



Faculty of Sciences

Department of Biological Sciences and Biotechnology

**CARRY-OVER EFFECTS OF ACUTE AND CHRONIC
ENVIRONMENTAL STRESS AS DRIVERS OF FITNESS IN
SPODOPTERA FRUGIPERDA (J.E SMITH)**

by

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DECLARATION REGARDING THE WORK AND COPYRIGHT

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Thesis Titled: CARRY-OVER EFFECTS OF ACUTE AND CHRONIC ENVIRONMENTAL STRESS AS DRIVERS OF FITNESS IN *SPODOPTERA FRUGIPERDA* (J.E Smith)

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Date: 19 April 2023

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I, the Candidate's **Primary Supervisor**, hereby confirm that I have inspected the above titled thesis, and, to the best of my knowledge, it is based on the original work of the candidate.



Signed:

Date: 19 April 2023

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ABSTRACT

Global increases in mean temperatures and changes in precipitation patterns due to climate change, coupled with anthropogenic pathways, have intensified biological pest insects' invasions. Continuous exposure to bouts of acute and chronic heat and fasting stresses (during e.g., droughts) might improve performance under recurring stresses, therefore enhancing/reducing fitness within- or across- life stages (i.e., within generation plasticity or carry-over effects). Here, I examined developmental acclimation effects in the invasive fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) - a highly invasive economic insect pest of cereal crops, particularly maize using standardized heat tolerance metrics. Specifically, I assessed (1) the effects of acute (3h) and chronic (3 days) heat treatments (at 32 °C, 35 °C, 38 °C), as well as fasting (48h), on 3rd instar larvae, and tested fitness traits (critical thermal maxima [CT_{max}] and heat knockdown time [HKDT]) at a later life stage (4th/5th larval instar). Acclimation to heat stress and fasting had significant fitness costs (lower CT_{max}) across majority of treatments. However, both heat and fasting acclimation improved HKDT (except for 35 and 38 °C [acute acclimation] and 35 °C [chronic acclimation]). The results suggest context-specific developmental acclimation costs and benefits in *S. frugiperda*. In particular, heat and fasting acclimation potentially have fitness costs and benefits for subsequent developmental stages facing high temperature stress. Second, I tested whether FAW critical low temperature activity limits likely adaptively tracked the prevailing temporal ambient environments. Using field collected wild FAW populations, temporal changes (over two years/seasons) in larval low temperature tolerance traits were tested using standardised protocols *vis* critical thermal minima (CT_{min}), chill coma recovery time (CCRT) and supercooling points (SCP). Results varied but generally showed that FAW low temperature responses tracked environmental temperature. Winter collected FAW generally had higher cold tolerance (lower CT_{min} and CCRT). These results show rapid responses to changing temperature environments in FAW suggesting that both long-term (season) and short-term (conditions prevailing just before organismal collection [e.g., rapid hardening responses]) may shape FAW thermal traits and overall ecology. These results are important in informing FAW spatial adaptation and may help inform context tailored pest management strategies.

CHAPTER ONE

1.1 Background and ecology of *Spodoptera frugiperda*

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is an invasive crop pest of cereal crops (Sparks, 1979; Kenis *et al.*, 2022). It prefers maize as its host plant; however it is a polyphagous insect feeding on over 353 plant species across different families (Montezano *et al.*, 2018). This makes it one of the most harmful pest insects threatening annual crops in tropical regions (Stokstad, 2017). FAW is a migratory pest that was first detected in Africa in early 2016 in Nigeria (Goergen *et al.*, 2016; Stokstad, 2017). Within a year, it had already invaded the whole of southern Africa and parts of eastern Africa (Day *et al.*, 2017; Stokstad 2017). In Botswana FAW was detected in 2017: temperatures in Botswana are quite warm which supports FAW spread and growth (Makale *et al.*, 2022). In most countries including Botswana response to FAW outbreak by farmers has been limited to excessive use of pesticides (Stokstad 2017; FAO & CABI, 2019; Makale *et al.*, 2021). A study by Nagoshi *et al.*, (2012) found that the migration of FAW from South America to other parts of the world is a behavioral adaptation to extend its geographical range into novel areas to evade inter- and –intra specific competition (Nagoshi *et al.*, 2012).

Duration of the FAW life cycle is dependent on environmental temperatures. It takes about 30 days during summer when temperatures are high and 80 to 90 days during winter. Adult females lay up to 200 eggs per batch usually on the underside of maize leaves (CABI, 2019). Eggs are cream white in colour, but change to light brown when nearing hatching, which takes approximately 2-3 days under optimal temperature conditions (Prasanna *et al.*, 2018). Adult average lifespan is 10 days but it can live up to 21 days (CABI, 2019). Females can lay up to 2 000 eggs in their lifetime (Igyuve *et al.*, 2018). Adults can fly thousands of kilometers to new environments as a way of avoiding competition and harsh weather conditions. Larvae have six instars which take 14-20 days to complete under optimal conditions. Larvae cause economic damage by feeding on maize leaves, whorl, and kernels. When insects reach 4th instar only one or two will occupy a single maize stalk as they become cannibalistic. Since larvae are the main feeding stage in FAW life cycle, they are most susceptible to starvation stress. From 4th instar FAW larvae are distinctively identified by an inverted ‘Y’ mark on the forehead and four large dots that form a square on the second to last segment (FAO, 2018). When larvae prepares for pupation they cease feeding and burrow 2 - 8 cm into the soil or maize reproductive parts. The pupae stage takes 8-9 days to enclose during summer and up to 30 days during colder seasons (CABI, 2019).

Fall armyworm is native to South America. It is chill susceptible thus does not survive periods of prolonged cold winter or sub-zero temperatures (Du Plessis *et al.*, 2020; Keosentse *et al.*, 2021). Due to its impaired cold tolerance its distribution during winter in America is restricted to Florida and Texas where winters are warmer (Sparks, 1979). To date FAW has invaded 47 countries in Africa and 18 countries in Asia (CABI, 2019; Jing *et al.*, 2021; Kenis *et al.*, 2022), and was recently detected in Australia in 2020 (Kearns *et al.*, 2020) (**Fig. 1**). Studies have shown that FAW has high heat tolerance with temperatures of 35.5 °C supporting development and survival (Ali *et al.*, 1990). Owing to its high heat tolerance, it has successfully invaded Asia with climatic conditions in some regions of South and Southeast Asia supporting its year round survival (Yang *et al.*, 2021).

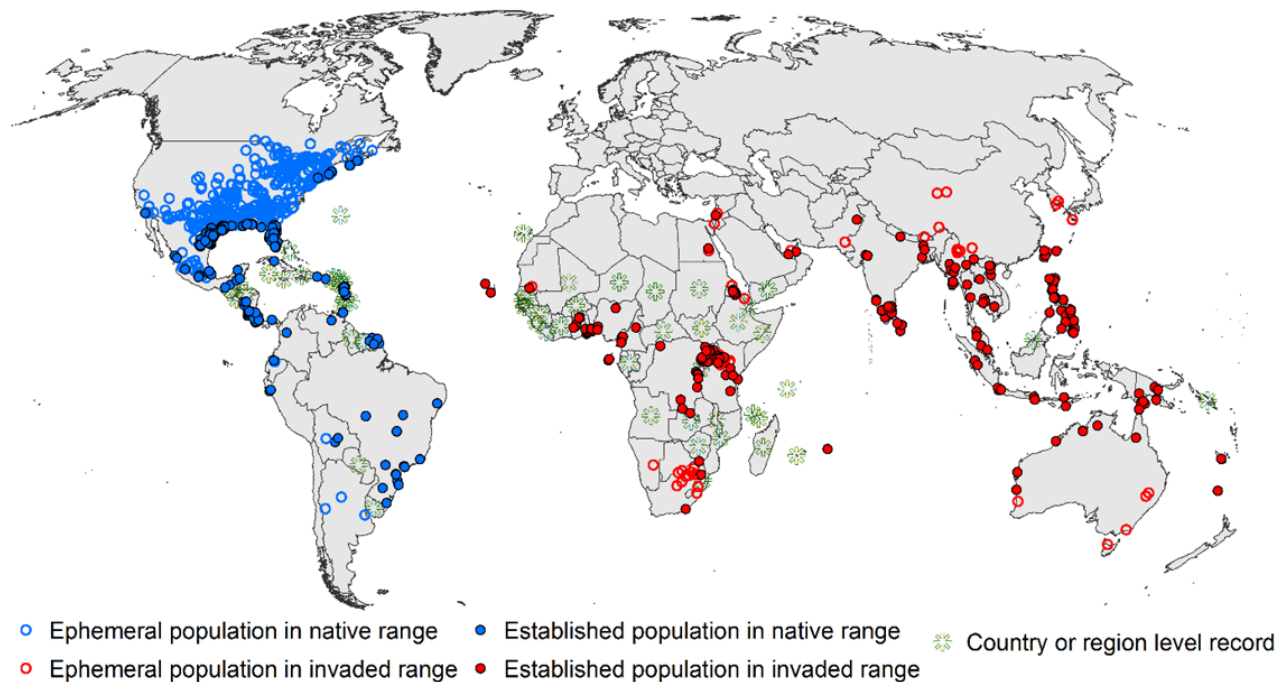


Figure 1: Global distribution of *Spodoptera frugiperda* (Kenis *et al.*, 2022).

Global increase in temperatures and inconsistent limited rainfall due to climate change transform previously inhabitable locations into novel environments for invasive pest establishment and success (Nyamukondiwa *et al.*, 2010; Nyamukondiwa *et al.*, 2022). This is likely one of the reasons for the global surge of invasive species and indeed the recent invasion and successful spread of the FAW. Previous studies on another invasive species, *Tuta absoluta* (Meyrick) (see Machekano *et al.*, 2018) propose that high basal thermal tolerance, plasticity and off-season availability of wild host plants enhance successful spread, habitat permeability and niche occupation by invasive insect species. Highly

invasive organisms have been observed to have flexible morphology and physiological stress tolerances due to seasonal and spatial changes in environmental factors (Winkler *et al.*, 2016). Exposure to stressful events such as high, low, fluctuating temperature or starvation due to limited or total unavailability of resources across seasons in one developmental stage/generation may likely affect fitness metrics of the subsequent life stage/generation in the next season e.g. survival and reproduction, in a phenomenon known as carry-over effects) (Harrison *et al.* 2011). A study by Ezeakacha and Yee (2019) on *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) showed that temperature affects life history of developing larvae under competitive interactions and can also alter adult fitness traits such as fecundity. There are few studies that looked at carry-over effects in invertebrates (but see Ezeakacha and Yee, 2019), particularly within generation. Thus, cross-over effects in many insect species remain largely unknown despite playing a significant ecological role in maintaining fitness under changing environments. Unraveling this phenomenon in model and other economic species like the FAW will help mechanistic models looking at invasive species biogeographic patterns and population dynamics. Furthermore, these mechanisms are important in determining how species may react to climate change thus help in pest control early warning systems through precise forecasting of potential invasion areas.

1.1 Justification of study

Spodoptera frugiperda is a tropical insect pest that has invaded the African continent. Given its tropical region, survival at high temperature and mechanisms involved thereof may be crucial to its invasive potential. It is already documented that this species does not do well at low temperatures (Sparks, 1979; Keosentse *et al.*, 2021). Thus, the long-term effects of environmental high temperature stress on fitness of *S. frugiperda* are key to its pest status. Abiotic stress in one instar or generation may improve or compromise environmental fitness in the subsequent instar/generation (carry-over effects). Given that FAW developmental stages may occupy spatially different environments/niches, it is thus critical to assess how potential carry-over effects contribute to the invasion of *S. frugiperda* under current and projected climate change scenarios. This knowledge will help in *S. frugiperda* early warning systems thus allow for timely and economic use of integrated pest management (IPM) interventions. Early detection will help minimize the excessive use of pesticides that have several negative effects on biodiversity, ecological services, public health, production economics and the environment at large.

1.2 Aim

The overall aim of this study was to examine innate thermal plasticity carry-over effects for *S. frugiperda*.

1.3 Objectives

1. Investigate the carry over effects of acute and chronic high temperature and fasting stress of the 3rd instar on the fitness of 4th and 5th instars in *S. frugiperda*.
2. Assess how seasonal changes in environmental conditions affect stress resistance (critical thermal minima, chill coma recovery time, and supercooling points) across *Spodoptera frugiperda* ontogeny.

1.4 Hypotheses

1. Temperature and fasting stress in one instar have heat tolerance fitness costs and/or benefits across other subsequent non-acclimated developmental stages through carry-over effects in *S. frugiperda*.
2. *Spodoptera frugiperda* thermal traits likely track prevailing seasonal/ambient conditions- temporal adaptation

CHAPTER 2: Literature review

2.1 Insect response to environmental stress

Insects experience various stressors in nature which affect metabolic and physiological processes (Chown & Nicolson, 2004; Furlong & Zalucki, 2017). These stressors may be biotic such as exposure to predators, pathogens and parasites, unavailability of host plants and abiotic factors such as extreme temperature incidences, desiccation and drought conditions (Chown & Nicolson, 2004; Ong'wen *et al.*, 2020). When exposed to these stressors, insects use behavioral, morphological, and physiological strategies in order to survive (Sheikh *et al.*, 2017). Physiological strategies are employed after behavioral and morphological strategies have failed to cope with stressful environments. Behavioral strategies include migration where insects move away from the stressful environments to more benign ones. Migration could be going to another, less stressful niche within the same ecosystem or even long distance often cross-continental movements. Long distance migration is generally seasonal and characteristic of winged insects, particularly insects of the order: Lepidoptera, Orthoptera and Odonata (Sheikh *et al.*, 2017). Another behavioral strategy is hibernation, a common strategy to evade low winter temperatures in insects such as houseflies and ladybirds (Sheikh *et al.*, 2017). When insects hibernate, they enter a state of diapause which involves lowering metabolic rates and energy consumption (Andrewartha, 1952). Stimulation and end of diapause is induced by environmental cues such as change in duration of photoperiod or prevailing ambient temperatures to signal whether conditions are favorable or not for growth (Tauber *et al.*, 1984; Tougeron, 2019). Morphological adaptations include melanism, which is the darkening of insect body colour (Saenko *et al.*, 2012). Melanism is a response to several stressors such as low temperature stress to allow greater absorption of heat thus improving thermoregulation (Trullas *et al.*, 2007). It is also a response to predation stress, increases immunity against pathogens releasing cytotoxic intermediates and causing oxidative damage to microorganisms at infected sites (Christensen *et al.*, 2005; Barillas-Mury, 2007).

Insects also employ basal and plastic stress tolerance to cope and survive various stressors (Nyamukondiwa *et al.*, 2022). Some insects inherently have physiological mechanisms in place to overcome certain stressors (basal tolerance) while others have to develop physiological strategies to adapt and compensate for genetic variation (Noh *et al.*, 2017). Basal tolerance is heritable and is independent of prior exposure to stressful environmental conditions (Zhao *et al.*, 2017). On the other hand, plastic responses are not genetic but involve physiological changes that improve survival under extreme environmental conditions preceding exposure to mild stress (Zhao *et al.*, 2017; Ma *et al.*,

2021). Even though it does not bring about genetic change, plasticity has an influence on genetic pathways (Beldade *et al.*, 2011). For example, it may result in increase or decrease in sensitivity to environmental conditions (Moore & Martin, 2019).

Insects can acquire plastic tolerance after short term exposure of a few minutes to hours (hardening) or longer durations of days and weeks (acclimation) to a particular stressor (Zhao *et al.*, 2017; Ma *et al.*, 2021). Plastic tolerance may manifest over wide time frames for example in developmental stages exposed to stress, later life stages (carry-over effects) or later generations (trans-generational) (Ma *et al.*, 2021). Carry-over effects is a form of developmental plasticity in which environmental conditions experienced by an individual in its earlier life stage affects life-history traits of following life stages (O'Connor *et al.*, 2014; Moore & Martin, 2019). Studies done on mosquitoes have found out that temperature (Alto & Bettinardi, 2013), diet (Telang *et al.*, 2012), predator exposure (Buxton *et al.*, 2020) and microbial symbiotic interactions (Dickson *et al.*, 2017) in mosquito larvae influence life history traits for the adults.

2.2 Effects of thermal stress on ectotherms

Development and biology of insects is largely influenced by environmental temperatures. Different insect species have a thermal optimum at which development is optimal and mortality is lowest (Cui *et al.*, 2018; Du Plessis *et al.*, 2020). Deviations from the thermal optimum may result in decreased developmental rate, increased exposure of vulnerable developmental stages to predators and parasites, and irreversible injury and death (Fields *et al.*, 2012; Aguilon & Velasco, 2015). The effect of temperature on insects is best described by the thermos-biological scale (Vannier, 1994) (see **Fig. 2**). The optimum temperature is the temperature range at which physiological performance and activity is at the best. It differs from one insect species to another and can vary within species developmental stages (Ricalde *et al.*, 2012). When temperature decreases or increases from the thermal optimum range, an insect undergoes a physiological state called heat stupor or cold stupor, related to heat and cold stress respectively (**Fig. 2**). Heat and cold stupors are termed the critical thermal limits (CTLs), comprising the critical thermal maxima (CT_{max}) (for high temperature stress) and critical thermal minima (CT_{min}) (for low temperature stress). CTLs have both been defined as the endpoint temperatures (both low and high), allowing insect activity, resulting in loss of response to mild stimuli (Angilletta, 2009; Nyamukondiwa & Terblanche 2009). CT_{max} and CT_{min} have been used as a measure of insect heat tolerance (Chown & Nicolson, 2004; Nyamukondiwa *et al.*, 2013; Chidawanyika *et al.*, 2017) and cold tolerance (Gotcha *et al.*, 2017; Tarusikirwa *et al.*, 2021) respectively. They are both easy to measure, ecologically relevant and closely approximate insect biogeographic distribution (Andersen *et al.*, 2015).

Further increasing or decreasing temperature beyond either CT_{max} or CT_{min} , an insect will enter into heat or chill coma respectively. Heat coma and chill coma are used as measures of heat and cold tolerance respectively (Andersen *et al.*, 2015). Heat tolerance using heat coma is measured as the time taken by an insect to lose physical activity (knockdown) due to acute heat stress termed as heat knockdown time (HKDT) (see Sørensen *et al.*, 2001; Chown & Nicolson, 2004; Weldon *et al.*, 2016; Machekano *et al.*, 2021). However for chill coma, cold tolerance is measured as the time taken for an insect to recover from chill coma associated with low temperature, termed chill coma recovery time (CCRT) (see Karl *et al.*, 2014; Scharf *et al.*, 2015). At chill coma, insects are immobilized, however the coma state is reversible once temperatures increase (Mellanby, 1939; Findsen *et al.*, 2014). Temperature increase or decrease beyond chill coma results in irreversible injury and eventually death (Vannier, 1994; Chown & Nicolson, 2004) (see Fig. 2).

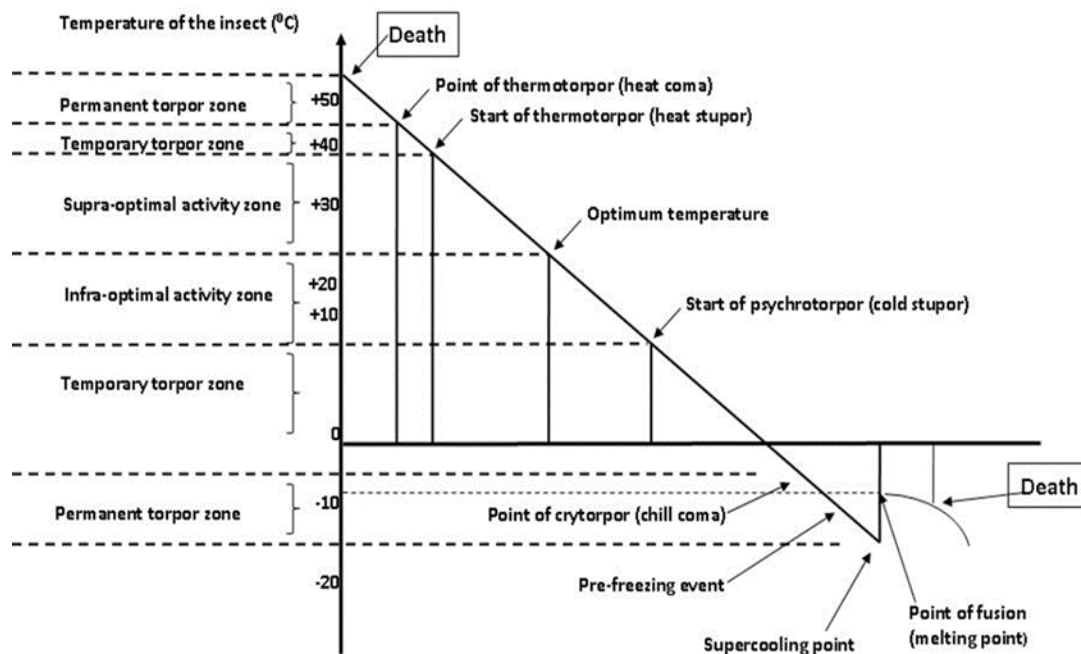


Figure 2: The thermo-biological scale (redrawn from Vannier, 1994).

2.3 Insect starvation resistance and adaptation to starvation stress

In nature, insects at times go through periods of low food resource availability where there is little to no food resources or available food fails to meet nutritional requirements. Unavailability of food for prolonged periods negatively affects several physiological processes such as reproduction, development, immunity, stress responses and eventually leads to insect death (Bauerfeind & Fischer,

2005; Yang *et al.*, 2021). To combat this stressor and prevent death insects have developed behavioral and physiological mechanisms to circumvent limited food resources. The two main behavioral strategies are migration to more food rich regions and diapause (Zhang *et al.*, 2019). Diapause is enforced cessation of growth and development (Gill *et al.*, 2017). One example is early and prolonged pupation in some insect species (Ballard *et al.*, 2008). Other behavioral strategies include cannibalism and trading off other life processes e.g. trading off reproduction (Zhang *et al.*, 2019). Reproduction is an energy costly process (Speakman, 2008). Thus, to save energy and restrict population sizes some insects reduce acceptance to mating (Churchill *et al.*, 2019), and reduce sizes of reproductive organs (Wang *et al.*, 2016).

Physiological adaptations to starvation have been poorly studied in insects, particularly invasive pests such as FAW. Based on literature, the main physiological mechanisms in which insects increase starvation resistance are: sequestering greater energy reserves, reduction in consumption of energy reserves during starvation, reduction in the minimal energy level required for organ function (Rion & Kawecki, 2007). Insects alternatively use other macromolecules such as proteins and glycogen and trehalose which are converted to glucose to provide energy during starvation (Helland *et al.*, 2003; Tang *et al.*, 2014). Increase in energy reserves, mainly lipids, has been observed in laboratory setups in response to starvation or nutrient poor diets in *Drosophila* (Harshman *et al.*, 1999; Piper *et al.*, 2005). Frugality in consumption of energy reserves by lowering of metabolic rate are not clearly understood but are identified as some of the mechanisms of plasticity to starvation stress (Marron *et al.*, 2003; Rion & Kawecki, 2007). Nerve signals and hormones such as histone methyltransferase regulate insect behavior and improve stress resistance during starvation (Shimaji *et al.*, 2017; Zhang *et al.*, 2019).

2.4 Insect cold tolerance

Cold tolerance or hardiness is the ability of insects to survive low temperatures (Zachariassen, 1985; Andreadis & Athanassiou, 2017). Factors which determine whether an insect is cold tolerant or intolerant are unknown, hemispheric origin defined by latitudinal diversity gradients of insects has an influence on their cold tolerance (Sinclair *et al.*, 2003). Recent arguments are that the classification of insects as either freeze tolerant or freeze avoiding is not inclusive and only represents a minority of insects and thus does not accurately represent the overall effects of low temperatures in insects (Bale, 2002). Insects that live in tropical and subtropical zones hardly experience sub-zero temperatures or temperatures below or close to their freezing point (supercooling point [SCP]). Exposure to cold (above freezing point) in these insects causes irreversible damage and even death (Bale, 2002). This has led to the creation of other inclusive classifications which are: chill tolerance, chill susceptible and

opportunistic survival. All three categories of insects experience mortality from above freezing point temperatures (Bale, 1993).

2.4.1 Freeze tolerance

These are insects that can survive internal ice formation. This is achieved by constraining ice crystal formation to the extracellular space to avoid cell and organ injury (Ramløy, 2000; Bale, 2002). During winter ice nucleating agents initiate freezing in the haemolymph. Water then moves out of the cells by osmosis to the haemocoel to create equilibrium. Freeze tolerant insects are the most cold tolerant and are usually located in very cold places (often temperate regions) characterized by harsh freezing winters (Bale, 1996). Characteristically, freeze tolerant insects have low SCPs that prevent sudden freezing and ice formation at low temperatures (Lee & Denlinger, 2010).

2.4.2 Freeze avoidance

Freeze avoiding insects employ various strategies to prevent death from internal body freezing. Insects avoid freezing by increasing their SCPs, expulsion of ice nucleating agents from the body, mainly in the digestive tract and haemolymph and reduction in body water content (Lee *et al.*, 1996, Brown *et al.*, 2004). Another strategy is the increase in low molecular weight antifreeze agents called polyols and sugars that reduce SCPs. Polyols also act as cryoprotectants, preventing protein and enzyme denaturation at low temperatures (Bale, 1996).

2.4.3 Chill tolerance

These are organisms that can survive subzero chilling temperatures but are killed by temperatures above their SCPs. They survive winter low temperatures by reducing their SCPs and can survive for extended number of periods in their supercooling state (Bale, 1996).

2.4.4 Chill susceptible

This is a subset of organisms that can survive low temperatures of up to 0 °C and are usually more vulnerable to low chilling temperatures than chill tolerant organisms. They are usually killed by a further depression of temperatures between -5 to -10 °C (Bale, 1996).

2.4.5 Opportunistic survivors

Opportunistic survival insects are non-cold hardy insects that experience mortality when exposed to temperatures below their low temperature developmental thresholds. Their survival at low temperatures is dependent on finding niches or microhabitats with warmer temperatures (Bale, 1996 Sinclair, 1999).

2.5 Pest Forecasting models

Pest forecasting models assist in monitoring and predicting potential distributions. Development of efficient models involves incorporating environmental factors necessary for a species to complete its life cycle, and the pest's biological characteristics which may affect its distribution patterns (Early *et al.*, 2018). Examples of environmental factors which have been incorporated in FAW prediction models include precipitation, soil moisture and minimum temperature, global mean temperatures and how they affect insect growth and developmental in terms of degree days (Macfadyen *et al.*, 2018; Van den Berg *et al.*, 2020). Biological characteristics of the pest such as the life table data and low and high temperature threshold for development and growth are also important factors in creating pest forecasting models (Van den Berg *et al.*, 2020). Similarly, critical thresholds to activity, e.g. CTLs have become important in models predicting insect biogeographic patterns (Chown & Nicolson 2004). Another factor is phenology events such as flowering of host plants which also affect pest populations (Tonnang *et al.*, 2017). Despite this, pest forecasting models do not take into account potential influence of carry-over effects and plasticity partly due to lack of that type of data. As such, investigation into carry-over effects of plastic responses in pest insects may help refine models for invasive pest species and may assist in improving efficacy of pest management strategies, including outbreak forecasting models.

2.6 The economic importance of *Spodoptera frugiperda*

Fall armyworm causes major economic losses directly through losses during maize production and indirectly through the money spent on chemical pesticides in efforts to control it (Kassie *et al.*, 2020). Maize is also a staple crop in most African countries with majority of subsistence farmers planting maize for food (Day *et al.*, 2017). Estimated lossess of maize due to fall armyworm amount up to 47% in Kenya, and over 134 000 tons in Ethiopia (Beemer 2018; Kumela *et al.*, 2019). Fall armyworm is thus a threat to food security (Tambo *et al.*, 2021),

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CHAPTER 3

Thermal fitness costs and benefits of developmental acclimation in fall armyworm*

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3.1 Introduction

Biological invasions are a growing ecological and economic threat worldwide (Bellard *et al.*, 2016; Diagne *et al.*, 2021). The success and impact of invasive alien species may interact synergistically with environmental changes, such as increasing temperature for improved survival chances of invasive species, but potential synergies between these processes remain largely unknown (Ricciardi *et al.*, 2021). Insects are among the most impactful taxa worldwide when they invade, owing to a myriad of introduction pathways and rapid human-mediated dispersal, driving some of the highest economic costs worldwide among invasive species (Cuthbert *et al.*, 2021; Venette & Hutchinson, 2021). The fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) is an economic invasive insect pest of South America origin (Sparks, 1979) that attacks cereal crops, particularly maize. It first invaded the African region through west Africa in 2016 (Goergen *et al.*, 2016; Stokstad, 2017), and by 2017, it had spread to the whole of eastern Africa and parts of southern Africa including Botswana (Day *et al.*, 2017; Stokstad 2017), before spreading to the Middle East and Asia (India) in 2018 (Sharanabasappa *et al.*, 2019; EPPO, 2020) (Fig. 1).

FAW is a highly multivoltine polyphagous pest, feeding on over 350 plant species across different families (Midega *et al.*, 2018; Montezano *et al.*, 2018; 2019), with a high preference for maize. Larvae feed on leaves, stems, and economic parts of plants e.g., maize cobs, thereby causing economic damage (Rwomushana, 2019). To reach the adult stage, they go through six instar stages, that may take as little as ~11 days (at 32 °C) (Du Plessis *et al.*, 2020). However, FAW larva has striking host plasticity, varying from five to ten instar stages depending on host plant (Ali *et al.*, 1990; Murúa *et al.*, 2003); reportedly having more instars on sub-optimal hosts. This host plasticity facilitates development on less favourable or drought stressed hosts (Esperk *et al.*, 2007). Given this host plasticity, African invasion by the FAW represent a substantial biosecurity threat. For example, FAW has threatened maize production across the world, e.g., the pest accounted for ~17.7 million tons in maize losses across 12 African countries to date (FAO, 2021). Maize remains nevertheless, a staple food for over 200 million people globally (Nuss & Tanumihardjo, 2010) and accounts for 40% of the cereal production in sub-Saharan Africa (FAOSTAT, 2016) with economic, social and political significance. Thus, FAW continental invasion and associated crop losses exacerbates the food security crises in Africa (see e.g., Sasson, 2012).

Bio-physical environmental conditions experienced during early life stages of an organism are significant determinants of many key fitness life history traits (Chown & Nicolson, 2004). In particular, factors such as individual diet, feeding frequency, temperature and relative humidity (RH) during early-

life stages have significant effects on subsequent developmental stages (Mutamiswa *et al.*, 2019; Sasmita *et al.*, 2019; Sgro *et al.*, 2016). Determining the effects of within-developmental stage phenotypic plasticity has been the focus of research for decades (West-Eberhard, 2003; Chown & Nicolson, 2004; Sgro *et al.*, 2016). However, the effects across developmental stages within-generation remain scarce (but see Terblanche & Chown, 2006; Zeilstra & Fischer, 2005), despite their ecological significance. For example, insect developmental stages may be spatially separated, raising questions about how environmental history may differentially shape fitness of subsequent developmental stages within the same generation, but across new environments (e.g., Nyamukondiwa & Terblanche 2010). Indeed, FAW developmental stages may also occupy ≥ 2 spatially distinct bio-physical environments (Melo *et al.*, 2014) resulting in likely different fitness consequences in subsequent instars. Through adult flight migrations (Nagoshi *et al.*, 2012) and larval silking (FAO, 2018), FAW uses this behavioral adaptation to extend its geographical range into novel areas to circumvent inter- and intra-specific competition. However, it is not known whether prior environmental conditions/stress affects fitness of subsequent developmental stages, or aids invasiveness of FAW. Previous studies have nevertheless documented that overcoming environmental barriers, e.g., of temperature and desiccation tolerance, are critical for invasion success (Richardson & Pysek 2006). For this reason, invasive insects often have high basal stress tolerance, phenotypic plasticity (Nyamukondiwa *et al.*, 2010; Kelley, 2014; Wan & Yang, 2016; Machezano *et al.*, 2018) and metabolic flexibility (Smit *et al.*, 2021).

Other external stressors, such as lack of food, have also been documented to influence insect thermal tolerance in complex, and often unpredictable ways (Nyamukondiwa & Terblanche, 2009). Fasting can occur when there is a lack of adequate food (due to various environmental perturbations) to meet the energy requirements for biological processes in the insect's body (McCue., 2010). This food deprivation stress is presumed to result in a trade-off in insect thermal tolerance (Scharf *et al.*, 2016) due to cross talk or cross tolerance (Sinclair *et al.*, 2013). Several studies have supported this notion, as fasting pre-treatment often impairs cold tolerance in insects (Gotcha *et al.*, 2018; Kenny *et al.*, 2008). However, fasting acclimation appears not to have any direct effects on heat tolerance (see Gotcha *et al.*, 2018; Scharf *et al.*, 2016). Nevertheless, how a different stressful trait in one developmental stage may directly or indirectly interact with stress tolerance of the subsequent developmental stages and environments remains unknown in FAW, despite evidence for phenotypic plasticity within and across ontogeny (see e.g., West-Eberhard, 2003; Sgrò *et al.*, 2016) and across different developmental stages and seasons/environments (i.e., 'carry-over' effects) (Norris, 2005; Harrison *et al.*, 2011; Fayet *et al.*, 2016; Ezeakacha & Yee, 2019).

Despite overwhelming evidence of the effects of prior environment on insect fitness (Nyamukondiwa & Terblanche, 2010; Fayet *et al.*, 2016; Ezeakacha & Yee, 2019), few studies have investigated developmental acclimation effects for invasive insects with a view of making inferences for pest invasiveness (Cao *et al.*, 2018; Parkash *et al.*, 2021). Nevertheless, investigation of the effect of environmental history has large ecological implications for organismal fitness under changing environments. Unravelling developmental acclimation is important in determining how species may react to changes in environment across developmental stages and seasons, and how this may shape their fitness and by extension, their population dynamics. This has downstream implications on designing pest control strategies e.g., through development of early warning systems. Here, I thus examined innate within-generation developmental temperature and fasting acclimation effects on heat tolerance in *S. frugiperda* following high temperature acute and chronic acclimation, as well as fasting. Given its tropical origin, heat tolerance may remain a key trait that facilitates invasion, and more-so in arid and semi-arid environments such as Botswana. I hypothesise that temperature and food deprivation stress in one instar may have heat tolerance fitness costs or benefits across other subsequent non-acclimated developmental stages (developmental acclimation). Confirmation of positive adaptive developmental acclimation effects may have implications on *S. frugiperda* invasiveness under heterogeneous stressful environments associated with changing climate. This knowledge is important in informing spatially-dependant *S. frugiperda* pest management strategies.

3.2 Methods

3.2.1 Insect rearing and maintenance

Field populations of *S. frugiperda* were collected as 2nd-5th instar larvae from infested maize crops in two commercial farms; Talana farms (S22°.13467; E28°.59468) and Motloutse River farm, Bobonong Village, Central district of Botswana, and placed in 50 ml vials containing a meridic artificial diet containing both crude and chemically defined ingredients (Table 1), which was adopted from Tefera *et al.* (2010). Both collection areas are within the same region and experience similar climatic environments. Specimens were reared in Memmert climate chambers (Memmert GmbH + Co. KG, Schwabach, Germany) in the laboratory at optimal conditions (28±1 °C, 65±10% RH) and fed on the same artificial insect diet aforementioned (see Tefera *et al.* 2010). Both pupae and moths were kept in Bugdorm cages (Megaview Science Co., Ltd, Taichung, Taiwan) in climate chambers. All adult moths were fed on 10% sucrose solution *ad libitum*. Moths were mated in oviposition cages containing a 4-week-old maize plant (as oviposition substrate). Following oviposition, eggs were incubated and hatched on the host plant. After hatching, 1st instar neonates were subsequently transferred to vials

containing the artificial diet (see Table 1). Each 50 ml vial comprised three larvae, reared up to 3rd instar after hatching in the laboratory. However, following moulting to the 3rd instar stage, all larvae were transferred into individual vials in preparation for the experiments (Fig. 4a) and simultaneously to circumvent larval cannibalism, which is usually more apparent from the 3rd instar onwards (see Rwomushana, 2019). Experiments were run using these lab-reared 3rd instar specimens from F₁ to F₄ generations, randomised across the treatments. I assumed that laboratory adaptation had insignificant effects on thermal fitness across the three tested generations, as has been observed in similar experiments (Opperman 2018; but see Hoffman *et al.*, 2001). Acclimation treatments were done following moulting of 3rd instar larvae, and heat tolerance traits (i.e., critical thermal maxima [CT_{max}] and heat knockdown time [HKDT]) were tested on 4th instar larvae following acute acclimation and 5th instar larvae following chronic acclimation (as majority of the larvae moulted twice during the 3-day chronic acclimation plus one day recovery period).

Table 2: List of ingredients used in the formulation of FAW diet used in the study.

	Ingredient	Quantity
Fraction A	Distilled water	1600 ml
	Brewer's yeast	90 g
	Sorbic acid	2.6 g
	Ethyl phydroxy	4.0 g
	Ascorbic acid	10 g
	Vitamin E capsules	8.4 g
	Sorghum leaf powder	100 g
	Bean powder	350 g
	Sucrose	140 g
Fraction B	Agar	50 g
	Distilled water	1600 ml
Fraction C	Formaldehyde	4 ml
	Antibiotic	5 g

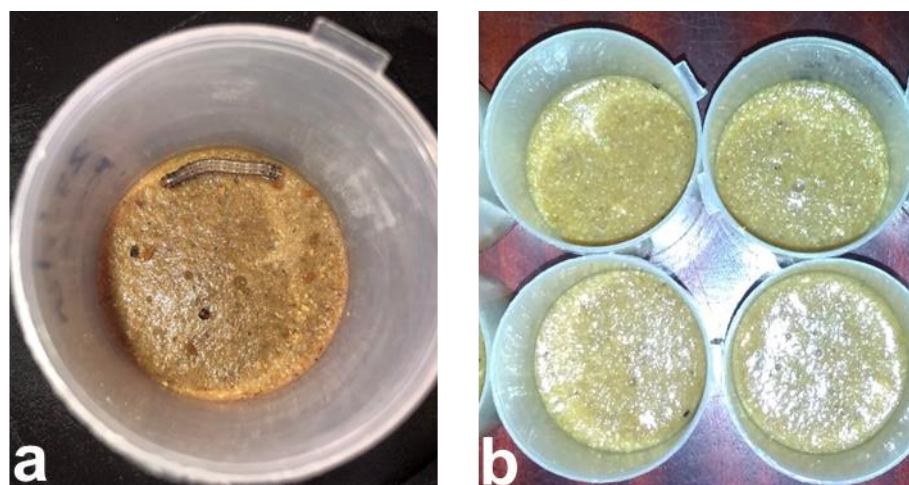


Figure 4: a) FAW larvae in 50ml container with diet b) Newly prepared FAW diet in 50 ml plastic vial containers.

3.2.2 Acclimation experiments

Experimental treatments (acclimation) comprised acute and chronic sub-lethal high temperature acclimation and fasting. This was undertaken in 3rd instar larvae by exposing insects to temperatures of 28.0 (control) 32.0, 35.0 and 38.0 $\pm$ 1.0 $^{\circ}$ C (each under 65 $\pm$ 10% RH) for 3 hours (acute) and 3 days (chronic) acclimation treatments (see Fig. 4) in Memmert climate chambers. Temperatures selected for acclimation were ecologically relevant and based on a previous study that showed temperatures across Botswana to reach up to 42 $^{\circ}$ C during heat waves (Moses, 2017; see Fig. 4), and considering that the optimal temperature range of *S. frugiperda* is 26-30 $^{\circ}$ C (Du Plessis *et al.*, 2020). From the optimum temperature range, 28 $^{\circ}$ C was selected as the control temperature and 3-4 $^{\circ}$ C were added to establish mild high temperatures for acclimation, based on modified protocols from Mutamiswa *et al.* (2019). Control insects were kept at optimal environmental conditions of 28 $\pm$ 1 $^{\circ}$ C and 65 $\pm$ 10% RH during experimental treatments before measuring thermal fitness traits (Fig. 4). Following both acute and chronic acclimation, insects were allowed to recover at optimal conditions (28 $\pm$ 1 $^{\circ}$ C and 65 $\pm$ 10% RH) for 24 hours before measuring physiological traits.

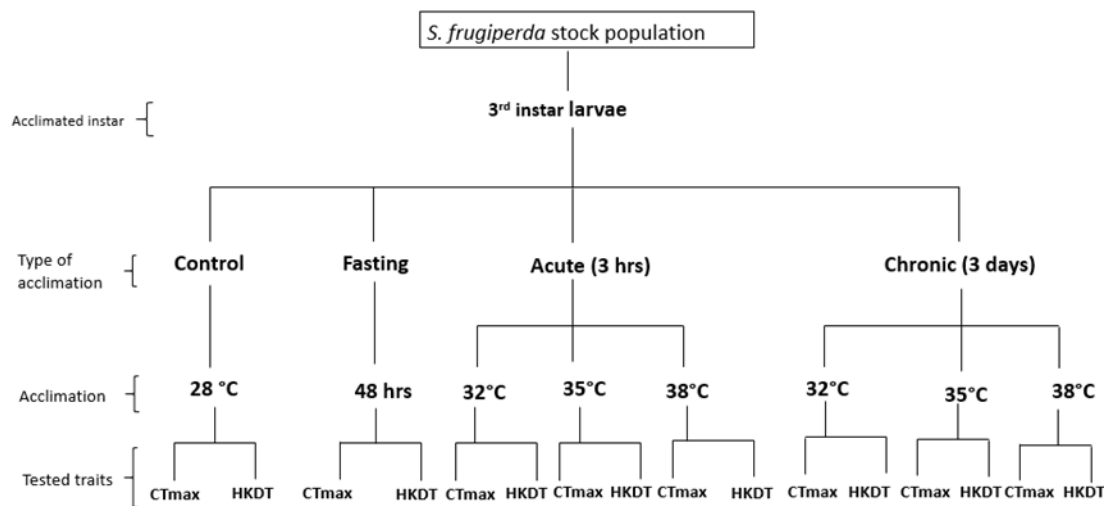


Fig. 4: Experimental flow diagram indicating acclimated developmental stages, type of acclimation (acute versus chronic), acclimation temperatures (28 [control]; 32; 35 and 38 $^{\circ}$ C) and the traits tested (HKDT and CT_{max}). Traits were tested on instar 4 in acute acclimation and instar 5 in chronic acclimation pre-treatments. Heat knockdown temperatures were acclimation-treatment temperature-specific, and were derived from average CT_{max} values, following methods by Hoffman *et al.* (2003), CT_{max} = critical thermal maximum; HKDT = heat knockdown time.

To determine the carry-over effects of feeding status on the thermal fitness of *S. frugiperda*, 3rd instar larvae were deprived of food (fasted) for 48 hours. All fasting acclimations were done using a constant time period (48 hours), and results were directly compared to those of acute and chronic temperature treatments. Larvae were removed from artificial diet at 3rd instar and individually placed into empty 50 ml vials without any food, but with a water source (cotton wad, to prevent desiccation associated mortality) for 48 hrs. The larvae were kept under benign conditions (28 ± 1 °C, $65 \pm 10\%$ RH; 12L: 12D) to ensure that food deprivation was the only limiting factor. Post 48 hrs, larvae were returned to individual vials with access to food (artificial diet) and water *ad libitum* for 24 hours to allow recovery. Measurement of thermal traits was conducted 24 hours post-recovery following methods by Gotcha *et al.* (2018). Control larvae were provided with artificial diet and kept at optimal temperatures and RH (28 ± 1 °C and $65 \pm 10\%$) throughout prior to running experiments

3.2.3 Heat tolerance metrics

To test the effects of heat and fasting acclimation on heat tolerance, (i) CT_{max} – the maximum temperature allowing insect activity, measured as a loss of response to mild stimuli (Chown & Nicolson, 2004, Nyamukondiwa & Terblanche, 2009), and (ii) HKDT – the time taken to knock down an insect following acute heat stress, were measured (Chown & Nicolson, 2004). Both traits are ecologically sound heat tolerance indices (Lutterschmidt & Hutchison, 1997; Huey & Kearney, 2020) and correlate well with insect biogeographical patterns. For CT_{max}, individual 4th instar (for acute acclimation) and 5th instar (for chronic acclimation) larvae were placed into an insulated double jacketed chamber with ten ‘organ pipes’ connected to a programmable bath filled with 1:1 water: propylene glycol, which regulates the flow of liquid around the chamber (Grant GP200-R4, Grant Instruments, UK) (Nyamukondiwa & Terblanche, 2009). Critical thermal maxima experiments started at 28 °C (FAW optimum temperature) from which temperature was gradually increased using a ramping rate of 0.25 °C /min until the larvae reached upper temperature limit of activity (CT_{max}) (Nyamukondiwa & Terblanche, 2009) (Table 2). This ramping rate is faster than the natural diel increase in temperature, but nevertheless ecologically more relevant as it is lower than other ramping rates used in literature e.g., 0.5 °C/minute (reviewed in Chown & Nicolson, 2004). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05 °C) was inserted into the middle organ pipe to record the chamber temperature. The experimental procedure was repeated 3 times to yield n ~ 30 larvae per treatment (30 replications). In this study, CT_{max} was defined as the temperature at which an individual larva lost co-ordinated muscle function (self-righting) and ability to respond to mild prodding using a thermally inert object.

Heat knockdown time was assessed on 4th instar larvae (following acute acclimation) and 5th instar larvae (following chronic acclimation) using standardized protocols (Nyamukondiwa & Terblanche, 2009). I used treatment-specific heat knockdown temperatures, derived from each treatment's CT_{max} value plus 2 °C. This heat knockdown temperature is ecologically sufficient to elicit heat knockdown effects in insects (see e.g., Hoffman *et al.*, 2003; Mutamiswa *et al.*, 2019). Thus, knockdown temperatures of 53.0, 50.0, 51.4, and 50.7 °C were used as acute knockdown temperatures for 28 (control) 32, 35, and 38 °C acclimation pre-treatments respectively, whereas 53.0, 51.6, 51.9 and 50.8 °C were used as chronic knockdown temperatures for 28 (control) 32, 35, and 38 °C acclimation pre-treatments, respectively. Ten individual larvae were placed in 1.5 ml microcentrifuge tubes and placed in a Memmert climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at various temperatures as indicated above. Temperatures above CT_{max} cause heat coma in insects and are often used in HKDT assays (see Nyamukondiwa *et al.*, 2011). A video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) linked to a computer was connected to the climate chamber and used to monitor knockdown activity and timing. Heat knockdown time was defined as the time (in minutes) at which an individual larva lost activity following acute heat stress.

3.2.4 Data analysis

Data analyses were all performed using R, version 4.1.1 (R Development Core Team, 2021). The residuals were first checked for normality and variance homogeneity using Shapiro–Wilks and Levene's tests, respectively, and were found to violate normality and homogeneity of variance assumptions. Kruskal-Wallis tests were thus used to examine CT_{max} and HKDT as a function of treatment for each respective acute and chronic exposure treatment (i.e., four separate models). Dunn tests were used post-hoc for pairwise comparisons, with p-values adjusted via the Holm method (Ogle *et al.*, 2021).

I summarized the magnitude of both CT_{max} and HKDT following acclimation using methods by Tarusikirwa *et al.* (2020) and Mutamiswa *et al.* (2019). Specifically, I calculated the magnitude of change in thermal fitness conferred by the acclimation treatment using the formula below, where the mean heat tolerance (CT_{max} / HKDT) after each treatment was divided by the mean control CT_{max} or HKDT results of which were tabulated into Table 1.

$$\text{Magnitude of change} = \frac{\text{Final CT}_{\text{max}} \text{ (or HKDT) for each treatment}}{\text{Control CT}_{\text{max}} \text{ (or HKDT)}}$$

where a value > 1 denotes beneficial acclimation effects, whereas a value < 1 denotes potentially deleterious acclimation effect (see Leori *et al.*, 1994; Marais & Chown, 2008).

3.3 Results

For acute exposures, CT_{max} differed significantly among control group, 32 °C, 35 °C and fasting acclimation treatments ($\chi^2 = 58.397$, $df = 4$, $p < 0.001$). Control group 4th instar CT_{max} was significantly higher than 32 °C, 35 °C and fasting acclimation treatments (all $p < 0.01$), but not 38 °C ($p > 0.05$). In turn, 38 °C treatment CT_{max} was significantly greater than 32 °C, 35 °C and fasting acclimation groups (all $p < 0.05$). CT_{max} following fasting acclimation significantly exceeded the 32 °C treatment ($p < 0.05$) (Fig. 3a). In addition, heat tolerance (CT_{max}) for acute treatments was significantly lower than chronic exposure treatments ($\chi^2 = 35.070$, $df = 4$, $p < 0.001$). For chronic acclimation, control group CT_{max} was again highest, and significantly greater than 32 °C, 38 °C and fasting acclimation treatments (all $p < 0.05$), but not 35 °C ($p > 0.05$). CT_{max} at the 35 °C treatment was also significantly greater than 38 °C and following fasting acclimation (both $p < 0.05$) (Fig. 5b).

Following acute 3rd instar acclimation exposures, HKDT differed significantly among treatment groups ($\chi^2 = 51.220$, $df = 4$, $p < 0.001$). Control HKDT was significantly lower than 32 °C and fasting acclimation groups (both $p < 0.05$), but not 35 °C or 38 °C (both $p > 0.05$). Fasting acclimation and 32 °C treatment groups had the highest HKDT, which significantly exceeded 35 °C and 38 °C (all $p < 0.001$) (Fig. 5c). For chronic exposures, HKDT again significantly differed among treatments ($\chi^2 = 32.332$, $df = 4$, $p < 0.001$). Control group HKDT was significantly lower than 32 °C, 38 °C and fasting acclimation groups (all $p < 0.01$), but not 35 °C ($p > 0.05$), whereas 38 °C significantly exceeded 35 °C ($p < 0.05$) (Fig. 3d).

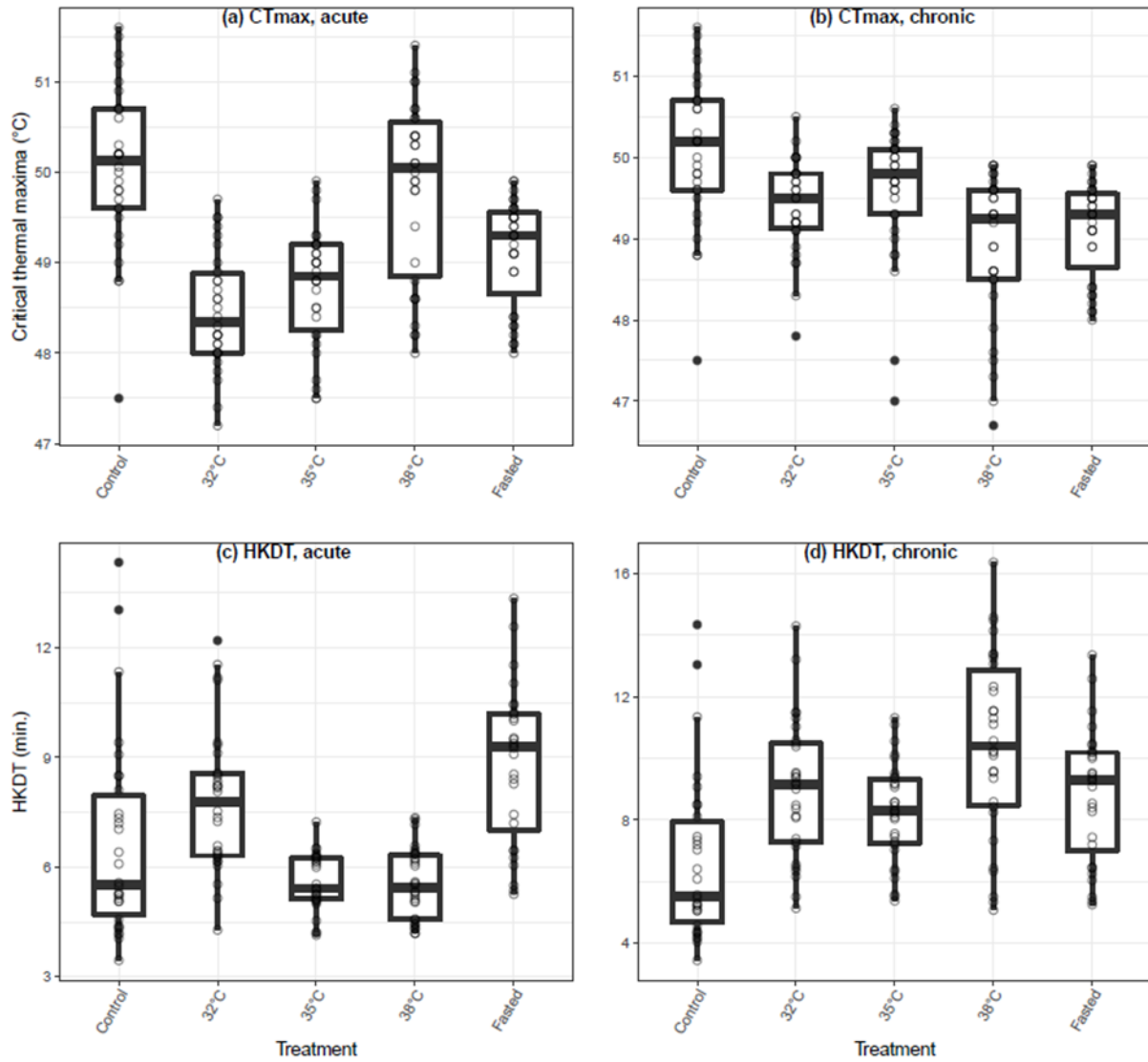


Fig. 5: Critical thermal maxima (CT_{max} [a, b]) and heat knockdown time (HKDT [c, d]) following acute (a, c) and chronic (b, d) temperature treatments. For all fasting pre-treatments, organisms were deprived of food for 48h. In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges, and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. Points are raw data. Acute and chronic represents acute and chronic acclimations

Chronic and acute acclimation had contrasting results on the direction of both CT_{max} and HKDT plasticity (Table 2). Both acute and chronic acclimation, as well as fasting, yielded negative deleterious plastic effects (i.e., negative magnitude; see Table 2) for CT_{max}, whereas positive effects on HKDT were exhibited following chronic exposure and fasting, but not acute exposures beyond 32 °C.

Table 2: Magnitude and direction of change of critical thermal maxima and heat knockdown time.

Type of acclimation	Acclimation temperature (°C)	*Magnitude of change (CT _{max}) (folds)	*Magnitude of change (HKDT) (folds)
Acute	32	0.968	1.175
	35	0.974	0.840
	38	0.995	0.832
Chronic	32	0.991	1.290
	35	0.099	1.240
	38	0.995	1.572
Fasting	28	0.981	1.130

*Magnitude < 1 indicates deleterious (negative) effects of acclimation, whereas magnitude > 1 indicates beneficial (positive) effects of acclimation

3.4 Discussion

Population dynamics of individuals that may occupy multiple spatially-distinct habitat environments can be highly complex (Webster *et al.*, 2002). As such, sub-lethal stressful conditions experienced during early developmental stages e.g., early instars of the larvae, may be important in determining key life history traits, either manifesting as beneficial through e.g., developmental acclimation effects (see Chown & Nicolson, 2004), or as deleterious (see e.g., Marais & Chown, 2008). Results showed that fasting and high temperature acclimation at the 3rd instar larval stage had mixed effects on the heat tolerance of subsequent developmental stages (4th instar larval stage) in FAW, tested as CT_{max} and HKDT, and following both acute and chronic acclimation. In particular, fasting, acute and chronic high temperature acclimation of 3rd instars reduced CT_{max} of 4th instar larval stage of *S. frugiperda*. This may point to a deleterious effect of developmental acclimation conditions tested here on *S. frugiperda* heat tolerance. Thus, the prior exposure to sub-lethal environmental stressors early in life may have negative consequences for subsequent developmental stages, synonymous to deleterious acclimation (Marais & Chown, 2008), and suggest that both the acclimation treatment and CT_{max} stress test effects are additive

(Jorgensen *et al.*, 2021). Although chronic acclimation was lower than control, it generally had higher CT_{max} than the acute acclimation treatments at the same temperatures. However, HKDT assays yielded contrasting results from those of CT_{max} , with both fasting and specific high temperature acclimation treatments increasing heat tolerance (HKDT) for both acutely and chronically acclimated 3rd instar *S. frugiperda*. Results, thus, confirm that acclimatory exposure of one developmental stage may have fitness costs and benefits to subsequent developmental stages, and that these costs and benefits depend on the context of the fitness metric being tested (e.g., CT_{max} vs HKDT). Thus, exposure to sub-lethal stress during development may have fitness and survival consequences on later life stages (Klockmann *et al.*, 2017), and by inference, could affect the propensity of invasive species establishment success in novel environments.

While acclimatory conditions experienced during one life stage of an organism can have fitness costs and benefits within that life stage, acclimatory effects can also manifest across developmental stages. Developmental acclimation effects are adaptive and may presumably facilitate ‘carry-over’ stress resistance under novel stressful environments. Results showed that most acute and chronic heat acclimation treatments, as well as fasting, significantly depressed CT_{max} . This result means that sub-lethal food deprivation and heat stress at the 3rd instar stage larvae may have negative CT_{max} fitness consequences on 4th and 5th instar larvae and probably by extension, other subsequent developmental stages. Climate change is often associated with episodes of acute and chronic heat stress, and prolonged droughts that may limit food resources (IPCC, 2014; Stillman, 2019). Thus, the deleterious effects of acclimation treatments recorded here may mean that frequent episodes of environmental heat and food deprivation stress faced in nature may offset heat tolerance of subsequent developmental stages, affecting population dynamics of invasive species. The reason for the negative effects of heat acclimation reported here are largely unknown. However, it may point to the notion that the stress faced during acclimation treatment and CT_{max} assays is additive (see Jorgensen *et al.*, 2021). Moreover, I may have missed certain acclimatory cues or specific treatment combinations that likely elicit CT_{max} acclimation responses in *S. frugiperda*, specifically. Thus, future studies may need to explore differential temperature and time combinations at all higher instar stages of the larvae that may elicit acclimation. One more interesting result observed here is that a treatment to one stress may also have negative effects on a divergent stress. For example, 3rd instar acute and chronic fasting acclimation had deleterious consequences on 5th instar larval CT_{max} . This may point to the notion that injury associated with diverging environmental stresses may be the same (see e.g., Shen *et al.*, 2015; Farahani *et al.*, 2020), and that divergent stress effects may thus be additive. Nevertheless, the lack of beneficial

acclimation effects for heat acclimation reported here is consistent with studies on *Tuta absoluta* that reported no beneficial acclimation effects following chronic high temperature acclimation (Tarusikirwa *et al.*, 2020).

By contrast, both acute and chronic 3rd instar larvae acclimation had beneficial effects on subsequent larval HKDT, albeit for specific treatments (32 °C and fasting for acute acclimation, and 32, 38 °C and fasting for chronic acclimation). Conditions eliciting acclimation responses are highly complex and often context-dependent (Chown & Nicolson, 2004; Sgro *et al.*, 2016; Mutamiswa *et al.*, 2019). This agrees with my results, that observed positive acclimation responses were specific to certain heat acclimation groups and corroborates with previous reports suggesting that conditions conferring acclimation responses are highly context-dependent (see Mutamiswa *et al.*, 2019). Similarly, acute heat acclimation at 35 and 38 °C, and chronic heat acclimation at 35 °C, had no significant effects on HKDT. This result means that heat wave episodes associated with climate change on 3rd instar larvae of *S. frugiperda* may have positive or neutral effects on subsequent developmental stages in the context of HKDT but not CT_{max}. Thus, *S. frugiperda* may have fitness benefits under projected heat stress in terms of enduring long durations of mild to high temperature stress associated with changing climates, potentially translating into greater invasiveness and resilience in high temperature tropical habitats.

Comparisons for HKDT experimental traits have often been investigated using a more constant temperature (see Chown & Nicolson 2004). However, here, I used different heat knockdown temperatures to investigate HKDT across different acute and chronic temperature treatments. Thus, differences in results reported here may also be partly due to the treatment-specific heat knockdown temperature methodology used in my study. Furthermore, the results also showed the positive beneficial effects of acclimation to a divergent stress trait (fasting) on a different stressor (heat tolerance [HKDT]). Such cross tolerance represents shared co-evolutionary response mechanisms to stress traits involved (see Gotcha *et al.*, 2018), and represent another facet that may help invasive species survive highly variable stressful environments, e.g., through integrated stress resistance (see discussions in Renault *et al.*, 2015). Cross tolerance results reported here are nevertheless in contrast with reports on *Ceratitis rosa*, where fasting resulted in increased CT_{max} and reduced HKDT (Gotcha *et al.*, 2018). These results indicate that fasting has dissimilar effects on different traits used to measure heat tolerance, and that elicitation of acclimation responses are trait dependant. Results obtained here thus mean that food deprivation (i.e., temporary absence of host plants) during mid-season droughts, and other plant-damaging natural disasters such as hailstorms, may provide a benefit in periods of rapid heat shock, such as heat waves, through cross tolerance developmental acclimation effects.

Increased heat tolerance (HKDT) reported here for certain treatments may represent context-specific expression of heat shock proteins and other hormonal heat stress regulators (Hoffmann *et al.*, 2003; King & Macrae, 2015). Heat shock proteins and hormonal activation due to heat stress usually occur over a certain temperature threshold, which are context-dependent but usually range from 39 – 41 °C (Qazi *et al.*, 2019). Similar results were observed in *Drosophila mojavensis* (Patterson & Crow, 1940), where high temperature acclimation at one developmental stage increased HKDT in subsequent developmental stages (Diaz *et al.*, 2021). Indeed, sublethal high temperature stress may influence physiological traits in addition to other life history traits e.g., fecundity and longevity (Nguyen & Amano, 2010). A study on *Plutella xylostella* (L.) showed that high temperature acclimation on larval stages had effects on adult stages, affecting oviposition patterns and adult life span (Zhang *et al.*, 2015). Results thus indicate that timing of sub-lethal high temperature stress such as heat waves may enhance thermal fitness and survival of other life stages of invasive pest insects.

Therefore, survival of *S. frugiperda* may increase in hot tropical areas even during high temperature incidences. Inclusion of carry-over effects of extreme high temperature incidences in pest management forecasts will increase accuracy of invasive species biogeographical patterns (Skendžić *et al.*, 2021). Likewise, processes and events at one developmental stage and in a particular season may have far-reaching consequences for other developmental stages in a different season; a phenomenon called ‘carry-over’ effects (Harrison *et al.*, 2011). While this phenomenon has largely been explored in migratory birds, little is known on how this affects the fitness of migratory insects in general (but see Galarza *et al.*, 2019), and invasive insects in particular. Future work should accordingly examine carry-over effects in *S. frugiperda* and assess how they may shape population dynamics and pest invasiveness. I also observed that acclimation responses were trait-dependant and observed such positive effects only for HKDT; a more acute heat stress metric compared to CT_{max}. I thus speculate, with caveats, that the differences in chronic exposure to stress during CT_{max} and HKDT assays may also have affected differential acclimation responses reported here. Future studies should thus look at more acute CT_{max} ramping methodologies e.g., 0.5 or 1 °C/minute (see e.g., Terblanche *et al.*, 2007) to try and capture potential acclimation cues that I may have missed. Failure to provide larvae with water during starvation experiments might have affected results. However, some environmental stressors are related and act interactively in nature (Nyamukondiwa *et al.*, 2022). It is rare to find food without water; therefore, normally insects would be exposed to both fasting and low relative humidity/water stress simultaneously. Herbivorous insects such as FAW get most of their water from plant sap as opposed to insects that inhabit or feed on dry products such as stored product pests (Mellanby & French, 1958).

Costs and benefits of plastic acclimation are difficult to predict and are not uniform across species, metrics tested and acclimation treatments. A study done by Alemu *et al.*, (2017) showed that age, increasing rate of temperature change and duration of heat hardening resulted in a benefit or increase in CT_{max} . Factors such as body mass have also been shown to have a cost on HKDT (Nyamukondiwa *et al.*, 2018). Results thus show mixed but context (trait)-dependent effects of both acute and chronic developmental acclimation. Specifically, I first show that while both heat and fasting acclimation at one developmental stage negatively affected heat tolerance measured as CT_{max} , it contrastingly improved another heat tolerance trait (HKDT), suggesting differential trait related acclimation responses. The positive beneficial acclimation responses for HKDT were, however, treatment dependant, suggesting specific cues eliciting acclimation for the HKDT trait. Second, I showed that while heat acclimation had negative effects (additive stress effects [see Jørgensen *et al.*, 2021]) for CT_{max} (same stressor), acclimation to a divergent stress (food deprivation) may also have additive stress effects on a different stress (heat tolerance [CT_{max}]). These results support the hypothesis that abiotic stress in one developmental stage may have fitness costs and benefits in the subsequent developmental stages (e.g., Ezeakacha & Yee, 2019) and that a divergent stressor (fasting) may have positive heat tolerance (HKDT) effects. I could, however, not find acclimation responses for CT_{max} and other treatments for HKDT. I speculate that I may have missed exact temperature-time treatment combinations that elicit acclimation response for CT_{max} and HKDT. Thus, future work should consider more comprehensive acclimation treatment combinations. Forecasting of insect pest invasions are usually done through prediction of how changes in mean temperature affect insect pest population dynamics (Zhang *et al.*, 2015). The results provide a step into understanding costs and benefits of developmental acclimation in *S. frugiperda* and how it may affect pest population dynamics. Future pest forecasting models should thus incorporate developmental acclimation responses and by extension, ‘carry-over’ and integrated stress resistance effects (see e.g., Norris & Taylor, 2006; Nyamukondiwa *et al.*, 2022) to potentially improve accuracy of model outputs and pest early warning systems.

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CHAPTER 4

Rapid responses are essential: adaptive temporal variation in cold tolerance of the invasive Fall armyworm

4.1 Introduction

Temperature plays an important role in ectotherms' growth and development and thus ecological structure of insects (Mellanby, 1939; Taylor, 1981; Sinclair *et al.*, 2015). For example, temperature affects insect phenology (Glazaczow & Bogdziewicz, 2016), fecundity and longevity (Ma *et al.*, 2017; Garrad *et al.*, 2016) and activity e.g. mate finding and ecosystem services (Lee & Denlinger, 2010; Gotcha *et al.*, 2021). As such, temperature may singly be the biggest environmental barrier for invasive insects' establishment and success in invaded environments (Richardson & Pyšek, 2006; Nyamukondiwa *et al.*, 2022). Given the increase in extreme temperatures with climate change (Johnson *et al.*, 2018), insects are thus continually exposed to sub-optimal low temperatures at different diurnal and seasonal timescales. Low temperatures limit insects' activity: prolong development, and in extreme cases may cause cell and tissue injury and death. To survive this, insects thus have evolved various strategies to overcome these stressors while maintaining key functional traits at low temperatures (Block, 1990). Insects may overwinter through using different mechanisms including diapause, migration, reduction of supercooling points (SCP) and directed internal body freezing (Bale & Hayward, 2010; Gullan & Cranston, 2014). The role of diapause and migration in overwintering has extensively been discussed in previous literature and helps organisms escape extreme low temperatures *in situ* and *ex situ* respectively (Bale & Hayward, 2010; Chapman *et al.*, 2015). The reduction of SCPs allows organisms to depress freezing temperatures and thus prevent bodily fluids from freezing injury (Zachariassen, 1985). Similarly, directed body freezing involves osmotic sequestering of water molecules to the extracellular space thus limiting organ and tissue damage from ice crystals. This strategy is used by a few insects that can withstand very low winter temperatures (Ramløy, 2000; Bale, 2002). Diapause and migration are widely used by insects and represent two key strategies that explain overwintering survival. For insects overwintering *in situ*, tracking environmental ambient temperature and adjusting stress responses is critical. Thus, rapid cold hardening – the ability to improve low temperature tolerance in the short-timescales is critical to surviving short bursts of low temperatures (Lee *et al.*, 1987). Empirical studies have shown that short- and long-term thermal acclimation improves thermal phenotypes in many insects (Sgrò *et al.*, 2016; Segaiso *et al.*, 2022). This mechanism may increase survival in thermally variable environments and compensate for more prolonged natural selection (Basson *et al.*, 2012; Tarusikirwa *et al.*, 2020). Previous studies have shown that for FAW, rapid cold hardening increased glycerol content and improved SCPs. However, several other factors such as permeability of cells to cryoprotectants also affects the ability of insects to rapidly adjust their low temperature tolerance phenotypes (Vatanparast & Park, 2022).

Several ecologically relevant traits have been proposed to measure insect cold tolerance (MacMillan & Sinclair, 2011). Chill coma recovery time (CCRT) measures the time taken for an organism to regain self-righteousness following chill coma while critical thermal minima (CT_{min}) is the minimum temperature that allows organismal activity (MacMillan & Sinclair, 2011; Sinclair *et al.*, 2015). Supercooling point represents the temperature at which bodily fluids freeze and is a critical trait especially for organisms inhabiting sub-zero temperature environments (Block, 1990; Renault *et al.*, 2002).

Climate change has resulted in more benign to warmer winters, particularly at higher altitudes (MacDonald, 2010). Furthermore, this has caused shifts in seasons, in particular earlier springs, extended growing seasons for plants and changes in phenological syncs (Marshall *et al.*, 2020; Liu *et al.*, 2018). Despite the warmer winters, there is an increased trend of acute extreme low temperature events even in areas or seasons that are not characterized by sub-zero temperatures (Kug *et al.*, 2015; van Der Walt & Fitchett, 2021; Macquarrie *et al.*, 2009). This means that insects may be subjected to a greater degree of cold stress (Marshall *et al.*, 2020) as well as high temperature variations (Marshall *et al.*, 2020). These episodes of seasonal high and low temperature variations allow insects to recover when temperatures are warmer and may thus favor continuous insect development and activity (Renault, 2011; Colinet *et al.*, 2018). However even though fluctuating temperatures may not cause immediate mortalities, repeated exposure to low temperature may result in energetic and fitness costs and change in phenology ultimately affecting winter survival (Marshall & Sinclair, 2015). In *Choristoneura fumiferana* frequent exposure to cold had a greater effect on survival compared to duration and intensity of the cold (Marshall & Sinclair, 2015). Insects thus should track micro ambient environmental temperature to safeguard against rapid temperature fluctuations and prevent mortality associated with acute changes in ambient environments (Halsch *et al.*, 2021).

As shown in previous chapters, the fall armyworm is an invasive pest of cereal crops including its main host maize (Midega *et al.*, 2018). It originates from South America but has invaded most of Africa and Asia due to similar warm climates found in the continents and high flight mobility (Johnson, 1987; Sparks, 1979; Nagoshi *et al.*, 2012; FAO, 2020). Climate change is partly characterized by extreme temperature anomalies within seasons and high variations temporally (Davis-Reddy & Vincent, 2017; Engelbrecht & Monteiro, 2021). Insects thus survive these extreme temperature events through overwintering – e.g. via cold hardiness strategies. While Africa is predominantly tropical (Davis-Reddy & Vincent, 2017), spatial differences in winter conditions exist, with several localities experiencing sub-zero winter temperatures (van Der Walt & Fitchett, 2021). Thus, while several localities may

present benign winter temperatures to facilitate continual development and activity during the cold season, overwintering strategies for FAW may be highly spatially dependent. For example, invasion of the FAW in China has been restricted to the southern region. Because of its low overwintering capacity, FAW cannot survive the low winter temperatures in southern China and other Asian countries such as Japan and Korea thus, it re-invades these areas post-winter (Early *et al.*, 2018; Li *et al.*, 2020). Similarly, in South Africa, FAW overwinters in Northern Limpopo and KwaZulu-Natal, where they re-invade other parts of the country, including the Eastern Cape and Western Cape (Van den Berg *et al.*, 2020). Modeling using CLIMEX has indicated that Southern Africa has suitable climate for population growth of FAW and it is predicted that Southern Africa will suffer chronic year round infestation of FAW in future (Du Plessis *et al.*, 2018; Abrahams *et al.*, 2017). However, the model does not explicitly indicate whether FAW is able to track habitat microclimates of the tropical seasons to survive the harshly variable temperature.

Unlike other insects, FAW does not diapause. It survives winter by migration of adults to warmer environments and reinvades colder environments post winter (Sparks, 1979; Nagoshi *et al.*, 2012, Westbrook *et al.*, 2016). It is predicted that similar to its winter migration to South Florida & Texas and re-invasion in North America post winter, in future FAW will migrate to east China during winter and reinvade south China from the east after winter (Luginbill, 1928; Li *et al.*, 2020). Data related to these FAW migration patterns for tropical Africa are lacking. Most research to date has focused on high temperature tolerance and plasticity and its effects on insect population dynamics, ignoring the low temperature components (Keosentse *et al.*, 2022; Huang *et al.*, 2021; Segaiso *et al.*, 2022) that may relate to overwintering capacity for seasonal survival. To my knowledge, no work has looked at the ability/inability of *S. frugiperda* to display seasonal plastic changes in cold tolerance traits, despite its importance in rapidly changing environments. Here, I thus investigated if FAW critical low temperature activity traits likely adaptively tracked the seasonal prevailing temperature. Specifically, I tested seasonal changes in larval low temperature tolerance traits *vis* CT_{min}, CCRT and SCPs in two seasons (each replicated twice) in Botswana. I hypothesised that FAW cold tolerance will be higher as temperatures plummet through winter and that it will be lower during high summer temperatures.

4.2 Methods

4.2.1 Field collection of insects

Field populations of *S. frugiperda* were collected as 3rd instar larvae from commercial and subsistence farms within Central District, Botswana: Bobonong (S 22°13'46.7"; E 28°59'46.8"), Machaneng (S 23°04'32.0"; E 027°31'8.74") Dikabeya (S 22°28'37.2"; E 027°13'49.8"), Serowe (S 22°26'00.3" E 026°49'05.6"). These three sampling areas are separated by ~214 km (furthest point), are environmentally homogeneous and have more or less similar vegetation and experiencing similar climate conditions. For both summer seasons sampling was done in December (4th December in summer 2020 and 2nd – 12th December in summer 2021). Sampling was done on the 11th of June for winter 2021 and on the 4th and 9th of May for winter 2022). Summer months in Botswana are between November to March while winter is from May to August (Jain *et al.*, 2006). Insects were collected and individually placed in 50 ml vials containing artificial diet, adopted from Tefera (2010). In the laboratory insects were kept in Memmert climate chambers (Memmert GmbH + Co. KG, Schwabach, Germany) set at 28 °C, 65% relative humidity (RH) and photocycle period of 12 h light and 12 h dark. Insects were kept for a period not exceeding 24 hr before experiments were run to circumvent effects associated with laboratory adaptation.

4.2.2 Cold tolerance metrics

4.2.2.1 Critical thermal minima (CT_{min})

Critical thermal minima were measured using protocols by Nyamukondiwa & Terblanche (2009). Individual 3rd instar larvae were placed in organ pipes in an insulated double-jacketed chamber connected to a programmable water bath. The water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG) filled with 1:1 water: propylene glycol was set at a ramping rate of 0.25 °C/min at which the insects were subjected to a gradual temperature decrease in the chamber. The temperature at which larvae lost coordinated muscle function and did not respond to gentle probing by a thermally inert object was recorded as the CT_{min} (Mutamiswa *et al.*, 2017). The temperature was recorded using a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05°C) inserted in the control organ pipe. The experiment was run twice yielding n = 20 (20 replications per treatment).

4.2.2.2 Chill coma recovery time (CCRT)

Chill coma recovery time experiments were conducted using established protocols (Mutamiswa *et al.*, 2018; Weldon *et al.*, 2011). Ten insects were individually placed in 2.0 ml microcentrifuge tubes and placed in two water tight ziplock bags layered one on top of the other. A thermocouple (type K, 36

SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05°C) was inserted into a separate 2.0 ml microcentrifuge tube and placed together with the insects in the ziplock bags. The Ziplock bag with the insects and thermocouple were submerged into a water bath (Systronix, Scientific, South Africa) filled with absolute alcohol and set at 0 °C for an hour. It has been previously shown that this temperature and time treatment elicits chill coma in FAW and other related insect taxa (Keosentse *et al.*, 2021; Tarusikirwa *et al.*, 2020). After 1 hour, the insects were quickly transferred to a climate chamber (Memmert GmbH + Co. KG, Schwabach, Germany) set at optimal 28 °C and 65% RH for recovery. Data was recorded using a video recording camera (HD Covert Network Camera, DS2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) connected to the climate chamber. The time (in minutes) it took for insects to regain consciousness i.e. self-righteousness through e.g. movement after being chilled was described as the CCRT (Tarusikirwa *et al.*, 2020). The experiment was performed twice, yielding n = 20 (20 replications).

4.2.2.3 **Supercooling point (SCP)**

Supercooling points were measured using protocols outlined by Nyamukondiwa *et al.*, (2013). Insects were individually placed in 2.0 ml microcentrifuge tubes, each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762–1121, Cambridge, UK). To ensure that the insect and the thermocouple stayed in contact they were secured using cotton wool. Thermocouples were connected to an 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) interfaces and temperatures were recorded at 1 second intervals using PicoLog software for windows (Pico Technology). Insects were first subjected to a temperature of 15 °C for 10 minutes followed by a gradual decrease in temperature at rate of 0.25 °C/min until their SCPs were recorded. SCP for each organism was determined as the lowest temperature recorded before a spike in temperature due to latent heat of crystallization (Nyamukondiwa *et al.*, 2013).

Natural ambient temperature and rainfall data for the specific sampling sites for the two seasons were obtained from the Department of Meteorological Services, Botswana.

4.2.3 **Data analyses**

Data was analyzed using Statistica 14.0.0.15 (Statsoft Inc., Tulsa, Oklahoma). The data was tested for normality using Shapiro–Wilks test which indicated that data was not normally distributed; therefore, non-parametric analyses techniques were adopted. Kruskal-Wallis non-parametric test was used to compare CT_{min}, CCRT and SCP of the FAW populations collected in the different seasons. Kruskal-Wallis post-hoc was used to separate statistically heterogeneous medians. Because no significant

differences were observed across treatments for SCPs, a frequency distribution curve was done using Statistica 14.0.0.15 (Statsoft Inc., Tulsa, Oklahoma). Temperature and rainfall data were plotted in OriginPro 8, version 8E (Origin Lab Corporation, Massachusetts, USA).

4.3 Results

Temporal seasonal temperature changes had significant effects on FAW low temperature tolerance limits measured as CT_{min} (Kruskal-Wallis test: $H(4, N=101) = 38.82554, p < 0.0001$) (**Fig. 6**). The CT_{min} for all the winter seasons' larvae were generally low and not statistically different from each other. Specifically, the FAW collected in both winter seasons as well as summer 2021 had lowest CT_{min} (the highest cold tolerance) compared to summer 2020 and the controls (except winter 2022 for the latter). Larval CT_{min} for summer 2020 was significantly higher than all other seasons ($p < 0.05$) but was statistically similar to the control group (**Fig. 6**).

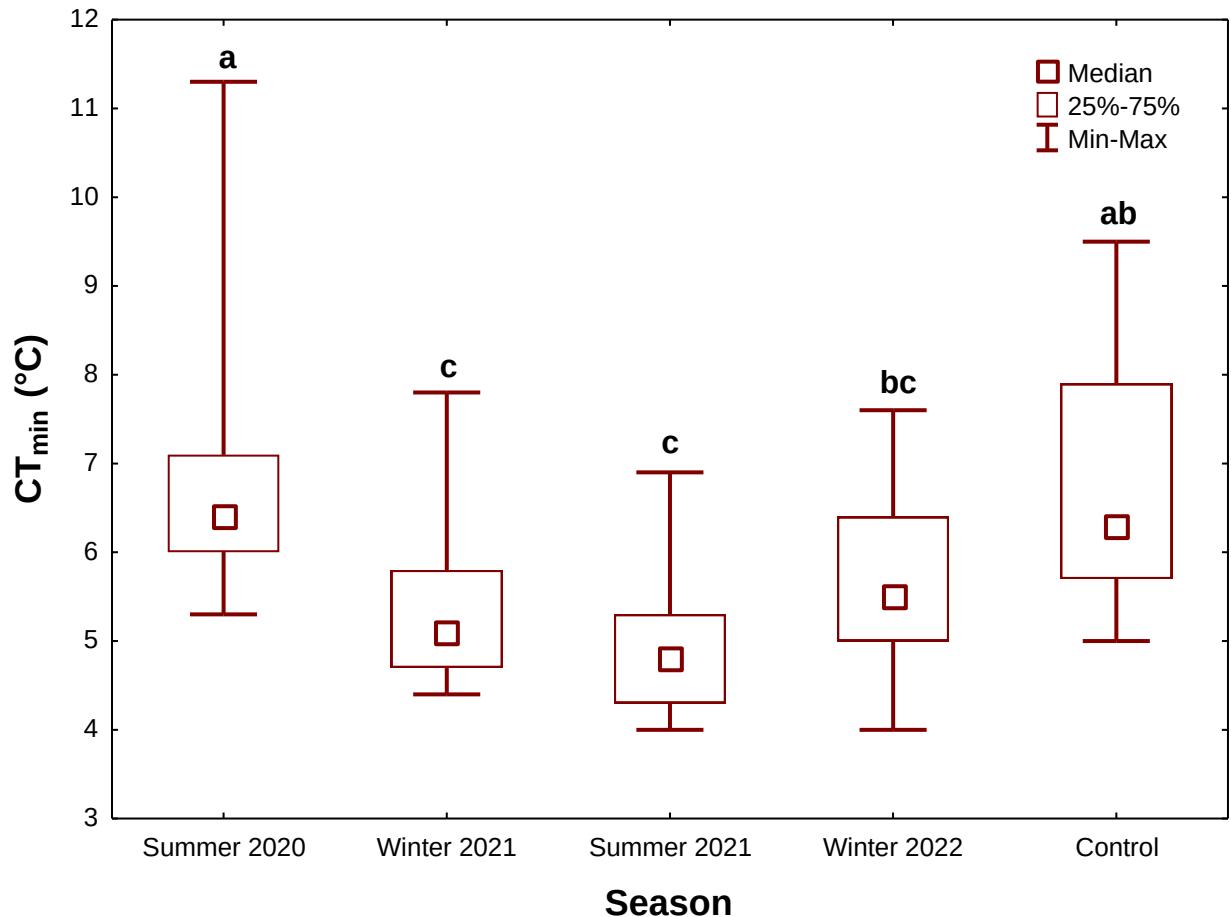


Figure 6: Critical thermal minima (CT_{min}) for the field populations of *S. frugiperda* 3rd instar larvae collected at different seasons compared to laboratory reared ($28 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH) controls ($n = 20$).

Values and boxes indicate medians and interquartile ranges respectively. Kruskal-Wallis post-hoc test was used to separate significantly different groups at $p = 0.05$. Bars with different letters are statistically different from each other. Winter and summer test organisms were collected in the months of May-June and December respectively and subsequently for the second seasons.

The sampling season had a significant effect on FAW cold tolerance measured as CCRT (Kruskal-Wallis test: $H(4, N=100) = 33.28580$, $p < 0.001$) (**Fig. 7**). The CCRT for winter sampled larvae was not significantly different from the controls in both sampling seasons/years. However, the summer collected larvae showed mixed results, i.e., summer 2021 larvae had significantly lower CCRT than control ($p > 0.001$), while summer 2020 collected larvae had CCRT that showed no significant difference with the control (**Fig. 7**). In addition, the CCRT for winter sampled larvae showed significant differences between the two seasons, with winter 2022 sampled larvae having significantly lower CCRT (higher cold tolerance) than winter 2021 collected specimens ($p < 0.05$). Similarly, cold tolerance of the two summers collected larvae was significantly different from each other. Larvae collected in summer 2020 had significantly higher CCRT (lower cold tolerance) than those collected in 2021 ($p < 0.05$) with the latter having CCRT significantly lower than the control ($p < 0.05$). This is consistent with the CT_{min} results where summer 2021 larvae had significantly lower CT_{min} than both the summer 2020 and the control larvae.

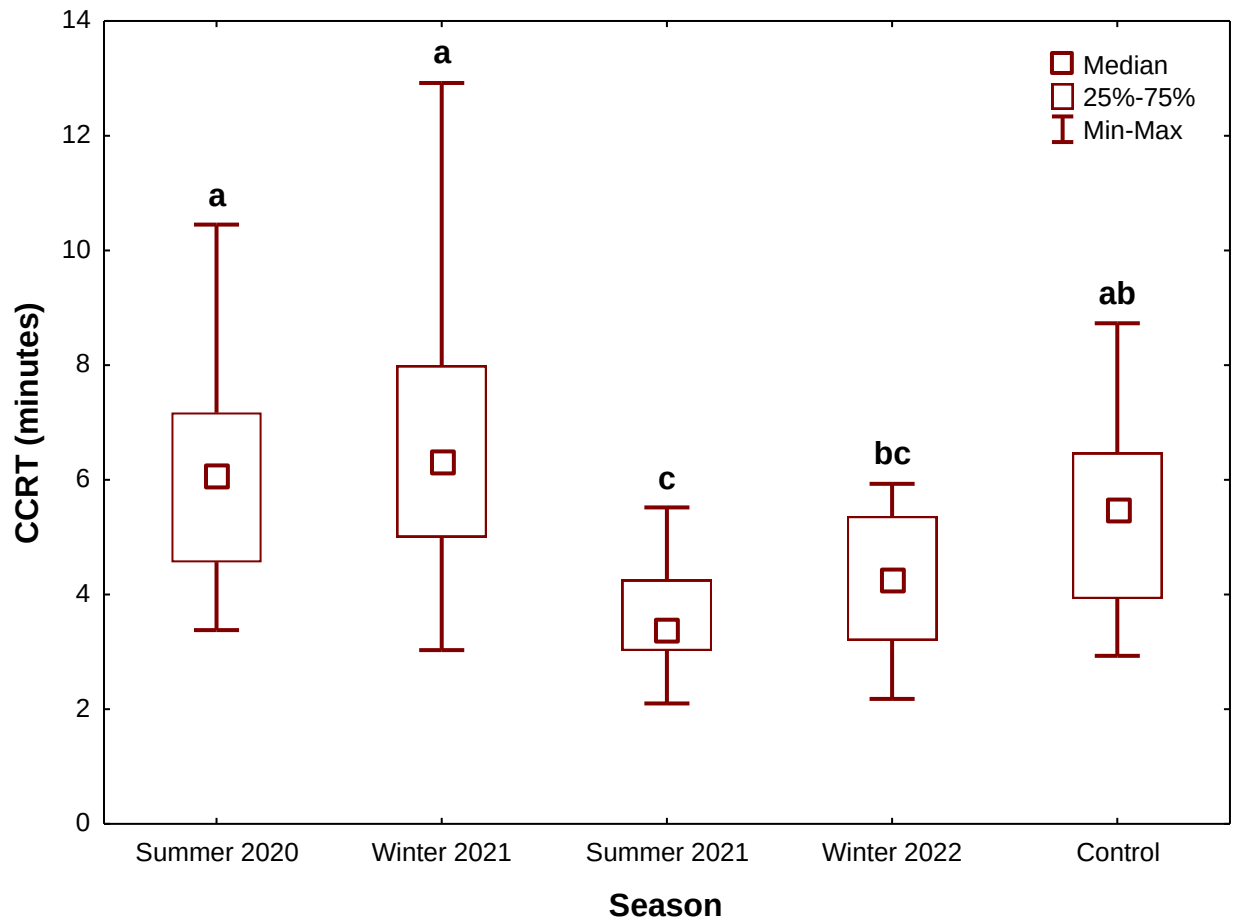


Figure 7: Chill coma recovery time (CCRT) for the field populations of *S. frugiperda* 3rd instar larvae collected at different seasons and compared to lab-reared (28 ± 1 °C, $65 \pm 10\%$ RH) control population ($n = 20$). Values and boxes indicate medians and interquartile ranges respectively. Kruskal-Wallis post-hoc test was used to separate significantly different groups at $p = 0.05$. Groups with different letters are statistically different from each other. Winter and summer test organisms were collected in the months of May-June and December respectively in each of the years.

Contrary to CT_{min} and CCRT results, there were no significant differences in the SCPs of *S. frugiperda* larvae across all seasons sampled and compared to the control larvae (**Fig. 8a**). Generally, SCPs ranged from -8.7 to -11.64 °C with a median of -10.21 ± 0.296 °C for summer 2020, winter 2021, summer 2021, and winter 2022 and controls respectively and followed a unimodal distribution curve (**Fig. 8b**).

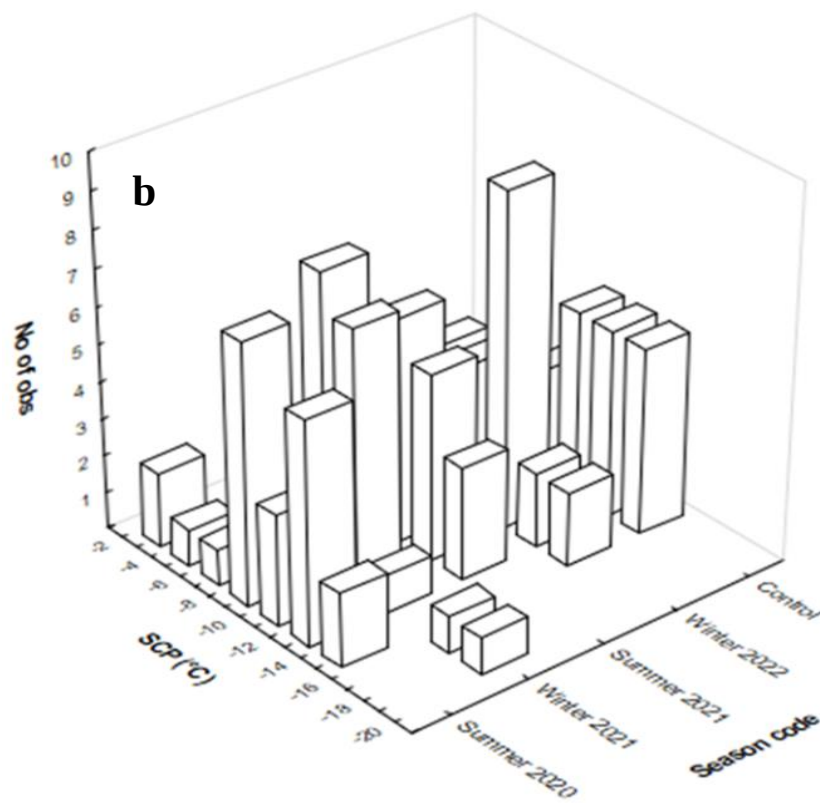
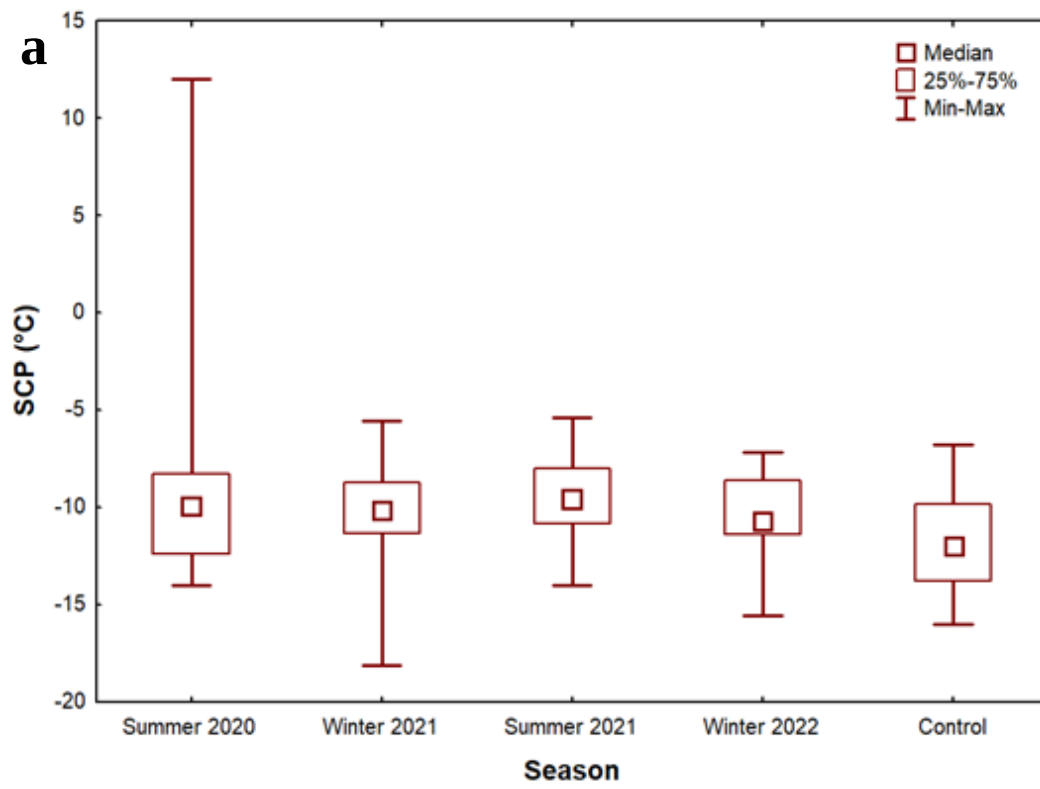


Figure 8: Supercooling points (SCPs) for field populations of *S. frugiperda* 3rd instar larvae collected in different seasons compared to lab reared (28 ± 1 °C, $65 \pm 10\%$ RH) control population ($n = 20$). Winter and summer test organisms were collected in the months of May and December respectively in each of the years. a) Box & whisker plot of supercooling points. Values and boxes indicate medians and interquartile ranges respectively. Kruskal-Wallis post-hoc test was used to separate significantly different groups at $p = 0.05$. b) Frequency distribution graph of supercooling points in larval FAW across different seasons showing a unimodal distribution curve.

Minimum and maximum environmental temperature data collected from October 2020 to May 2022 within the specimen sampling areas showed that winters had expectedly lower temperatures than summer seasons. Temperatures in winter (May-August) ranged from -0.5 to 32.8 °C, with sub-zero temperatures being recorded thrice within the two winter seasons. However, although summers (November-March) generally had high temperatures, summer 2021 was characterized by significant incidences of low temperature episodes, including unusually low subzero minimum temperatures of -8 °C on the 16th of December 2021 (**Fig. 9a**). Maximum temperature recorded during the two seasons in summer from October to March was 38.7 °C and the lowest minimum temperature was -8 °C (**Fig. 9a**). Similarly, low to no rainfall was recorded in both winter seasons; however, summer seasons had higher rainfall ranging from 0 - 98.5 mm. Apart from unusual temperatures, summer 2021 was also characterized by unusually high rainfall relative to 2020, reaching 98.5 mm in December, 2021 (see **Fig. 9b**).

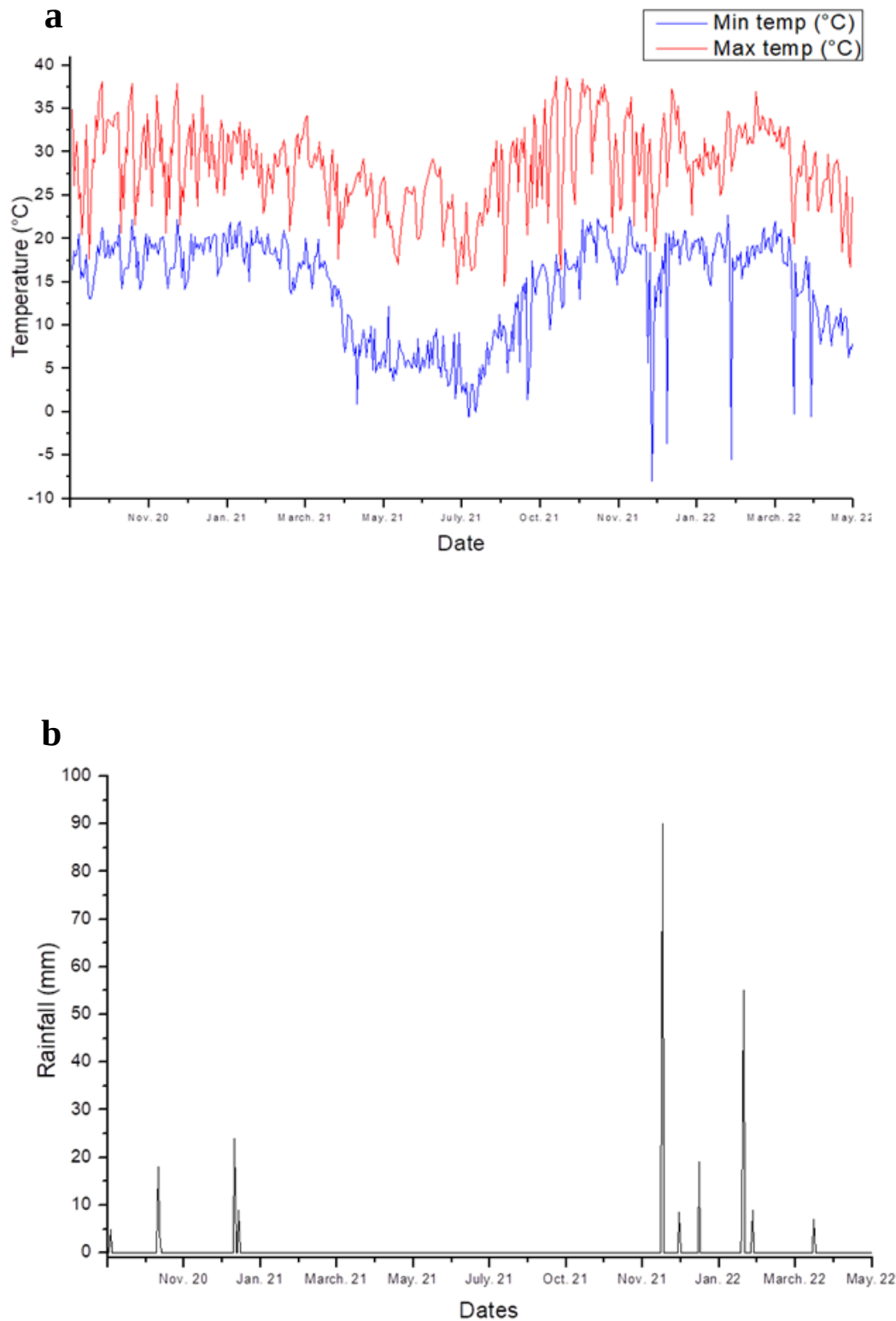


Figure 9b: Climate data records a)Average minimum and maximum temperatures recorded under natural conditions and experienced by fall armyworm in (Mahalapye), Botswana from 2020 to 2022

showing the minimum and maximum temperatures for all the sampled seasons b) Average rainfall (ml) recorded in Mahalapye, Botswana from October 2020 to May 2022 (Department of Meteorological Services, Botswana).

4.4 Discussion

Most insects are ectothermic and as such, their body temperatures always track ambient conditions. Given fluctuating thermal environments in nature, the ability of organisms to quickly adapt to thermally stressful events through rapid homeostatic adjustments in response to changing environmental temperatures is key to insect survival and ecological success. Mechanisms employed by insects to survive low stressful temperatures range from behavioral i.e., sheltering in cooler microclimates understory (reviewed in Pincebourde and Woods, 2020) and migrating to warmer climates, but can also be adaptation *in situ* through physiological or biochemical means. For example prolonged low temperature-induced seasonal acclimatization has been shown to improve thermal tolerance in insects (see Bale & Hayward, 2010; Driedzic & Gesser, 1994). To test FAW's ability to track ambient environments through shifting its low temperature critical limits to activity, I tested seasonal changes in larval low temperature tolerance traits *vis* CT_{min}, CCRT and SCP. Although this study showed mixed results to an extent, but generally, there was a clear trend that FAW low temperature responses track ambient environmental temperature seasonally. I recorded no significant difference in the SCPs across all treatment seasons and controls. For both traits of CT_{min} and CCRT, winter collected larvae generally had higher cold tolerance, suggesting that long term acclimatization through diurnally changing low temperatures may elicit some cold tolerance responses. However, I recorded a statistically significant difference for CT_{min} between the two summers- but not between winter -seasons, suggesting that both long- and short -term acclimatization may affect cold tolerance. Similarly, for similar traits, I recorded significant differences between the same seasons. For example summer 2020 collected larvae had higher CT_{min} than those collected in summer 2021, and likewise both season treatments for CCRT showing the interplay between long- and short -term acclimation in dictating field cold tolerance.

Summer collected FAW had significantly higher CT_{min} (lower cold tolerance) except for summer 2021 treatment. However, as expected, winter collected organisms showed significantly lower CT_{min} values (higher cold tolerance). Since insects do not often experience sub-optimal low temperatures during summer, cold tolerance may not be selected for over traits that are more influential for reproduction and survival during summer (Behrman *et al.*, 2015). A study done on *Drosophila melanogaster* yielded similar results with adults collected during summer having lower cold tolerance relative to those collected during the fall season (Noh *et al.*, 2017). Seasonal shifting of the lower thermal limits has

also been reported in mosquito communities. Seven mosquito species mainly from the genus *Aedes* and *Culex* increased their CT_{min} with increase in temperatures from spring to summer and decreased their CT_{min} when environmental temperatures decreased from summer to autumn (Oliveira *et al.*, 2021). Similarly, Nyamukondiwa *et al.* (2013) also showed that insects shift their thermal limits diurnally with shifting temperatures. For example, insects sampled early in the morning where temperatures are lower had higher cold tolerance than those sampled during the afternoon where temperatures are higher suggesting the ability of insects to track their ambient environments. Cyclic variation in seasonal environmental conditions provides efficient selection pressure for certain traits over others (Hoffmann & Sgrò, 2011; Chevin *et al.*, 2010). Change in critical thermal limits is more evident in insects from temperate and sub-tropical zones that experience large disparities in environmental temperatures between seasons and thus shifting critical thermal limits is critical for survival (Janzen, 1967; Oliveira *et al.*, 2021). However, even for tropical insects, insects significantly accumulate carbohydrate cryoprotectants as temperatures fall and day length shortens with winter (Teets & Denlinger, 2013). For example, insects have higher glycerol content in winter than summer (Storey, 1990) and this may help them survive low winter temperatures. While I did not test these biochemical mechanisms in this study, I speculate, with caveats that such mechanisms may explain the higher cold tolerance of FAW populations collected in winter. I thus recommend that future research may look into some of the biochemical mechanisms associated with temporary low temperature adaptation in the FAW.

Larvae collected in summer 2021 showed superior cold tolerance traits than those collected in both winter treatment seasons. Microclimate weather conditions during the sampling timing showed that these organisms experienced unusually low temperatures during summer. Sub-zero temperatures, reaching minimum of -8°C (**Fig. 9a**) and high rainfalls during the same time (**Fig. 9b**) may account for this anomaly. Thus, hypothetically larvae experienced a short-term low temperature exposure and tracked it despite the short duration of the cold snaps within the summer season. Indeed, (Nyamukondiwa & Terblanche, 2010) showed that both long- and short-term acclimation modulates critical thermal limits in insects. This may mean that both long term changes in ambient temperature environment with season and more acute temperature conditions experienced just before insect sampling may both have effects on FAW results. Here, I thus speculate that FAW larvae shifted its CT_{min} phenotype in the short term, synonymous to rapid hardening responses (see Sgro *et al.*, 2016; Rodrigues & Beldade, 2020). Within generation change in traits in response to low environmental temperatures (phenotypic plasticity) is one method which insects use to cope with rapid changes in environmental temperature (Brakefield & Reitsma, 1991). Several laboratory acclimation studies

performed on other Lepidoptera species such as *Chilo partellus*, *Busseola fusca* and *Tuta absoluta* have shown that short-term exposure to sub lethal low temperatures improves cold tolerance (Mutamiswa *et al.*, 2018b; Tarusikirwa *et al.*, 2020). Contrary to my results, in one study 3rd instar FAW larvae showed a lack of cold tolerance plasticity as rapid cold hardening did not influence CT_{min} (Keosentse *et al.*, 2021). However, exact experimental methodology such as length of acclimation period can affect acclimation responses (Rezende *et al.*, 2011; Rohr *et al.*, 2018).

Summer 2020 collected FAW larval cold tolerance was not significantly different from control insects for both CT_{min} and CCRT. Temperatures experienced during summer generally ranged between 26 and 30 °C (see **Fig. 9a**) which is generally consistent with the control rearing temperature (28 °C) and within the optimum temperature range for FAW development (Du Plessis *et al.*, 2020). Despite occasional bouts of temperatures above 30 °C recorded during summer 2020, temperatures experienced by insects may be slightly lower (add reason and REF), thus explaining the lack of difference in thermal responses. There are wide temperature differences in insect fine scale microclimates which affect thermal limits and traits (Pincebourde *et al.*, 2016; Pincebourde & Wood, 2020). Ideally cold tolerance measures fitness traits after exposure to cold stress (Overgaard & MacMillan, 2017). From the weather data experienced in the natural environments (**Fig. 9a**), summer 2020 lacked critical low temperatures that likely elicit acclimation responses and thus, may explain the lack of significant differences with the control.

Seasonal responses to low temperature were mixed; contrary to the insignificant differences observed for CT_{min} between winter seasons, winter 2022 displayed lower CCRT than winter 2021. Cold tolerance traits affect multiple organ systems and are thus used to indirectly determine the effect of cold in these systems (Davis *et al.*, 2021). Chill coma recovery time does not measure recovery from CT_{min} thus may give varying results (Andersen & Overgaard, 2019). Critical thermal minima results in an increase in extracellular potassium concentrations in neurons due to poor regulation of ion pumps which results in neural depolarization in the central nervous system (Andersen & Overgaard, 2019; Armstrong *et al.*, 2012; Rodgers *et al.*, 2010). Chill coma recovery time however measures the time it takes for insects to recover ion and water homeostasis in muscles when benign temperatures resurface following chill coma onset (MacMillan *et al.*, 2014; MacMillan *et al.*, 2012). Supercooling point on the other hand measures the lowest temperature at which insects can be cooled before internal body fluids freeze (Lee *et al.*, 1996). It is thus not surprising that the cold tolerance traits measured in this study gave varying results. Previous studies that worked with laboratory reared and wild insect populations indicated that CCRT and CT_{min} are independent of each other and thus may display divergent results across

populations and genotypes (Davis *et al.*, 2021; Garcia *et al.*, 2020). Similarly, a study on *T. absoluta* showed contrasting results for traits of cold tolerance, and that rapid cold hardening improved CT_{min} while compromising CCRT (Tarusikirwa *et al.*, 2020).

My results for SCPs showed no significant differences across all seasons and treatments contrary to other cold tolerance metrics of CT_{min} and CCRT. SCPs are affected by many factors including species, feeding status, body water content, and availability of other ice nucleating agents and related (Lee *et al.*, 1996). For example, insects often clear their gut contents in winter in preparation for lower temperatures. A related study showed that seasonal change of *Cydia pomonella* SCPs were in part attributed to feeding status and diet (Khani & Moharramipour, 2010; Maes *et al.*, 2015). Increased gut content increases the possibility of having ice nucleating agents which support the creation of ice crystals (Koch *et al.*, 2004; Zachariassen, 1982). In this study one instar was used across experiments and prior to sampling larvae fed on maize plants, thus the availability of- and similarity in diet and gut size is assumed to have contributed to the SCP results reported here. However, control population exclusively fed on a sorghum-leaf based artificial diet, but this did not have a consequence on SCP contrary to similar studies (Sprangers *et al.*, 2017). Production of carbohydrate cryoprotectants such as glycerol has been characterized in several insect species and attributed to high supercooling in freeze-avoiding insects (Storey & Storey, 2012). However, several reports suggest that supercooling ability may be more ecologically relevant for more temperate than tropical organisms, since the former experience more extreme low temperatures. As such, the ecological relevance of SCPs for tropical FAW here is a subject of debate. Developmental stage may also have a significant effect on cold tolerance traits. Previous studies have indicated that cold tolerance of FAW is life stage dependent, with adults being generally more cold tolerant than earlier developmental stages (Keosentse *et al.*, 2021; Zhang *et al.*, 2021). However, for SCPs, these studies were contradictory with adult FAW having higher SCP in one study (Keosentse *et al.*, 2021), while having lower SCP than other life stages in another (Zhang *et al.*, 2021). This difference may be due to clinal variation. Therefore, lack of change in the SCP across seasons for a period of 2 years could mean that other intrinsic or inherent factors (likely heritable), had more influence on FAW SCP. Further studies may need to test this over a high number of both winter and summer seasons.

In conclusion, the results show that FAW cold tolerance tracks seasonal environmental temperature. First, I show that response to seasonal change in temperatures in FAW larvae varies with the cold tolerance matrix being measured. While CT_{min} increased with rise in temperatures from winter to

summer, the season had an opposite effect on FAW CCRT. Secondly, I showed that FAW cold tolerance tracks the temperature not the season, through rapid non-seasonal responses to environmental temperature changes. Indeed, summer 2021 collected larvae had higher cold tolerance than summer 2020, and similar to the winter collected organisms owing to the unusually short-term bouts of low temperatures experienced during that summer (2021). Thirdly the results showed a lack of seasonal plasticity in FAW SCP suggesting that either SCPs may not be ecologically significant for this tropical species, or further studies are required. These results provide evidence that FAW has long- to short-term responses to seasonally fluctuating environmental temperatures. This may be beneficial for FAW survival during both short- and long-term cold snaps which have increased in frequency due to climate change aiding both its survival and invasion. Thus, both long- and short-term temperatures modulate insect population dynamics. Incorporating such data into predictive models may be important in improving current pest forecast and distribution models.

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CHAPTER 5
General Discussion

Insects being predominantly ectothermic, they are highly sensitive to changing environmental ambient temperature conditions (Du Plessis *et al.*, 2020; Robinet & Roques, 2010). Thus, the ability of insects to withstand temperature extremes and maintain functionality under different magnitudes of stressful temperature fluctuations is important for insect fitness and survival (Nyamukondiwa *et al.*, 2018). This is especially important under current climatic conditions which are characterized by increased temperatures and increased incidences of heat waves and cold snaps due to global warming (Stillman, 2019; WMO, 2020). These changes and associated impacts continue to increase in the 20th century (Clusella-Trullas, 2022), causing re-organisation of ecological structures, population and species biogeography as well as associated ecological impacts (Harvey *et al.*, 2022; Jørgensen *et al.*, 2022), e.g., increased invasive species (Nyamukondiwa *et al.* 2022). Looking into the contribution of global warming to pest fitness, distribution and survival is imperative for insect pest management through early warning systems and forecasting potential future invasion areas. This study thus focused on understanding the effect of prior exposure to stressful factors on thermal fitness subsequent life stages of *Spodoptera frugiperda*. I thus examined innate within-generation developmental temperature and fasting acclimation effects on heat tolerance through measuring critical thermal maxima (CT_{max}) and heat knock-down time (HKDT) in *S. frugiperda* following high temperature acute and chronic acclimation, as well as fasting (Chapter 3; Segaiso *et al.*, 2022). I further assessed seasonal changes in environmental stress resistance (critical thermal minima, chill coma recovery time, and supercooling points) across *S. frugiperda* ontogeny (Chapter 4). Unraveling the effects of prior environments may be important for organisms occupying different environments/niches within-generation and may explain the phenotypes of subsequent developmental stages. Incorporating such data in predictive models may help refine the forecasting algorithms through insights on how species respond to different environments.

My results showed that heat and fasting acclimation potentially have context-specific fitness costs and benefits for subsequent *S. frugiperda* developmental stages facing high temperature stress (Chapter 3). Specifically, fasting, acute and chronic, and high temperature acclimation of 3rd instars reduced critical thermal maxima (CT_{max}) of 4th instar larval stage. Fasting and high temperature acclimation had an opposite effect on HKDT with the tested stage having improved HKDT after acclimation. These results provide evidence that exposure to sub-lethal stress in one developmental stage may have fitness costs and benefits on subsequent developmental stages depending on the trait being measured. Despite both acute and chronic acclimation having deleterious effects on *S. frugiperda* CT_{max}, chronically acclimated *S. frugiperda* larvae generally had higher CT_{max} than acute acclimated larvae. Chronic exposure to

stress may have activated biochemical and molecular mechanisms that enhance heat tolerance thus explaining the difference in results (Hasanuzzaman *et al.*, 2013). My results also showed that acclimation to a different stress (fasting) may also have additive stress effects on a different stress (heat tolerance [CT_{max}]). Overall, results indicated that timing of sub-lethal high temperature stress such as heat waves may enhance thermal fitness and survival of other life stages of *S. frugiperda*. I also showed a divergent stressor like enforced insect fasting due to mid- or full- season droughts associated with climate change may enhance thermal stress tolerance through cross talk/cross tolerance. Therefore, survival of *S. frugiperda* may increase in hot, drought prone tropical areas even during high temperature or drought incidences. Thus, this study looked at effects of heat and fasting acclimation treatments on within-generation developmental stages (Chapter 3).

Overwintering is an important strategy in insects to survive winter low temperatures (Chapman *et al.*, 2015). Due to global warming, there has been an increase in winter temperatures which may aid redistribution of tropical insect pests into previously cooler environments (MacDonald *et al.*, 2004). Despite the rise in winter temperatures there has been an increase in extreme low temperature incidence resulting in increased temperature fluctuations (Kug *et al.*, 2015; Marshall *et al.*, 2020). Thus, ability to track ambient temperatures to avoid sudden injury or death may be a beneficial trait for insect success and survival. My results showed that *S. frugiperda* had mixed seasonal responses to low temperature (Chapter 4). For critical thermal minima (CT_{min}) except for the cold summer 2021 sampled larvae, winter collected larvae generally had lower CT_{min} (greater cold tolerance) than summer collected larvae. Weather data showed that winter sampling months had significantly lower minimum temperatures than summer sampling months. This indicates that *S. frugiperda* larvae reduced their CT_{min} during winter (increased cold tolerance) in response to the low environmental temperatures. Shifting of critical thermal limits in the short time-scales, i.e., diurnally and seasonally with change in temperatures has been reported in previous studies (Oliveira *et al.*, 2021; Nyamukondiwa *et al.* 2013). However for chill coma recovery time (CCRT), no similarity was found in CCRT recorded during summer seasons as well as those recorded during winter seasons. Chill coma recovery time and CT_{min} are independent of each other thus may display differing results. Larvae collected in summer 2021 showed similar to superior cold tolerance (lower CT_{min} and CCRT) to those collected in both winter seasons. Microclimate weather data showed that these organisms experienced unusually low temperatures and high rainfall during summer which may have influenced their cold tolerance metrics. I speculate that upon exposure to these weather conditions *S. frugiperda* larvae shifted their CT_{min} and CCRT to avoid

chill injury (Chapter 4). This may mean that both short- and long-term acclimatory capacity modulate insect field fitness (see discussions in Nyamukondiwa & Terblanche, 2010).

My study looked at singular effects of temperature and starvation stress on *S. frugiperda* thermal fitness. However, insects experience multiple stressors at the same time in nature. Thus, future studies must investigate the combined effect of these stressors. I may have also missed exact treatment combinations that elicit acclimation response for CT_{max} and these specific treatments for HKDT. Thus, future work should consider more comprehensive acclimation treatment combinations that may potentially elicit responses (Chapter 3). This study also did not go in depth to study biochemical mechanisms that influenced higher cold tolerance of FAW populations collected in winter. I thus recommend that future research look into some of the biochemical mechanisms associated with temporary low temperature adaptation in FAW (Chapter 4).

In conclusion, my thesis results show that (i) within-generation, thermal stress in one life stage reduces heat stress tolerance (fitness) in the next life stage in the FAW through carryover effects, (ii) a divergent stressor (fasting) experienced in one life stage, improves heat tolerance (fitness) in the next life stage, (iii) larvae track the short-term diurnal/seasonal changes in environmental temperature. Despite the season, the short-term diurnal changes in temperature matter more than the seasonal average for FAW thermal adjustments. The ability to track short term environmental temperatures and shift critical thermal limits in response to rapid temperature fluctuations may increase its invasion success across different global climates and seasons including. In addition, exposure to thermal and fasting stress in one developmental stage has detrimental and beneficial effects respectively on the thermal fitness of *S. frugiperda* larvae. Thus, global warming may have mixed effects on pest invasion and success and studying these effects is significant in explaining responses of invasive species to changing climatic environments. The results of this study indicate that heat and fasting acclimation experienced during one developmental stage had differential effects on the heat tolerance traits of subsequent developmental stages. Incorporation of these results into predictive models may be important in improving accuracy of current pest forecasts, distribution models and pest early warning systems.

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