2	26.6	<0.001
Life stage x Age	60.4	4	15.1	8.51	<0.001
Life stage x Sex	4.8	2	2.4	1.36	0.26
Age x Sex	13.6	2	6.8	3.83	0.024
Life stage x Age x Sex	7.5	4	1.9	1.06	0.38

In mated insects, CT_{max} varied significantly across age and sex ($P < 0.001$) (Table 4.3; Fig. 4.4). There was a significant interaction effect between life stage and age ($P < 0.001$) (Table 4.3). Like in virgin insects, mated females also recorded higher CT_{max} than males across all heat shocked developmental stages (Fig. 4.4). Critical thermal maxima of adult males and females also decreased with age following developmental heat shock at 4^{th} and 6^{th} instar stages with fluctuations recorded following heat shock at pupal stage (Fig. 4.4).

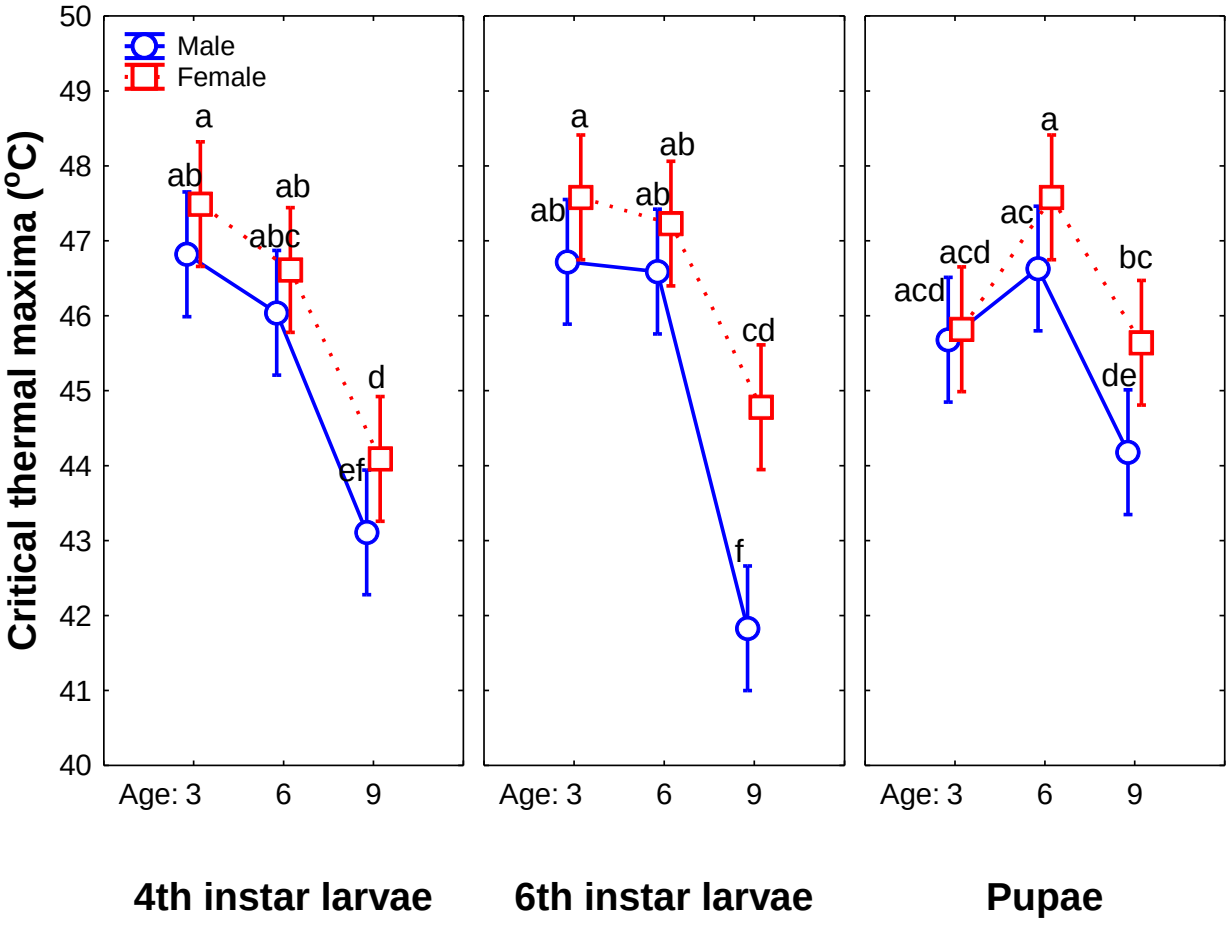

Figure 4. 5 Effects of juvenile heat shock (40oC for 2 hr) on basal heat tolerance (CTmax) of mated adult *Spodoptera frugiperda* (N=20). Error bars represents 95% confidence limits and means with the same letter are not significantly different. CTmax = critical thermal maxima.

4.3. Discussion

In the present study, heat tolerance of *Spodoptera frugiperda* was influenced by age, sex and mating status in agreement with other studies, which attributed the factors as key for mediating thermaltolerance (Li et al., 2022). Females recorded higher CT_{max} than males following adult heat shock. This indicates that females are more heat tolerant and exhibit higher level of plasticity than males which is consistent with Cui et al. (2008) who reported high temperature tolerance levels in *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B females than males. Our results therefore suggest that *S. frugiperda* females have a fitness and possibly survival advantage when encountering transient heat stress in nature. An improvement in insect thermal tolerance can come at a negative cost of other physiological parameters and can be dependent on somatic condition or age (Shen et al., 2014; Chen et al., 2018). In this study, heat tolerance in virgin females increased with age while decreasing with age in mated females suggesting a link between reproductive fitness and tolerance to heat stress, but mediated by age. While the reason is currently unknown, these results show some trade-off between thermal tolerance and reproduction. It is however plausible that variation inenergy reserves and resource allocation after mating could have influenced the tolerance to heat (van Noordwijk & De Jong, 1986; Johnson & Stahlschmidt, 2020). This warrants further investigation to fully elucidate this variation. These results indicate that mating may significantly affect heat tolerance of adult *S. frugiperda* during aging. Males and females responded differently to high temperatures such that mated females had a high basal heat tolerance than virgin males. A similar result in different taxon has been reported, where mating in *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) influenced thermal tolerance such that mated females had higher basal thermal tolerance (CT_{max}) than virgins (Xue & Ma, 2020). These results suggest that mated female adults may have evolved adaptive strategies to cope with high temperatures.

Previous studies have reported that heat stress experienced in juvenile life-stages may have variable effects on other later developmental stages (Olsen et al., 2006; Zhang et al., 2015a,b; Mutamiswa et al.,

2022). In heat shocked juveniles (4th and 6th instars), CT_{max} of adult males and females decreased with age such that older adults recorded lower CT_{max} than younger ones (Fig. 4.2 and 4.3). Such trends may be associated with developmental 'carry-over' effects (Bowler & Terblanche, 2008). According to evolutionary theories of ageing, strength of natural selection declines as organisms' age, since reproduction occurs at young stages (Halleet al., 2015). In addition, as insects age, they often accumulate abnormal proteins over time which may become less functional resulting in increased susceptibility to heat stress (Niedzwiecki et al., 1991; Li et al., 2022). Conversely, Li et al. (2022) reported otherwise in older *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) adults recording higher CT_{max} than younger ones. This suggests that evolutionary responses to thermal tolerance are highly genetically conserved and species- specific. Further comparison of larval and pupal heat shocked insects in our study showed females from pupal heat shock recording higher CT_{max} than those from larval heat shock (Fig. 4.3). This supports the notion that immobile life-stages (eggs and pupae) are more phenotypicallyplastic in their thermal tolerance due to limited capacity to behaviorally thermoregulate (Chown & Terblanche, 2006; Enriquez & Colinet, 2017). As a result, the pupal fitness advantage was passed to the emerging adults through cross over effects.

In virgin and mated insects, females recorded higher heat tolerance than males following larvaland pupal heat shock. However, Mutamiswa et al. (2022), recently reported otherwise in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) males and females following pupal heat stress. This indicates that thermal tolerance following juvenile stress maybe species dependent. In addition, it indicates that females are more heat resistant than males which also gives them a survival advantage and population perpetuation under changing climate.

Microclimatic field temperature data recorded in an agroecosystem where *S. frugiperda*inhabits indicate that hot days are regularly observed under field conditions (see Keosentse et al., 202; Mbande et al., 2023). These climatic conditions may be worsened by ongoing climate variabilities (Chen et al., 2018). However, the duration of these suboptimal temperature conditions is currently unknown. As a species with overlapping and short generations, *S. frugiperda* life stages may likely experience these stressful conditions in agroecosystems. Given the differential responsesof *S. frugiperda* following juvenile and adult heat shock

and its capacity to tolerate high temperature conditions, it indicates that this invasive insect pest has a potential to spread and establish in most agroecosystems in sub Saharan Africa through population perpetuation underchanging environments. As a result, this may negatively affect crop production and household food security. While this study focused on fixed heat shock temperature and duration of exposure, future studies should also focus on fluctuating temperatures and durations of exposure effects on physiological responses of this invasive insect pest.

In conclusion, this study shows that age, sex and mating status plays a crucial role in thermal tolerance and persistence of *S. frugiperda* under climate change. Juvenile heat shock demonstrated adult "carry over" effects with aged adults showing a decline in CT_{max}. Heat shock at pupal stage was advantageous in thermal tolerance at adult stage and females showed fitness and survival advantage when exposed to heat stress. Females fitness may show population persistence since females may continue to oviposit when males are long dead. This study suggests that plasticity of thermal tolerance may be an important mechanism for *S. frugiperda* population persistence in novel environments.

4.4 References

Ali, A., Luttrell, R.G. & Schneider, J.C. 1990. Effects of temperature and larval diet on development of the fall armyworm (Lepidoptera: Noctuidae). Annals of the Entomological Society of America, 83(4), 725-733.

Cornelissen, T. 2011. Climate change and its effects on terrestrial insects and herbivory patterns. Neotropical entomology, 40,155-163.

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J. & Gomez, J. 2017. Fall armyworm: impacts and implications for Africa. Outlooks on Pest Management, 28(5), 196-201.

Du Plessis, H., Schlemmer, M.L. & Van den Berg, J. 2020. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects, 11(4), 228.

Ekpa, O., Palacios-Rojas, N., Kruseman, G., Fogliano, V. & Linnemann, A.R. 2018. Sub-Saharan African maize-based foods: technological perspectives to increase the food and nutrition security impacts of maize breeding programmes. Global Food Security, 17, 48-56.

Enriquez, T. & Colinet, H. 2017. Basal tolerance to heat & cold exposure of the spotted wing drosophila, *Drosophila suzukii*. Peer J, 5, e3112.

Feldmann, F., Rieckmann, U. & Winter, S. 2019. The spread of the fall armyworm *Spodoptera frugiperda* in Africa—What should be done next? Journal of Plant Diseases & Protection, 126(2), 97-101.

Garcia, A.G., Ferreira, C.P., Godoy, W.A. & Meagher, R.L. 2019. A computational model to predict the population dynamics of *Spodoptera frugiperda*. Journal of Pest Science, 92(2), 429-441.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks ofthe fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien pest in West and Central Africa. Plos one, 11 (10), p.e0165632.

Halle, S., Nowizki, A. & Scharf, I. 2015. The consequences of parental age for development, body mass and resistance to stress in the red flour beetle. Biological Journal of the Linnean Society, 115(2), 305-314.

Hansen, P.J. 2009. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1534), 3341-3350.

Hoffmann, A.A. 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses & genetic variation. Journal of Insect Physiology, 36, 885–891.

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

Keosentse, O., Mutamiswa, R., Du Plessis, H. & Nyamukondiwa, C. 2021. Developmental stage variation in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) low temperature tolerance: Implications for overwintering. Austral Entomology, 60*(*2), 400-410.

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

Krebs, R.A. & Loeschcke, V. 1995. Resistance to thermal stress in pre-adult *Drosophila buzzatii*: variation among populations and changes in relative resistance across life stages. Biological Journal of the Linnean Society, 56(4), 517-531.

Krebs, R.A. & Thompson, K.A. 2005. A genetic analysis of variation for the ability to fly after exposure to thermal stress in *Drosophila mojavensis.* Journal of Thermal Biology, 30(4), 335-342.

Kristensen, T.N., Hoffmann, A.A., Overgaard, J., Sørensen, J.G., Hallas, R. & Loeschcke, V. 2008. Costs and benefits of cold acclimation in field-released Drosophila. Proceedings of the National Academy of Sciences, 105(1), 216-221.

Li, C., Addeo, N. F., Rusch, T. W., Dickerson, A. J., Tarone, A. M., Hu, W. & Tomberlin, J. K. 2022. Impact of age, sex, & size on the thermal tolerance of the adult black soldier fly (Diptera: Stratiomyidae). Journal of Insects as Food and Feed, 8(6), 681-691.

Lockwood, B.L., Gupta, T. & Scavotto, R. 2018. Disparate patterns of thermal adaptation between lifestages in temperate vs. tropical *Drosophila melanogaster*. Journal of Evolutionary Biology, 31(2), 323-331.

M.J. 2018. Experimental evolution with an insect model reveals that male homosexualbehaviour occurs due to inaccurate mate choice. Animal Behavior, 139, 51-59.

Machekano, H., Mutamiswa, R. & Nyamukondiwa, C. 2018. Evidence of rapid spread and establishment of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in semi-arid Botswana. Agriculture and Food Security, 7(1), 1-12.

Malawey, A.S., Zhang, H., McGuane, A.S., Walsh, E.M., Rusch, T.W., Hjelmen, C.E., Delclos, P.J., Rangel, J., Zheng, L., Cai, M. & Yu, Z. 2021. Interaction of age and temperature on heat shock protein expression, sperm count, and sperm viability of the adult black soldier fly (Diptera: Stratiomyidae). Journal of Insects as Food & Feed, 7(1), 21-33.

Markow, T.A. & Toolson, E.C. 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. In Ecological & evolutionary genetics of *Drosophila* Springer. Boston, MA. 315-331.

Mbande, A., Mutamiswa, R., Nyamukondiwa, C. & Chidawanyika, F. 2023. Contrasting effects of acute heat shock on physiological and ecological performance of the fall armyworm. Entomologia Experimentalis et Applicata, 171, 525–534.

Midega, C.A., Pittchar, J.O., Pickett, J.A., Hailu, G.W. & Khan, Z.R. 2018. A climate-adapted push-pull system effectively controls, *Spodoptera frugiperda* (JE Smith), in maize in EastAfrica. Crop Protection, 105, 10-15.

Munday, P.L., Warner, R.R., Monro, K., P&olfi, J.M. & Marshall, D.J. 2013. Predicting evolutionaryresponses to climate change in the sea. Ecology Letters, 16(12), 1488-1500.

Mutamiswa, R., Chidawanyika, F. & Nyamukondiwa, C. 2018. Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. Physiological Entomology, 43(2), 108-119.

Mutamiswa, R., Machekano, H., Chidawanyika, F. & 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.

Mutamiswa, R., Tarusikirwa, V.L., Nyamukondiwa, C., Cuthbert, R.N. & Chidawanyika, F. 2022. Thermal stress exposure of pupal oriental fruit fly has strong & trait-specific consequences in adult flies. Physiological Entomology,48(1), 35-44.

Niedzwiecki, A., Kongpachith, A.M. & Fleming, J.E. 1991. Aging affects expression of 70-kDa heat shock proteins in Drosophila. Journal of Biological Chemistry, 266(14), 9332-9338. Nyamukondiwa, C. & Terblanche, J.S. 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, sex and feeding status. Journal of Thermal Biology, 34(8), 406-414.

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

Nyamukondiwa, C., Machekano, H., Chidawanyika, F., Mutamiswa, R., Ma, G. & Ma, C.S. 2022. Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance & other complementary traits in the tropics. Current Opinion in Insect Science, 100878.

Olsen, A., Vantipalli, M.C. & Lithgow, G.J. 2006. Lifespan extension of *Caenorhabditis elegans* following repeated mild hormetic heat treatments. Biogerontology, *7*, 221-230.

Roux, O., Le Lann, C., Van Alphen, J.J.M. & van Baaren, J. 2010. How does heat shock affect the

life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)? Bulletin of Entomological Research, 100(5), 543-549.

Sales, K., Trent, T., Gardner, J., Lumley, A.J., Vasudeva, R., Michalczyk, Ł., Martin, O.Y. & Gage, M.J. 2018. Experimental evolution with an insect model reveals that male homosexual behaviour occurs due to inaccurate mate choice. Animal Behaviour, *139*, 51-59.

Sales, K., Vasudeva, R. & Gage, M.J. 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. Royal Society Open Science, 8(3), 201717.

Sgrò, C.M., Terblanche, J.S. & Hoffmann, A.A. 2016. What can plasticity contribute to insect responses to climate change. Annual Review of Entomology, 61(1), 433-451.

Shen, Y., Gong, Y.J., Gu, J., Huang, L.H. & Feng, Q.L. 2014. Physiological effect of mild thermal stress and its induction of gene expression in the common cutworm, *Spodoptera litura*. Journal of Insect Physiology, 61, 34-41.

Simberloff, D., 2000. Global climate change and introduced species in United States forests. *Science of the total environment*, *262*(3), 253-261.Sørensen, J.G. Kristensen, T.N. & Overgaard, J. 2016. Evolutionary and ecological patterns of thermal acclimation capacity in Drosophila: is it important for keeping up with climate change? Current Opinion in Insect Science, 17, 98-104.

Soroye, P. Newbold, T. & Kerr, J. 2020. Climate change contributes to widespread declines among bumble bees across continents. Science, 367(6478), 685-688.

Sparks, A.N. 1979. A review of the biology of the fall armyworm. Florida Entomologist, 62(2), 82-87.

Westbrook, J.K., Nagoshi, R.N., Meagher, R.L., Fleischer, S.J. & Jairam, S. 2016. Modeling seasonalmigration of fall armyworm moths. International Journal of Biometeorology, 60(2), 255- 267.

Xue, Q. & Ma, C.S. 2020. Aged virgin adults respond to extreme heat events with phenotypic

plasticityin an invasive species, *Drosophila suzukii*. Journal of Insect Physiology. 121, 104016.

Zhang, W., Chang, X.Q., Hoffmann, A., Zhang, S. & Ma, C.S. 2015a. Impact of hot events at differentdevelopmental stages of a moth: the closer to adult stage, the less reproductive output. Scientific Reports, 5(1), 1-9.

Zhang, W., Rudolf, V.H.W. & Ma, C.S. 2015b. Stage-specific heat effects: timing and duration of heatwaves alter demographic rates of a global insect pest. Oecologia. 179, 947–957.

Zheng, R., Cheng, L., Peng, J., Li, Q., Yang, F., Yang, D., Xia, Y. & Tang, Q. 2023. Comparative analysis of gut microbiota and immune genes linked with the immune system of wild and captive *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Developmental & Comparative Immunology, 138, 104530.

Zizzari, Z.V. & Ellers, J. 2011. Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. Journal of Insect Physiology. 57(3), 421-426.

Chapter 5

Conclusions and Recommendations

5.1 General conclusion and recommendations

Extreme weather events will occur more frequently and abruptly due to climate change (Easterling et al., 2000; Fischer & Knutti, 2015; Ma et al., 2015; Bailey & van de Pol, 2016; Kingsolver & Buckley, 2017). These extreme climatic events may impact organisms at an individual, population and community level (Bailey & van de Pol, 2016). Climate change effects on agro-ecosystems are becoming apparent with seasonal and long term variations influencing insect pest population dynamics, abundance and activities of natural enemies andefficacy of pest management (Easterling et al., 2000; Chidawanyika et al., 2012; Selvaraj et al., 2013). Biological traits such as physiology and life history traits are influenced by extreme weather events and the gradual rise in average temperatures (Chown & Nicolson, 2004; Mutamiswa, 2017; Shumo et al., 2019). Understanding how invasive agricultural pests respond and how their thermal variability evolves is crucial under global climate change since these may have serious effects on human food security.

This study investigated various responses to thermal stress in *Spodoptera frugiperda* within and across generations. My results indicated that the longevity, fecundity, egg hatching success of *S. frugiperda* were significantly reduced by heat shock (Chapter 2). Acute temperature stressbrought on by global climate change has become the new normal, and has impacted weather patterns such as extreme temperatures which influence organisms. This study showed contrasting physiological and ecological traits of *S. frugiperda* after exposure to sub lethal heat stress. Prior acute heat shock led to reduction in heat tolerance (CT_{max}) while impairing cold tolerance (CT_{min}) but CT_{min} at 38°C increased (Chapter 2). This shows how acute heat stress offsets important physiological characteristics and impacted reproduction. Females showed high CT_{max} and plasticity than males, showing a survival advantage over males in natural heat stress (Chapter 2, 3 and 4). This was consistent with Nyamukondiwa & Terblanche (2009) who showed sex- related differences in heat tolerance. The differences in basal and plastic differences observed in this study are likely explained by lipids which have been suggested to play a crucial role inthermal tolerance (Chown & Nicolson, 2004). The relationship between sex and

lipid content differences and their role in CT_{max} variation should be further investigated.

The present study detailed life stage related variation in *S. frugiperda*'s repeatability of cold tolerance and my findings show that cold tolerance traits are repeatable and important in predicting responses to future extreme cold conditions in natural environments (Chapter 3). Insects avoid extreme temperature by means of behavioral avoidance, migration, and diapause or in a highly altered physiological condition (Perkins, 1979; Du Plessis et al., 2020). *Spodoptera frugiperda* does not diapause and, therefore, migrate to regions which have more favorable environmental conditions (Du Plessis et al., 2020). *Spodoptera frugiperda* larvae and pupae have been reported to be unable to survive temperatures below 13℃ at the overwintering sites (Perkins, 1979). Therefore, this study offers proof that cold hardening may be crucial defense mechanisms for *S. frugiperda* against repeated short-term exposure to cold conditions. In this study, Body Water Content and Body Lipid Content (BWC & BLC) did not correlate with cold tolerance, hence these adaptations may help species endureprolonged cold conditions in nature leading to population persistence under changing environmental conditions (Chapter 3). The ability of many cold-hardened insects to maintain basal metabolism at low temperatures depends on the water content remaining unfrozen (Blocket al., 2003; Colinet et al., 2006; Alfaro-Tapia et al., 2021). Cold hardening may be an important mechanism to cope with repeated cold exposure over the short-term. These cold tolerance responses may help the species survive repeated cold conditions. Cold tolerance traitsare significant in determining responses when *S. frugiperda* face extreme conditions in nature.

Cold tolerance significantly decreased after heat shock while heat tolerance improved, showingthat heat and cold tolerance were responsive to heat shock in *S. frugiperda* (Chapter 2). There were also sex variations in physiological and ecological responses to heat shock, following heat shock with females recording high CT_{max} than males, while males recorded higher longevity (Chapter 2 and Chapter 4). Both sexes showed phenotypic plasticity after short-term exposure to heat stress (Chapter 2). According to the beneficial hypothesis, acclimation to one stress may increase survival of acclimated individuals compared to unacclimated individuals (Wilson & Franklin, 2002). Several studies also found significant higher expression of heat shock proteins (HSP90 and HSP70) in heat shocked individuals compared to

unacclimated individuals showing the function of heat shock proteins in thermal tolerance (heat tolerance) (Zhang, 2005; Adams, 2023). Therefore, the increase in CT_{max} after heat shock is not surprising.

Juvenile life stages are the most sensitive and crucial life stages during development and are often influenced by temperature (Pottier et al., 2022). Early juvenile environmental experiences shape thermal tolerance such as developmental plasticity. Examining whether thermal tolerance is influenced\shaped by life stage or early thermal environments has a critical implications in ecophysiological modelling and experimental research. Ontogenetic variation in thermal sensitivity has been documented (Bowler & Terblanche, 2008). Heat shock also influenced mating status, age and sex in *S. frugiperda* (Chapter 4). Juvenile exposure to heat stress positively impacted *S. frugiperda* at adult stage and different life stages showed varying thermal sensitivity to heat stress (Chapter 4). Individual thermal tolerance is highly variable and is largely age-dependent, such that thermal tolerance reduce in later life stages (Bowler & Terblanche, 2008). Variable responses to fluctuating temperature conditions across ontogeny exists. In the natural environment, insects have an option to seek microclimatic relief from heat stress. *Spodoptera frugiperda* is known for its migratory behavior and this may be one of themechanisms that aided its spread.

The male reproductive system is known to be more sensitive to thermal stress than any other life histories traits (Nguyen et al., 2013). While in males, heat shock may reduce mating success and indirectly injure testes and sperms resulting in reduction in fertility (Krebs & Loeschke, 1994; Sales et al., 2021), the decrease in fecundity and hatching success with increasing heat shock temperatures may be due to the cumulative effects of damage to tissues (Chown & Nicholson, 2004). Although *S. frugiperda* showed capacity for rapid CT_{max} adjustment showing plasticity under heat stress, this study showed that this may come at a cost of reduced fecundity. Thus plasticity may aid activity in the short term but population level fitness maybecompromised through poor reproductive success. Thus my findings highlight the importance of tracking both individual and population level traits in investigating the impacts of climate variability on insects as short term benefits maybe off set by trade-offs in the long term. This study shows that FAW exhibits sufficient plasticity to potentially allow it to establish in most maize-growing areas of South Africa, even under an altered future climate, although appropriate forecasting distribution models would be needed

to be certain about this. Future studies should include area-wide & early warning systems for early seasonal detection and effective control strategies. It is worthy to note that thermal tolerance limits are useful predictors and have been studies extensively, evidence shows that these matrices are not perfect predictors of climate change vulnerability (Crusella-Trullas et al., 2021). Phenotypes of an individuals are not fixed through time and this may be challenging for biologists in generating predictive models for organismal responses to climate change. Therefore, there is a need to study thermal tolerance of FAW in the field based insects where temperatures fluctuate, since under climate change thermal stresses are likely to occur in combination with other environmental stressors. This study also contributes new theoretical knowledge to the field of insect thermal tolerance and variations between different life stages.

5.2 References

Adams, V., 2023. HSP70 Upregulation during Fall-like Acclimation of Gryllus veletis.

Alfaro-Tapia, A., Alvarez-Baca, J.K., Tougeron, K., Lavandero, B., Le Lann, C. & Van Baaren, J. 2021. Overwintering strategies and life-history traits of different populations of *Aphidius platensis* along a latitudinal gradient in Chile. Entomologia Generalis, 42(1), 127-145.

Bailey, L.D. & van de Pol, M. 2016. Tackling extremes: challenges for ecological and evolutionary research on extreme climatic events. Journal of Animal Ecology, 85(1), 85-96.

Block, W. 2003. Water or ice? The challenge for invertebrate cold survival. ScienceProgress, 86(1-2), 77–101.

Chidawanyika, F., Mudavanhu, P. & Nyamukondiwa, C. 2012. Biologically based methods for pest management in agriculture under changing climates: challenges and future directions. Insects, 3(4), 1171-1189.

Chown, S.L., & Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns.* Oxford University Press, UK.

Colinet, H., Hance, T. & Vernon, P. 2006. Water relations, fat reserves, survival, and longevity of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidiinae). Environmental Entomology, 35(2), 228–236.

Du Plessis, H., Schlemmer, M.L. & Van den Berg, J. 2020. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects, 11(4), 228.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. 2000. Climate extremes: observations, modelling, and impacts. Science, 289(5487), 2068-2074.

Fischer, E.M. & Knutti, R. 2015. Anthropogenic contribution to global occurrence of heavy-

precipitation and high-temperature extremes. Nature climate change, 5(6), 560-564.

Krebs, R.A. & Loeschcke, V. 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Functional Ecology, 730-737.

Ma, G., Rudolf, V.H. & Ma, C.S. 2015. Extreme temperature events alter demographic rates,relative fitness, and community structure. Global Change Biology, 21(5), 1794-1808.

Mutamiswa, R. 2017. Impacts of climate variability on stemborers-natural enemies trophiclevel interaction: implications for biological control under global change. PhD Thesis. Botswana International University of Science and Technology. Palapye. Botswana.

Nguyen, T.M., Bressac, C. & Chevrier, C. 2013. Heat stress affects male reproduction in a parasitoid wasp. Journal of Insect Physiology, 59(3), 248-254.

Nyamukondiwa, C. & Terblanche, J.S. 2009. Thermal tolerance in adult Mediterranean andNatal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, sex and feeding status. Journal of Thermal Biology, 34(8), 406-414.

Nyamukondiwa, C. & 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.

Perkins, W.D. 1979. Laboratory rearing of the fall armyworm. Florida Entomologist, .87-91.

Sales, K, Vasudeva, R. & Gage, M.J. 2021. Fertility and mortality impacts of thermal stressfrom experimental heatwaves on different life stages and their recovery in a model insect. Royal Society Open Science, 8(3). 201717.

Selvaraj, S., Ganeshamoorthi, P. & Pandiaraj, T. 2013. Potential impacts of recent climate change on biological control agents in agro-ecosystem: A review. International Journal of Biodiversity and Conservation, 5(12), 845-852.

Shumo, M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K., Subramanian, S., Ekesi, S., Van Huis, A. & Borgemeister, C. 2019. Influence of temperature on selected life-history traits of black

soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. Animals, 9(3), 79.

Sparks, A.N. 1979. A review of the biology of the fall armyworm. Florida Entomologist, 62(2), 82-87.

Tarusikirwa, V.L., Cuthbert, R.N., Mutamiswa, R. & Nyamukondiwa, C., 2022. Context- dependent integrated stress resistance promotes a global invasive pest. Insect Science, 1–15.

Wilson, R.S. & Franklin, C.E. 2002. Testing the beneficial acclimation hypothesis. Trends in Ecology & Evolution, 17(2), 66-70.

Zhang, C. 2005. Isolation of genes from cold acclimated *Poncirus trifoliata and Citrus unshiu*. Auburn University.

Appendix 1

Received: 23 January 2023 | Accepted: 28 March 2023

DOI: 10.1111/eea.13310

Entomologia Experimentalis et Applicata

ORIGINAL ARTICLE

Special Issue: Fall Armyworm

Contrasting effects of acute heat shock on physiological and ecological performance of the fall armyworm

Abongile Mbande[1] | Reyard Mutamiswa[1,2,3] | Casper Nyamukondiwa[3,4] | Frank Chidawanyika[1,5]

[1]Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa

[2]Tugwi-Mukosi Multidisciplinary Research Institute, Midlands State University, Gweru, Zimbabwe

[3]Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

[4]Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

[5]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya

Correspondence
Frank Chidawanyika, University of the Free State - Zoology and Entomology, 205 Nelson Mandela, Drive Bloemfontein 9301, South Africa.
Email: fchidawanyika@icipe.org

Funding information
National Research Foundation; SACTA maize trust bursary; Universiteit van die Vrystaat

Abstract

Temperature is a critical factor that influences the behavior, physiology, and development of ectothermic organisms. This has become even more important as acute temperature stress associated with global climate change becomes the new norm. Using the invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), we assessed its physiological and ecological responses following acute heat stress, synonymous to heat waves associated with recent climate change. Specifically, we measured the effects of short-term exposure (for 2 h) to heat shock (at 32, 35, and 38 °C) on physiological responses, such as critical thermal minima (CT_{min}) and maxima (CT_{max}), and life-history traits, such as reproductive success (fecundity and hatching success) and longevity, using virgin adults. Our results showed that prior acute heat shock compromised cold tolerance (CT_{min}) while enhancing heat tolerance (CT_{max}). In addition, heat shock reduced fecundity and hatching success and had dramatic effects on adult longevity. We conclude that acute heat stress associated with shifting environmental conditions may generally offset key physiological traits, affect reproduction and thus population persistence, and simultaneously have complex effects on adult lifespan.

KEYWORDS

acute temperature stress, climate change, ectothermic organism, fall armyworm, heat shock, invasive species, Lepidoptera, life-history traits, longevity, Noctuidae, *Spodoptera frugiperda*, thermal plasticity

INTRODUCTION

Ectotherms' body temperatures are always closely related to prevailing ambient conditions (Angilletta, 2009). Given the role of temperature in organismal activity, function, and biochemical processes (Chown & Nicolson, 2004), it thus plays a key role in determining overall animal fitness (Bale et al., 2002; Mutamiswa et al., 2017). Insects are highly responsive to temperature because they are ectotherms. Consequently, climate change-associated heat waves are expected to affect their performance. For example, climate stress may negatively affect native species and benefit invasive alien species (Nyamukondiwa et al., 2022), and coupled with failed natural pest suppression under climate change (Mironidis & Savopoulou-Soultani, 2010; Chidawanyika et al., 2019), continuous bouts of heat waves may thus favor invasive pest proliferation. However, species responses to thermal variation at both organismal and population level are dynamic and may be mediated by several basal, plastic, and genetically determined factors that are environmentally dependent (Angilletta, 2009; Sgrò et al., 2016).

Organisms have evolved various adaptive mechanisms to buffer against stressful environments (Hoffmann et al., 2013). This adaptation can be in two forms: (1) phenotypic plasticity (occurring at individual level or within generation), and (2) genetic changes through natural selection (evolution of stress traits that may favor the fittest) (Angilletta, 2009). Phenotypic plasticity involves changes in an organism's behavior,

morphology, and/or physiology in response to changes in environmental conditions (Chown & Nicolson, 2004). Phenotypic plasticity takes two forms, i.e., hardening in the short term and acclimation on longer timescales (Sgrò et al., 2016). Both forms allow individuals to respond rapidly to stress outside their thermal limits (Chidawanyika & Terblanche, 2011; Sgrò et al., 2016). Phenotypic plasticity of a genotype plays a critical role in many evolutionary processes such as selection within and among species (Salamin et al., 2010) and the establishment of barriers of reproduction between and within populations. Although the phenomenon is nearly ubiquitous in insects and is mainly adaptive (Chown & Nicolson, 2004; Angilletta, 2009), phenotypic plasticity can also be maladaptive. Maladaptive plasticity occurs when a population encounters an environment that induces the production of phenotypes away from the local optimum, resulting in a negative relationship between the direction of plasticity and that of adaptive evolution (Ghalambor et al., 2015). For example, Svensson et al. (2020) showed that phenotypic plasticity is maladaptive to both *Calopteryx splendens* (Harris) and *Calopteryx virgo* (L.).

Heat stress may also have fitness costs and benefits to insects (Segaiso et al., 2022). However, these costs and benefits are dependent on the level of stress. Although many studies have suggested that a single heat wave may negatively affect insect life-history traits – e.g., through reduced fecundity, egg hatchability, and prolonged development (Liang et al., 2014; Zheng et al., 2017) – considerable studies have also shown the contrary, that sub-lethal acute heat stress may increase fitness, e.g., through increased longevity, reproduction, and heat tolerance (Roux et al., 2010; Sarup et al., 2016). Thus, taxonomic differences in the costs and benefits of heat stress on life-history traits warrant a more thorough investigation. At organismal level, sustained exposure to heat stress has fitness consequences due to injuries at cellular level mediated by protein denaturation (Chown & Nicolson, 2004). Injuries sustained can manifest acutely within the same organisms or, in some cases, may appear in subsequent developmental stages and/or generations (Lu et al., 2014; Su et al., 2021). For example, exposure of larval *Drosophila melanogaster* Meigen to sub-lethal heat stress resulted in both mutations and pupation failure (Cui et al., 2008). Furthermore, parental sub-lethal heat shock decreased egg hatchability in *Mononychellus mcgregori* (Flechtmann & Baker) (Lu et al., 2014) and *Plutella xylostella* (L.) (Zhang et al., 2013). Similarly, in *Ephestia cautella* (Walker), sub-lethal heat shock reduced overall fecundity (Silbermann & Tatar, 2000), consistent with results on *Helicoverpa armigera* (Hübner) (Mironidis & Savopoulou-Soultani, 2010) and *Tribolium castaneum* (Herbst) (Sales et al., 2021). Such reduction in fecundity following heat shock stress has been associated with injury to the oocytes and ovaries (Mironidis & Savopoulou-Soultani, 2010). Similarly, in males, heat stress may reduce fertility through direct injury to the testes and sperm (Rinehart et al., 2000; Sales et al., 2021) and may reduce mating success in other insect species (Jerbi-Elayed et al., 2015). Heat shock may also reduce female longevity among adults (Chen et al., 2015; Zhang et al., 2016) thereby minimizing their full reproduction potential. Subjection to heat stress is known to induce the production of heat shock proteins that prevent protein denaturation and repair damaged proteins (Nyamukondiwa et al., 2010; Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2017). Thus, heat stress may be a significant determinant to survival and potentially mediate insect population dynamics in nature. As such, an understanding of its effects on ecological and physiological responses is critical in elucidating invasive insect population dynamics, establishment, and spread under changing environments (Chen et al., 2019; Tarusikirwa et al., 2022).

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), native to South America, is a highly invasive insect pest that threatens food security (Kenis et al., 2023). It is highly damaging in maize, a staple food in most of sub-Saharan Africa (Day et al., 2017; Kasoma et al., 2021), and substantially damages other economically important crops due to its polyphagous and often opportunistic feeding nature (Day et al., 2017; Montezano et al., 2018; Kenis et al., 2023). Adult FAW has high dispersal capacity, which is the major contributor to its long-distance spread and migration (Nagoshi et al., 2019). Given that FAW adults may occupy thermally divergent habitats, coupled with increasing acute temperature stress, no studies have investigated the effects of previous thermally stressful environments on the eco-physiology of FAW. Under optimum temperature conditions of ca. 28 °C, an adult female usually deposits eggs for 4–5 days, in batches of 100–200 each day – so, total fecundity is up to 1000 eggs (Kumela et al., 2019). Fall armyworm larvae have 5–10 instars (six optimally) (Ali et al., 1990; reviewed in Kenis et al., 2023). First and second instars feed on one side of the leaf skeletonizing it in the process, whereas the later instars feed on most plant parts damaging them in the process (Kumela et al., 2019). The larval stage may last up to 14 days in summer and up to 30 days in winter, highly suggestive of temperature-dependent development (Nagoshi et al., 2019). Cognizant of its economic importance and the adult migratory nature, the effects of thermal environments on FAW fitness thus warrant investigation. We assessed the effects of sub-lethal heat shock on eco-physiological performance of this invasive insect pest. Specifically, we determined whether sub-lethal heat shock may influence (1) cold tolerance (critical thermal minima, CT_{min}) and heat tolerance (critical thermal maxima, CT_{max}), (2) reproductive capacity (fecundity and hatching success), and (3) longevity. We hypothesized that adult heat shock stress may influence physiological and ecological performance of FAW adults.

MATERIALS AND METHODS

Plant and insect culture

Maize plants, *Zea mays* L. (Poaceae), were grown individually in plastic pots (20 cm diameter) with loamy soil mixed with potting mix (Gromor, Cato Ridge,

KwaZulu-Natal province, South Africa) as it is the preferred host of *S. frugiperda* (Assefa, 2018). The plants were watered daily and fertilized weekly with 30 g of fertilizer to maintain the same levels of fertilizer (Lawn & Leaf 7:1:3; Wonder, Isando, South Africa) for 4 weeks before FAW inoculation. The initial *S. frugiperda* larval culture (maize strain) was obtained from the Agricultural Research Council – Plant Health Protection (ARC-PHP) in Pretoria, South Africa, and maintained in the insectary under optimum conditions at 28 ± 2 °C, 65 ± 5% r.h., and L12:D12 photoperiod. They were allowed to grow into adults and newly laid (F_1 generation) eggs were incubated until larval emergence in cages with 2-week-old fresh maize plants. Thereafter, third larvae were individually transferred into vials containing artificial diet to prevent cannibalism until they pupated. Pupae were then sexed using differences in genitalia and transferred into 1-L plastic tubs containing moist soil until adult eclosion.

Adult sub-lethal heat shock treatments

Newly emerged adults of *S. frugiperda* (24 h old) were individually heat-shocked in propylene vials at 32, 35, or 38 °C for 2 h in a programmable water bath (model Tx150; Grant Instruments, Shepreth, UK) using the plunge protocol (e.g., Chidawanyika and Terblanche, 2011). These non-lethal high temperature–time combinations for heat shock were chosen based on preliminary trials in which survival was assessed under similar static thermal regimes. In addition, microclimatic conditions experienced in one of the maize-growing areas in South Africa range from −13.7 to 45.6 °C (see Results). A piece of moist cotton wool was placed at the bottom of each vial to prevent desiccation-related mortality. Controls were maintained under optimum conditions (28 ± 2 °C, 65 ± 5% r.h., L12:D12) in cages in the insectarium before measuring life-history traits. All metrics were measured just after heat shock, i.e., 0 h post heat shock treatment and using 24- to 48-h-old moths.

Effects of heat shock on low and high temperature tolerance

Critical thermal limits (CTLs; CT_{min} and CT_{max}) were assessed following Nyamukondiwa & Terblanche (2010). Ten adult FAW from heat shock treatment and controls were individually placed into a double jacketed 'organ pipe' chamber comprising 11 separate 200-mm tubes, connected to a programmable Grant Tx150 water bath filled with a 1:1 mixture of water and propylene glycol and subjected to constant heating and/or cooling. In the 'organ pipe', insects were given 10 min first to equilibrate at 28 °C (optimum developmental temperature) before ramping temperature up (CT_{max}) or down (CT_{min}) at a rate of 0.25 °C per min. This was repeated to yield n = 20 per treatment (20 replications). A thermocouple (type K 36 SWG; Fluke Corporation, Everett, WA, USA) connected to a digital thermometer (53/54IIB; Fluke) was inserted into the control chamber to monitor the chamber temperatures. Critical thermal minima (CT_{min}) and maxima (CT_{max}) were defined as the lower and upper temperatures, respectively, at which the adults lost muscle coordination, which was regarded as a lack of response to mild prodding (e.g., Nyamukondiwa & Terblanche, 2010).

Effects of heat shock on female fecundity and hatching success

Following heat shock, virgin individual adult females and males were paired as follows: treated female × untreated male, treated female × treated male, and untreated female × treated male (20 pairs each). Each pair was transferred into a rearing cage with potted fresh 4-week-old maize plants and a cotton wad soaked in 25% sugar water for oviposition and food provision, respectively. Untreated adults (male and female) were maintained at 28 °C as controls. The plants were monitored daily and leaves with newly deposited eggs were cut from the plant and transferred into Petri dishes and incubated under optimum conditions. Eggs were counted under a Nikon SMZ 645 dissecting microscope in order to determine fecundity. The process was repeated until no newly deposited eggs were observed (up to day 15). Fecundity was defined as the total number of eggs per pair during 15 days.

Hatchlings were counted and removed daily until no more larvae were observed. Hatching success was defined as the total number of larvae that hatched vs. the total number of eggs from each pair.

Effects of heat shock on longevity

Twenty replicates of heat-shocked virgin male and female adults were placed individually in propylene vials with screw cap lids (20 males and 20 females). A moist cotton wick dipped in 10% sugar solution was placed in each vial for provision of food and water. Thereafter, the vials were maintained in the insectary under optimum conditions (28 °C, 65 ± 5% r.h., L12:D12). Controls (virgin males and females) were maintained individually in cages as treatments under optimum conditions before measuring longevity. Mortality was recorded once per day until all the adults were dead.

Microclimate data recording

Microclimatic data were recorded using model DS1920 Thermocron iButtons (0.5 °C accuracy, 0.5 h sampling frequency; Dallas Semiconductors, Dallas, TX, USA) in

Clifton, Bloemfontein, Free State province, South Africa (29.1128°S, 26.2312°E) during the period March 2019 to March 2020 to determine the environmental conditions experienced by *S. frugiperda* in the field. Most *S. frugiperda* host plants are grown in this area at subsistence and commercial level. The iButtons were placed under a tree canopy (shaded environment), 1 m above the ground.

Data analysis

Data were analyzed in STATISTICA v.13.5.0 (TIBCO, Palo Alto, CA, USA) and R v.4.1.2 (R Core Team, 2021). Data were first checked for normality and equality of variances using the Shapiro–Wilk and Hartley–Bartlett tests, respectively. For CTLs, linear model assumptions of constant variance and normal errors were met, whereas longevity, fecundity, and hatching success data did not conform to assumptions of ANOVA. Therefore, CTLs results were analyzed using full-factorial ANOVA with sex and treatment as categorical factors, and CT_{min} and CT_{max} as dependent variables. Longevity, fecundity, and hatching success data were considered as count data and were analyzed using a generalized linear model (GLM) assuming a Poisson distribution and a log link function in R. A quadratic term was added to the temperature term, to improve model fit based on the Akaike Information Criterion. Tukey–Kramer post-hoc tests were used to separate means.

RESULTS

Effects of sub-lethal heat shock stress on low and high temperature tolerance

Sub-lethal heat shock significantly influenced low-temperature tolerance (CT_{min}) in both adult females and males (Table 1). The interaction effects between sex and treatment were highly significant for CT_{min} (Table 1). Sub-lethal heat shock stress at the highest treatment temperature (38 °C) significantly increased CT_{min} (reduced cold tolerance) for both males (6.43 ± 0.09 °C) and females (7.02 ± 0.06 °C) (Figure 1A). Control treatment showed a significant difference in low-temperature tolerance between females and males with females recording higher CT_{min} than males (Figure 1A).

Similarly, heat shock significantly influenced high-temperature tolerance (CT_{max}) in both adult females and males (Table 1). It improved CT_{max} in both sexes at 32 °C heat shock treatment with females recording 49.69 ± 0.06 °C and males 46.82 ± 0.19 °C, showing plasticity towards heat stress tolerance (Figure 1B). The interaction between treatments was significantly different for high temperature tolerance (Table 1). Control treatment had no significant response for CT_{max} for both sexes (Figure 1B).

Effects of heat shock on female fecundity and hatching success

Fecundity and hatching success varied significantly across pairs following heat shock treatments (Table 2, Figure 2). There was a significant interaction effect between temperature and pair for both fecundity and hatching success (Table 2). Fecundity and hatching success decreased with increase in heat shock temperature across all pairs (Figure 2). Following heat shock treatments at 32 to 38 °C, fecundity ranged from 409 to 144 (treated male × untreated female), from 369 to 132 (treated female × untreated male), and from 342 to 81 eggs (treated male × treated female) (Figure 2A). Likewise, following the same heat shock treatment conditions, hatching success ranged from 332 to 103 (treated male × untreated female), from 287to 99 (treated female × untreated male), and from 301 to 81 eggs (treated male × treated female) (Figure 2B). Fecundity and hatching success at 35 °C differed significantly between both sexes treated vs. treated male × untreated female (Figure 2). At 38 °C, a significant difference in fecundity was recorded between the same pairs, but not in hatching success (Figure 2). The treated male × treated female pair recorded

TABLE 1 Full factorial ANOVA of the effects of sub-lethal 2-h heat shock treatment at four temperatures on critical thermal minimum (CT_{min}) and maximum (CT_{max}) of *Spodoptera frugiperda* males and females.

Trait	Effect	SS	d.f.	MS	F	P
CT_{min}	Intercept	1114.61	1	1114.61	2590.63	<0.001
	Sex	3.22	1	3.22	7.49	<0.01
	Temperature	894.02	3	298.01	692.64	<0.001
	Sex*temperature	7.51	3	2.50	5.82	<0.001
	Error	65.40	152	0.43		
CT_{max}	Intercept	345820.5	1	345820.5	77318.17	<0.001
	Sex	51.4	1	51.4	11.50	<0.001
	Temperature	251.3	3	83.8	18.73	<0.001
	Sex*temperature	48.8	3	16.3	3.63	0.014
	Error	679.8	152	4.5		

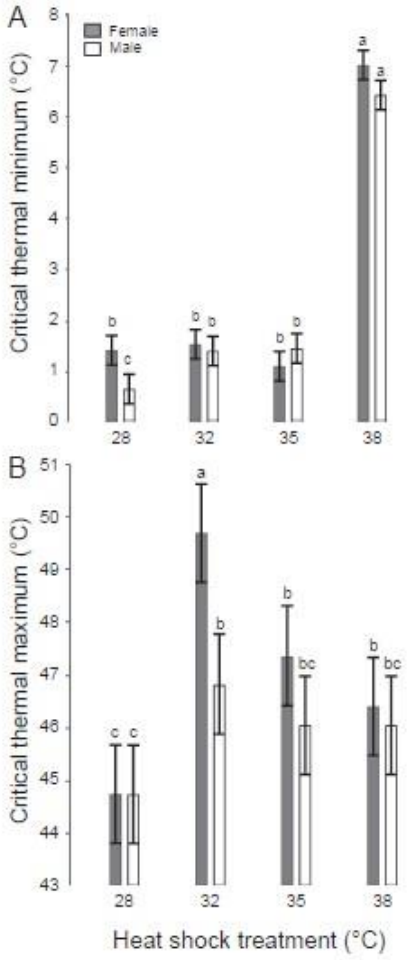

FIGURE 1 Effect of sub-lethal 2-h heat shock treatment at 28, 32, 35, and 38 °C on mean (+ 95% confidence interval; n = 20) critical thermal (A) minimum (CT_{min}) and (B) maximum (CT_{max}) of *Spodoptera frugiperda* females and males. Means within a panel capped with the same letter are not significantly different (Tukey–Kramer test: P > 0.05).

the lowest fecundity and hatching success at 35 and 38 °C (Figure 2).

Effects of heat shock on longevity

Overall, longevity was marginally higher in males (z = 2.496, P = 0.013) and initially increased and later decreased with increasing heat shock (z = 3.821, P < 0.001), as was the case with females (Figure 3). However, there were no significant sex*temperature effects across treatments (z = 1.789, P = 0.074) (Figure 3).

TABLE 2 Factorial analysis using generalized linear model (GLM) of the effects of sub-lethal 2-h heat shock treatment at three temperatures on fecundity and hatching success of male × female *Spodoptera frugiperda* pairs.

Trait	Effect	d.f.	χ^2	p
Fecundity	Temperature	2	1496754	<0.001
	Pair[1]	3	5289052	<0.001
	Temperature*pair	6	550537	<0.001
Hatching success	Temperature	2	1144322	<0.001
	Pair[1]	3	5094727	<0.001
	Temperature*pair	6	428048	<0.001

[1]Treated or untreated females were paired with treated or untreated males (four types of pairs, 20 pairs per type).

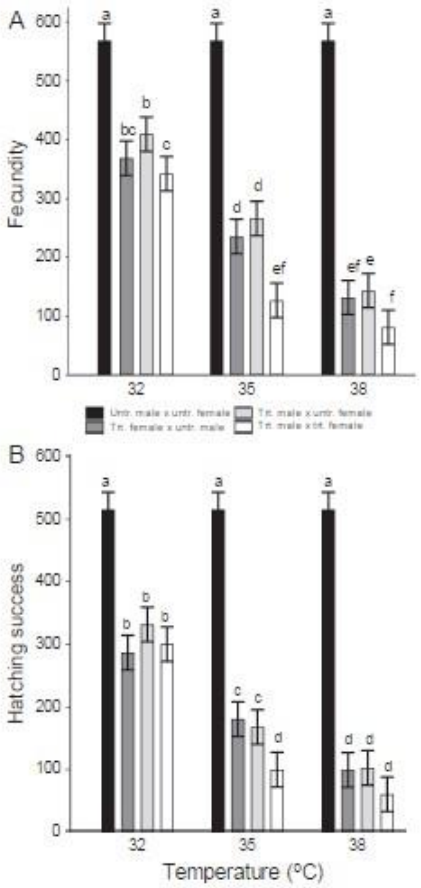

FIGURE 2 Effect of sub-lethal 2-h heat shock treatment at 32, 35, and 38 °C on mean (+ 95% confidence interval; n = 20 pairs) (A) fecundity (no. eggs deposited in 15 days) and (B) hatching success (total no. hatched larvae) of pairs of treated (trt.) or untreated (untr.) *Spodoptera frugiperda* males and females. Means within a panel capped with the same letter are not significantly different (Tukey–Kramer test: P > 0.05).

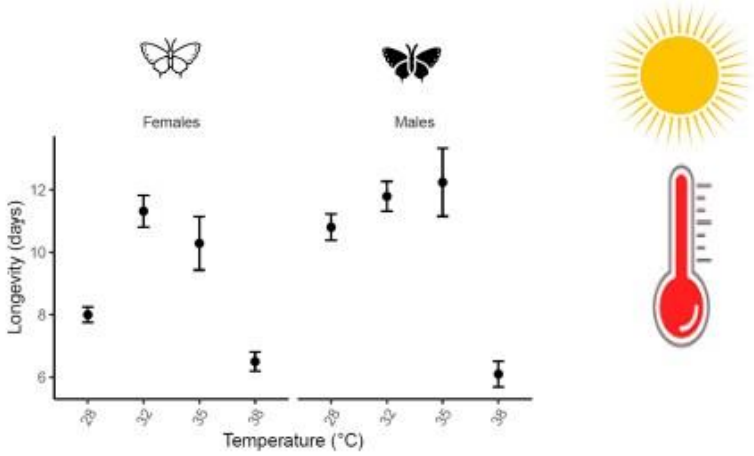

FIGURE 3 Effects of sub-lethal 2-h heat shock treatment at 28, 32, 35, and 38 °C on the longevity (days) of *Spodoptera frugiperda* males and females (n = 20 pairs). Nodes represent individual data, the lines denote microhabitat temperature.

Microclimate data recordings

Temperature between March 2019 and March 2020 in Clifton, Bloemfontein, South Africa, one of the areas where maize is grown, ranged from −13.7 to 45.6 °C (Figure 4A). Analysis of the temperature severity showed that acute heat stress episodes are common under natural conditions (Figure 4B). Temperature stress conditions of 30–45 °C were experienced at least 1000×, whereas temperatures of 45–50 °C were experienced ca. 250× in a calendar year (Figure 4B).

DISCUSSION

In nature, insects experience adverse abiotic stress, and the ability to survive these stressors may determine success (Nyamukondiwa et al., 2022). The current study showed contrasting physiological and ecological responses of *S. frugiperda* following sub-lethal heat shock stress. We found that sub-lethal heat shock treatment compromised cold tolerance (CT_{min}) whereas it improved heat tolerance (CT_{max}) across all treatments. Furthermore, similar to CT_{min} results, we also found that acute sub-lethal heat stress during development may come at the cost of adult survival and fitness, manifested as reduced longevity and reproductive success (lower fecundity and egg hatching success).

Sub-lethal heat stress significantly reduced cold tolerance in our study, showing evidence of cross-susceptibility between heat and cold stress (Tarusikirwa et al., 2022). Although previous studies demonstrated the importance of cross-tolerance in response to heat and cold (Chidawanyika & Terblanche, 2011; Sinclair et al., 2012, 2015), our study showed no evidence for this relationship. This may mean that heat stress can exert cumulative effects leading to poor thermal tolerance, perhaps due to cumulative tissue injuries. Our results are also in agreement with Mutamiswa et al. (2018) and Tarusikirwa et al. (2020) who reported reduced cold tolerance (CT_{min}) following heat stress in the lepidopteran species *Busseola fusca* (Fuller) and *Tuta absoluta* (Meyrick).

There was improved heat tolerance (CT_{max}) following sub-lethal heat shock treatments in both sexes indicating the ability to shift CT_{max} phenotypes, otherwise termed phenotypic plasticity. The increase in CT_{max} following heat shock treatment is not surprising as several studies reported significantly higher expression of heat shock proteins (HSP90 and HSP70) in heat shocked relative to untreated individuals, suggesting their role in heat tolerance (reviewed in Feder & Hofmann, 1999; King & McRae, 2015). Upregulation of heat shock proteins, which act as molecular chaperones against protein denaturation following heat stress, may explain our current results (Lopez-Martinez & Denlinger, 2008; Al-Ghzawi et al., 2022; Xing & Zhao, 2022). As heat shock protein levels were not measured in the current study, this warrants further investigation to fully elucidate mechanisms eliciting these plastic responses. Females had higher heat tolerance (CT_{max}) and plasticity thereof than males, conferring them a survival advantage over males in natural heat stress environments. This is consistent with sex-related differences in heat tolerance reported by Nyamukondiwa & Terblanche (2009). Females often have higher lipid content, owing to higher reproductive investment. Lipids play a key role in thermal tolerance (see Chown & Nicolson, 2004); thus, lipids may explain differences in basal and plastic differences observed here. However, sex-related lipid content differences and their role in the CT_{max} variation should be investigated further to test this hypothesis. For invasive pests in the tropical regions,

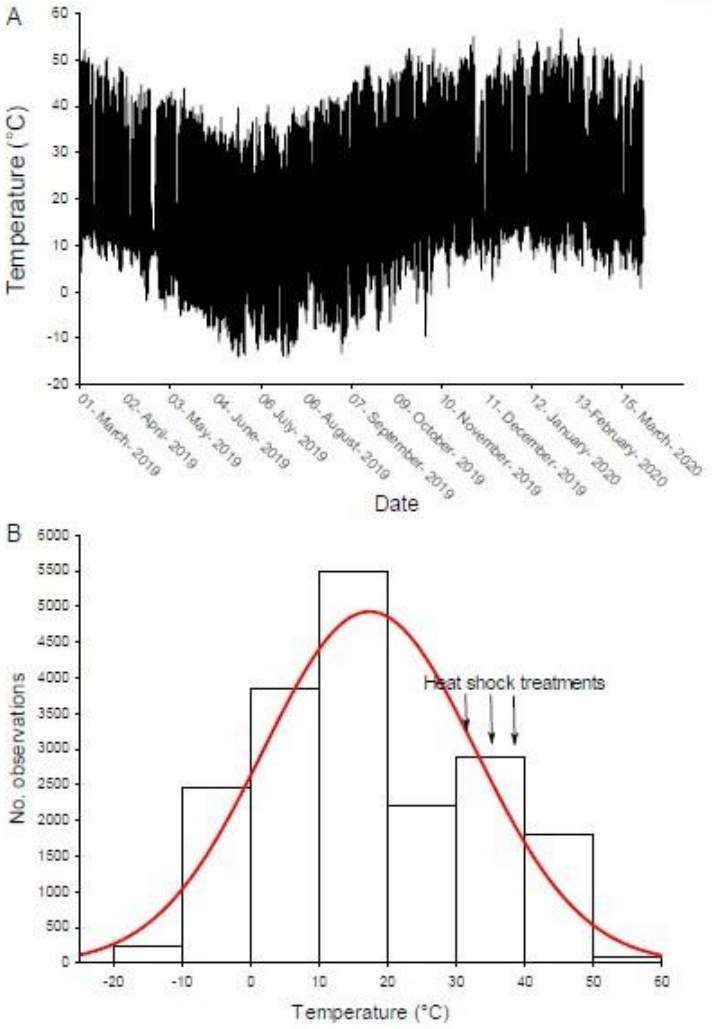

FIGURE 4 (A) Microhabitat temperature recorded using Thermocron iButtons at 0.5-h sampling frequency under a tree canopy, 1 m above the ground at Clifton, Free State Province, South Africa, from March 2019 to March 2020. (B) Temperature frequency distribution during the same period. Arrows indicate the heat shock temperatures that were used in laboratory experiments.

counter-adaptive physiological adjustments against heat stress sustain dispersal propensity through preservation of key life-history traits such as mating and locomotion (Bujan et al., 2021; Ma & Ma, 2022; Nyamukondiwa et al., 2022). Here, FAW showed capacity for rapid CT_{max} adjustment under heat stress, suggesting plasticity. This plasticity may likely aid its invasiveness, consistent with reports on related species (e.g., Nyamukondiwa et al., 2010; Tarusikirwa et al., 2022).

Fecundity and hatching success decreased with increase in sub-lethal heat shock temperature underlying the cumulative effects of tissue damage (Chown & Nicolson, 2004). Thus, acute heat waves may reduce the severity of FAW invasions and the damage larvae cause to crops in the short term due to poor FAW reproductive success. However, the net invasiveness of FAW will depend on how FAW compensate for heat stress-associated costs of reproduction. The mechanisms behind reduced fecundity and hatching success are not clear. Heat stress is known to elicit dehydration and denaturalization of proteins including enzymes resulting in reduced survival, fecundity, or offspring viability (Somero, 1995).

Furthermore, the female reproductive system is known to be sensitive to thermal stress due to disturbance of the neurohormonal regulation of reproduction (Munoz-Valencia et al., 2013). Studies have reported that heat shock can elicit injury to oocytes and ovarian development leading to a decrease in egg production, whereas in males, heat shock can reduce mating success by directly injuring testes and sperms, resulting in reduced fertility (Krebs & Loeschcke, 1994; Rinehart et al., 2000; Cui et al., 2008; Sales et al., 2021). In addition, spermatozoa are generally sensitive to even negligible variation in temperature such that heat-exposed sperm incurs DNA breakage and chromatin decondensation leading to blastocyst and embryo failures (Burfening et al., 1970; Sales et al., 2021). Given that the present study treated both males and females, heat shock may have influenced oogenesis, spermatogenesis, and embryogenesis which culminated in reduced fecundity and hatching success. As this study did not explore reproductive system responses to heat stress (e.g., sperm and egg counts and viability), nor assess whether the reduction in fecundity was a result of damage to the reproductive system of both sexes or only one sex, this warrants further investigation. Furthermore, given repeated episodes of acute heat stress with climate change (e.g., Stillman, 2019), future studies should also look into the 'chronic' effects of repeated heat stress exposure on the physiology and ecology of insects.

Our results showed that the mean longevity increased with increased heat shock treatments for both sexes, but significantly decreased beyond 32 °C treatments, suggesting temperature-related effects on *S. frugiperda* longevity. This result is in agreement with Zhou et al. (2011) who reported a drastic decline in *Ophraella communa* LeSage adult longevity following exposure (for 2 h) to temperatures above 35 °C. Males lived longer than females indicating a higher cost of acute heat stress treatment in females. Such differential sex responses to heat stress may reduce the full reproduction potential of this invasive pest.

Microclimate data revealed that heat shock conditions that were employed in this study are regularly experienced in nature, and that even more severe heat stress conditions are common. Heat stress may thus realistically influence FAW physiological and ecological success (reproduction and longevity), which may manifest through, e.g., changes in population phenology and dynamics. Further work is necessary to fully test the capabilities of different life-stages to mount plastic responses to diverse stressful environments.

In conclusion, our study shows contrasting effects of acute sub-lethal heat stress on FAW physiological and ecological traits. Cold tolerance was reduced following heat treatment whereas heat tolerance was improved, suggesting cross-susceptibility between heat and cold stress. Sex-related differences in physiological and ecological responses were shown following acute heat stress treatment; for example, females had higher CT_{max} whereas males had higher longevity. This sex-related fitness and survival advantage may partly enhance population persistence under changing environment. We also show that acute heat stress during development may manifest as reduced adult fecundity, hatching success, and longevity. Reduced reproduction and survival following heat stress will have implications for FAW population persistence amidst increased heat waves with climate change. Future investigations are necessary to elucidate the physiological mechanisms that underlie the variations elicited by heat stress on the fitness and life-history traits of *S. frugiperda*. In addition, transgenerational plasticity studies are also recommended, as well as studies of the effects of more chronic and repeated episodes of heat stress. This information is significant in pest risk assessment and in the development of *S. frugiperda* management options for sustainable crop production under changing climate.

AUTHOR CONTRIBUTIONS

Abongile Mbande: Formal analysis (equal); investigation (lead); methodology (equal); writing – original draft (lead). **Reyard Mutamiswa:** Formal analysis (equal); methodology (equal); validation (equal); visualization (equal); writing – review and editing (equal). **Casper Nyamukondiwa:** Validation (equal); visualization (equal); writing – review and editing (equal). **Frank Chidawanyika:** Conceptualization (equal); data curation (equal); funding acquisition (lead); methodology (equal); project administration (lead); resources (lead); supervision (lead); validation (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

We are very grateful for the National Research Foundation (NRF) and SACTA NPC trust PhD bursaries to AM and the support from NRF to FC. We also acknowledge valuable support from University of the Free State (UFS) to AM, FC, and RM, Botswana International University of Science and Technology (BIUST) to CN, International Centre of Insect Physiology and Ecology to FC, as well as Midlands State University to RM. The authors would like to further acknowledge Dr Gerald Chikowore for microclimatic data and Dr Ross N. Cuthbert for statistical advice.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Casper Nyamukondiwa https://orcid.org/0000-0002-0395-4980
Frank Chidawanyika https://orcid.org/0000-0002-4601-768X

REFERENCES

AL-Ghzawi AAA, Al-Zghoul MB, Zaitoun S, Al-Omary IM & Alahma NA (2022) Dynamics of heat shock proteins and heat shock factor expression during heat stress in daughter workers in pre-heat-treated

(rapid heat hardening) *Apis mellifera* mother queens. *Journal of Thermal Biology 104*: 103194.

Ali A, Luttrell RG & Schneider JC (1990) Effects of temperature and larval diet on development of the fall army worm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America 83*: 725–733.

Angilletta MJ (2009) *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, UK.

Assefa F (2018) Status of fall armyworm (*Spodoptera frugiperda*), biology and control measures on maize crop in Ethiopia: a review. *International Journal of Entomological Research 6*: 75–85.

Assefa F & Ayalew D (2019) Status and control measures of fall armyworm (*Spodoptera frugiperda*) infestations in maize fields in Ethiopia: a review. *Cogent Food & Agriculture 5*: 1641902.

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM et al. (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology 8*: 1–16.

Bujan J, Charavel E, Bates OK, Gippet JM, Darras H et al. (2021) Increased acclimation ability accompanies a thermal niche shift of a recent invasion. *Journal of Animal Ecology 90*: 483–491.

Burfening PJ, Elliott DS, Eisen EJ & Ulberg LC (1970) Survival of embryos resulting from spermatozoa produced by mice exposed to elevated ambient temperature. *Journal of Animal Science 30*: 578–582.

Chen H, Solangi GS, Zhao C, Yang L, Guo J et al. (2019) Physiological metabolic responses of *Ophraella communa* to high temperature stress. *Frontiers in Physiology 10*: 1053.

Chen W, Li D, Zhang M, Zhao Y, Wu W & Zhang G (2015) Cloning and differential expression of five heat shock protein genes associated with thermal stress and development in the polyphagous predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Experimental and Applied Acarology 67*: 65–85.

Chidawanyika F, Mudavanhu P & Nyamukondiwa C (2019) Global climate change as a driver of bottom-up and top-down factors in agricultural landscapes and the fate of host-parasitoid interactions. *Frontiers of Ecology and Evolution 7*: 80.

Chidawanyika F & Terblanche JS (2011) Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology 57*: 108–117.

Chown SL & Nicolson SW (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford, UK.

Cui X, Wan F, Xie M & Liu T (2008) Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. *Journal of Insect Science 8*: 24.

Day R, Abrahams P, Bateman M, Beale T, Clottey V et al. (2017) Fall armyworm: impacts and implications for Africa. *Outlooks on Pest Management 28*: 196–201.

Feder ME & Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology 61*: 243–282.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN & Hughes KA (2015) Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature 525*: 372–375.

Goergen G, Kumar PL, Sankung SB, Togola A & Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE 11*: e0165632.

Griffith TC, Paterson ID, Owen CA & Coetzee JA (2019) Thermal plasticity and microevolution enhance establishment success and persistence of a water hyacinth biological control agent. *Entomologia Experimentalis et Applicata 167*: 616–625.

Hodgins KA, Bock DG & Rieseberg LH (2018) Trait evolution in invasive species. *Annual Plant Reviews Online 1*: 459–496.

Hoffmann AA, Chown SL & Clusella-Trullas S (2013) Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology 27*: 934–949.

Hulme PE (2017) Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews 92*: 1297–1313.

Janowitz SA & Fischer K (2011) Opposing effects of heat stress on male versus female reproductive success in *Bicyclus anynana* butterflies. *Journal of Thermal Biology 36*: 283–287.

Jerbi-Elayed M, Lebdi-Grissa K, Foray V, Muratori F & Hance T (2015) Using multiple traits to estimate the effects of heat shock on the fitness of *Aphidius colemani*. *Entomologia Experimentalis et Applicata 155*: 18–27.

Jørgensen LB, Malte H, Ørsted M, Klahn NA & Overgaard J (2021) A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. *Scientific Reports 11*: 12840.

Kasoma C, Shimelis H & Laing MD (2021) Fall armyworm invasion in Africa: implications for maize production and breeding. *Journal of Crop Improvement 35*: 111–146.

Kenis M, Benelli G, Biondi A, Calatayud PA, Day R et al. (2023) Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomologia Generalis*. https://doi.org/10.1127/entomologia/2022/1659

King AM & McRae TH (2015) Insect heat shock proteins during stress and diapause. *Annual Review of Entomology 60*: 59–75.

Krebs RA & Loeschcke V (1994) Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. *Functional Ecology 8*: 730–737.

Kumela T, Simiyu J, Sisay B, Likhayo P, Mendesil E et al. (2019) Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *International Journal of Pest Management 65*: 1–9.

Liang LN, Zhang W, Ma G, Hoffmann AA & Ma CS (2014) A single hot event stimulates adult performance but reduces egg survival in the oriental fruit moth, *Grapholitha molesta*. *PLoS ONE 9*: e116339.

Lopez-Martinez G & Denlinger DL (2008) Regulation of heat shock proteins in the apple maggot *Rhagoletis pomonella* during hot summer days and overwintering diapause. *Physiological Entomology 33*: 346–352.

Lu F, Chen Q, Chen Z, Lu H, Xu X & Jing F (2014) Effects of heat stress on development, reproduction, and activities of protective enzymes in *Mononychellus mcgregori*. *Experimental and Applied Acarology 63*: 267–284.

Ma G & Ma CS (2022) Potential distribution of invasive crop pests under climate change: incorporating mitigation responses of insects into prediction models. *Current Opinion in Insect Science 49*: 15–21.

Mironidis GK & Savopoulou-Soultani M (2010) Effects of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults. *Journal of Thermal Biology 35*: 59–69.

Montezano DG, Sosa-Gómez DR, Specht A, Roque-Specht VF, Sousa-Silva JC et al. (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *African Entomology 26*: 286–300.

Munoz-Valencia V, Diaz-Gonzalez F, Manzano-Martínez MDR, Toro-Perea N & Cárdenas-Henao H (2013) Basal and induced thermotolerance to heat shocks in *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae). *Revista Colombiana de Entomología 39*: 18–25.

Mutamiswa R, Chidawanyika F & Nyamukondiwa C (2017) Dominance of spotted stemborer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) over indigenous stemborer species in Africa's changing climates: ecological and thermal biology perspectives. *Agricultural and Forest Entomology 19*: 344–356.

Mutamiswa R, Chidawanyika F & Nyamukondiwa C (2018) Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. *Physiological Entomology 43*: 108–119.

Nagoshi RN, Dhanani I, Asokan R, Mahadevaswamy HM, Kalleshwaraswamy CM & Meagher RL (2019) Genetic characterization of fall armyworm infesting South Africa and India indicate recent introduction from a common source population. *PLoS ONE 14*: e0217755.

Nyamukondiwa C, Kleynhans E & Terblanche JS (2010) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of

Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology 35*: 565–575.
Nyamukondiwa C, Machekano H, Chidawanyika F, Mutamiswa R, Ma G & Ma CS (2022) Geographic dispersion of invasive crop pests: the role of basal plastic climate stress tolerance and other complementary traits in the tropics. *Current Opinion in Insect Science 50*: 100878.
Nyamukondiwa C & Terblanche JS (2009) Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *Journal of Thermal Biology 34*: 406–414.
Nyamukondiwa C & Terblanche JS (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*: 255–264.
R Core Team (2021) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
Rinehart JP, Yocum GD & Denlinger DL (2000) Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. *Physiological Entomology 25*: 330–336.
Roux O, Le Lann C, van Alphen JJM & van Baaren J (2010) How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)? *Bulletin of Entomological Research 100*: 543–549.
Salamin N, Wüest RO, Lavergne S, Thuiller W & Pearman PB (2010) Assessing rapid evolution in a changing environment. *Trends in Ecology and Evolution 25*: 692–698.
Sales K, Vasudeva R & Gage MJ (2021) Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *Royal Society Open Science 8*: 8201717.
Sarup P, Petersen SMM, Nielsen NC, Loeschcke V & Malmendal A (2016) Mild heat treatments induce long-term changes in metabolites associated with energy metabolism in *Drosophila melanogaster*. *Biogerontology 17*: 873–882.
Segaiso B, Machekano H, Ross CC & Nyamukondiwa C (2022) Thermal fitness costs and benefits of developmental acclimation in fall armyworm. *Scientific African 17*: e01369.
Sgrò CM, Terblanche JS & Hoffmann AA (2016) What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology 61*: 433–451.
Silbermann R & Tatar M (2000) Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. *Evolution 54*: 2038–2045.
Sinclair BJ, Alvarado LEC & Ferguson LV (2015) An invitation to measure insect cold tolerance: methods, approaches, and workflow. *Journal of Thermal Biology 53*: 180–197.
Sinclair BJ, Williams CM & Terblanche JS (2012) Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology 85*: 594–606.
Somero GN (1995) Proteins and temperature. *Annual Review of Physiology 57*: 43–68.
Stillman JH (2019) Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems, and human communities. *Physiology 34*: 86–100.
Su Y, Li X, Zhang W, Zhang B & Ma CS (2021) Dynamics of heat shock protein responses to thermal stress changes after metamorphosis in a lepidopteran insect. *Archives of Insect Biochemistry and Physiology 107*: e21791.
Svensson EI, Gomez-Llano M & Waller JT (2020) Selection on phenotypic plasticity favors thermal canalization. *Proceedings of the National Academy of Sciences of the USA 117*: 29767–29774.
Tarusikirwa VL, Cuthbert RN, Mutamiswa R & Nyamukondiwa C (2022) Context-dependent integrated stress resistance promotes a global invasive pest. *Insect Science 29*: 1790–1804.
Tarusikirwa VL, Mutamiswa R, English S, Chidawanyika F & Nyamukondiwa C (2020) Thermal plasticity in the invasive South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Thermal Biology 90*: 102598.
Xing K & Zhao F (2022) Acclimation effects of natural daily temperature variation on longevity, fecundity, and thermal tolerance of the diamondback moth (*Plutella xylostella*). *Insects 13*: 309.
Zhang GH, Li YY, Zhang KJ, Wang JJ, Liu YQ, Liu H (2016) Effects of heat stress on copulation, fecundity and longevity of newly-emerged adults of the predatory mite, *Neoseiulus barkeri* (Acari: Phytoseiidae). *Systematic and Applied Acarology 21*: 295–306.
Zhang W, Zhao F, Hoffmann AA & Ma CS (2013) A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS ONE 8*: e75923.
Zheng J, Cheng X, Hoffmann AA, Zhang B & Ma CS (2017) Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress? *Journal of Insect Physiology 102*: 36–41.
Zhou ZS, Guo JY, Min L & Wan FH (2011) Effect of short-term high temperature stress on the development and fecundity of *Ophraella communa* (Coleoptera: Chrysomelidae). *Biocontrol Science and Technology 21*: 809–819.

How to cite this article: Mbande A, Mutamiswa R, Nyamukondiwa C & Chidawanyika F (2023) Contrasting effects of acute heat shock on physiological and ecological performance of the fall armyworm. *Entomologia Experimentalis et Applicata 00*: 1–10. https://doi.org/10.1111/eea.13310

Appendix 2

Bulletin of Entomological Research

cambridge.org/ber

Ontogenetic responses of physiological fitness in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in response to repeated cold exposure

Abongile Mbande[1], Reyard Mutamiswa[1,2,3] and Frank Chidawanyika[1,4]

[1]Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa; [2]Tugwi-Mukosi Multidisciplinary Research Institute, Midlands State University, Gweru, Zimbabwe; [3]Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa and [4]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya

Research Paper

Cite this article: Mbande A, Mutamiswa R, Chidawanyika F (2023). Ontogenetic responses of physiological fitness in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in response to repeated cold exposure. *Bulletin of Entomological Research* 1–7. https://doi.org/10.1017/S0007485323000111

Received: 13 October 2022
Revised: 14 February 2023
Accepted: 23 February 2023

Keywords:
Climate change; fall armyworm; plasticity-tradeoff; thermal tolerance

Author for correspondence:
Frank Chidawanyika,
Email: fchidawanyika@icipe.org

Abstract

In this era of global climate change, intrinsic rapid and evolutionary responses of invasive agricultural pests to thermal variability are of concern given the potential implications on their biogeography and dire consequences on human food security. For insects, chill coma recovery time (CCRT) and critical thermal minima (CT_{min}), the point at which neuromuscular coordination is lost following cold exposure, remain good indices for cold tolerance. Using laboratory-reared *Spodoptera frugiperda* (Lepidoptera: Noctuidae), we explored cold tolerance repeated exposure across life stages of this invasive insect pest. Specifically, we measured their CT_{min} and CCRT across four consecutive assays, each 24 h apart. In addition, we assessed body water content (BWC) and body lipid content (BLC) of the life stages. Our results showed that CT_{min} improved with repeated exposure in 5th instar larvae, virgin males and females while CCRT improved in 4th, 5th and 6th instar larvae following repeated cold exposure. In addition, the results revealed evidence of cold hardening in this invasive insect pest. However, there was no correlation between cold tolerance and BWC as well as BLC. Our results show capacity for cold hardening and population persistence of *S. frugiperda* in cooler environments. This suggests potential of fall armyworm (FAW) to withstand considerable harsh winter environments typical of its recently invaded geographic range in sub-Saharan Africa.

Introduction

Repeatability or reproducibility experiments are profound tools that were originally developed for independent testing of the precision of experimental protocols. In biological research, the repeatability of observational data can be used to track organismal plastic and genetic responses to stress factors at the individual or population level at various temporal scales (Avargues-Weber *et al.*, 2015; Niemelä and Dingemanse, 2017; Näslund, 2021). Given the escalated attention on climate change in recent years, repeatability studies (though controversial) can be pivotal in investigating basal and plasticity of thermal tolerance (Morgan *et al.*, 2018; O'Donnell *et al.*, 2020; O'Neill *et al.*, 2021) where both environmental and genetic phenotypic variation effects can be used to determine within-individual trait variability (Grinder *et al.*, 2020). If the thermal tolerance of a tested organism is consistent over time, denoting high repeatability, it indicates that the adaptive potential of the trait is high while the converse is true for low repeatability (Morgan *et al.*, 2018).

For insects, body temperature depends on ambient conditions mediating biochemical and physiological processes therein (Chown and Nicolson, 2004; Sinclair *et al.*, 2015). Subsequently, such organismal responses mediate development and can cascade to population level through factors such as seasonality, geographic distribution and voltinism (Du Plessis *et al.*, 2020; Phophi *et al.*, 2020; Tarusikirwa *et al.*, 2020; Nyamukondiwa *et al.*, 2022). Of interest is how the magnitude and frequency of thermal extremes in the form of heat waves and cold snaps wrought by the changing climates influence pest physiology, survival and key life-history traits (Tollefson, 2014) as it has direct implications on their population dynamics (Chidawanyika *et al.*, 2012, 2020) and ultimately food security (Gregory *et al.*, 2009). Thus, apart from magnitude of thermal exposure, insects experience different mode of thermal fluctuations (e.g. acute vs. chronic, rapid vs. slow fluctuations and/or repeated exposures) typical of diel and seasonal changes (Colinet *et al.*, 2007). Such extremes, and not average temperatures drive several organismal responses including evolutionary adaptations within and across generations (Cox *et al.*, 2010; Travis 2014; Buckley and Huey, 2016) and define geographic ranges *via* various demographic tipping points (Lynch *et al.*, 2014).

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

Indeed, insects have evolved diverse morphological, physiological and behavioural adaptations to withstand and colonize otherwise lethal novel environments (Bale, 2002; Neal *et al.*, 2021). For example, overwintering insects are known to survive stressful low temperatures through employing cold tolerance strategies such as rapid cold hardening (RCH), freeze tolerance and freeze avoidance (Sinclair *et al.*, 2015; Feng *et al.*, 2018). Freeze-tolerant insects survive intracellular ice formation through use of cryoprotectants, removal of ice nucleators and anti-freeze heat shock proteins synthesis (Elnitsky *et al.*, 2008; Storey and Storey, 2012; Toxopeus *et al.*, 2019). On the contrary, freeze-intolerant/avoidant insects cannot withstand internal ice formation but survive through keeping their body fluids under a supercooled condition (Sinclair *et al.*, 2015; Andreadis and Athanassiou, 2017). RCH, a form of phenotypic plasticity, confers survival advantages at low lethal temperature after brief pre-treatment to a prior sub-lethal temperature shock (Lee *et al.*, 1987; Teets and Denlinger, 2013). Over longer time scales such prior exposure to sublethal temperatures also confer advantages to identical future identical thermal stress in what is referred to as beneficial acclimation (Leroi *et al.*, 1994).

In nature, insects may thus face multiple stressors including repeated cold stress during diel and seasonal thermal fluctuations (Marshall and Sinclair, 2010) where the above-mentioned plastic responses play a role (Nyamukondiwa *et al.*, 2018). Mimicking such repeated thermal exposure in manipulative experiments allows investigation of the relationship between repeatability and adaptive responses (Boake, 1989; Morgan *et al.*, 2018; Grinder *et al.*, 2020). In this study, we used common measures of cold tolerance in critical thermal minimum (CT_{min}) and chill coma recovery time (CCRT) as proxies for cold hardiness (Andersen *et al.*, 2015; Mutamiswa *et al.*, 2018, 2019; Izadi *et al.*, 2019).

CT_{min} is an organism's lower thermal tolerance limit where an insect is incapacitated due to compromised neuromuscular activity (Sinclair *et al.*, 2015; Izadi *et al.*, 2019). If low temperature conditions persist, CT_{min} is followed by chill coma where paralysis due to complete loss of neuromuscular function occurs (Hazell and Bale, 2011; O'Neill *et al.*, 2021). The time that an insect requires to regain neuromuscular function following chill coma is what is then regarded as CCRT (Sinclair *et al.*, 2015). Given their ubiquitous occurrence in nature and capacity to define limits for organismal activity, these key indices provide valuable ecologically relevant measures of insect cold tolerance. Thus, understanding the evolutionary capacity following repeated exposure provides important information on their adaptive capacity and potential geographic range expansion in invasive insects such as *Spodoptera frugiperda*.

S. frugiperda is a highly invasive insect pest native to the tropics and sub-tropics of America (Goergen *et al.*, 2016). The larvae of this polyphagous insect cause significant economic losses in several important crops but inflict the most damage in the Poaceae family (Lu and Adang, 1996; Nboyine *et al.*, 2020). In Africa, *S. frugiperda* was first detected in Nigeria before rapidly spreading to 47 countries across the African continent (Goergen *et al.*, 2016; Cock *et al.*, 2017; Early *et al.*, 2018; Nboyine *et al.*, 2020). It is highly destructive to maize, *Zea mays*, which is a staple food in many parts of Africa (Day *et al.*, 2017; Kasoma *et al.*, 2021).

S. frugiperda does not diapause, instead it is known to migrate to environments with favourable conditions for survival (Du Plessis *et al.*, 2020; Vatanparast and Park, 2022). It has been reported to survive in Africa, all year-round due to prevailing conducive biophysical environment (Early *et al.*, 2018; Du Plessis *et al.*, 2020; Keosentse *et al.*, 2021). The upregulation of glycerol-3-phosphate dehydrogenase and glycerol kinase genes for increased synthesis of the cryoprotectant glycerol has been attributed to the key physiological response to withstand cold environments in *S. frugiperda* (Vatanparast and Park, 2022). However, survival has been reported to be limited in some cases in Asia where harsh winters decimate seasonal populations while annual reinvasions provide new propagules (Vatanparast and Park, 2022). Nevertheless, little is known about the role of acquired/induced cold tolerance in the fitness of *S. frugiperda* following prior exposure. Yet, induced cold tolerance can play a key role in preserving and improving key life-history activities at acute temporal scales.

Here, we examined the consequences of repeated cold exposure on low thermal tolerance (CT_{min} and CCRT) of *S. frugiperda* life stages across 72 h. We hypothesized that CT_{min} and CCRT are repeatable traits and may change over time because of cold hardening. Since body water and lipid content is associated with basal and induced cold tolerance in insects (or lack thereof), we subsequently assessed the two parameters following thermal exposure to draw inferences on the performance of *S. frugiperda* and subsequent management.

Materials and methods

Insect culture and maintenance

The initial colony of *S. frugiperda* was obtained as larvae from the Agricultural Research Council, Plant Health Protection (ARC-PHP) Pretoria, South Africa. Thereafter, the insects were maintained on an artificial diet in the insectary under optimum conditions of 28°C, 65 ± 5% relative humidity (RH) and 12L:12D photoperiod. Since cannibalism is reportedly predominant among late larval instars (Chapman *et al.*, 1999), each third instar larva was individually placed in a separate 100 ml plastic vial with perforated screw-cap lid and soybean wheat germ artificial diet (Southland Products Inc., Lake Village, Arkansas, USA) until pupation. Pupae were maintained in open Petri dishes (30 × 30 × 30 cm^3) in collapsible rearing cages made of mesh cloth until adult eclosion. Adults were provided with 25% sugar-water from a moistened cotton wool placed in a Petri dish. At least two maize plants (3–4 weeks old) were placed in each rearing cage as oviposition substrate for gravid females. After hatching, the 1st instar larvae were transferred to an artificial diet for subsequent rearing. For all the experiments F_1 generation of 4th, 5th, 6th instar larvae and 24–48 h old virgin adults were used.

CT_{min} and repeated cold exposure assays

To the relationship between CT_{min} and repeated cold exposure, larvae and adults (males and females) of *S. frugiperda* underwent repeated cold tolerance (CT_{min}) assays at 0 (control), 24, 48 and 72 h intervals. CT_{min} were assayed using standardized dynamic and ecologically relevant protocols (Chidawanyika and Terblanche, 2011; Chidawanyika *et al.*, 2017). Ten replicate larvae and adults were individually placed randomly in a series of 200 mm glass tubes ('organ pipes') connected to an insulated double-jacketed chamber linked to a programmable water bath (Grant model Tx150; Grant Instruments, UK) filled with 1:1 water:propylene glycol. In the 'organ pipes', insects were allowed to equilibrate for 10 min at 28°C (optimum temperature) before

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

decreasing the temperature at a rate of 0.25°C min^{-1} until their CT_{min} were recorded. This was repeated twice for each life stage to yield sample sizes of $n = 20$ per treatment. To record chamber temperature, a thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, Everett, Washington, USA) was inserted into a control (centre) glass tube of the organ pipes. After each assay, insects were given time to recover before repeating the same assay across 24, 48 and 72 h intervals using the same batch of insects. CT_{min} was considered as the temperature at which insects did not respond to gentle prodding (e.g. Nyamukondiwa and Terblanche 2009).

Influence of repeated cold exposure on CCRT

CCRT was assessed following Mutamiswa *et al.* (2018). A total of ten replicate larvae and adults were placed individually in 7 ml screw-cap glass vials with 1 mm diameter holes pierced through cap for ventilation. The vials were then placed into a large zip-lock bag which was subsequently submerged into a water bath (Grant LTC40 model TX150) filled with a 1:1 water:propylene glycol mixture and set at 0°C for 1 h. After 1 h at chill-coma temperature, the tubes were removed from the water bath and transferred to a Memmert climate chamber (HPP 260, Memmert GmbH+ Co.KG, Schwabach, Germany) set at 28°C, 65% RH for recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, Hangzhou, Zhejiang, China) that was linked to a computer where observations were recorded. This was repeated twice for each life stage to yield sample sizes of $n = 20$ per treatment. After each assay, insects were exposed to the same treatment and CCRT measured across 24, 48 and 72 h intervals using the same batch of insects. CCRT was defined as the time (in min) required for an adult to stand upright on its legs (Milton and Partridge, 2008).

Determination of body water content (BWC)

After 72 h interval following CT_{min} and repeated cold exposure assays, BWC of the insects were determined. Larvae (4th, 5th and 6th instar) and adults were individually placed in a pre-weighed 50 ml Eppendorf tubes and the initial mass of each insect before oven drying was measured (to 0.0001 g) on a Scout Pro (DHAUS) microbalance (model: Scout Pro SPU 123, Parsippany, USA). Thereafter, insects were placed in a Memmert drying oven (UL50, Memmert, Schwabach, Germany) set at 60°C for 72 h. Insects were allowed to cool under laboratory temperature conditions of 28°C for 30 min thereafter, dry mass was measured (to 0.0001 g) on a microbalance. To determine BWC, dry mass was subtracted from the initial mass following Bazinet *et al.* (2010) and Weldon *et al.* (2018).

Determination of body lipid content (BLC)

Following BWC assays, the tested insects were further oven dried for another 72 h at 60°C. Thereafter, the insects were individually washed in 1.5 ml diethyl ether and then gently agitated at 250 rpm for 24 h at 37°C using ST 5 CAT orbital shaker (model: Zipperer GmbH, D 79219 Staufen, Germany) following the methods of Mitchell *et al.*, (2017). The diethyl ether was then removed from the tubes and insects were oven dried again at 60°C for 24 h, before reweighing. The lipid content for each individual was calculated by subtracting the lipid-free dry mass from the initial dry mass. Controls were exposed to the same conditions before measuring their lipid content.

Data analysis

Data analyses were carried out in STATISTICA, 13.5.0 version (Statsoft Inc., 2021) and R version 4.1.2 (R Development Core Team, 2021). Normality and equality of variances were first checked using the Shapiro–Wilk and Hartley–Bartlett tests, respectively. Data for CCRT was linear and met the conditions for normality and equality of variances ($W = 0.83$, $P = 0.12$) and were analysed using generalized linear models assuming a Gaussian distribution and an identity link function in R. The CT_{min} data also met the linear model assumptions and were analysed using repeated measures analysis of variance. Tukey–Kramer's *post-hoc* tests were used to separate statistically heterogeneous means. The relationship between CT_{min} and BWC and BLC were examined using linear regression in STATISTICA.

Results

CT_{min} and repeated cold exposure assays

CT_{min} significantly varied across life stages following repeated cold exposure ($F_{16,\ 282} = 134.59$, $P < 0.001$) (fig. 1). In 5th instar and virgin adults, cold tolerance (CT_{min}) improved with repeated cold exposure (fig. 1). However, 6th instar larvae showed compromised cold tolerance with CT_{min} increasing with repeated exposure (fig. 1). Virgin females recorded the lowest CT_{min} across all assays relative to other life stages (fig. 1).

CCRT and repeated cold exposure assays

As in CT_{min} assays, CCRT varied significantly across life stages with repeated cold exposure ($F_{16,\ 282} = 4.06$, $P < 0.001$) (fig. 2). CCRTs of tested instars (4th, 5th and 6th instar) decreased with repeated cold exposure (fig. 2). In adults (virgin males and females), CCRT improved following repeated exposure at 24 h interval and was compromised after 48 and 72 h intervals (fig. 2).

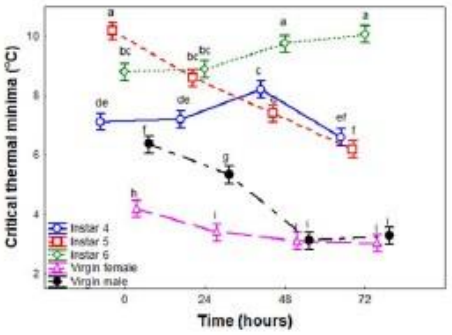

Figure 1. CT_{min} in adult (virgin male and female) and larval stages of *S. frugiperda* following repeated cold exposure. Data points represent means of $n = 20$ while error bars denote 95% confidence limits for each gender and life stage. Different letters above error bars denote significant differences.

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

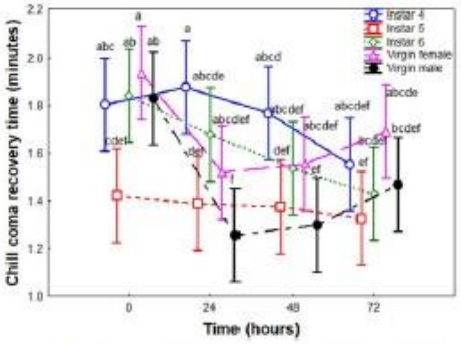

Figure 2. CCRT in adult (virgin male and female) and larval stages of *S. frugiperda* following repeated cold exposure. Data points represent means of $n = 20$ while error bars denote 95% confidence limits for each gender and life stage. Different letters above error bars denote significant differences.

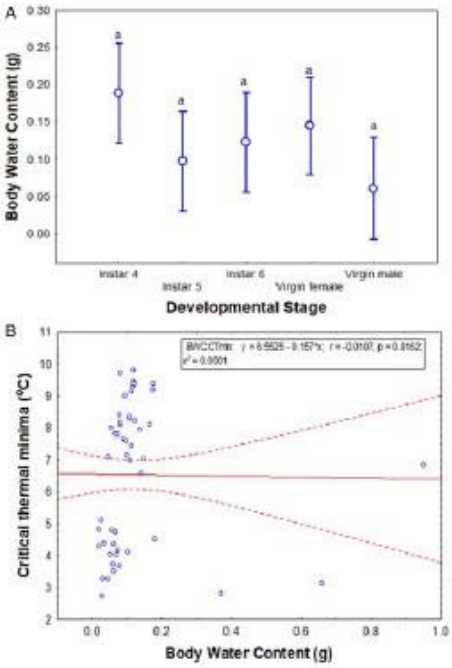

Figure 3. BWC (g) across different life stages (A) and relationship between BWC and CT_{min} (B) in *S. frugiperda*.

Body water and lipid content

BWC did not vary significantly among life stages ($F_{4,\ 95} = 2.01$, $P = 0.98$) (fig. 3A). There was no significant difference in BWC between all tested life stages (fig. 3A). Nevertheless, BWC was not significantly correlated with low temperature tolerance (measured as CT_{min}) (fig. 3B).

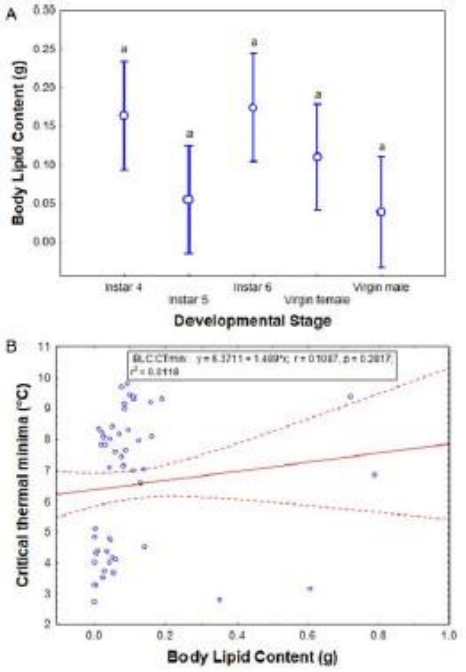

Figure 4. BLC (g) across different life stages (A) and the relationship between BLC and CT_{min} (B) in *S. frugiperda*.

Similar to BWC, BLC did not significantly vary among life stages ($F_{4,\ 95} = 2.94$, $P = 0.24$) (fig. 4A). As in BWC, BLC was not significantly correlated with low temperature tolerance such that CT_{min} decreased with BLC (fig. 4B).

Discussion

Insect physiological and behavioural adaptations are very important for determining survival and population dynamics in both transient and seasonal cold spells (Chown and Nicolson, 2004; Terblanche *et al.*, 2011; Andrew and Kemp, 2016). As expected, our results showed that repeated cold exposure influences the fitness of *S. frugiperda* (determined as CT_{min} and CCRT). While insects may face multiple temperature variabilities in winter season, the repeated cold exposures can trigger responses that may set the insect on a different physiological path relative to a single exposure (Marshall and Sinclair, 2010, 2012). In the current study, CT_{min} improved with repeated exposure in 5th instar larvae, virgin males and females in agreement with Renault *et al.* (2004) who reported improved survival in beetles that were exposed to repeated cold exposure. A similar trend was reported in *Drosophila melanogaster*, with low temperature tolerance improving following repeated cold exposure in tested insects (Le Bourg, 2007). However, compromised and fluctuating CT_{min} were recorded in 6th instar and 4th instar larvae, respectively. Given this variation across life stages, it therefore indicates that repeated thermal exposure impacts on CT_{min} are life-stage dependent.

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

While 5th instar larvae, virgin males and females showed enhanced CT_{min} across subsequent exposures, virgin females recorded the lowest CT_{min} across treatment intervals indicating that they were the most thermally tolerant. This gives them a fitness and survival advantage when they encounter extreme cold conditions in nature.

In the present study, repeated thermal exposure improved CCRT in 4th, 5th and 6th instar larvae and this is in consonance with Andersen *et al.* (2017) who reported improved chill-coma recovery, cellular survival and cold tolerance in *Locusta migratoria* following brief cold exposure periods. However, compromised CCRTs were recorded in adults (males and females) in keeping with van Dooremalen *et al.* (2011) who reported CCRT decrease in *Orchesella cincta* following repeated cold exposure. The variations in the current study underlie that CCRT responses are life-stage dependent. Although CCRT and CT_{min} are measures of cold tolerance, surprisingly, 6th instar larvae recorded compromised CT_{min} and enhanced CCRT indicating that responses also vary across traits, thus can be trait dependent.

The changes in cold tolerance across consecutive measurements provide insight into potential benefits of short-term acclimation to extreme cold events through cold hardening. Our results showed evidence of cold hardening in *S. frugiperda* as indicated by improved cold tolerance in some of the life stages. This suggests significant adaptive potential for cold tolerance in this invasive insect species and that individuals may also respond directly to low temperature extremes through phenotypic plasticity. While *S. frugiperda* has been reported to overwinter and survive all year round in Africa (Kebede and Shimalis, 2018; Prasanna *et al.*, 2018; Keosentse *et al.*, 2021), the results indicate its potential to adapt to variable thermal extremes in winter and this may give it fitness and survival advantage in the face of climate change. Insects reportedly enhance their cold tolerance through carbohydrate cryoprotectants accumulation, antifreezes synthesis, lipid membranes reordering and either removal (freeze avoiding) or retaining (freeze tolerant) of ice nucleators (Lee, 2010). Therefore, differential life-stage responses shown in this study following repeated exposure assays may be a result of variation in these physiological components of cold hardiness. However, this warrants further investigation to fully elucidate the responses.

Cold tolerance is dependent on the water content remaining unfrozen in many cold hardened insects by allowing basal metabolism to continue at low temperature levels (Colinet *et al.*, 2007; Alfaro-Tapia *et al.*, 2021). Reports have shown that reduction in BWC and subsequent increase in solute concentration may increase cold tolerance in insects (Worland, 1996). In the current study there was no relationship between cold tolerance and BWC. This may be because insects in our assays did not experience repeated cold conditions that trigger any water loss and subsequent solute concentration increase. While Keosentse *et al.* (2021) reported that BWC increased with larval stage in *S. frugiperda*, our results report otherwise on CT_{min} following repeated exposure. This may be because our present study measured BWC following plastic responses while Keosentse *et al.* (2021) measured basal BWC. Given these responses, it indicates that *S. frugiperda* may trade-off basal BWC for plasticity of thermal tolerance.

Lipid content plays a vital role in cold tolerance as they can serve as anti-freezers in the insect haemolymph (Sinclair and Marshall, 2018; Trenti *et al.*, 2022). In winter, most insects do not feed and may face the unreplaced energy consumption, water loss and low temperatures (Sinclair *et al.*, 2013; Williams *et al.*, 2015). Low temperature is one of the stressors which affect neutral lipid fluidity and mobilization and energy drain, since lipids are the primary overwintering source of fuel (Sinclair and Marshall, 2018). As such, most overwintering insects end winter with fewer lipid stores than at the beginning (Sinclair, 2015). For example, in laboratory-reared colonies of *D. melanogaster*, glycogen levels decreased following repeated cold exposure (Marshall and Sinclair, 2010). In addition, there was a positive correlation between BLC and cold tolerance in *Drosophila* spp. (Hoffmann *et al.*, 2001; Kaczmarek and Boguś, 2021). However, in the current study, our results showed no significant correlation between BLC and cold tolerance in *S. frugiperda*. A recent study attributed glycerol as the key cryoprotectant used by *S. frugiperda* (Vatanparast and Park, 2022). This therefore suggests that the influence of BLC on cold tolerance may be species dependent and glycerol maybe more important in this species.

In conclusion, the current study documents life-stage-related variation in cold tolerance for *S. frugiperda* following repeated thermal exposure. Our results suggest that repeated cold exposure differentially influences the fitness of *S. frugiperda* in nature where vulnerability is life-stage and trait dependent. In addition, the study provides evidence that cold hardening may be an important mechanism for *S. frugiperda* to cope with repeated cold exposure over the short term. These cold tolerance responses may provide temporal fitness benefits following repeated cold conditions in nature hence population persistence under changing environments. The results also have direct implications on the geographic distribution of the pest under climate change scenarios where warming winter seasons will lead to even further spatial expansion and multivoltinism due to favourable conditions. For a polyphagous pest such as *S. frugiperda* this will be critical as alternative hosts will support multiple generations enough to exert pest pressure on the main crop in the subsequent season (Vatanparast and Park, 2022). In such cases, management practices should consider area-wide monitoring of the pest populations even during off-season for early integrated pest management practices. This may include improved phytosanitary measures and reduction of alternative hosts on-farm. More importantly, augmentative releases to boost parasitoid populations during this period will also be a feasible option to suppress the pest populations to reduce the pressure in the main crop in the impending season. This will greatly reduce pest pressure, but costs are associated with control of the outbreak pest using synthetic pesticides on-season. Future studies should therefore determine the intensity of such parasitoid levels to maintain pest pressure well below economic injury levels.

Acknowledgements. The authors are very grateful for the National Research Foundation (NRF) and SACTA NPC trust PhD bursaries to AM and the support from the University of the Free State and NRF to FC. The authors also appreciate support from Midlands State University and Rhodes University to RM. In addition, the authors acknowledge Tsabang Mashigo and Lugisani Mulaudzi for technical assistance. The authors are also grateful to Hannelene du Plessis for kindly providing the stock culture of the fall armyworm.

References

Alfaro-Tapia A, Alvarez-Baca JK, Tougeron K, Lavandero B, Le Lann C and Van Baaren J (2021) Overwintering strategies and life-history traits of different populations of *Aphidius platensis* along a latitudinal gradient in Chile. *Entomologia Generalis* **42**, 127–145.

Andersen JL, Manenti T, Sørensen JG, MacMillan HA, Loeschcke V and Overgaard J (2015) How to assess *Drosophila* cold tolerance: chill coma

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

temperature and lower lethal temperature are the best predictors of cold distribution limits. *Functional Ecology* **29**, 55–65.

Andersen MK, Folkersen R, MacMillan HA and Overgaard J (2017) Cold acclimation improves chill tolerance in the migratory locust through preservation of ion balance and membrane potential. *Journal of Experimental Biology* **220**, 487–496.

Andreadis SS and Athanassiou CG (2017) A review of insect cold hardiness and its potential in stored product insect control. *Crop Protection* **91**, 93–99.

Andrew SC and Kemp DJ (2016) Stress tolerance in a novel system: genetic and environmental sources of (co)variation for cold tolerance in the butterfly *Eurema smilax*. *Austral Ecology* **41**, 529–537.

Avargues-Weber A, Lihoreau M, Isabel G and Giurfa M (2015) Information transfer beyond the waggle dance: observational learning in bees and flies. *Frontiers in Ecology and Evolution* **3**, 24.

Bale JS (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**, 849–862.

Bazinet AL, Marshall KE, Macmillan HA, Williams CM and Sinclair BJ (2010) Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. *Journal of Insect Physiology* **56**, 2006–2012.

Boake CR (1989) Repeatability: its role in evolutionary studies of mating behavior. *Evolutionary Ecology* **3**, 173–182.

Buckley LB and Huey RB (2016) How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integrative and Comparative Biology* **56**, 98–109.

Chapman JW, Williams T, Escribano A, Caballero P, Cave RD and Goulson D (1999) Age-related cannibalism and horizontal transmission of a nuclear polyhedrosis virus in larval *Spodoptera frugiperda*. *Ecological Entomology* **24**, 268–275.

Chidawanyika F and Terblanche JS (2011) Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology* **57**, 108–117.

Chidawanyika F, Mudavanhu P and Nyamukondiwa C (2012) Biologically based methods for pest management in agriculture under changing climates: challenges and future directions. *Insects* **3**, 1171–1189.

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**, e0169371.

Chidawanyika F, Chikowore G and Mutamiswa R (2020) Thermal tolerance of the biological control agent Neolema abbreviata and its potential geographic distribution together with its host Tradescantia fluminensis in South Africa. *Biological Control* **149**, 104315.

Chown SL and Nicolson SW (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford, UK: Oxford University Press.

Cock MJ, Beseh PK, Buddie AG, Cafá G and Crozier J (2017) Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. *Scientific Reports* **7**, 4103.

Colinet H, Nguyen TTA, Cloutier C, Michaud D and Hance T (2007) Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. *Insect Biochemistry and Molecular Biology* **37**, 1177–1188.

Cox J, Schubert AM, Travisano M and Putonti C (2010) Adaptive evolution and inherent tolerance to extreme thermal environments. *BMC Evolutionary Biology* **10**, 1–11.

Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, Colmenarez Y, Corniani N, Early R, Godwin J and Gomez J (2017) Fall armyworm: impacts and implications for Africa. *Outlooks on Pest Management* **28**, 196–201.

Du Plessis H, Schlemmer ML and Van den Berg J (2020) The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects* **11**, 228.

Early R, González-Moreno P, Murphy ST and Day R (2018) Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. *NeoBiota* **40**, 25–50.

Elnitsky MA, Hayward SA, Rinehart JP, Denlinger DL and Lee Jr, RE (2008) Cryoprotective dehydration and the resistance to inoculative freezing in the Antarctic Midge, Belgica Antarctica. *Journal of Experimental Biology* **211**, 524–530.

Feng Y, Zhang L, Li W, Yang X and Zong S (2018) Cold hardiness of overwintering larvae of *Sphenoptera* sp. (Coleoptera: Buprestidae) in Western China. *Journal of Economic Entomology* **111**, 247–251.

Goergen G, Kumar PL, Sankung SB, Togola A and Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE* **11**, e0165632.

Gregory PJ, Johnson SN, Newton AC and Ingram JS (2009) Integrating pests and pathogens into the climate change/food security debate. *Journal of Experimental Botany* **60**, 2827–2838.

Grinder RM, Bassar RD and Auer SK (2020) Upper thermal limits are repeatable in *Trinidadian guppies*. *Journal of Thermal Biology* **90**, 102597.

Hazell SP and Bale JS (2011) Low temperature thresholds: are chill coma and CT_{min} synonymous? *Journal of Insect Physiology* **57**, 1085–1089.

Hoffmann AA, Hallas R, Sinclair C and Partridge L (2001) Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* **55**, 436–438.

Izadi H, Mohammadzadeh M and Mehrabian M (2019) Cold tolerance of the *Tribolium castaneum* (Coleoptera: Tenebrionidae), under different thermal regimes: impact of cold acclimation. *Journal of Economic Entomology* **112**, 1983–1988.

Kaczmarek A and Boguś M (2021) The metabolism and role of free fatty acids in key physiological processes in insects of medical, veterinary and forensic importance. *PeerJ* **9**, e12563.

Kasoma C, Shimelis H and Laing MD (2021) Fall armyworm invasion in Africa: implications for maize production and breeding. *Journal of Crop Improvement* **35**, 111–146.

Kebede M and Shimalis T (2018) Out-break, distribution and management of fall armyworm, *Spodoptera frugiperda* JE Smith in Africa: the status and prospects. *Academy of Agricultural Journal* **3**, 551–568.

Keosentse O, Mutamiswa R, Du Plessis H and Nyamukondiwa C (2021) Developmental stage variation in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) low temperature tolerance: implications for overwintering. *Austral Entomology* **60**, 400–410.

Le Bourg E (2007) Hormetic effects of repeated exposures to cold at young age on longevity, aging and resistance to heat or cold shocks in *Drosophila melanogaster*. *Biogerontology* **8**, 431–444.

Lee RE Jr. (2010) A primer on insect cold-tolerance. In Denlinger DL and Lee RE (eds), *Insect Low Temperature Biology* New York: Cambridge University Press, pp. 3–34.

Lee RE Jr, Chen C-P and Denlinger DL (1987) A rapid cold-hardening process in insects. *Science (New York, N.Y.)* **238**, 1415–1417.

Lenoi AM, Bennett AF and Lenski RE (1994) Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences* **91**, 1917–1921.

Lu Y and Adang MJ (1996) Distinguishing fall armyworm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Florida Entomologist* **79**, 48–55.

Lynch HJ, Rhainds M, Calabrese JM, Cantrell S, Cosner C and Fagan WF (2014) How climate extremes not means define a species' geographic range boundary via a demographic tipping point. *Ecological Monographs* **84**, 131–149.

Marshall KE and Sinclair BJ (2010) Repeated stress exposure results in a survival–reproduction trade-off in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences* **277**, 963–969.

Marshall KE and Sinclair BJ (2012) The impacts of repeated cold exposure on insects. *Journal of Experimental Biology* **215**, 1607–1613.

Milton CC and Partridge L (2008) Brief carbon dioxide exposure blocks heat hardening but not cold acclimation in *Drosophila melanogaster*. *Journal of Insect Physiology* **54**, 32–40.

Mitchell KA, Boardman L, Clusella-Trullas S and Terblanche JS (2017) Effects of nutrient and water restriction on thermal tolerance: a test of mechanisms and hypotheses. *Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology* **212**, 15–23.

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

Morgan R, Finnøen MH and Jutfelt F (2018) CT_{max} is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports* **8**, 1–8.

Mutamiswa R, Machekano H, Chidawanyika F and Nyamukondiwa C (2018) Thermal resilience may shape population abundance of two sympatric congeneric Cotesia species (Hymenoptera: Braconidae). *PLoS ONE* **13**, e0191840.

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.

Näslund J (2021) Behavioural repeatability in larval *Limnephilus lunatus* Curtis, 1834 (Trichoptera) in an open-field test. *Aquatic Insects* **42**, 62–77.

Nboyine JA, Kusi F, Abudulai M, Badii BK, Zakaria M, Adu GB, Haruna A, Seidu A, Osei V, Alhassan S and Yahaya A (2020) A new pest, Spodoptera frugiperda (JE Smith), in tropical Africa: Its seasonal dynamics and damage in maize fields in northern Ghana. *Crop Protection* **127**, 104960.

Neal AS, Diaz R, Qureshi JA and Cave RD (2021) Adult cold tolerance and potential North American distribution of *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae). *Biological Invasions* **23**, 3719–3731.

Niemelä PT and Dingemanse NJ (2017) Individual versus pseudo-repeatability in behaviour: lessons from translocation experiments in a wild insect. *Journal of Animal Ecology* **86**, 1033–1043.

Nyamukondiwa C and Terblanche JS (2009) Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *Journal of Thermal Biology* **34**, 406–414.

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**, e0198610.

Nyamukondiwa C, Machekano H, Chidawanyika F, Mutamiswa R, Ma G and Ma CS (2022) Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance and other complementary traits in the tropics. *Current Opinion in Insect Science* **50**, 100878.

O'Donnell MJ, Regish AM, McCormick SD and Letcher BH (2020) How repeatable is CT_{max} within individual brook trout over short- and long-time intervals? *Journal of Thermal Biology* **89**, 102559.

O'Neill E, Davis HE and MacMillan HA (2021) A lack of repeatability creates the illusion of a trade-off between basal and plastic cold tolerance. *Proceedings of the Royal Society B* **288**, 20212121.

Phophi MM, Mafongoya P and Lottering S (2020) Perceptions of climate change and drivers of insect pest outbreaks in vegetable crops in Limpopo province of South Africa. *Climate* **8**, 27.

Prasanna BM, Huesing JE, Peschke Eddy R (2018) *Fall armyworm in Africa: A Guide for Integrated Pest Management*, 1st edition. Mexico, CDMX: CIMMYT.

R Core Team (2021) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/.

Renault D, Nedved O, Hervant F and Vernon P (2004) The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. *Physiological Entomology* **29**, 139–145.

Sinclair BJ (2015) Linking energetics and overwintering in temperate insects. *Journal of Thermal Biology* **54**, 5–11.

Sinclair BJ and Marshall KE (2018) The many roles of fats in overwintering insects. *Journal of Experimental Biology* **221**, jeb161836.

Sinclair BJ and Marshall KE (2018) The many roles of fats in overwintering insects. *Journal of Experimental Biology* **221**, jeb161836. doi: 10.1242/jeb.161836.

Sinclair BJ, Ferguson LV, Salehipour-Shirazi G and MacMillan HA (2013) Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integrative and Comparative Biology* **53**, 545–556.

Sinclair BJ, Alvarado LEC and Ferguson LV (2015) An invitation to measure insect cold tolerance: methods, approaches, and workflow. *Journal of Thermal Biology* **53**, 180–197.

Storey KB and Storey JM (2012) Insect cold hardiness: metabolic, gene, and protein adaptation. *Canadian Journal of Zoology* **90**, 456–475.

Tarusikirwa VL, Mutamiswa R, Chidawanyika F and Nyamukondiwa C (2020) Cold hardiness of the South American tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae): both larvae and adults are chill-susceptible. *Pest Management Science* **77**, 184–193.

Teets NM and Denlinger DL (2013) Physiological mechanisms of seasonal and rapid cold hardening in insects. *Physiological Entomology* **38**, 105–116.

Terblanche JS, Hoffmann AA, Mitchell KA, Rako L, le Roux PC and Chown SL (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology* **214**, 3713–3725.

Tollefson J (2014) The case of the missing heat. *Nature* **505**, 276–278.

Toxopeus J, Koštál V and Sinclair BJ (2019) Evidence for non-colligative function of small cryoprotectants in a freeze-tolerant insect. *Proceedings of the Royal Society B* **286**, 20190050.

Travis WR (2014) Weather and climate extremes: pacemakers of adaptation? *Weather and Climate Extremes* **5**, 29–39.

Trenti F, Sandron T, Guella G and Lencioni V (2022) Insect cold tolerance and lipidome: membrane lipid composition of two chironomid species differently adapted to cold. *Cryobiology* **106**, 84–90.

van Dooremalen C, Suring W and Ellers J (2011) Fatty acid composition and extreme temperature tolerance following exposure to fluctuating temperatures in a soil arthropod. *Journal of Insect Physiology* **57**, 1267–1273.

Vatanparast M and Park Y (2022) Cold tolerance strategies of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Science Reports* **12**, 1–16.

Weldon CW, Nyamukondiwa C, Karsten M, Chown SL and Terblanche JS (2018) Geographic variation and plasticity in climate stress resistance among southern African populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Scientific Reports* **8**, 9849.

Williams CM, Henry HA and Sinclair BJ (2015) Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biological Reviews* **90**, 214–235.

Worland MR (1996) The relationship between water content and cold tolerance in the Arctic collembolan *Onychiurus arcticus* (Collembola: Onychiuridae). *European Journal of Entomology* **93**, 341–348.

https://doi.org/10.1017/S0007485323000111 Published online by Cambridge University Press

Appendix 3:

Phytoparasitica
https://doi.org/10.1007/s12600-023-01095-6

RESEARCH

Thermal adaptation in Lepidoptera under shifting environments: mechanisms, patterns, and consequences

Reyard Mutamiswa · Abongile Mbande · Casper Nyamukondiwa · Frank Chidawanyika

Received: 21 April 2023 / Accepted: 20 July 2023

Abstract Thermal adaptation is a key facet safeguarding organismal function among ectothermic organisms. In this era of rapidly changing environments, understanding the diverse mechanisms mediating organismal climate stress resistance have become a priority given contrasting effects on organisms, *vis* declines in keystone species and an increase in invasive pest species. Here, we review mechanisms and patterns of thermal adaptation among shifting climates, specifically focusing on Lepidoptera, an economically significant insect order owing to its importance in agriculture and conservation. Lepidoptera are highly distinct, comprising species of diverse and unique morphology, ontogenetic development, habitat types and diets. Similarly, the diversity of adaptive responses ensuring survival under diverse thermal niches is equally remarkable. We therefore outline the mechanisms underpinning the success of Lepidoptera, mainly focusing on the important families and species which have quite attracted research attention in that order. We conclude by highlighting future studies for better understanding of lepidopteran species thermal adaptation under climate change. Understanding such adaptation will assist in accurate predictions and management of pest insect species and help conservation efforts in keystone species of the order Lepidoptera.

Keywords Behavioural thermoregulation · Climate change · Insect conservation · Morphology · phenotypic plasticity · Thermal stress

R. Mutamiswa · A. Mbande · F. Chidawanyika
Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa

R. Mutamiswa
Tugwi-Mukosi Multidisciplinary Research Institute, Midlands State University, Gweru, Zimbabwe

R. Mutamiswa · C. Nyamukondiwa
Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

C. Nyamukondiwa
Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

F. Chidawanyika (✉)
International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
e-mail: fchidawanyika@icipe.org

Published online: 03 August 2023

Introduction

The order Lepidoptera is the second largest, most diverse and widespread in the class Insecta, including more than150,000 species (Altermatt, 2009; Perveen & Khan, 2017; Rabieh, 2018). It consists of ~ 126 families and 46 superfamilies comprising butterflies, skippers, micro and macro moths (Perveen & Khan, 2017). This order is of high economic significance since it includes pests of agricultural importance

(Vreysen et al., 2016), keystone species for studies on climate change responses (Hufnagel & Kocsis, 2011) ecological services provider including pollinators, trophic interactions (Ghazanfar et al., 2016) and providers of economic bioproducts e.g. silk (Fedic et al., 2002). Due to their extensive polyphagy necessitated by elaborate larval biting and chewing mouthparts, the order is one of the most economically important in both natural and agroecosystems (Rabieh, 2018). Although lepidopterans ecological niches vary, with each species occupying microhabitat which adequately supports its survival, they are highly susceptible to environmental changes (e.g. temperature and relative humidity [RH]) (Maurer et al., 2018; Woestmann & Saastamoinen, 2016). It is thus not surprising that the order Lepidoptera has become a model taxon in conducting research on insect responses to global climate change (Perveen & Khan, 2017).

Anthropogenic activities have rapidly shifted habitat environments by increasing average and magnitude of variation of temperatures and frequency of extreme weather events (e.g. heatwaves, cold snaps, floods and drought) (IPCC, 2014; Harris et al., 2018). If mitigation measures against climate induced changes fail, global concentrations of carbon dioxide are projected to increase by 540–970 ppm, coupled with consequent mean surface temperature increase of ~ 1.4 to 5.8 °C by 2100 (Engelbrecht et al., 2015). Moreover, rainfall patterns will become more erratic, and southern Africa is expected to be drier (Engelbrecht et al., 2015). Such changes in habitat environments may offset the integrity of the ecosystem which may negatively impact on biodiversity, ecosystem function and services (Bale et al., 2002; Bodlah et al., 2017; Karuppaiah & Sujayanad, 2012). Due to habitat loss and fragmentation, the challenge of tracking favourable environmental conditions in time and space in most species is further compounded (Chevin et al., 2010). As a result, thermal adaptation may be key to survival. Without adaptive mechanisms to cope with changing environments, many insect species may be highly susceptible to extirpation. Thermal adaptation, a result of natural selection, is the ability of the insect to adjust to temperature variability (Sheikh et al., 2017). The thermal adaptation hypothesis posits that warm, aseasonal tropical environments produce insect populations with higher and narrower critical thermal limits (Kaspari et al., 2015). However, organisms from more temperate environments are thought to have broader thermal breaths owing to the climate variability hypothesis (Gutiérrez-Pesquera et al., 2016). An insect with a genotype that enhances its selective advantage in a certain environment is considered to have adapted to that particular environment (Sheikh et al., 2017). Given the ectothermic nature of most insects and diversity of microclimatic conditions in any ecosystem (Kaspari et al., 2015; Pincebourde & Woods, 2020), some may emerge 'winners' while others emerge as 'losers' due to detrimental effects (Mutamiswa et al., 2017a).

Temperature, one of the key abiotic factors plays a significant role in influencing insect development, reproduction, seasonal phenology, population dynamics, distribution, survival, and abundance (Colinet et al., 2015; Nguyen et al., 2014; Noh et al., 2017). Insect performance is dependent on body temperature, and it varies with species and taxa (Foray et al., 2014). Insects generally have a developmental thermal window and optimum temperature at which all activities are optimal. Hence any deviation from the optimal may have negative consequences on fitness and survival (Foray et al., 2014). In nature insects often encounter heterogeneous overlapping environmental stressors (e.g. heat, cold, desiccation, starvation) which may negatively affect fitness and survival (Bauerfeind & Fischer, 2013; reviewed in Zhang et al., 2019). To adapt to these adverse conditions, insects have evolved a suite of anti-stress mechanisms such as behavioural, morphological and physiological for survival (Bodlah et al., 2017; Chevin et al., 2010; Chown & Nicolson, 2004; Mutamiswa et al., 2017b).

Behavioural mechanisms such as behavioural thermoregulation, microhabitat selection and migration are the first line of responses to increased thermal stress, due to low energy costs. In nature, this is evidenced by variations in timing of daily activities and/or different microhabitats explorations (Kleckova & Klecka, 2016). As a behavioural thermoregulation technique, some insects are known to change their body orientations, bask in the sun or move between shade and sun and vice versa (Sanborn, 2008). Body orientation changes involves resting in an exposed position to acquire radiant heat from the sun when body temperature is low or seek shelter in a shade to increase heat loss to the environment when body temperature is high (Sanborn, 2008). Due to the high surface area to volume ratio, there is faster heat exchange between the insect and the environment.

In addition, insects such as beetles and grasshoppers usually press their bodies against warm ground surface for uptake of heat through conduction when their body temperatures are low (Sanborn, 2008). However, when their body temperatures rise, they extend their legs and elevate their bodies to avoid contact with the warm ground surface, a process called stilting. Microhabitat selection common in desert insects involves vertical migration in which insects move away from the ground when it becomes warmer and move back to the ground when it is cool and windy to seek warmth (Sanborn, 2008). To evade freezing temperatures, monarch butterflies have been reported to undergo long distance migration from North America to Mexico (reviewed in Reppert & de Roode, 2018). Similarly, a total of ten British butterfly species have reportedly extended northwards in the past decades owing to high temperatures (Bryant et al., 1997; Pollard & Yates, 1992).

Morphologically, pigmentation plays a pivotal role in insect adaptation to thermal stress (Sanborn, 2008). The thermal melanism hypothesis posits that under low ambient temperature conditions, dark organisms have a fitness and survival advantage relative to light organisms due to faster body warming rate at any given level of solar radiation (Clusella-Trullas et al., 2007). On the other hand, adaptation to warmer climates may be dependent on higher colour lightness to minimize the negative effects of overheating. For example, an enhanced fitness due to melanism has been reported in butterflies resulting in increased activity, fecundity and egg maturation rates as well as avoidance of predators (Clusella-Trullas et al., 2007; Ellers & Boggs, 2004; Roland, 2006). In addition, some insects have bodies covered with hairs (insulation that allows heat retention in the body) to survive cool temperatures (Davis et al., 2005; Sanborn, 2008). Moreover, to the plasticity of melanisation (see e.g. Stoehr & Goux, 2008), this shows that body morphology plays a key role in insect fitness under stressful environments.

Physiologically, insects survive extreme thermal conditions through genetic adaptation (Karl et al., 2014) and phenotypic plasticity (Sgrò et al., 2016). Phenotypic plasticity is the ability of an organism of the same genotype to remodel its phenotype based on a change in environment (Whitman & Ananthakrishnan, 2009). It involves a number of mechanisms such as hardening (rapid cold and heat hardening) (in the short-term) acclimation under controlled laboratory conditions or acclimatization in the field (longer-term) (Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2018a; Nyamukondiwa et al., 2013). For example, rapid cold hardening (RCH) improved survival in lepidopterans such as *Thaumatotibia leucotreta* (Meyrick, 1913) (Stotter & Terblanche, 2009) and *Chilo partellus* (Swinhoe, 1885) (Mutamiswa et al., 2018a). On the other hand, rapid heat hardening (RHH) improved survival in *Cydia pomonella* Linnaeus, 1758 (Chidawanyika & Terblanche, 2011) and *C. partellus* (Mutamiswa et al., 2018a). Similarly, acclimation improved fitness in *Bicyclus anynana* (Butler, 1879), *Tuta absoluta* (Meyrick, 1917) and *C. partellus* (Fischer et al., 2010; Mutamiswa et al., 2018a; Tarusikirwa et al., 2020a). For example, low temperature increased egg size in *B. anynana* (Fischer et al., 2003a). In addition, developmental acclimation (acclimation from the larval and pupal stages) improved cold and heat tolerance in *C. partellus* (Mutamiswa et al., 2019). Phenotypic plasticity also appears to be 'heritable', through epigenetic gene expression, a phenomenon called transgenerational plasticity (Cavieres et al., 2020). For example, ambient temperature experienced by parents improved heat resistance in *Drosophila melanogaster* (Meigen, 1830) offspring (Crill et al., 1996). Similarly, low temperature exposure of *Drosophila serrata* (Malloch, 1927) mothers increased fecundity in offspring (Magiafoglou & Hoffmann, 2003). For lepidopterans with a migratory history (Reppert & de Roode, 2018), the processes and environmental effects in one habitat may explain the fitness characteristics of individuals upon arrival at a different environment, in a phenomenon called carry-over effects (Zheng et al., 2017). This mechanism has been observed to improve oviposition rate and longevity in different insect species including *Plutella xylostella* (Linnaeus, 1758) (Zhang et al., 2015a, b) and *Grapholita molesta* (Busck, 1916) (Zheng et al., 2017).

This background illustrates that adaptation to temperature variation in various insect taxa is essential for survival and success of the class Insecta under changing environments. However, there are few studies on thermal adaptation of agriculturally important pests, which consequently mask the effectiveness of pest management options under climate change (Hoffmann, 2017; Qi et al., 2019).

In this review, we highlight thermal adaptation in Lepidoptera, covering the mechanisms behind

successful establishment under rapidly changing environments. We summarise thermal adaptation from key aspects such as behavioural, evolutional, morphological and physiological and discuss how insects in this order enhance their thermal tolerance. We highlight future research directions based on the knowledge obtained to date on thermal adaptation in this key order. Understanding these adaptations will help in accurate predictions and management of some economically important insects among lepidopterans.

Behavioural adaptation

As the first line of defence, behavioural adaptation involves the actions that an organism employs either individually or in a group in response to stress (Karl et al., 2008; Sheikh et al., 2017). The key behavioural mechanisms employed by insects include behavioural thermoregulation techniques such as microhabitat selection, basking, spatio-temporal activity cycle modifications and endothermy as well as long-distance migration (Bodlah et al., 2017; Kleckova & Klecka, 2016). Lepidopteran species may use a single or a combination of these strategies within or across developmental stages to avoid stress (see Table 1). To raise their body temperatures above that of the environment, some lepidopteran species bask in the sun to absorb solar radiation. This activity is common in cool environments and has been reported in various insect species (see Table 1). Adult butterfly species often exhibit three basking mechanisms such as lateral, dorsal and reflectance (Kemp & Krockenberger, 2004). Lateral and dorsal basking involves strategic body orientation such that either ventral or dorsal wings respectively lie perpendicular to solar radiation plane resulting in body temperature being raised in preparation for key activities such as flight, mating and food searching (Kemp & Krockenberger, 2004; Tsai et al., 2020). On the other hand, reflectance basking common in *Pierinae* species involves reflection of solar radiation through the use of dorsal wings surfaces (Kingsolver, 1985; Kemp & Krockenberger, 2004). Most migratory lepidopteran species are from the Nymphalidae and Pieridae families (Sheikh et al., 2017).

In Lepidoptera, migration involves two key strategies which include movement in one direction without returning to the area of origin and movement to hibernating or aestivating sites where insects undergo overwintering in a diapause state before returning to the area of origin (Scoble, 1995). For example, to avoid extreme winter conditions associated with extended sub-zero temperatures in temperate regions, *Danaus plexippus* (Linnaeus, 1758) migrate annually in Autumn (late September and October) from North eastern America to overwintering sites in central Mexico where cool and humid conditions are prevalent (Larsen & Lee, 1994). Most of these butterflies will be in a reproductive diapause state, associated with lipid content increase and undeveloped ovaries (Gill et al., 2017). However, during migration, these butterflies encounter unfavourable climatic conditions such as frost, heavy dews and sub-zero temperatures (Larsen & Lee, 1994), hence adaptation remains a key strategy in surviving these conditions until they reach their destination. As a result, heat gaining and other thermoregulation behaviours play a pivotal role in facilitating survival. For example, heat production through shivering allows butterflies to increase their thoracic temperatures thus permitting them to crawl up vegetation to bask as well as maintaining flight temperatures during partly cloudy conditions (Masters et al., 1988). In addition, *Cnaphalocrocis medinalis* (Guenée, 1854) adults are known to migrate from Southwest to North eastern China during spring and summer seasons as a way of evading long-term heat stress in the southern parts of China (Bodlah et al., 2017; Zhang et al., 1981). During migration, *C. medinalis*, *Mythimna separata* (Walker, 1865) and *Autographa gamma* (Linnaeus, 1758) have been reported to use multi-stop migration strategy (Table 1). This involves flying at dusk for ~ 300 km, rest at dawn and then fly again at the next dusk (Alerstam et al., 2011; Wang et al., 2017). When resting, these species replenish their energies for the next flight through nectar feeding (Wang et al., 2017).

In addition to migration, microhabitat selection, defined as selection in the short term of thermally favourable microclimates, plays a significant role in thermal adaptation in lepidopteran mobile developmental stages (larvae and adults) (Kührt et al., 2005; Pincebourde & Woods, 2020). However, this behavioural technique is not possible in other immobile life stages (Bodlah et al., 2017; Kührt et al., 2005). Consequently, habitat selection by females and larvae

Table 1 Some behavioural strategies employed by various developmental stages of lepidopteran species in response to thermal stress. The list may not be exhaustive but was compiled using literature available at the time of writing

Species	Developmental Stage	Type of stress	Behavioural strategy used	Reference
Cnaphalocrocis medinalis	Adult	High and low temperatures	Multi-stop migration	Chang et al., 1980; Zhang et al., 1981
	Adult	High temperature	Microhabitat selection	Bodlah et al., 2017
	Larva	High temperature	Leaf folding	Bodlah et al., 2017
	Larva	High temperature	Microhabitat selection	Bodlah et al., 2017
Cydia pomonella	Larva	High temperature	Microhabitat selection	Kührt et al., 2005
Gonimbrasia belina	Larva	High temperature	Microhabitat selection	Frears et al., 1997
	Larva	High temperature	Hanging	Frears et al., 1997
Hyles lineata	Larva	High temperature	Microhabitat selection	Casey, 1976
Manduca sexta	Larva	High temperature	Microhabitat selection	Casey, 1976
Plutella xylostella	Adult	High temperature	Microhabitat selection	Talekar and Shelton, 1993
Aglais urticae	Larva	High temperature	Microhabitat selection	Bryant et al., 1997
Agrotis infusca	Adult	High temperature	Annual migration	Hill, 2007; Sheikh et al., 2017
Danaus plexippus	Adult	Low temperature	Annual migration Shivering wings	Reppert and de Roode, 2018
Danaus plexippus	Larva	High temperature	Microhabitat selection	Rawlins and Lederhouse, 1981
Malacosoma americanum	Larva	Low temperature	Microhabitat selection	Knapp and Casey, 1986
Mythimna separata	Adult	High and low temperatures	Multi-stop migration	Chapman et al., 2010
Autographa gamma	Adult	High and low temperatures	Multi-stop migration	Chapman et al., 2010
Bombyx mori	Larva	High temperature	Increased cocoon spinning	Ramachandra et al., 2001
Pararge aegeria	Adult	Low temperature	Basking	Berwaerts et al., 2001
Euphydryas aurinia	Larva	Low temperature	Basking	Porter, 1982
Pieris occidentalis	Adult	Low temperature	Basking	Kingsolver, 1985
Pieris rapae	Adult	Low temperature	Basking	Ohsaki, 1986
Parrhasius m-album	Adult	Low temperature	Lateral basking	Tsai et al., 2020
Satyrium caryaevorus	Adult	Low temperature	Lateral basking	Tsai et al., 2020
Satyrium Favonius	Adult	Low temperature	Lateral basking	Tsai et al., 2020
Pieris melete	Adult	Low temperature	Microhabitat selection	Ohsaki, 1986
Pieris napi	Adult	Low temperature	Microhabitat selection	Ohsaki, 1986
Colias butterflies	Adult	Low temperature	Basking Microhabitat selection	Sherman and Watt, 1973
Orgyia antiqua	Larva	High temperature	Microhabitat selection	Sandre et al., 2014
Hypolomnas bolina	Adult	Low temperature	Basking Microhabitat selection	Kemp and Krockenberger, 2004
	Larva	High and low temperatures	Seeking refuge in ground mass cover	Sherman and Watt, 1973
Parnassius apollo	Larva	High and low temperatures	Microhabitat selection	Ashton et al., 2009
Battus philenor	Larva	High and low temperatures	Microhabitat selection	Nice and Fordyce, 2006
Deilephila nerii	Adult	Low temperature	Endothermy	Dorsett, 1962
Helicoverpa punctigera	Adult	Low temperature	Endothermy	Coombs, 1993
Helicoverpa armigera	Adult	Low temperature	Endothermy	Coombs, 1993
Hyles euphorbia	Adult	Low temperature	Endothermy	Heinrich and Casey, 1973
Deilephila elpeno	Adult	Low temperature	Endothermy	Heinrich and Casey, 1973
Erebia aethiops	Adult	High temperature	Flying under shade	Kleckova and Klecka, 2016

for oviposition and pupation respectively determines fitness and survival of offspring (Bodlah et al., 2017). For instance, third instar larvae of *C. medinalis* seek shade on the lower parts of rice leaves when they encounter high temperatures (Bodlah et al., 2017). Towards pupation, larvae also move to the cool and moist underside of rice leaves under heat stress since pupae do not have capacity to behaviourally thermoregulate due to immobility (Bodlah et al., 2017). Some lepidopteran species use regional endothermy associated with thorax heat generation as a mechanism of gaining heat to facilitate behavioural activities such as flight. For example, endothermic warm up as a result of wing shivering in *Helicoverpa armigera* (Hübner, 1808) and *H. punctigera* (Wallengren, 1860) by simultaneous contracting of their main upstroke and downstroke muscles raised thoracic temperature to flight threshold (Coombs, 1993; Heinrich, 1987), and excess heat is used to raise temperatures of the head and abdomen through haemolymph convection. This endothermic mechanism was also reported in other insect species such that vibration and beating of wing warmed up flight muscles to temperatures enough to facilitate flight (see Table 1). Consequently, these behavioural attributes may facilitate fitness of insects and facilitate their success in natural and managed ecosystems.

Morphological adaptation

This form of adaptation involves change in physical features that promote survival to stressful environments (Sheikh et al., 2017) such as changing body colouration. Morphological changes are determined by genetic and environmental cues and are key strategies for thermal adaptation in lepidopteran species (Solensky & Larkin, 2003). An analysis of 473 European butterfly and dragonfly species showed that dark-coloured and light-coloured species favour cooler and warmer climates respectively and biogeographical shifts from 1988 to 2006 resulted in species assemblages becoming lighter in warming regions (MacLean et al., 2016; Zeuss et al., 2014). Various studies have reported how decreased melanism and variable colour patterns morphologically influence thermal adaptation in lepidopterans under variable thermal conditions (Forsman et al., 2016; Hill et al., 2021; Scriber, 2020). For example, in cooler microclimates, darker and more melanic wings reportedly enhanced heat absorption in butterflies while in high elevation areas, longer thoracic setae helped in retaining heat and increasing body temperatures (Hill et al., 2021; Kingsolver & Moffat, 1982). In addition, *Colias meadii* butterflies exhibited increased wing melanism and longer setae at high elevation areas following pupal developmental acclimation at cooler temperatures indicating adaptive phenotypic plasticity (MacLean et al., 2016). The butterflies of North America and European temperate regions exhibit a dark colouration compared to those from warmer regions (Stelbrink et al., 2019). In addition, dark-coloured wings are known to absorb more solar radiation in basking *Colias* butterflies enabling them to raise their body temperature in preparation for flight (Ellers & Boggs, 2002). Most moths are covered by a coat of dense hairs (derived from scales) which plays a significant role in retardation of heat loss (Heinrich, 1987). For example, *Malacasoma americanum* (Fabricius, 1793) moths survive low temperature conditions during summer nights with the aid of hairs (Heinrich, 1987). Larvae of *Aglais urticae* Linnaeus, 1758 are black and yellow in colour, hence this colouration has enabled them to raise body temperature when basking (Bryant et al., 1997). In *B. philenor* larvae, dark phenotype is mostly sustained in cooler microclimates to adapt to extreme thermal conditions (Nice & Fordyce, 2006). In addition, *D. plexippus* larvae exhibit black, white and yellow colouration bands which also enable thermoregulation and survival under suboptimal thermal conditions (Solensky & Larkin, 2003). Furthermore, dark larvae of *Papilio polyxenes* Fabricius, 1775 tend to take advantage of shorter sunlight periods in autumn through optimising solar radiation absorption resulting in increased body temperature and larval growth (Hazel, 2002). Thus, morphological adaptation, acting complimentary or synergistically to other adaptation mechanisms may help lepidopteran species to occupy variable thermal environments. Moreover, morphological adaptations are also critical for other ecologically significant traits that determine survival. For example, lepidopteran body colouration helps in aposematic mimicry, camouflage and warning (Ellers & Boggs, 2002; Solensky & Larkin, 2003), key traits in natural selection.

Physiological adaptation

This comprises all suites of internal body mechanisms employed by insects to evade extreme stressful conditions (Sheikh et al., 2017). Several physiological mechanisms are used by lepidopteran species in averting thermal stress among different developmental stages. Some of the key physiological mechanisms include basal stress tolerance, phenotypic plastic responses mediated by upregulation of heat shock proteins, accumulation of cryoprotectants (e.g. polyols, sugars), cross tolerance, cold hardiness and metabolic responses (Fig. 1).

Basal stress tolerance

Basal stress tolerance is an inherent ability of an organism and its cell structures to withstand stressful environmental conditions without prior acclimation (Munoz-Valencia et al., 2013; Park & Yun, 2013). Insect species with superior basal stress tolerance have a higher likelihood of surviving projected climate warming thereby accelerating their spread and establishment (Parmesan, 2006; Zerebecki & Sorte, 2011; Ju et al., 2013). Given that heat stress often occurs together with desiccation or starvation stress, insects with a higher capacity to withstand these conditions may emerge 'winners' in the environment. Critical thermal limits (CT_{min} and CT_{max}), heat knockdown time (HKDT), chill coma recovery time (CCRT), lethal temperatures (lower and upper lethal temperatures), starvation and desiccation tolerance are key physiological traits commonly used to assess insect basal thermal tolerance under projected climate change (Käfer et al., 2020; Sinclair et al., 2015). For example, many lepidopteran species exhibited enhanced cold and high temperature tolerance and these responses indicate inherent thermal adaptation as well as fitness advantage when they encounter cold and high temperature conditions in nature (see Table 2). Other abiotic stress resistance traits play a pivotal role in lepidopteran thermal adaptation. For example, in arid environment, *Sitotroga cerealella* adults exhibited a reduced water loss rate which enhances its survival and pest status under changing environment (Machekano et al., 2018a). Similarly,

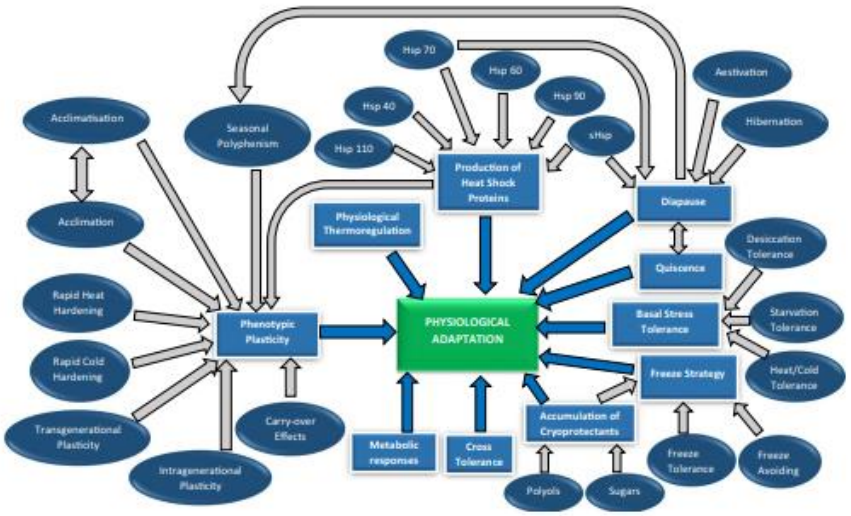

Fig. 1 Summary of the key physiological adaptation mechanisms likely used by Lepidoptera in response to stressful environments

Table 2 Basal stress tolerance in different lepidopteran species. CCRT = chill coma recovery time, HKDT = heat knockdown time, CT_{min} = critical thermal minimum, CT_{max} = critical thermal maximum, LLT = lower lethal temperature, ULT = upper lethal temperature. The list may not be exhaustive but was compiled using literature available at the time of writing

Species	Life stage	Tolerance Trait	Trait Record	Reference
Cydia pomonella	adult	CT_{min}	1.1 °C	Chidawanyika and Terblanche, 2011
		LLT	−20 °C−−5 °C	Chidawanyika and Terblanche, 2011
		ULT	32 °C−47 °C	Chidawanyika and Terblanche, 2011
Chilo partellus	larva	CT_{max}	48.5 °C	Mutamiswa et al., 2017a, 2018b
	adult	CT_{max}	47.8 °C	Mutamiswa et al., 2017a, 2018b
Bicyclus dorothea	adult	CT_{max}	46 °C	Dongmo et al., 2021
Busseola fusca	larva	CT_{min}	2.7 °C	Mutamiswa et al., 2018b
	adult	CT_{min}	1.5 °C	Mutamiswa et al., 2017a
Sesamia calamistis	larva	CT_{max}	48.2 °C	Mutamiswa et al., 2018b
	adult	CT_{min}	1.8 °C	Mutamiswa et al., 2018b
Sitotroga cerealella	adult	CT_{max},	46.1 °C	Machekano et al., 2018a
		HKDT	7.97 min	Machekano et al., 2018a
Heliconius spp		HKDT	15.9 min	Montejo-Kovacevich et al., 2020
Plutella xylostella	larva	CT_{max}	45.6 °C	Machekano et al., 2018b
		CT_{min}	−2.4 °C	Machekano et al., 2018b
	adult	CT_{max}	46.6 °C	Machekano et al., 2018b
	larva	ULT	31 °C−47 °C	Machekano et al., 2018b
		LLT	−20 °C−2 °C	Machekano et al., 2018b
Tuta absoluta	larva	LLT	−17 °C−0 °C	Tarusikirwa et al., 2020b
	adult	CT_{min}	−5.2 °C	Machekano et al., 2018c
Spodoptera frugiperda	adult	CT_{min},	1.9 °C	Keosentse et al., 2021
		CCRT	3.5 min	Keosentse et al., 2021
Carminda paeon	adult	CT_{max}	53.4 °C	Silva et al., 2020
Eunica cuvierii	adult	CT_{max}	60.5 °C	Silva et al., 2020
Junonia evarete	adult	CT_{max}	69 °C	Silva et al., 2020
Memphis appias	adult	CT_{max}	64 °C	Silva et al., 2020
Moneuptychia walhbergi	adult	CT_{max}	54 °C	Silva et al., 2020
Yphthimoides angularis	adult	CT_{max}	58.5 °C	Silva et al., 2020
Zaretis isidora	adult	CT_{max}	57 °C	Silva et al., 2020
Colobura dirce	adult	CT_{max}	52 °C	Silva et al., 2020
Doxocopa laurentia	adult	CT_{max}	59 °C	Silva et al., 2020
Eryphanis reevesii	adult	CT_{max}	53.6 °C	Silva et al., 2020

S. frugiperda larvae exhibited water loss rate reduction under desiccating conditions (Keosentse et al., 2022). On the other hand, starvation improved basal heat tolerance in *C. partellus* larvae (Mutamiswa et al., 2018b; Nyamukondiwa et al., 2022). Recent trends have shown *C. partellus* expanding from low to mid and high altitude and humid transitional areas (Khadioli et al., 2014; Mutamiswa et al., 2017a). This has been necessitated by its inherent high potential to withstand environmental stresses (e.g., starvation and desiccation stress) (Mutamiswa et al., 2018b; Nyamukondiwa et al., 2022).

Rapid physiological thermoregulation

Pre-flight warm up is a significant component of physiological thermoregulation in Lepidoptera. It is generally initiated by dorso-longitudinal and dorsoventral muscles which are responsible for flipping down and up wings respectively during flight

(Heinrich, 1987; Neve & Hall, 2016). The thoracic temperature is highly dependent on environmental temperature and heat generation through muscle movements (Neve & Hall, 2016). For example, *Papilo*, *Colias* and *Pieris* species require thoracic temperatures ranging from 28 °C to 42 °C for flight whereas 33–38 °C thoracic temperatures restrict rigorous flight (Kingsolver, 1985; Neve & Hall, 2016). The lowest thoracic temperatures ranging from 17 °C to 20 °C for flight have been recorded in large butterfly species such as *Parnassius phoebus* Fabricius, 1793 (Guppy, 1986). Through this endothermic thoracic heat generation, excess heat can be used to maintain other body regions through haemolymph counter current exchange (Tsai et al., 2020; Tsuji et al., 1986). Coupled with behavioural thermoregulation, endothermy may act synergistically with behaviour to optimise insect body temperature for flight and other life defining activities. Maintenance of flight at low ambient temperatures also optimises key life history activities such as mating, oviposition, dispersal and food searching (Kingsolver, 1983).

However, fitness and survival can be reduced if thoracic temperature exceeds ambient temperature due to high risk of overheating. For instance, in *Colias* butterflies thoracic temperature higher than 40 °C may be detrimental (Kingsolver & Watt, 1983). Insects however have several mechanisms for maintenance of optimal temperature following endothermic heat generation. For example, they can use behaviour (Kingsolver & Watt, 1983), convective cooling through cooler ambient air exchange (Tsai et al., 2020) and evaporative cooling (Prange, 1996; Sanborn, 2008). Therefore, an understanding of physical constraints to thermal tolerance in lepidopteran species is of paramount importance in determining its impact on fitness, survival and ability to disperse (Neve & Hall, 2016). Moreover, understanding the regulation of endothermy is also significant given the increasing temperatures associated with climate change.

Phenotypic plasticity

Organisms can remodel phenotypes generated from the same genotypes based on environment, in a near ubiquitous mechanism in insects. Different forms of plasticity, including RCH, RHH and acclimation have been reported as key mechanisms of thermal adaptation in Lepidoptera (Fig. 1). Rapid cold hardening/rapid heat hardening is defined as a rapid improvement in survival at a low/high lethal temperatures respectively after brief pre-treatment to sub-lethal temperature shock (Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2018a). For example, RCH reportedly enhanced cold tolerance in *Busseola fusca* (Fuller, 1901), *C. partellus* and *T. absoluta* (Mutamiswa et al., 2018b; Tarusikirwa et al., 2020a) (Table 3). Similarly, RHH improved cold tolerance in *B. fusca* and *C. partellus* (Mutamiswa et al., 2018b) (Table 3). Acclimation is defined as an improvement in survival following pre-conditioning at sub-lethal conditions (Andrew et al., 2013; Chidawanyika & Terblanche, 2011; Nyamukondiwa & Terblanche, 2010). It is a facultative phenotypic response of organisms to environmental changes that occur over a period of days, months, seasons or years (Münzbergová & Hadincová, 2017). For example, acclimation improved cold and heat tolerance in *B. anynana* and *C. partellus* (Fischer et al., 2010; Mutamiswa et al., 2017a, 2018a; 2019) (Table 3).

Intragenerational plasticity refers to non-genetic effects of one life stage phenotype on the phenotypes of other developmental stages in that particular life cycle (Sgrò et al., 2016). For example, environmental conditions experienced in early developmental stage may influence performance and plasticity in later stages of life (Gray, 2013; Sgrò et al., 2016). On the other hand, transgenerational/cross-generational plasticity refers to the epigenetic effects of parental phenotype or environment on offspring phenotypes (Qi et al., 2019). It is generally beneficial if environmental conditions experienced by the parents are similar to those faced by the offspring (Sgrò et al., 2016). For instance, high temperature parental exposure resulted in improved hatching success in *Pararge aegeria* (Linnaeus, 1758) (Gibbs et al., 2010). Similarly, low temperature parental exposure improved egg size, hatching success, larval hatching mass and larval developmental time in *B. anynana* (Fischer et al., 2003b; Geister et al., 2009; Steigenga & Fischer, 2007) whereas in *P. aegeria* it improved egg mass and embryonic developmental time (Gibbs et al., 2010). Nevertheless, there are limited studies on intragenerational and transgenerational plasticity in Lepidoptera. Given their importance (intragenerational and transgenerational plasticity), there is need for researchers to evaluate plastic response variations

Table 3 Forms of plasticity in different lepidopteran species in response to stress. RCH = rapid cold hardening, RHH = rapid heat hardening, CCRT = chill coma recovery time, HKDT = heat knockdown time, CT_{min} = critical thermal minima, CT_{max} = critical thermal maxima, SCP = supercooling point, H/ARR = Hardening/Acclimation Response Ratio. The list may not be exhaustive but was compiled using literature available at the time of writing

Form of Plasticity	Species	Life Stage	Improved Trait	H/ARR	Reference
Acclimation	*Chilo partellus*	Adult	CT_{min}	0.25	Mutamiswa et al., 2017a
		Adult	CT_{max}	0.056	Mutamiswa et al., 2017a
		Adult	CCRT	0.14	Mutamiswa et al., 2018a
		Larva	CT_{min}	0.35	Mutamiswa et al., 2018a
		Larva	CCRT	0.3	Mutamiswa et al., 2018a
		Larva	SCP	0.41	Mutamiswa et al., 2018a
		Larva	CT_{max}	0.13	Mutamiswa et al., 2018a
		Larva	HKDT	0.68	Mutamiswa et al., 2018a
		Adult	HKDT	0.22	Mutamiswa et al., 2018a
		Pupa	CCRT	0.08	Mutamiswa et al., 2019
		Pupa	HKDT	0.43	Mutamiswa et al., 2019
	Busseola fusca	Larva	CT_{min}	0.1	Mutamiswa et al., 2018b
	Sesamia calamistis	Larva	CT_{min}	0.17	Mutamiswa et al., 2018b
	Danaus plexippus	Adult	Low temperature survival	–	Larsen and Lee, 1994
	Bicyclus anynana	Adult	CCRT	–	Fischer et al., 2010
		Adult	HKDT	–	Fischer et al., 2010
	Pringleophaga marioni	Larva	Low temperature survival	–	Sinclair and Chown, 2003
	Phthorimaea operculella	Larva	Low temperature survival	–	Hemmati et al., 2014
		Pupa	Low temperature survival	–	Hemmati et al., 2014
	Tuta absoluta	Larva	CT_{min}	0.15	Tarusikirwa et al., 2020a
			HKDT	–	Tarusikirwa et al., 2020a
		Adult	HKDT	0.33	Tarusikirwa et al., 2020a
RCH	*Chilo partellus*	Larva	CT_{min}	0.1	Mutamiswa et al., 2018b
	Busseola fusca	Larva	CT_{min}	0.025	Mutamiswa et al., 2018b
	Tuta absoluta	Larva	CT_{min}	0.05	Tarusikirwa et al., 2020a
			HKDT	0.3	Tarusikirwa et al., 2020a
	Spodoptera exigua	Egg	Low temperature survival	–	Kim and Kim, 1997
		Larva	Low temperature survival	–	Kim and Kim, 1997
		Pupa	Low temperature survival	–	Kim and Kim, 1997
	Danaus plexippus	Adult	Low temperature survival	–	Larsen and Lee, 1994
	Cydia pomonella	Adult	Low temperature survival	–	Chidawanyika and Terblanche, 2011
RHH	*Chilo partellus*	Larva	CT_{min}	0.21	Mutamiswa et al., 2018b
	Busseola fusca	Larva	CT_{min}	0.13	Mutamiswa et al., 2018b
	Cydia pomonella	Adult	High temperature survival	–	Chidawanyika and Terblanche, 2011
	Pringleophaga marioni	Larva	Low temperature survival	–	Sinclair and Chown, 2003
	Phthorimaea operculella	Larva	Survival	–	Hemmati et al., 2014
		Pupa	Survival	–	Hemmati et al., 2014
Transgenerational	*Pararge aegeria*	Adult	Hatching success	–	Gibbs et al., 2010
		Adult	Egg mass	–	Gibbs et al., 2010
			Embryonic developmental time	–	Gibbs et al., 2010
	Bicyclus anynana	Adult	Egg size	–	Steigenga and Fischer, 2007

Table 3 (continued)

Form of Plasticity	Species	Life Stage	Improved Trait	H/ARR	Reference
		Adult	Hatching success	–	Fischer et al., 2003b
		Adult	Larval hatching mass	–	Geister et al., 2009
		Adult	Larval developmental time	–	Fischer et al., 2003b
Seasonal polyphenism	*Bicyclus anynana*	Adult	High temperature	–	Woestmann and Saastamoinen, 2016
	Polygonia c-album	Adult	Low temperature	–	Woestmann and Saastamoinen, 2016
	Pararge aegeria	Adult	Low temperature	–	Van Dyck and Wiklund, 2002
	Araschnia levana	Adult	Low temperature	–	Friberg and Karlsson, 2010
Carry-over effects	*Grapholita molesta*	Adult	Longevity	–	Zheng et al., 2017
		Adult	Survival	–	Zheng et al., 2017
	Plutella xylostella	Egg	Oviposition rate	–	Zhang et al., 2015a, b
		3rd instar larva	Oviposition rate	–	Zhang et al., 2015a, b

within ontogenetic stage in the order Lepidoptera. Although plasticity of thermal tolerance plays a pivotal role in buffering thermal stress in Lepidoptera, its costs to fitness should not be ignored. Therefore, costs of thermal plasticity on performance of lepidopteran species deserve further investigation. Developmental life stages are interdependent such that thermal stress experienced by one life stage in insect's life cycle may influence subsequent life stages within or across generations (Zheng et al., 2017) (Table 3). For example, heat stress (40 °C) on *P. xylostella* eggs and 3rd instar larvae resulted in faster oviposition rate in adults (Zhang et al., 2015a, b). Similarly, adult heat resistance and longevity significantly increased following heat stress exposure of 35 °C on *G. molesta* pupae (Zheng et al., 2017).

Seasonal polyphenism, a form of phenotypic plasticity arising from a single genotype, is a change in colour or pattern of butterfly larvae, pupae and adults in response to variations in environmental conditions such as temperature, humidity and photoperiod (Koi & Daniels, 2017; Villagra & Frías-Lasserre, 2020; Woestmann & Saastamoinen, 2016). Seasonal polyphenism which is also induced by diapause improve survival in lepidopterans through thermoregulation (see Gill et al., 2017; Koi & Daniels, 2017). For example, in *B. anynana*, it has been reported as an adaptive response to wet-dry seasonal environments. In temperate regions, seasonal polyphenism improved cold hardiness in *Polygonia c-album* (Linnaeus, 1758), *P. aegeria* and *Araschnia levana* (Linnaeus, 1758) through overwintering (diapausing) (Van Dyck & Wiklund, 2002; Woestmann & Saastamoinen, 2016). In addition, a key thermoregulatory mechanism in *Pieris occidentalis* (Reakirt, 1866) involved enhancement of a higher magnitude of melanisation in spring than summer as strategy of survival in temperate regions (Kingsolver, 1995; Kingsolver & Wiernasz, 1991).

Moreover, looking at hardening/acclimation response ratio (H/ARR), lepidopteran species such as *C. partellus*, *B. fusca*, *Sesamia calamistis* Hampson and *T. absoluta* seem to have higher H/ARR indicating stronger hardening and acclimation responses (see Table 3). This suggests that acclimation and hardening may allow these lepidopteran species to thermally adapt consequently being cushioned under changing environments.

Mechanisms mediating phenotypic plasticity

Production of heat shock proteins Heat shock proteins (Hsps) production is a key physiological response to environmental stress in insects such as heat, cold, overcrowding, starvation, desiccation and anoxia (Gu et al., 2019). They are categorised into several key families such as sHsp (small heat shock proteins), Hsp 40, Hsp 60, Hsp 70, Hsp 90 and Hsp 110 basing on molecular mass and protein homology (Garczynski et al., 2011; King & MacRae,

2015; Parsell & Lindquist, 1993) (see Fig. 1). The sHsps which are independent of adenosine triphosphate (ATP) are the first line of defence responsible for prevention of substrate proteins denaturation when cells face thermal stress (Basha et al., 2012). On the other hand, the ATP-dependent Hsps (Hsp 60, Hsp 70, Hsp 90 and Hsp 110) act as molecular chaperones and are responsible for folding, degrading, disaggregation, and localisation of denatured proteins (Cai et al., 2017; Gu et al., 2019). Production of Hsps in Lepidoptera is triggered by heat or cold shock and their induction result in improved thermal tolerance (Gu et al., 2019). For example, in *Spodoptera litura* (Fabricius, 1775) Hsp 60 and Hsp 90 were highly expressed in adult females (Shu et al., 2011) whereas in *Spodoptera exigua* (Hübner, 1808) and *Chilo suppressalis* (Walker, 1863) the highest expression of Hsp 70 was recorded in first and second instar larvae (Lu et al., 2014; Xu et al., 2011). Similarly, Hsp 90, Hsp 70 and Hsp 60 protected *C. suppressalis* larvae from extreme cold conditions through maintaining structural proteins and/or main metabolic enzymes under winter low temperatures (Lu et al., 2013). In addition, Hsp 70 was also highly expressed in *Helivoverpa zea* (Boddie, 1850) pupae (Zhang & Denlinger, 2010). In *Melitaea cinxia* (Linnaeus, 1758) heat shock at 40 °C triggered production of higher levels of Hsp 70 mRNA (Luo et al., 2015). Similarly, in *T. leucotreta* changes in Hsp 70 following pre-chilling treatments resulted in improved low temperature tolerance (Boardman et al., 2015). Exposure of *C. medinalis* larvae to heat shock at 37 and 41 °C induced increased levels of Hsp 70 and Hsp 90 mRNA resulting in enhanced heat tolerance (Gu et al., 2019). In *C. pomonella* sHsps (Hsp 19.8, Hsp 19.9 and Hsp 22.2) were upregulated in various developmental stages following heat shock at 42 °C indicating their significance in high temperature tolerance (see Garczynski et al., 2011). In addition, upregulation of sHsp genes improved cold tolerance in *S. litura* larvae (Shen et al., 2011).

The upregulation of Hsps during diapause also plays a pivotal role in improving cold hardiness in Lepidoptera (Rinehart et al., 2007). For example, sHsps genes upregulation has been reported in *G. molesta* during diapause (Zhang et al., 2015a, b) whereas Hsp 20.8 is closely linked to overwintering (diapause) in *Sesamia nonagrioides* (Lefebvre, 1827) larvae resulting in improved cold hardiness (Gkouvitsas et al., 2008). Diapause entry has been reported to upregulate Hsp 70 in embryonic diapause of *Lymantria dispar* (Linnaeus, 1758.) (Yocum et al., 1991), larval diapause in *Ostrinia nubilalis* (Hübner, 1796) and pupal diapause in *Manduca sexta* (Linnaeus, 1763) (Rinehart et al., 2007). *Grapholita molesta* is known to experience sub-lethal temperatures across seasons with low temperatures being encountered at night during early spring and autumn (Zhang et al., 2015a, b). As a result, adult moths may regulate expression of genes like GmHsp19.6 and GmHsp21.7 for survival (Zhang et al., 2015a, b). Response to high temperatures in summer may involve expression of genes such as GmHsp19.9 and GmHsp24.8 (Zhang et al., 2015a, b). Expression of Hsp 70 was observed in *Xestia c-nigrum* (Linnaeus, 1758) larvae and pupae following heat and cold shock thereby corroborating with high larval temperature tolerance and cold resistance in larvae and pupae (Wang et al., 2015). Several exoskeleton proteins have also been reported to aid thermal stress resistance in insects. For example, Nguyen et al. (2009) reported proteins 944 1109, 1367 and 1448 to improve heat resistance in winged and wingless aphids *Macrosiphum euphorbiae* Thomas, 1878 respectively. Similarly, these cuticle proteins have also been observed in Lepidoptera, likely facilitating thermal stress resistance (Quan et al., 2020). These mechanisms show that heat shock and cuticle proteins are the key drivers of thermal adaptation in Lepidoptera.

Freeze strategies The capacity of an insect to survive suboptimal low temperatures is termed cold hardiness. The phenomenon varies with species, developmental stage, diapause development, seasonality as well as severity and duration of cold exposure (Izadi et al., 2019; Sinclair et al., 2015). At sub-zero temperatures, there is high risk of ice formation, hence insects employ freeze tolerance and freeze avoidance as key strategies in surviving these extreme conditions (Feng et al., 2018) (Fig. 2). For example, in the northern hemisphere, most insects are predominantly freeze avoidance while in the southern hemisphere freeze tolerant insects are more predominant (Sinclair et al., 2003). Supercooling point (SCP), defined as the temperature at which ice begins to form within the body fluids after exposure to sub-zero temperature (also called the temperature of crystallization) is a significant indicator of the type of freeze strategy

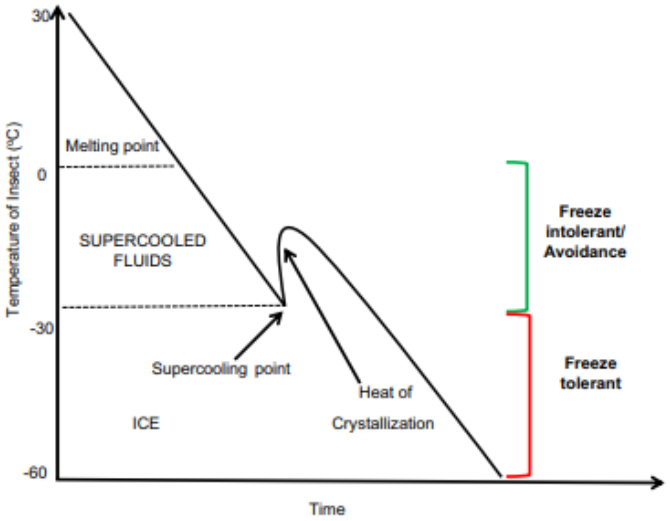

Fig. 2 Freeze strategy in Lepidoptera, redrawn from (Denlinger & Lee, 2010). Supercooling point represents the lowest temperature reached before release of the latent heat of crystallization. Freeze intolerant organisms (green) cannot tolerate freezing while freeze tolerant insects (red) can withstand internal ice formation

insects use (Feng et al., 2018; Hance et al., 2007) (Fig. 2).

Freeze avoidance or intolerant insects survive sub-zero temperatures in the absence of internal ice formation, but may be killed by any internal ice within their tissues (Sinclair et al., 2003). In that respect, to avoid lethal effects of freezing, these insects select favourable overwintering sites, increase body fat content and maintain their bodily fluids in a supercooled or liquid state below their melting point through accumulation of cryoprotectants and ice nucleation prevention (Andreadis & Athanassiou, 2017; Sinclair et al., 2015). Several lepidopteran species use this technique in enhancing their cold hardiness (see Table 4). In contrast, freeze tolerant insects survive ice formation within their tissues hence avoiding desiccation (Sinclair et al., 2003). Physiologically this may be accomplished through inoculative freezing, production of ice nucleating proteins, crystalloid proteins, crystalloid compounds and microbes (Lee & Costanzo, 1998). Nevertheless, chill susceptible insects which are killed by cold in the absence of internal ice formation are highly dependent on extensive supercooling capacity which facilitates survival under moderate low temperatures ranging from 0 °C to 5 °C (Sinclair et al., 2015; reviewed in Andreadis & Athanassiou, 2017). Many lepidopteran species survive sub-zero temperatures through freeze tolerance while a few survive via freeze avoidance and extensive supercooling (chill susceptible) (see Table 4).

Cross-tolerance and cross talk Lepidopteran species experience multiple abiotic and biotic stressors simultaneously in nature (Sinclair et al., 2013). These species may respond to these stressors through protective mechanisms (cross-tolerance) or shared signalling pathways (cross talk) (Sinclair et al., 2013). Cross talk is a mechanism under which a single stress triggers different signalling pathways which lead to physiological responses that offer protection against various stressors in nature (Sinclair et al., 2013). On the other hand, cross-tolerance is physiological adaptation whereby mechanisms protecting against a single stress also protect against another at cellular level (Sinclair et al., 2013). An understanding of whether physiological responses are a result of cross talk or cross-tolerance is significant in predicting outcomes of interactions among stressors (Sinclair et al., 2013) and co-occurring environmental stressors. Signalling pathways in response to stress have been well investigated in plants, with limited studies in insects (Knight & Knight, 2001).

Table 4 Freeze strategy in different developmental stages of lepidopteran species. The list may not be exhaustive but was compiled using literature available at the time of writing and is meant to show freeze strategy in some of the species and developmental stages within the order Lepidoptera

Species	Life Stage	Freeze Strategy	Reference
Ostrinia nubilalis	Larva	Freeze tolerance	Panko, 2017
Gynaephora groenlandica	Adult	Freeze tolerance	Panko, 2017
Sesamia nonagrioides	Larva	Freeze tolerance	Gillyboeuf et al., 1994
Ecpantheria scribonia	Larva	Freeze tolerance	Jack and Layne, 2004
Cisseps fulvicollis	Larva	Freeze tolerance	Fields and McNeil, 1986
Ctenucha virginica	Larva	Freeze tolerance	Fields and McNeil, 1988
Pyrrharctia isabella	Larva	Freeze tolerance	Goettel and Philogene, 1980
Hypercompe scribonia	Larva	Freeze tolerance	Jack et al., 2008
Aporia crataegi	Larva	Freeze tolerance	Li, 2016
Dendrolimus superans sibiricus	Larva	Freeze tolerance	Li, 2016
Cossus cossus	Larva	Freeze tolerance	Li, 2016
Pringleophaga marioni	Larva	Freeze tolerance	Marshall and Sinclair, 2011
Sesamia inferens	Larva	Freeze tolerance	Sun et al., 2014
Papilio polyxenes	Pupa	Freeze tolerance	Hazel, 2002
Tineola bisselliella	Egg	Freeze tolerance	Andreadis and Athanassiou, 2017
Cydia pomonella	Larva	Freeze avoidance	Neven, 1999; Khani et al., 2007
Hyphantria cunea	Larva	Freeze avoidance	Li et al., 2001
Epiblema scudderiana	Larva	Freeze avoidance	Rickards et al., 1986
Apatele psi	Pupa	Freeze avoidance	Li, 2016
Phthorimaea operculella	Larva	Freeze avoidance	Hemmati et al., 2014
	Pupa	Freeze avoidance	Hemmati et al., 2014
Thaumatotiba leucotreta	Larva	Chill susceptible	Boardman et al., 2012
Spodoptera exigua	Larva	Chill susceptible	Atapour and Moharramipour, 2014
Ephestia kuehniella	Pupa	Chill susceptible	Andreadis and Athanassiou, 2017
	Adult	Chill susceptible	Andreadis and Athanassiou, 2017
Plodia interpunctella	Pupa	Chill susceptible	Andreadis and Athanassiou, 2017
	Adult	Chill susceptible	Andreadis and Athanassiou, 2017
Chilo partellus	Larva	Chill susceptible	Mutamiswa et al., 2017b
	Pupa	Chill susceptible	Mutamiswa et al., 2017b
	Adult	Chill susceptible	Mutamiswa et al., 2017b
Tuta absoluta	Larva	Chill susceptible	Tarusikirwa et al., 2020b
	Adult	Chill susceptible	Tarusikirwa et al., 2020b

In Lepidoptera, cross-tolerance has been reported in cereal stemborers (Mutamiswa et al., 2018b). For example, RCH improved cold tolerance (measured as CT_{min}) in *C. partellus* larvae (Mutamiswa et al., 2018b). In addition, starvation and desiccation acclimation improved CT_{min} in *C. partellus*, *B. fusca* and *S. calamistis* larvae indicating cross-tolerance effects against cold stress (Mutamiswa et al., 2018b). Similarly, pre-treatment of *P. operculella* larvae and adults at 40 °C for 2 h significantly improved survival following exposure to sub-zero temperatures (Hemmati et al., 2014). Although there are limited studies on cross talk in Lepidoptera, there is need to explore this physiological mechanism in different species of this order and how this may influence adaptation in the face of climate change.

Metabolic responses Thermal stress in insects is a key factor responsible for induction of reactive oxygen species (ROS) resulting in protein dysfunction,

lipid peroxidation and oxidative damage (Ali et al., 2016; Liu et al., 2017). As a result, insects have evolved defence mechanisms such as upregulation of antioxidant enzymes which play a pivotal role in removal of ROS from biological systems. For example, increased production of ROS in *M. separata* following stressful high temperatures resulted in significant increase in primary antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) (Ali et al., 2016). Given its migration habit, this physiological adaptation mechanism has facilitated its fitness under warmer conditions resulting in optimisation of survival (Ali et al., 2016).

Similarly, SOD, peroxidase (POD) and thioredoxin were upregulated in *Glyphodes pyloalis* Walker, 1859 following heat stress (40 °C) (Liu et al., 2017). In addition, the induction of cytochrome P450s resulted in higher heat resistance in *G. pyloalis*. Moreover, two mRNAs for aldehyde dehydrogenase (ALDH) were upregulated following heat shock indicating enhanced heat resistance through metabolic pathway (Liu et al., 2017).

Digestion, absorption, detoxification of harmful substances and maintenance of water, ion and osmotic pressure balance in insects primarily take place in the mid gut (Lei et al., 2014; Valencia et al., 2016). As a result, the interplay between the insect and environmental conditions is governed by the mid gut (Liu et al., 2017). For instance, the mid gut transcriptome analyses in *G. pyloalis* showed that detoxification and vitamin digestion and absorption pathways were the key molecular mechanisms responsible for its heat stress resistance (Liu et al., 2017). Recent study on transcriptome analysis of *C. medinalis* to heat acclimation showed more differentially expressed genes (DEGs) expression in heat-acclimated than unacclimated larvae in response to heat stress (Quan et al., 2020). These genes are linked to structural components of cuticle and eye lens as well as development of sensory organs, key in heat stress tolerance. In addition, DEGs activated various pathways linked to longevity regulation, endoplasmic reticulum protein processing and immune systems in response to heat stress (Quan et al., 2020). These metabolic and molecular responses play a pivotal role in thermal adaptation of these aforementioned lepidopteran species under rapidly changing environments.

Developmental and ontogenetic responses

The developmental period for each life stage is dependent on lower developmental temperature and degree days required for development and this may influence population dynamics across seasons. For example, in a multivoltine sugarcane stemborer *Chilo auricilius* Dudgeon, 1905 larval developmental time is at least eightfold longer in winter than summer period indicating faster development during the latter than former (Kingsolver & Buckley, 2020). The temperature size rule (TSR) stipulates that exposure of an organism to high environmental thermal conditions during ontogeny results in rapid growth but smaller adult body size (Atkinson, 1994; Higgins et al., 2015). For example, heat treatments accelerated development in two different populations of *Colias eriphyle* Edwards, 1876 during the second to fourth developmental instars, albeit this effect disappeared following pupation (Higgins et al., 2015). This response indicates a potential for rapid population growth of *C. eriphyle* under changing environments due to considerable reduction in time to reproductive maturity.

Previous studies have reported variation in thermal sensitivity across developmental stages (reviewed in Kingsolver & Buckley, 2020). For instance, heat resistance in *B. anynana* increased with body size across ontogeny such that pupae and adults exhibited more resistance than eggs and larvae (Klockmann et al., 2017). This indicates a fitness and survival advantage of pupae and adults relative to eggs and larvae under rapidly changing environments. Similarly, the developmental times (days) for *T. absoluta* life stages decreased with increasing temperature such that eggs and pupae exhibited lower developmental times than larvae (de Campos et al., 2021). In addition, Li et al. (2013) reported a decrease in developmental duration of *A. lepigone* life stages as temperature increased from 18 to 30° C. Nevertheless, more research should extend to other lepidopteran species in both temperate and tropical regions to fully

understand the pattern of ontogenetic responses to thermal stress in the face of climate change.

Diapause and quiescence

Diapause is a physiological state of arrested growth and development that is genetically determined, neurohormonally mediated and often triggered by environment (Hance et al., 2007). It is common in insects and is usually induced by changes in photoperiod and temperature (Diniz et al., 2017). It can be categorically defined basing on the effects of environmental factors (facultative and obligatory), life stage (embryonic, larval, pupal and adult) and seasonal variations that is hibernation (winter diapause) or aestivation (summer diapause) (Gill et al., 2017). Aestivation is defined as summer or dry season dormancy for survival of arid environmental conditions (Storey & Storey, 2012). For example, in various tropical areas such as East Africa, Australia, India and Amazonia, dry seasons may be associated with extremely high temperatures resulting in butterfly species diurnal activities being affected (Hoskins, 2019). As such, most of the species in the family Nymphalidae are known to survive through aestivation, with moths initiating a robust suppression of metabolism, retention of body water, energy conservation and nitrogen metabolism before hiding in moist, cool dark places during dry mid-summer months (Storey & Storey, 2012). Some butterfly species in South America such as *Marpesia berania* (Hewitson, 1852) are known to hang from tree branches in a cluster of at least 60 moths (Hoskins, 2019). Similarly, in South-East Asia, *Danaus misippus* (Linnaeus, 1764) and *Euploea core* (Cramer, 1780) as well as *Heliconius* species in South America hang in groups of 6–30 adults from dry twigs. In addition, *Methona*, *Melinaea* and *Mechanitis* species often congregate along dry river beds in densely shaded areas in the forest (Hoskins, 2019).

On the other hand, hibernation defined as winter season dormancy involves depression of metabolism and energy consumption during low temperature winter months. For example, *Gonepteryx rhamni* (Linnaeus, 1758) adults hibernate beneath bramble or ivy leaves in winter (Hoskins, 2019). In addition, *Inachis io* (Linnaeus, 1758) *Nymphalis antiopa* (Linnaeus, 1758) and *Nymphalis polychloros* (Linnaeus, 1758) are known to hibernate under logs, hollow tree trunks and dark places such as caves or animal burrows. *Polygonia c-album* hibernates in the open, hanging from tree branches or in leaf litter on the forest floor (Hoskins, 2019). Diapause usually occurs at various developmental stages depending on species. For example, most butterfly species in temperate regions are known to overwinter as larvae, others as eggs or pupae while a few overwinter as adults e.g. *I. io, P. c-album* and *G. rhamni* (Hoskins, 2019).

In tropical regions, the larval stage of some cereal stemborers is the main developmental stage that overwinters in crop residues (Kfir, 1991; Ofomata et al., 1999). For example, during the dry season, larval *B. fusca, S. calamistis*, and *Chilo orichalcociliellus* Strand, 1911 undergo obligatory diapause for several months (Kfir et al., 2002; Ofomata et al., 1999) whereas *C. partellus* larvae undergo facultative diapause under unfavourable climatic conditions (Ofomata et al., 1999). *Chilo partellus* overwinters mostly in the lower parts of cereal plant stems for ~ 6–8 months where it acquires protection from extreme environmental conditions and natural enemies (Gill et al., 2017; Kfir, 1991). In addition, *C. suppressalis* larvae enter facultative diapause during autumn in response to changes in photoperiod (short day conditions) (Xiao et al., 2010). *Bombyx mori* (Linnaeus, 1758) and *L. dispar* overwinter at embryonic and larval stages respectively (Gill et al., 2017). Moreover, recent study reported facultative diapause in *T. absoluta* pupae developing from larvae following exposure to low temperatures and short-day length across different periods (de Campos et al., 2021). Some lepidopteran species undergo prolonged or extended diapause (> 1 year). For example, *Prodoxus y-inversus* Riley, 1892 moths are known to eclose after 19 years of prepupal diapause (Powell, 1989). Some lepidopteran species survive extreme conditions through undergoing both winter and summer diapause. These include *H. armigera* (Jadhav et al., 2013), *Pieris melete* Ménétriés, 1857, *Pieris brassicae* (Linnaeus, 1758) and *Mamestra brassicae* (Linnaeus, 1758) (Spieth et al., 2011; Xiao et al., 2013). Previous studies have shown that the incidence and severity of extreme variation in temperatures and cold snaps are gradually increasing globally (Harris et al., 2018). Therefore, under these aforementioned climatic conditions, diapause may play a pivotal role in overwintering success of many lepidopteran species in temperate and cooler climates and may further

confer enhanced cold hardiness in the absence of low temperature acclimation, which often occurs naturally during the transition from summer to fall and winter (Skendžić et al., 2021).

Although *Spodoptera frugiperda* (JE Smith, 1797) is not resistant to severe winters, it often survives winters through overwintering in southern Florida and Texas resulting in annual infestations in East and central United States as well as southern Canada (Nagoshi et al., 2012). Recent study reported that *S. frugiperda* larval and adult developmental stages have the potential to overwinter in arid tropical African environments given their low temperature tolerance and inability to undergo diapause (Keosentse et al., 2021). Similarly, larvae and adults of *T. absoluta* do not diapause and are known to overwinter in the Mediterranean region (Van Damme et al., 2015) and Africa (Tarusikirwa et al., 2020b) owing to their superior thermal resilience.

Quiescence on the other hand, is a form of dormancy manifesting as slowed development and metabolic activity following short period of unfavourable environmental conditions (Gill et al., 2017). It can be induced by one environmental condition and terminated by another e.g. low temperature stress may induce quiescence whereas high temperature may terminate it (Gill et al., 2017). For example, despite diapause termination, larvae of *C. suppressalis* remained in quiescence for three months in spring due to unfavourable climatic conditions (low temperatures) (Lu et al., 2013).

Genetic and evolutionary adaptation

Genetic adaptation is a heritable characteristic that enhances fitness and survival traits in organisms (Solomon & Hussen, 2018). Most organisms tend to use genetic adaptation in coping with environmental stresses such as extreme heat and cold (Solomon & Hussen, 2018). Some insects have genes responsible for adjusting their biological clocks in surviving shorter or longer winters hence improving their chances of adapting to changes in climate (Gotthard & Wheat, 2019; Kozak et al., 2019; Pruisscher et al., 2018). Synchronisation of morphological, behavioural and other changes across seasons is of paramount importance in most insects' life cycles (Kozak et al., 2019). Researchers have found two key genes that are responsible for permitting some insects to survive climate variability through regulating their biological annual clocks (Gotthard & Wheat, 2019). For example, in Lepidoptera, the two circadian clock genes period (*per*) and pigment dispersing factor receptor (*Pdfr*) positioned within two epistatic quantitative trait loci (QTL) were discovered in *O. nubilalis* and are responsible for enabling this moth to adapt to climate change e.g. shorter or longer winters, through diapause termination (Kozak et al., 2019). These two genes interact with circadian pacemaker neurons in the moth brain, where they play a pivotal role in synchronising biological activities to daily cycles of day and night (Kozak et al., 2019; Li et al., 2014). They vary in frequency of alleles amongst individual insects pupating earlier or later and this variation is responsible for eliciting evolutional timing of diapause under rapidly changing environments (Gotthard & Wheat, 2019; Kozak et al., 2019). For example, *per* and *Pdfr* regulate larval diapause termination in *O. nubilalis* through stimulating prothoracicotropic hormone (PTTH) to initiate release of development hormone, ecdysone from prothoracic gland (Gelman et al., 1992). Due to global change, winter seasons are projected to be milder and shorter in the next century, hence the survival of insects may be determined by the ability to adjust to these conditions (Gotthard & Wheat, 2019; Kozak et al., 2019). This implies that the ability to emerge from dormant state (diapause) early in winter may permit moths with these genes to generate a larger population. Therefore, a strong selection on variations of these genes may be important in thermal adaptation, range expansion and long-term species persistence under climate change.

Evolutionary adaptation plays a significant role in lepidopteran species through buffering selection pressure of climate variability (Hoffmann & Sgrò, 2011). This may occur through changes in gene frequency as well as genetic structure (Qi et al., 2019). In response to climate variability, insects tend to evolve through taking advantage of novel environmental conditions as well as tolerating new conditions which the population may not yet have adapted to (Franks & Hoffmann, 2012). Evidence from various insect taxa indicates that species tend to undergo rapid evolutionary change in novel environments thereby enhancing thermal adaptation (Gilchrist & Lee, 2007). This implies that genetic heterogeneity in phenotypic traits may be inherently

present amongst early invaders at the same time being passed on to subsequent generations. For example, exotic *C. partellus* exhibited high basal thermal tolerance and plasticity relative to indigenous species *B. fusca* and *S. calamistis* (Mutamiswa et al., 2017a, 2018b). This indicates that *C. partellus* may thrive in the short term by having higher basal or inherent resistance to prevailing climate, or mounting acute responses to these thermal extremes thus evading potentially lethal effects.

Over short and long time-scales, rapid evolutionary adaptation may thus favour selection of novel phenotypes in *C. partellus*. The latitudinal hypothesis stipulates that developmental plasticity should increase from low to high latitude areas in response to thermal seasonality increase (Manenti et al., 2017). As a result, most insects have recently shifted their ranges to higher altitudes and latitudes due to global change (Hill et al., 2011). In this regard, geographic clines of these insects are a result of evolutionary adaptation to local environmental conditions (Hill et al., 2011). For example, *C. partellus* was previously limited to low and mid elevation areas (Zhou et al., 2001) and has now extended to high altitude and humid transitional areas (Khadioli et al., 2014) thereby displacing indigenous stemborer species (Kfir, 1997; Mutamiswa et al., 2017a). This has led to this species selecting for increased resistance to stressful conditions as well as mechanisms of circumventing these extremes relative to other species. As a result, this has enhanced adaptation to the novel environments thereby perpetuating the population. The biogeography of some lepidopteran species is strongly correlated with their basal thermal tolerance and plasticity patterns. For example, basal low temperature tolerance in *Lycaena tityrus* (Poda, 1761) varied across altitudes such that CCRT decreased with increased altitude indicating an enhanced cold hardiness in higher altitude insects (Karl et al., 2008). This capacity to thermally adapt to low temperatures may enhance overwintering survival as well as permitting higher levels of activity such as development, mating, oviposition and dispersal (Karl et al., 2008). In contrast, HKDT decreased with increasing altitude such that insects of high altitude were knocked down earlier (low heat stress resistance) than those of low altitude. In addition, low altitude butterfly populations were more temperature plastic than those of high altitude. This implies that high altitude butterflies have a greater potential to adjust fast their heat stress resistance under extreme conditions thereby optimising key life history traits (e.g. mating and feeding) (Karl et al., 2008).

Prospects for future research

Global climate change may have negative effects on abundance and distribution of lepidopteran species hence thermal adaptation remains a key strategy in circumventing stress. The increasing rapid evolutionary responses in some insect species due to these changes show the significance of understanding adaptation mechanisms including the genetic basis to environmental stress adaptation (Franks & Hoffmann, 2012). However, in lepidopteran species these studies are limited.

Therefore, there is need to extend these studies to Lepidoptera through combining ecological approaches, molecular and quantitative genetics in unravelling their responses to changing climate environments. Epigenetic mechanisms such as methylation of genes may contribute to transgenerational plasticity in insects across environments (Sgrò et al., 2016). Given that methylation of genes may persist for between one and five generations due to changes in the environment, alteration of methylation patterns may substantially affect climate change adaptation (Sgrò et al., 2016).

Thermal stress may lead to epigenetic modifications in insects resulting in key genetic changes (Franks & Hoffmann, 2012) Despite these modifications playing a pivotal role in gene regulation and expression, their role in thermal stress adaptation in various insect populations remain unclear (Franks & Hoffmann, 2012). Thus, exploring their roles in Lepidoptera may be a fruitful endeavour.

It is also of paramount importance to understand how plastic responses may be affected by various environmental components e.g. temperature, rainfall and their fluctuations (Sgrò et al., 2016). Previous studies on plasticity of thermal tolerance mainly focused on constant temperatures and other conditions, with recent studies now incorporating fluctuating thermal conditions (Sgrò et al., 2016). However, there are no studies that have looked into both fluctuating temperature and relative humidity effects in Lepidoptera in the face

of climate change (but see Mutamiswa et al., 2019). With the incidence and severity of climate 'presses' and 'pulses' such as extreme variation in temperatures and rainfall, heatwaves, cold snaps, drought and floods expected to increase (Harris et al., 2018), future studies should incorporate these projected changes and their interactions thereof in Lepidoptera across short and longer time scales and how these insects may adapt to these environmental changes.

Although there has been increasing interest by insect physiologists in transgenerational effects, most studies have focused on plasticity of thermal response within ontogenetic stages and not across longer-time scales (Sgrò et al., 2016). In addition, there are limited studies on transgenerational plasticity in Lepidoptera. Furthermore, its effects on the capacity of species response to changes in climate are largely unexplored (Münzbergová & Hadincová, 2017). Therefore, there is need to explore the significance of transgenerational plasticity spanning across multiple generations and response to multiple environmental factors in Lepidoptera.

An understanding of geographic clinal variation in basal thermal tolerance and plasticity thereof between populations and variations amongst populations is significant in predicting insects' response to global change (Jensen et al., 2019). Geographic latitudinal clines in various quantitative traits have been reported in different *Drosophila* species indicating evidence of thermal adaptation in the environment (Hoffmann & Weeks, 2007). In Lepidoptera, basal low temperature tolerance varied in *L. tityrus* across altitudes (Karl et al., 2008). Therefore, future studies should extend to other key lepidopteran species in both tropical and temperate regions to fully understand their thermal adaptation under global change.

Conclusion

Thermal adaptation remains a critical mechanism for enhancing organismal function, fitness and survival in Lepidoptera. This review unravels adaptive behavioural, morphological, physiological and evolutionary mechanisms that promote survival in Lepidoptera. These adaptive strategies are key to maintaining fitness in both native and novel constantly shifting environments. For invasive species, this may impact on population level invasion potential. These attributes are significant for biodiversity conservation, biosecurity and preservation of keystone species. As a model taxon, future research and development should focus on epigenetic effects, transgenerational plasticity and latitudinal clines effects on thermal adaptation under global change. This information is significant in accurate predictions and refining future management of some of the insects of economic importance in this order.

Acknowledgements Authors would like to acknowledge valuable support from University of the Free State (UFS) to FC, AM and RM, Botswana International University of Science and Technology (BIUST) to CN, International Centre of Insect Physiology and Ecology to FC as well as Midlands State University and Rhodes University to RM and CN. We also thank several anonymous referees for the constructive comments in an earlier version of this manuscript.

Author contributions Reyard Mutamiswa: Writing, Visualisation
Abongile Mbande: Writing
Casper Nyamukondiwa: Conceptualization, Validation, Revision
Frank Chidawanyika: Conceptualization, Writing, Validation, Revision

Funding Open access funding provided by University of the Free State.

Data availability Not applicable

Declarations

Competing interests The authors declare no competing interests.

References

Alerstam, T., Chapman, J. W., Bäckman, J., Smith, A. D., Karlsson, H., Nilsson, C., Reynolds, D. R., Klaassen, R. H., & Hill, J. K. (2011). Convergent patterns of long-distance nocturnal migration. in noctuid moths and passerine birds. *Proceedings of the Royal Society B: Biological Sciences, 278*, 3074–3080.

Ali, A., Rashid, M. A., Huang, Q. Y., Wong, C., & Lei, C. L. (2016). Response of antioxidant enzymes in *Mythimna separata* (Lepidoptera: Noctuidae) exposed to thermal stress. *Bulletin of Entomological Research, 107*, 382–390.

Altermatt, F. (2009). Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society B: Biological Sciences, 277*, 1281–1287.

Andreadis, S. S., & Athanassiou, G. C. (2017). A review of insect cold hardiness and its potential in stored product insect control. *Crop Protection, 91*, 93–99.

Andrew, N. R., Hart, R. A., Jung, M. P., Hemmings, Z., & Terblanche, J. S. (2013). Can temperate insects take the heat? A case study of the physiological and behavioural responses in a common ant, *Iridomyrmex purpureus* (Formicidae), with potential climate change. *Journal of Insect Physiology, 59*, 870–880.

Ashton, S., Gutierrez, D., & Wilson, R. J. (2009). Effects of temperature and elevation on habitat use by a rare mountain butterfly: Implications for species responses to climate change. *Ecological Entomology, 34*, 437–446.

Atapour, M., & Moharramipour, S. (2014). Cold hardiness process of beet armyworm larvae, *Spodoptera exigua* (Lepidoptera: Noctuidae). *Journal of Crop Protection, 3*, 147–158.

Atkinson, D. (1994). Temperature and organism size – a biological law for ectotherms? In M. Begon & A. H. Fitter (Eds.), *Advances in ecological research* (pp. 1–58). Academic Press.

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., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. *Global Change Biology, 8*, 1–16.

Basha, E., O'Neill, H., & Vierling, E. (2012). Small heat shock proteins andα-crystallins: Dynamic proteins with flexible functions. *Trends in Biochemical Sciences, 37*, 106–117.

Bauerfeind, S. S., & Fischer, K. (2013). Increased temperature reduces herbivore host-plant quality. *Global Change Biology, 19*, 3272–3282.

Berwaerts, K., Van Dyck, H., Vints, E., & Matthysen, E. (2001). Effect of manipulated wing characteristics and basking posture on thermal properties of the butterfly *Pararge aegeria* (L.). *Journal of Zoology, 255*, 261–267.

Boardman, L., Grout, T. G., & Terblanche, J. S. (2012). False codling moth *Thaumatotiba leucotreta* (Lepidoptera, Tortricidae) larvae are chill-susceptible. *Insect Science, 19*, 315–328.

Boardman, L., Sørensen, J. G., & Terblanche, J. S. (2015). Physiological and molecular mechanisms associated with cross tolerance between hypoxia and low temperature in *Thaumatotibia leucotreta*. *Journal of Insect Physiology, 82*, 75–84.

Bodlah, M. A., Gu, L.-L., Tan, Y., & Liu, X.-D. (2017). Behavioural adaptation of the rice leaf folder *Cnaphalocrocis medinalis* to short-term heat stress. *Journal of Insect Physiology, 100*, 28–34.

Bryant, S. R., Thomas, C. D., & Bale, J. S. (1997). Nettle-feeding nymphalid butterflies: Temperature, development and distribution. *Ecological Entomology, 22*, 390–398.

Cai, Z., Chen, J., Cheng, J., & Lin, T. (2017). Overexpression of three heat shock proteins protects *Monochamus alternatus* (Coleoptera: Cerambycidae) from thermal stress. *Journal of Insect Science, 17*, 113.

Casey, T. M. (1976). Activity patterns, body temperature and thermal ecology in two desert caterpillars (Lepidoptera: Sphingidae). *Ecology, 57*, 485–497.

Cavieres, G., Rezende, E. L., Clavijo-Baquet, S., Alruiz, J. M., Rivera-Rebella, C., Boher, F., & Bozinovic, F. (2020). Rapid within- and transgenerational changes in thermal tolerance and fitness in variable thermal landscapes. *Ecology and Evolution, 10*, 8105–8113.

Chang, S. S., Lo, Z. C., Keng, C. G., Li, G. Z., Chen, X. L., & Wu, X. W. (1980). Studies on the migration of rice leaf roller *Cnaphalocrocis medinalis* Guenee. *Acta Entomologica Sinica, 23*, 130–140.

Chapman, J. W., Nesbit, R. L., Burgin, L. E., Reynolds, D. R., Smith, A. D., Middleton, D. R., & Hill, J. K. (2010). Flight orientation behaviors promote optimal migration trajectories in high-flying insects. *Science, 327*, 682–685.

Chevin, L. M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLOS Biology, 8*, e1000357.

Chidawanyika, F., & Terblanche, J. S. (2011). Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology, 57*, 108–117.

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

Clusella-Trullas, S., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms. *Journal of Thermal Biology, 32*, 235–245.

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology, 60*, 123–140.

Coombs, M. (1993). Endothermy and Flight Thresholds for *Helicoverpa punctigera* and *Helicoverpa armigera* (Lepidoptera, Noctuidae). *Australian Journal of Zoology, 41*, 577–587.

Crill, W., Huey, R., & Gilchrist, G. (1996). Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution, 50*, 1205–1218.

Davis, A. K., Farrey, B. D., & Altizer, S. (2005). Variation in thermally induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. *Journal of Thermal Biology, 30*, 410–421.

de Campos, M. R., Béarez, P., Amiens-Desneux, E., Ponti, L., Gutierrez, A. P., Biondi, A., Adiga, A., & Desneux, N. (2021). Thermal biology of *Tuta absoluta*: Demographic parameters and facultative diapause. *Journal of Pest Science, 94*, 829–842.

Denlinger, D. L., & Lee, R. E. Jr. (Eds.). (2010). *Low temperature biology of insects*. Cambridge University Press.

Diniz, D. F. A., de Albuquerque, C. M. R., Oliva, L. O., de Melo-Santos, M. A. V., & Ayres, C. F. J. (2017). Diapause and quiescence: Dormancy mechanisms that

contribute to the geographical expansion of mosquitoes and their evolutionary success. *Parasites & Vectors, 10*, 310.

Dongmo, M. A. K., Hanna, R., Smith, T. B., Fiaboe, K. K. M., Fomena, A., & Bonebrake, T. C. (2021). Local adaptation in thermal tolerance for a tropical butterfly across ecotone and rainforest habitats. *Biology Open, 10*, bio058619.

Dorsett, D. A. (1962). Preparation for flight by hawk-moths. *Journal of Experimental Biology, 39*, 579–588.

Ellers, J., & Boggs, C. L. (2002). The evolution of wing color in *Colias* butterflies: Heritability, sex linkage, and population divergence. *Evolution, 56*, 836–840.

Ellers, J., & Boggs, C. L. (2004). Functional ecological implications of intraspecific differences in wing melanization in *Colias* butterflies. *Biological Journal of the Linnean Society, 82*, 79–87.

Engelbrecht, F., Adegoke, J., Bopape, M. J., Naidoo, M., Garland, R., Thatcher, M., McGregor, J., Katzfey, J., Werner, M., Ichoku, C., & Gatebe, C. (2015). Projections of rapidly rising surface temperatures over Africa under low mitigation. *Environmental Research Letters, 10*, 085004.

Fedic, R., Zurovec, M., & Sehnal, F. (2002). The silk of Lepidoptera. *Journal of Insect Biotechnology and Sericology, 71*, 1–15.

Feng, Y., Zhang, L., Li, W., Yang, X., & Zong, S. (2018). Cold hardiness of overwintering larvae of *Sphenoptera* sp. (Coleoptera: Buprestidae) in Western China. *Journal of Economic Entomology, 111*, 247–251.

Fields, P. G., & McNeil, J. N. (1986). Possible dual cold hardiness strategies in *Cisseps fulvicollis* (Lepidoptera: Arctiidae). *Canadian Entomologist, 118*, 1309–1311.

Fields, P. G., & McNeil, J. N. (1988). The cold hardiness of *Ctenucha virginica* (Lepidoptera: Arctiidae) larvae, a freezing-tolerant species. *Journal of Insect Physiology, 34*, 269–277.

Fischer, K., Eenhoorn, E., Bot, A. N., Brakefield, P. M., & Zwaan, B. J. (2003a). Cooler butterflies lay larger eggs: Developmental plasticity versus acclimation. *Proceedings of the Royal Society B: Biological Sciences, 270*, 2051–2056.

Fischer, K., Brakefield, P. M., & Zwaan, B. J. (2003b). Plasticity in butterfly egg size: Why larger offspring at lower temperatures? *Ecology, 84*, 3138–3147.

Fischer, K., Dierks, A., Franke, K., Geister, T. L., Liszka, M., Winter, S., & Pflicke, C. (2010). Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus anynana*. *Plos One, 5*, e15284. https://doi.org/10.1371/journal.pone.0015284

Foray, V., Desouhant, E., & Gibert, P. (2014). The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps. *Functional Ecology, 28*, 411–423.

Forsman, A., Betzholtz, P.-E., & Franzén, M. (2016). Faster poleward range shifts in moths with more variable colour patterns. *Scientific Reports, 6*, 36265.

Franks, S. J., & Hoffmann, A. A. (2012). Genetics of climate change adaptation. *Annual Review of Genetics, 46*, 185–208.

Frears, S. L., Chown, S. L., & Webb, P. I. (1997). Behavioural thermoregulation in the mopane worm (Lepidoptera). *Journal of Thermal Biology, 22*, 325–330.

Friberg, M., & Karlsson, B. (2010). Life-history polyphenism in the map butterfly *Araschnia levana*: Developmental constraints versus season-specific adaptations. *Evolutionary Ecology Research, 12*, 603–615.

Garczynski, S. F., Unruh, T. R., Guedot, C., & Neven, L. G. (2011). Characterization of three transcripts encoding small heat shock proteins expressed in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *Insect Science, 18*, 473–483.

Geister, T. L., Lorenz, M. W., Hoffmann, K. H., & Fischer, K. (2009). Energetics of embryonic development. Effects of temperature on egg and hatchling composition in a butterfly. *Journal of Comparative Physiology B, 179*, 87–98.

Gelman, D. B., Thyagaraja, B. S., Kelly, T. J., Masler, E. P., Bell, R. A., & Borkovec, A. B. (1992). Prothoracicotropic hormone levels in brains of the European corn borer, *Ostrinia nubilalis*: Diapause vs the non-diapause state. *Journal of Insect Physiology, 38*, 383–395.

Ghazanfar, M., Malik, M. F., Hussain, M., Iqbal, R., & Younas, M. (2016). Butterflies and their contribution in ecosystem: A review. *Journal of Entomology and Zoology Studies, 4*, 115–118.

Gibbs, M., Van Dyck, H., & Karlsson, B. (2010). Reproductive plasticity, ovarian dynamics and maternal effects in response to temperature and flight in *Pararge aegeria*. *Journal of Insect Physiology, 56*, 1275–1283.

Gilchrist, G. W., & Lee, C. E. (2007). All stressed out and nowhere to go: Does evolvability limit adaptation in invasive species? *Genetica, 129*, 127–132.

Gill, H. K., Goyal, G., & Chahil, G. (2017). Insect diapause: A review. *Journal of Agricultural Science and Technology A, 7*, 454–473.

Gillyboeuf, N., Anglade, P., Lavenseau, L., & Peypelut, L. (1994). Cold hardiness and overwintering strategy of the pink maize stalk borer, *Sesamia nonagrioides* Lef (lepidoptera, noctuidae). *Oecologia, 99*, 366–373.

Gkouvitsas, T., Kontogiannatos, D., & Kourti, A. (2008). Differential expression of two small Hsps during diapause in the corn stalk borer *Sesamia nonagrioides* (Lef.). *Journal of Insect Physiology, 54*, 1503–1510.

Goettel, M. S., & Philogene, B. J. R. (1980). Further studies on the biology of the banded woolly bear, *Pyrrharctia (Isia) Isabella* (J.E. Smith) (Lepidoptera: Arctiidae). IV. Diapause development as influenced by temperature. *Canadian Journal of Zoology, 58*, 317–320.

Gotthard, K., & Wheat, C. W. (2019). Diapause: Circadian clock genes are at it again. *Current Biology, 29*, R1245–R1246.

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*, 1001–1007.

Gu, L.-L., Li, M.-Z., Wang, G.-R., & Liu, X.-D. (2019). Multigenerational heat acclimation increases thermal tolerance and expression levels of Hsp70 and Hsp90 in the rice leaf folder larvae. *Journal of Thermal Biology, 81*, 103–109.

Guppy, C. S. (1986). The adaptative significance of alpine melanism in the butterfly *Parnassius phoebus* (Lepidoptera: Papilionidae). *Oecologia, 70*, 205–213.

Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. Á., Duarte, H., Nicieza, A., & Solé, M. (2016). Testing the

climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography, 43*, 1166–1178.

Hance, T., van Baaren, J., Vernon, P., & Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology, 52*, 107–126.

Harris, R. M. B., Beaumont, L. J., Vance, T. R., Tozer, C. R., Remenyi, T. A., Perkins-Kirkpatrick, S. E., Mitchell, P. J., Nicotra, A. B., McGregor, S., Andrew, N. R., Letnic, M., Kearney, M. R., Wernberg, T., Hutley, L. B., Chambers, L. E., Fletcher, M. S., Keatley, M. R., Woodward, C. A., Williamson, G., … Bowman, D. M. J. S. (2018). Biological responses to the press and pulse of climate trends and extreme events. *Nature Climate Change, 8*, 579–587.

Hazel, W. N. (2002). The environmental and genetic control of seasonal polyphenism in larval color and its adaptive significance in a swallowtail butterfly. *Evolution, 56*, 342–348.

Heinrich, B. (1987). Thermoregulation in winter moths. *Scientific American, 256*, 104–111.

Heinrich, B., & Casey, T. M. (1973). Metabolic rate and endothermy in Sphinx Moths. *Journal of Comparative Physiology, 82*, 195–206.

Hemmati, C., Moharramipour, S., & Talebi, A. A. (2014). Effects of cold acclimation, cooling rate and heat stress on cold tolerance of the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *European Journal of Entomology, 111*, 487–494.

Higgins, J. K., Maclean, H. J., Buckley, L. B., & Kingsolver, J. G. (2015). Growth, developmental and stress responses of larvae of the clouded sulphur butterfly *Colias eriphyle* to repeated exposure to high, sub-lethal temperatures. *Physiological Entomology, 40*, 189–195.

Hill, L. (2007). *Agrotis* (Lepidoptera: Noctuidae) species in Tasmania including montane, summer aestivation of the bogong moth, *Agrotis infusca* (Boisduval, 1832). *Veteran Entomologist, 37*, 3–9.

Hill, J. K., Griffiths, H. M., & Thomas, C. D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annual Review of Entomology, 56*, 143–159.

Hill, G. M., Kawahara, A. Y., Daniels, J. C., Bateman, C. C., & Scheffers, B. R. (2021). Climate change effects on animal ecology: Butterflies and moths as a case study. *Biological Reviews, 96*, 2113–2126.

Hoffmann, A. A. (2017). Rapid adaptation of invertebrate pests to climatic stress? *Current Opinion in Insect Science, 21*, 7–13.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature, 470*, 479–485.

Hoffmann, A. A., & Weeks, A. R. (2007). Climatic selection on genes and traits after a 100 year-old invasion: A critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. *Genetica, 129*, 133. https://doi.org/10.1007/s10709-006-9010-z

Hoskins, A. (2019). *Learn About Butterflies: The complete guide to the world of butterflies and moths*. New Holland Publishers.

Hufnagel, L., & Kocsis, M. (2011). Impacts of climate change on Lepidoptera species and communities. *Applied Ecology and Environmental Research, 9*, 43–72.

IPCC. (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, pp 151.

Izadi, H., Mohammadzadeh, M., & Mehrabian, M. (2019). Cold tolerance of the *Tribolium castaneum* (Coleoptera:Tenebrionidae), under different thermal regimes: Impact of cold acclimation. *Journal of Economic Entomology, 112*, 1983–1988.

Jack, R., & Layne, Jr. (2004). Freeze tolerance and cryoprotection in caterpillars of the giant leopard moth (*Ecpantheria scribonia* Lepidoptera: Arctiidae). *Journal of Thermal Biology, 30*, 267–271.

Jack, R., Layne, Jr., & Leszczynski, F. (2008). Cold Hardiness and Postfreeze Metabolism in Caterpillars of *Hypercompe scribonia* (Arctiidae: Lepidoptera). *Environmental Entomology, 37*, 1069–1073.

Jadhav, D. R., Armes, N. J., & Bhatnagar, V. S. (2013). Incidence of winter and summer diapause in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Andhra Pradesh, India. *Asian Journal of Agricultural Sciences, 5*, 40–51.

Jensen, A., Alemu, T., Alemneh, T., Pertoldi, C., & Bahrndorff, S. (2019). Thermal acclimation and adaptation across populations in a broadly distributed soil arthropod. *Functional Ecology, 33*, 833–845.

Ju, R.-T., Gao, L., Zhou, X.-H., & Li, B. (2013). Tolerance to high temperature extremes in an invasive Lace bug, *Corythucha ciliata* (Hemiptera: Tingidae), in subtropical China. *Plos One, 8*, e54372.

Käfer, H., Kovac, H., Simov, N., Battisti, A., Erregger, B., Schmidt, A. K. D., & Stabentheiner, A. (2020). Temperature tolerance and thermal environment of European seed bugs. *Insects, 11*, 197. https://doi.org/10.3390/insects11030197

Karl, I., Janowitz, S. A., & Fischer, K. (2008). Altitudinal life-history variation and thermal adaptation in the copper butterfly *Lycaena tityrus*. *Oikos, 117*, 778–788.

Karl, I., Becker, M., Hinzke, T., Mielke, M., Schiffler, M., & Fischer, K. (2014). Interactive effects of acclimation temperature and short-term stress exposure on resistance traits in the butterfly *Bicyclus anynana*. *Physiological Entomology, 39*, 222–228.

Karuppaiah, V., & Sujayanad, G. K. (2012). Impacts of climate change on population dynamics of insect pests. *World Journal of Agricultural Sciences, 8*, 240–246.

Kaspari, M., Clay, N. A., Lucas, J., Yanoviak, S. P., & Kay, A. (2015). Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology, 21*, 1092–1102.

Kemp, D. J., & Krockenberger, A. K. (2004). Behavioural thermoregulation in butterflies: The interacting effects of body size and basking posture in *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae). *Australian Journal of Zoology, 52*, 229–239.

Keosentse, O., Mutamiswa, R., Du Plessis, H., & Nyamukondiwa, C. (2021). Developmental stage variation in

Spodoptera frugiperda (Lepidoptera: Noctuidae) low temperature tolerance: Implications for overwintering. *Austral Entomology, 60*, 400–410.

Keosentse, O., Mutamiswa, R., & Nyamukondiwa, C. (2022). Interaction effects of desiccation and temperature stress resistance across *Spodoptera frugiperda* (Lepidoptera, Noctuidae) developmental stages. *NeoBiota, 73*, 87–108.

Kfir, R. (1991). Duration of diapause in the stem borers, *Busseola fusca* and *Chilo partellus*. *Entomologia Experimentalis et Applicata, 61*(3), 265–270. https://doi.org/10.1111/j.1570-7458.1991.tb01559.x

Kfir, R. (1997). Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepdoptera: Pyralidae). *Annals of the Entomological Society of America, 90*, 619–624.

Kfir, R., Overholt, W. A., Khan, Z. R., & Polaszek, A. (2002). Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annual Review of Entomology, 47*, 701–713.

Khadioli, N., Tonnang, Z. E. H., Muchugu, E., Ong'amo, G., Achia, T., Kipchirchir, I., Kroschel, J., & Le Ru, B. (2014). Effect of temperature on the phenology of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), simulation and visualization of the potential future distribution of *C. partellus* in Africa under warmer temperatures through the development of life-table parameters. *Bulletin of Entomological Research, 104*, 809–822.

Khani, A., Moharramipour, S., & Barzegar, M. (2007). Cold tolerance and trehalose accumulation in overwintering larvae of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *European Journal of Entomology, 104*, 385–392.

Kim, Y., & Kim, N. (1997). Cold hardiness in *Spodoptera exigua* (Lepidoptera: Noctuidae). *Environmental Entomology, 26*, 1117–1123.

King, A. M., & MacRae, T. H. (2015). Insect heat shock proteins during stress and diapause. *Annual Review of Entomology, 60*, 59–75.

Kingsolver, J. G. (1983). Thermoregulation and flight in *Colias* butterflies: Elevational patterns and mechanistic limitations. *Ecology, 64*, 534–545.

Kingsolver, J. G. (1985). Butterfly thermoregulation: Organismic mechanisms and population consequences. *Journal of the Lepidopterists' Society, 24*, 1–20.

Kingsolver, J. G. (1995). Viability selection on seasonally polyphenic traits: Wing melanin pattern in western white butterflies. *Evolution, 49*, 932–941.

Kingsolver, J. G., & 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., & Moffat, R. J. (1982). Thermoregulation and the determinants of heat transfer in Colias butterflies. *Oecologia, 53*, 27–33.

Kingsolver, J. G., & Watt, W. (1983). Thermoregulatory strategies in *Colias* butterflies thermal stress and the limits to adaptation in temporally varying environments. *American Naturalist, 121*, 32–55.

Kingsolver, J. G., & Wiernasz, D. C. (1991). Seasonal polyphenism in wing-melanin pattern and thermoregulatory adaptation in *Pieris* butterflies. *The American Naturalist, 137*, 816–830.

Kleckova, I., & Klecka, J. (2016). Facing the heat: Thermoregulation and behaviour of lowland species of a cold-dwelling butterfly Genus *Erebia*. *PLoS ONE, 11*, e0150393.

Klockmann, M., Gunter, F., & Fischer, K. (2017). Heat resistance throughout ontogeny: Body size constraints thermal tolerance. *Global Change Biology, 23*, 686–696.

Knapp, R., & Casey, T. M. (1986). Thermal ecology, behavior, and growth of gypsy moth and eastern tent caterpillars. *Ecology, 67*, 598–608.

Knight, H., & Knight, M. R. (2001). Abiotic stress signalling pathways: Specificity and cross-talk. *Trends in Plant Science, 6*, 262–267.

Koi, S., & Daniels, J. (2017). Life history variations and seasonal polyphenism in *Eumaeus atala* (Lepidoptera: Lycaenidae). *Florida Entomologist, 100*, 219–229.

Kozak, G. M., Wadsworth, C. B., Kahne, S. C., Bogdanowicz, S. M., Harrison, R. G., Coates, B. S., & Dopman, E. B. (2019). Genomic basis of circannual rhythm in the European corn borer moth. *Current Biology, 29*, 3501–3509.

Kührt, U., Samietz, J., & Dorn, S. (2005). Thermoregulation behaviour in codling moth larvae. *Physiological Entomology, 30*, 54–61.

Larsen, K. J., & Lee, R. E. (1994). Cold tolerance including rapid cold-hardening and inoculative freezing of fall migrant Monarch butterflies in Ohio. *Journal of Insect Physiology, 40*, 859–864.

Lee, R. E., & Costanzo, J. P. (1998). Biological ice nucleation and ice distribution in cold-hardy ectothermic animals. *Annual Review of Physiology, 60*, 55–72.

Lei, Y., Zhu, X., Xie, W., Wu, Q., Wang, S., Guo, Z., Xu, B., Li, X., Zhou, X., & Zhang, Y. (2014). Midgut transcriptome response to a Cry toxin in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Gene, 533*, 180–187.

Li, N. G. (2016). Strong tolerance to freezing is a major survival strategy in insects inhabiting central Yakutia (Sakha Republic, Russia), the coldest region on earth. *Cryobiology, 73*, 221–225.

Li, Y. P., Goto, M., Ito, S., Sato, Y., Sasaki, K., & Goto, N. (2001). Physiology of diapause and cold hardiness in the overwintering pupae of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae) in Japan. *Journal of Insect Physiology, 47*, 1181–1187.

Li, L. T., Wang, Y. Q., Ma, J. F., Liu, L., Hao, Y. T., Dong, C., Gan, Y. J., Dong, Z. P., & Wang, Q. Y. (2013). The effects of temperature on the development of the moth *Athetis lepigone*, and a prediction of field occurrence. *Journal of Insect Science, 13*, 103.

Li, Y., Guo, F., Shen, J., & Rosbash, M. (2014). PDF and cAMP enhance PER stability in Drosophila clock neurons. *Proceedings of the National Academy of Sciences, 111*, E1284–E1290.

Liu, Y., Su, H., Li, R., Li, X., Xu, Y., Dai, X., Zhou, Y., & Wang, H. (2017). Comparative transcriptome analysis of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) reveals novel insights into heat stress tolerance in insects. *BMC Genomics, 18*, 974.

Lu, M. X., Cao, S. S., Du, Y. Z., Liu, Z. X., Liu, P., & Li, J. (2013). Diapause, signal and molecular characteristics of overwintering *Chilo suppressalis* (Insecta: Lepidoptera: Pyralidae). *Scientific Reports, 3*, 3211.

Lu, M. X., Liu, Z. X., Cui, Y. D., & Du, Y. Z. (2014). Expression patterns of three heat shock proteins in *Chilo suppressalis* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America, 107*, 667–673.

Luo, S., Ahola, V., Shu, C., Xu, C., & Wang, R. (2015). Heat shock protein 70 gene family in the Glanville fritillary butterfly and their response to thermal stress. *Gene, 556*, 132–141.

Machekano, H., Mvumi, B. M., & Nyamukondiwa, C. (2018a). Loss of coevolved basal and plastic responses to temperature may underlie trophic level host-parasitoid interactions under global change. *Biological Control, 118*, 44–54.

Machekano, H., Mvumi, B. M., & Nyamukondiwa, C. (2018b). *Sitotroga cerealella* (Olivier) resilience to extreme temperature and desiccation may explain its increasing pest status in changing climates. 12th International Working Conference on Stored Product Protection (IWCSPP) in Berlin, Germany.

Machekano, H., Mutamiswa, R., & Nyamukondiwa, C. (2018c). Evidence of rapid spread and establishment of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in semi-arid Botswana. *Agriculture and Food Security, 7*, 48.

MacLean, H. J., Kingsolver, J. G., & Buckley, L. B. (2016). Historical changes in thermoregulatory traits of alpine butterflies reveal complex ecological and evolutionary responses to recent climate change. *Climate Change Responses, 3*, 1–10.

Magiafoglou, A., & Hoffmann, A. A. (2003). Cross-generation effects due to cold exposure in *Drosophila serrata*. *Functional Ecology, 17*, 664–672.

Manenti, T., Sørensen, J. G., & Loeschcke, V. (2017). Environmental heterogeneity does not affect levels of phenotypic plasticity in natural populations of three Drosophila species. *Ecology and Evolution, 7*, 2716–2724.

Marshall, K. E., & Sinclair, B. J. (2011). The sub-lethal effects of repeated freezing in the woolly bear caterpillar *Pyrrharctia isabella*. *Journal of Experimental Biology, 214*, 1205–1212.

Masters, A. R., Malcom, S. B., & Brower, L. P. (1988). Monarch butterfly (*Danaus plexippus*) thermoregulatory behaviour and adaptations for overwintering in Mexico. *Ecology, 69*, 458–467.

Maurer, J. A., Shepard, J. H., Crabo, L. G., Hammond, P. C., Zack, R. S., & Peterson, M. A. (2018). Phenological responses of 215 moth species to inter annual climate variation in the Pacific Northwest from 1895 through 2013. *PLoS ONE, 13*, e0202850.

Montejo-Kovacevich, G., Martin, S. H., Meier, J. I., Bacquet, C. N., Monllor, M., Jiggins, C. D., & Nadeau, N. J. (2020). Microclimate buffering and thermal tolerance across elevations in a tropical butterfly. *Journal of Experimental Biology, 223*, jeb220426.

Munoz-Valencia, V., Diaz-Gonzalezi, F., Manzano-Martinezi, M. D., Toro-Perea, N., & Cardenas-Henao, H. (2013). Basal and induced thermotolerance to heat shocks in *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae). *Revista Colombiana De Entomologia, 9*, 18–25.

Münzbergová, Z., & Hadincová, V. (2017). Transgenerational plasticity as an important mechanism affecting response of clonal species to changing climate. *Ecology and Evolution, 7*, 5236–5247.

Mutamiswa, R., Chidawanyika, F., & Nyamukondiwa, C. (2017a). Dominance of spotted stemborer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) over indigenous stem borer species in Africa's changing climates: Ecological and thermal biology perspectives. *Agricultural and Forest Entomology, 19*, 344–356.

Mutamiswa, R., Chidawanyika, F., & Nyamukondiwa, C. (2017b). Comparative assessment of the thermal tolerance of spotted stemborer, *Chilo partellus* (Lepidoptera: Crambidae) and its larval parasitoid, *Cotesia sesamiae* (Hymenoptera: Braconidae). *Insect Science, 25*, 847–860.

Mutamiswa, R., Chidawanyika, F., & Nyamukondiwa, C. (2018a). Thermal plasticity potentially mediates the interaction between host *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) under rapidly changing environments. *Pest Management Science, 74*, 1335–1345.

Mutamiswa, R., Chidawanyika, F., & Nyamukondiwa, C. (2018b). Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. *Physiological Entomology, 43*, 108–119.

Mutamiswa, R., Machekano, H., Chidawanyika, F., & 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.

Nagoshi, R. N., Meagher, R. L., & Hay-Roe, M. (2012). Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. *Ecology and Evolution, 2*, 1458–1467.

Neve, G., & Hall, C. (2016). Variation of thorax flight temperature among twenty Australian butterflies (Lepidoptera: Papilionidae, Nymphalidae, Pieridae, Hesperiidae, Lycaenidae). *European Journal of Entomology, 113*, 571–578.

Neven, L. G. (1999). Cold hardiness adaptations of codling moth, *Cydia pomonella*. *Cryobiology, 38*, 43–50.

Nguyen, T. T. A., Michaud, D., & Cloutier, C. (2009). A proteomic analysis of the aphid *Macrosiphum euphorbiae* under heat and radiation stress. *Insect Biochemistry and Molecular Biology, 39*, 20–30.

Nguyen, C., Bahar, M. H., Baker, G., & Andrew, N. R. (2014). Thermal tolerance limits of diamondback moth in ramping and plunging assays. *PLoS ONE, 9*, e87535.

Nice, C. C., & Fordyce, J. A. (2006). How caterpillars avoid overheating: Behavioral and phenotypic plasticity of pipevine swallowtail larvae. *Oecologia, 146*, 541–548.

Noh, S., Everman, E. R., Berger, C. M., & Morgan, T. J. (2017). Seasonal variation in basal and plastic cold

tolerance: Adaptation is influenced by both long-and short-term phenotypic plasticity. *Ecology and Evolution, 7*, 248–5257.

Nyamukondiwa, C., & 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*, 255–264.

Nyamukondiwa, C., Weldon, C. W., Chown, S. L., le Roux, P. C., & 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*, 1199–1211.

Nyamukondiwa, C., Machekano, H., Chidawanyika, F., Mutamiswa, R., Ma, G., & Ma, C. S. (2022). Geographic dispersion of invasive crop pests: The role of basal, plastic climate stress tolerance and other complementary traits in the tropics. *Current Opinion in Insect Science, 50*, 100878.

Ofomata, V. C., Overholt, W. A., & Egwuatu, R. I. (1999). Diapause termination of *Chilo partellus* Swinhoe and *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae). *Insect Science and Its Application, 19*, 187–191.

Ohsaki, N. (1986). Body temperatures and behavioural thermoregulation strategies of three *Pieris* butterflies in relation to solar radiation. *Journal of Ethology, 4*, 1–9.

Panko, B. (2017). *What do insects do in winter*. Smithsonian.

Park, H. J., & Yun, D. J. (2013). New insights into the role of the small ubiquitin-like modifier (SUMO) in plants. *International Review of Cell and Molecular Biology, 300*, 161–209.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics, 37*, 637–669.

Parsell, D. A., & Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. *Annual Review of Genetics, 27*, 437–496.

Perveen, F. K., & Khan, A. (2017). Introductory Chapter: Lepidoptera, Lepidoptera, Farzana Khan Perveen, IntechOpen,: https://doi.org/10.5772/intechopen.70452.

Pincebourde, S., & Woods, H. A. (2020). There is plenty of room at the bottom: Microclimates drive insect vulnerability to climate change. *Current Opinion in Insect Science, 41*, 63–70.

Pollard, E., & Yates, T. J. (1992). The extinction and foundation of local butterfly populations in relation to population variability and other factors. *Ecological Entomology, 17*, 249–254.

Porter, K. (1982). Basking behaviour in larvae of the butterfly *Euphydryas aurinia*. *Oikos, 38*, 308–312.

Powell, J. A. (1989). Synchronized, mass-emergences of a Yucca moth, *Prodoxus y-inversus* (Lepidoptera: Prodoxidae), after 16 and 17 years in diapause. *Oecologia, 81*, 490–493.

Prange, H. D. (1996). Evaporative cooling in insects. *Journal of Insect Physiology, 42*, 493–499.

Pruisscher, P., Nylin, S., Gotthard, K., & Wheat, C. W. (2018). Genetic variation underlying local adaptation of diapause induction along a cline in a butterfly. *Molecular Ecology, 27*, 3613–3626.

Qi, X., Majeed, M. Z., Wei, Z., & Chun-sen, M. (2019). Adaptation of Drosophila species to climate change-A literature review since 2003. *Journal of Integrative Agriculture, 18*, 805–814.

Quan, P. Q., Li, M. Z., Wang, G. R., Gu, L. L., & Liu, X. D. (2020). Comparative transcriptome analysis of the rice leaf folder (*Cnaphalocrocis medinalis*) to heat acclimation. *BMC Genomics, 21*, 450. https://doi.org/10.1186/s12864-020-06867-6

Rabieh, M. M. (2018). Biodiversity of noctuid moths (Lepidoptera: Noctuidae) in the agroecosystems of Mashhad County. *Biodiversity International Journal, 2*, 147–151.

Ramachandra, Y. L., Bali, G., & Rai, S. P. (2001). Effect of temperature and relative humidity on spinning behaviour of silkworm (*Bombyx mori* L.). *Indian Journal of Experimental Biology, 38*, 87–89.

Rawlins, J. E., & Lederhouse, R. C. (1981). Developmental influences of thermal behavior on Monarch caterpillars (*Danaus plexippus*): An adaptation for migration (Lepidoptera: Nymphalidae: Danainae). *Journal of the Kansas Entomological Society, 54*, 387408.

Reppert, S. M., & de Roode, J. C. (2018). Demystifying monarch butterfly migration. *Current Biology, 28*, R1009–R1022.

Rickards, J., Kelleher, J., & Storey, K. B. (1986). Strategies of freeze avoidance in larvae of the goldenrod gall moth, *Epiblema scudderiana*: Winter profiles of natural population. *Journal of Insect Physiology, 33*, 443–450.

Rinehart, J. P., Li, A., Yocum, G. D., Robich, R. M., Hayward, S. A., & Denlinger, D. L. (2007). Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 1130–11137.

Roland, J. (2006). Effect of melanism of alpine *Colias nastes* butterflies (Lepidoptera: Pieridae) on activity and predation. *Canadian Entomologist, 138*, 52–58.

Sanborn, A. (2008). Thermoregulation in insects. In J. L. Capinera (Ed.), *Enyclopedia of entomology*. Dordrecht: Springer.

Sandre, S. L., Tamaru, T., Vanatoa, A., & Esperk, T. (2014). Maintenance of larval color polymorphism in *Orgyia antiqua* (Lepidoptera: Lymantriidae): Evaluating the role of thermal adaptation. *Environmental Entomology, 36*, 1303–1309.

Scoble, M. J. (1995). *Migration in the lepidoptera: Form, function and diversity* (pp. 68–71). Natural History Museum.

Scriber, J. M. (2020). Assessing ecological and physiological costs of melanism in North American *Papilio glaucus* females: Two decades of dark morph frequency declines. *Insect Science, 27*, 583–612.

Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology, 61*, 433–451.

Sheikh, A. Z., Rehman, N. Z., & Kumar, R. (2017). Diverse adaptations in insects: A review. *Journal of Entomology and Zoology Studies, 5*, 343–350.

Shen, Y., Gu, J., Huang, L. H., Zheng, S. C., Liu, L., Xu, W. H., Feng, Q. L., & Kang, L. (2011). Cloning and expression analysis of six small heat shock protein genes in the common cutworm, *Spodoptera litura*. *Journal of Insect Physiology, 57*, 908–914.

Sherman, P. W., & Watt, W. B. (1973). The thermal ecology of some *Colias* butterfly larvae. *Journal of Comparative Physiology, 83*, 25–40.

Shu, Y., Du, Y., & Wang, J. (2011). Molecular characterization and expression patterns of *Spodoptera litura* heat shock protein 70/90, and their response to zinc stress. *Comparative Biochemistry and Physiology A: Molecular & Integrative Physiology, 158*, 102–110.

Silva, V. D. E., Beirão, M. V., & Cardoso, D. C. (2020). Thermal tolerance of fruit-feeding butterflies (Lepidoptera: Nymphalidae) in contrasting mountaintop environments. *Insects, 11*, 278.

Sinclair, B. J., & Chown, S. L. (2003). Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera: Tineidae). *Journal of Insect Physiology, 49*, 45–52.

Sinclair, B. J., Addo-Bediako, A., & Chown, S. L. (2003). Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews, 78*, 181–195.

Sinclair, B. J., Ferguon, L. V., Salehipour-shirazi, G., & MacMillan, H. A. (2013). Cross-tolerance and cross-talk in the cold: Relating low temperatures to desiccation and immune stress in insects. *Integrative and Comparative Biology, 53*, 545–556.

Sinclair, B. J., Coello Alvarado, L. E., & Ferguson, L. V. (2015). An invitation to measure insect cold tolerance: Methods, approaches, and workflow. *Journal of Thermal Biology, 53*, 180–197.

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

Solensky, M. J., & Larkin, E. (2003). Temperature-induced variation in larval coloration in *Danaus plexippus* (Lepidoptera: Nymphalidae). *Annals of the Entomological Society of America, 96*, 211–216.

Solomon, T., & Hussen, E. (2018). Benefits of farm animals genetic adaptation: A review. *European Journal of Experimental Biology, 8*, 22. https://doi.org/10.21767/2248-9215.100063

Spieth, H. R., Porschmann, U., & Teiwes, C. (2011). The occurrence of summer diapause in the large white butterfly *Pieris brassicae* (Lepidoptera: Pieridae): A geographical perspective. *European Journal of Entomology, 108*, 377–384.

Steigenga, M. J., & Fischer, K. (2007). Within- and between- generation effects of temperature on life-history traits in a butterfly. *Journal of Thermal Biology, 32*, 396–405.

Stelbrink, P., Pinkert, S., Brunzel, S., Kerr, J., Wheat, C. W., Brandl, R., & Zeuss, D. (2019). Colour lightness of butterfly assemblages across North America and Europe. *Scientific Reports, 9*, 1760.

Stoehr, A. M., & Goux, H. (2008). Seasonal phenotypic plasticity of wing melanisation in the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). *Ecological Entomology, 33*, 137–143.

Storey, K. B., & Storey, J. M. (2012). Aestivation: Signaling and hypometabolism. *Journal of Experimental Biology, 215*, 1425–1433.

Stotter, R. L., & Terblanche, J. S. (2009). Low temperature tolerance of false codling moth *Thaumotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. *Journal of Thermal Biology, 34*, 320–325.

Sun, M., Tang, X. T., Lu, M. X., Yan, W. F., & Du, Y. Z. (2014). Cold tolerance characteristics and overwintering strategy of *Sesamia inferens* (Lepidoptera: Noctuidae). *Florida Entomologist, 97*, 1544–1553.

Talekar, N. S., & Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology, 38*, 275–301.

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

Tarusikirwa, V. L., Mutamiswa, R., Chidawanyika, F., & Nyamukondiwa, C. (2020b). Cold hardiness of the South American tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae): Both larvae and adults are chill-susceptible. *Pest Management Science, 77*, 184–193.

Tsai, C. C., Childers, R. A., Shi, N. N., Ren, C., Pelaez, J. N., Bernard, G. D., Pierce, N. E., & Yu, N. (2020). Physical and behavioral adaptations to prevent overheating of the living wings of butterflies. *Nature Communications, 11*, 1–14.

Tsuji, J. S., Kingsolver, J. G., & Watt, W. B. (1986). Thermal physiological ecology of *Colias* butterflies in flight. *Oecologia, 69*, 161–170.

Valencia, A., Wang, H., Soto, A., Aristizabal, M., Arboleda, J. W., Eyun, S. I., Noriega, D. D., & Siegfried, B. (2016). Pyrosequencing the midgut transcriptome of the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) reveals multiple protease-like transcripts. *PLoS One, 11*, 1–16.

Van Dyck, H., & Wiklund, C. (2002). Seasonal butterfly design: Morphological plasticity among three developmental pathways relative to sex, flight and thermoregulation. *Journal of Evolutionary Biology, 15*, 216–225.

Van Damme, V., Berkvens, N., Moerkens, R., Berckmoes, E., Wittemans, L., De Vis, R., Casteels, H., Tirry, L., & De Clercq, P. (2015). Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. *Journal of Pest Science, 88*, 533–541.

Villagra, C., & Frías-Lasserre, D. (2020). Epigenetic molecular mechanisms in insects. *Neotropical Entomology, 49*, 615–642.

Vreysen, M. J. B., Klassen, W., & Carpenter, J. E. (2016). Overview of technological advances toward greater efficiency and efficacy in sterile insect-inherited sterility programs against moth pests. *Florida Entomologist, 99*, 1–12.

Wang, L., Yang, S., Han, L., Zhao, K., & Ye, L. (2015). Expression profile of two HSP70 chaperone proteins in response to extreme thermal acclimation in *Xestia c-nigrum* (Lepidoptera: Noctuidae). *Florida Entomologist, 98*, 506–516.

Wang, F. Y., Yang, F., Lu, M. H., Luo, S. Y., Zhai, B. P., Lim, K. S., McInerney, C. E., & Hu, G. (2017). Determining the migration duration of rice leaf folder (*Cnaphalocrocis medinalis* Guenée moths using a trajectory analytical approach. *Scientific Reports, 7*, 39853.

Whitman, D. W., & Ananthakrishnan, T. N. (2009). *Phenotypic plasticity of insects: Mechanisms and consequences*. Science Publishers.

Woestmann, L., & Saastamoinen, M. (2016). The importance of transgenerational effects in Lepidoptera. *Current Zoology, 62*, 489–499.

Xiao, H. J., Mou, F. C., Zhu, X. F., & Xue, F. S. (2010). Diapause induction, maintenance and termination in the rice stem borer *Chilo suppressalis* (Walker). *Journal of Insect Physiology, 56*, 1558–1564.

Xiao, H., Wu, S., Chen, C., & Xue, F. (2013). Optimal low temperature and chilling period for both summer and winter diapause development in *Pieris melete*: Based on a similar mechanism. *PLos ONE, 8*, e56404. https://doi.org/10.1371/journal.pone.0056404

Xu, Q., Zou, Q., Zheng, H., Zhang, F., Tang, B., & Wang, S. (2011). Three heat shock proteins from *Spodoptera exigua*: Gene cloning, characterization and comparative stress response during heat and cold shocks. *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology, 159*, 92–102.

Yocum, G. D., Joplin, K. H., & Denlinger, D. L. (1991). Expression of heat shock proteins in response to high and low temperature extremes in diapausing pharate larvae of the gypsy moth, *Lymantria dispar*. *Archives of Insect Biochemistry and Physiology, 18*, 239–249.

Zerebecki, R. A., & Sorte, C. J. B. (2011). Temperature tolerance and stress proteins as mechanisms of invasive species success. *PLoS ONE, 6*, e14806.

Zeuss, D., Brandl, R., Brändle, M., Rahbek, C., & Brunzel, S. (2014). Global warming favours light-coloured insects in Europe. *Nature Communications, 5*, 3874.

Zhang, Q., & Denlinger, D. L. (2010). Molecular characterization of heat shock protein 90, 70 and 70 cognate cDNAs and their expression patterns during thermal stress and pupal diapause in the corn earworm. *Journal of Insect Physiology, 56*, 138–150.

Zhang, X., Geng, J., & Zhou, W. (1981). A study on ecological mechanism of the migration of rice leaf roller *Cnaphalocrocis medinalis* Guenée. *Journal of Nanjing Agricultural College, 4*, 40–51.

Zhang, W., Chang, X. Q., Hoffmann, A., Zhang, S., & Ma, C. S. (2015a). Impact of hot events at different developmental stages of a moth: The closer to adult stage, the less reproductive output. *Scientific Reports, 5*, 10436.

Zhang, B., Zheng, J., Peng, Y., Liu, X., Hoffmann, A. A., & Ma, C. S. (2015b). Stress responses of small heat shock protein genes in Lepidoptera point to limited conservation of function across phylogeny. *PLoS One, 10*, e0132700.

Zhang, D.-W., Xiao, Z.-J., Zeng, B.-P., Li, K., & Tang, Y.-L. (2019). Insect behavior and physiological adaptation mechanisms under starvation stress. *Frontiers in Physiology, 10*, 163.

Zheng, J., Cheng, X., Hoffmann, A. A., Zhang, B. O., & Ma, C. S. (2017). Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress? *Journal of Insect Physiology, 102*, 36–41.

Zhou, G., Overholt, W. A., & Mochiah, M. B. (2001). Change in the distribution of lepidopteran maize stem borer in Kenya from the 1950s to 1990s. *Insect Science and Its Application, 21*, 395–402.