IMPACTS OF CLIMATE VARIABILITY ON STEMBORERS-NATURAL ENEMIES TROPHIC LEVEL INTERACTION: IMPLICATIONS FOR BIOLOGICAL CONTROL UNDER GLOBAL CHANGE

by

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CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the Faculty of Science a thesis titled:

Impacts of Climate Variability on Stemborers-Natural Enemies Trophic Level Interaction: Implications for Biological Control Under Global Change, in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences (Applied Entomology) of BIUST.



……

Dr. Casper Nyamukondiwa

(Supervisor)

Date: 03 February 2018

DEDICATION

This thesis is dedicated to my family and my mom. Your prayers, support and love during the course of this study are highly appreciated.

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ABSTRACT

Lepidopteran stemborers are key destructive insect pests of cereal crops in sub-Saharan Africa with the indigenous noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the exotic crambid *Chilo partellus* Swinhoe accounting for ~5-75% of potential cereal crop yield losses. The main larval endoparasitoids comprising, indigenous *Cotesia sesamiae* and exotic *Cotesia flavipes* Cameron contribute to a significant extent in managing these economic herbivorous insect pests. However, efficacy of biological control under global change is currently under threat, and factors limiting trophic level abundance, distribution and population phenologies of these insect species under climate change remain unclear. Against this background, this study provides an understanding of the thermal ecophysiology of laboratory reared *B. fusca*, *S. calamistis* and *C. partellus* and their larval parasitoids, *C. sesamiae* and *C. flavipes* under variable climatic conditions and how their population phenologies are likely to be shaped by variable thermal regimes under global change scenarios. This study reports implications of these results thereof for biological control efficacy under climate change. Firstly, a comparative assessment of *C. partellus* and *C. sesamiae* thermal tolerance using dynamic and static protocols showed developmental stage differences in *C. partellus* thermal tolerance (with respect to lethal temperatures and critical thermal limits) and a compromised temperature tolerance of *C. sesamiae* relative to its host. This indicates potential asynchrony between host-parasitoid population phenology and consequently biocontrol efficacy under global change. Secondly, I investigated the short to medium term phenotypic plasticity of thermal tolerance of *C. partellus* developmental stages (larvae, pupae and adults) and its larval parasitoid *C. flavipes* (adults). Rapid cold hardening (RCH) and rapid heat hardening (RHH) effects in *C. partellus* larvae, pupae and adults and *C. flavipes* adults were highly significant ($P < 0.001$). In addition, high temperature acclimation (33°C) improved critical thermal limits [CTLs (CT_{min} and CT_{max})] and heat knock-down time (HKDT) for *C. partellus* larvae and *C. flavipes* adults ($P < 0.0001$) respectively while low temperature (23°C) acclimation enhanced supercooling point (SCP) for *C. flavipes* and chill-coma recovery time (CCRT) for both *C. partellus* larvae and *C. flavipes* adults ($P < 0.0001$) suggesting that the host is more plastic than the parasitoid. Thirdly, the study explored basal thermal tolerance of larval endoparasitoids, *C. sesamiae* and *C. flavipes* and how this may influence their geographical distribution and biological conservation. *Cotesia flavipes* showed a higher basal thermal tolerance (with respect to traits of temperature tolerance, CTLs,

HKDT and CCRT) than *C. sesamiae* indicating future non overlapping thermal environments of these species under climate change. Furthermore, since *C. flavipes* is exotic, its physiological advantage over indigenous *C. sesamiae* may likely mean exotic species may likely drive local species out of their native ranges, with significant consequences on biodiversity conservation and synergistic biocontrol efficacy. Lastly, the study investigated the effects of heterogeneous stressful environments on thermal tolerance *vis a vis* hardening (RCH and RHH), thermal (low, optimum and high), starvation and desiccation acclimation on thermal tolerance traits, measured as critical thermal limits on laboratory reared stemborers *B. fusca, S. calamistis* and *C. partellus* using dynamic (ramping) protocols. Thermal acclimation to low, optimum and high temperatures was highly significant for CT_{min} ($P < 0.001$) across all species. Similarly, across all species, RCH, RHH, starvation and desiccation acclimation were significant ($P < 0.001$) for CT_{min}. However, RHH, starvation and desiccation were not significant ($P > 0.05$) for CT_{max} across all species. These findings show differential thermal tolerances following exposure to heterogeneous environmental stress habitats. However, *Chilo partellus* was more plastic and more heat tolerant following starvation acclimation while *B. fusca* was more cold tolerant following RCH, starvation and desiccation acclimation. In summary, this work revealed asymmetrical variation in thermal ecophysiology across test species, appearing to be enhanced in *C. partellus* relative to *S. calamistis* and *B. fusca* and in *C. flavipes* relative to *C. sesamiae*. Therefore with projected climate change, *C. partellus* is highly likely to extend its geographical distribution relative to *S. calamistis* and *B. fusca* ultimately displacing these indigenous stemborer species. In addition, the larval parasitoids may not cope with the geographical expansion of their hosts due to variability in thermal tolerance, albeit indigenous *C. sesamiae* looks more vulnerable than exotic *C. flavipes*. This may have broad implications on biological control efficacy leading to more pest outbreaks. These results are useful in designing pest management options for invasive species and conducting pest risks assessments.

CHAPTER 1

General Introduction

1.0 Climate change and insect phenology

Global climate change is a transformation in the long term weather patterns and is evident from increase in global average temperature and its variability, changes in the rainfall pattern and extreme climatic events (Selvaraj *et al*., 2013). The global mean surface temperature is predicted to increase between 1.4 and 5.8°C by 2100 (Karuppaiah & Sujavanad, 2012). Climate changes have major impacts on the agroecosystem, and insects being ectotherms may be the most affected (Jaworski &Hilszczański, 2013). Such changes in climate could profoundly affect the population dynamics of insect pests, change their 'status' and consequently upset timing of control measures. Researchers have shown that global temperature increase may potentially accelerate development rate, survival, reproduction and population size of tropical and subtropical insects (Selvaraj *et al*., 2013) as well as altering their biogeography (Nyamukondiwa *et al*., 2011). Consequently, insects may be capable of completing higher numbers of generations per year (Deka *et al*., 2009) ultimately resulting in more crop damage (Yamamura & Kiritani, 1998), asynchrony in the timing of control strategies and as a result, affecting efficacy of control measures (Mutamiswa *et al*., 2017a). Future direction and rate of climate change as well as its direct and indirect effects are complex and may be difficult to predict (Gutierrez *et al*., 2008). Therefore, it is essential to understand the mechanisms behind climate influence on the interaction of ecosystem components and agricultural environment especially on insects of economic importance to agroecosystems (Jaworski & Hilszczański, 2013). Some insect pests which are already present but occurring at low densities in small areas, may be able to exploit the changing climatic conditions by spreading more widely and reaching damaging population densities (Karuppaiah & Sujavanad, 2012). As a consequence, there is a likelihood of farmers experiencing extensive impacts on insect management strategies under climate change.

Abiotic factors such as temperature and humidity have been reported to directly influence insect population dynamics through modulation of development rates, survival, fecundity, parasitism and dispersal (Selvaraj *et al*., 2013). Exposure to temperature extremes induces lethal and sub lethal damage to predators and parasitoids (Hance *et al*., 2007), whose ecological function is to keep the insects below economic injury level. Consequently, temperature extremes associated with global change may also likely offset the efficacy of biological control programmes (Selvaraj *et al*., 2013). Natural enemies and host insect populations may respond differently to changes in temperature. Apart from surviving thermal extremes, natural enemies

need to counter climate change by mating and then locating hosts effectively across a wider range of thermal and humidity conditions (Thomson *et al*., 2010). Parasitism may be reduced if host populations emerge and pass through vulnerable life stages before parasitoids emergence. Hosts may pass through vulnerable life stages more quickly at higher temperatures, reducing the window of opportunity for parasitism which may give great set back to the survival and multiplication of parasitoids (Fand *et al*., 2012). While natural enemies have some potential to deal with changes in climatic variability through acclimation and diapause, and by having some innate resistance of thermal extremes, climatic variability may influence their effectiveness in controlling pests (Thomson *et al*., 2010). Field data suggest that parasitoids are more sensitive to climatic variability than their hosts, possibly related to the greater intrinsic rate of population increase shown by hosts following mortality of both groups (Thomson *et al*., 2010).

1.1 Insect Thermal Biology and Physiology

Temperature can impact insect physiology and development directly or indirectly through the physiology or existence of hosts. Depending on the insect development "strategy", temperature has been reported to exert various effects (Bale *et al*., 2002; Petzoldt & Seaman, 2006). Some insect species such as cicadas and arctic moths take several years to complete one life cycle hence they tend to moderate variabilities in temperature over their entire life history (Bale *et al*., 2002). In addition, some insect pests develop more rapidly during time periods when temperatures are favourable (Petzoldt & Seaman, 2006). Day-Degree (DD) or phenology based models are therefore used to predict the emergence of these insects and their potential to damage crops.

Insect responses to temperature may be summarised using the performance curve (Figure 1.1) (Wharton, 2002; Piyaphongkul, 2013) and thermobiological scale (Figure 1.2) (Vannier, 1994). Performance curves are essential when elucidating capacity responses of insects (Chown & Terblanche, 2007). The lower and upper lethal temperature is the temperature required to kill a certain proportion of the population in a specified time period (Bale & Walters, 2001). At temperatures below and above the optimum, metabolism slows and eventually ceases due to the damaging and lethal effects of low and high temperatures. The upper lethal temperature (ULT) is normally higher than the critical thermal maxima (CT_{max}) and heat coma temperature (HCT) whilst lower lethal temperature (LLT) will be lower than the critical thermal minima (CT_{min}) and

chill coma temperature (CCT) (Figure 1.1). However, the difference between the heat coma temperature and ULT is sometimes small e.g. around 1˚C or less, and insect species experiencing heat coma may not be able to recover, so the heat coma temperature may effectively be the ULT (Hazell *et al*., 2010, Piyaphongkul, 2013). There are a range of methods used to measure the ULT and LLT including 'direct plunge' where insects are transferred directly to a potentially high and low temperature, and 'dynamic methods' where the temperature is increased or decreased gradually from a set point benign temperature and mortality is assessed at a sequence of increasingly higher and lower temperatures (Nyamukondiwa & Terblanche 2009; Piyaphongkul, 2013).

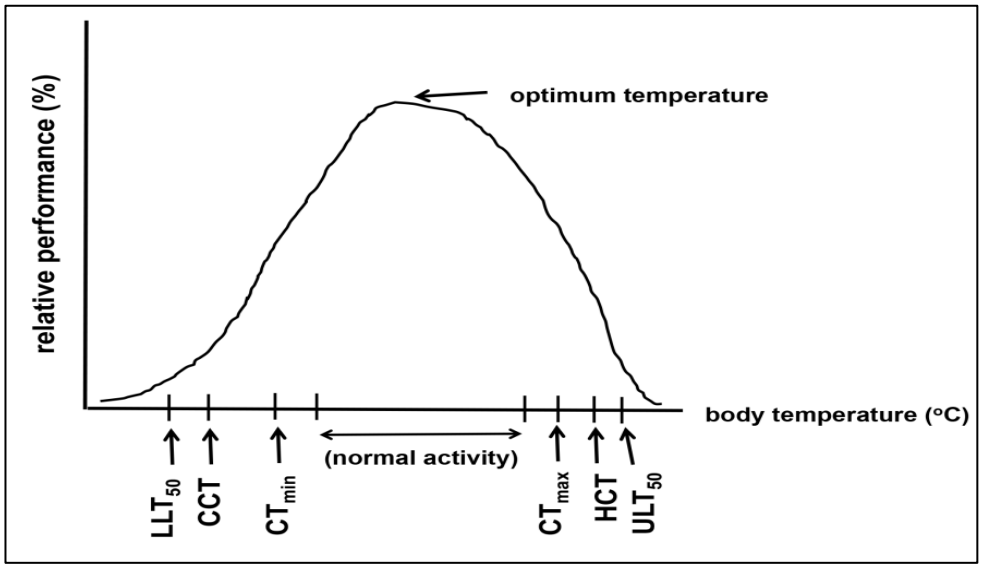


Figure 1.1: Responses to temperature in a hypothetical organism (Adapted from Wharton, 2002; Piyaphongkul, 2013). CT_{min}= critical thermal minima; CTmax = critical thermal maxima; CCT = chill coma temperature; HCT = heat coma temperature; LLT = lower lethal temperature; ULT = upper lethal temperature

The thermobiological scale is critical when considering resistance responses (Chown & Terblanche, 2007). In the middle of the thermobiological scale (Figure 1.2) is the optimum temperature for an insect's survival, growth and evolutionary fitness. At both ends of the scale, continuation of the change in temperature results first in knockdown of the insect (Chown & Nicholson, 2004), then in prolonged coma, and finally irreversible trauma and death (Figure 1.2).

119 At low temperatures, insects show a wider variety of responses to sub-lethal and potentially
120 lethal temperatures than they do at high temperatures, including responses to non-freezing
121 temperatures and those made in preparation for the decline of temperatures below the freezing
122 point of water (Chown & Nicholson, 2004).

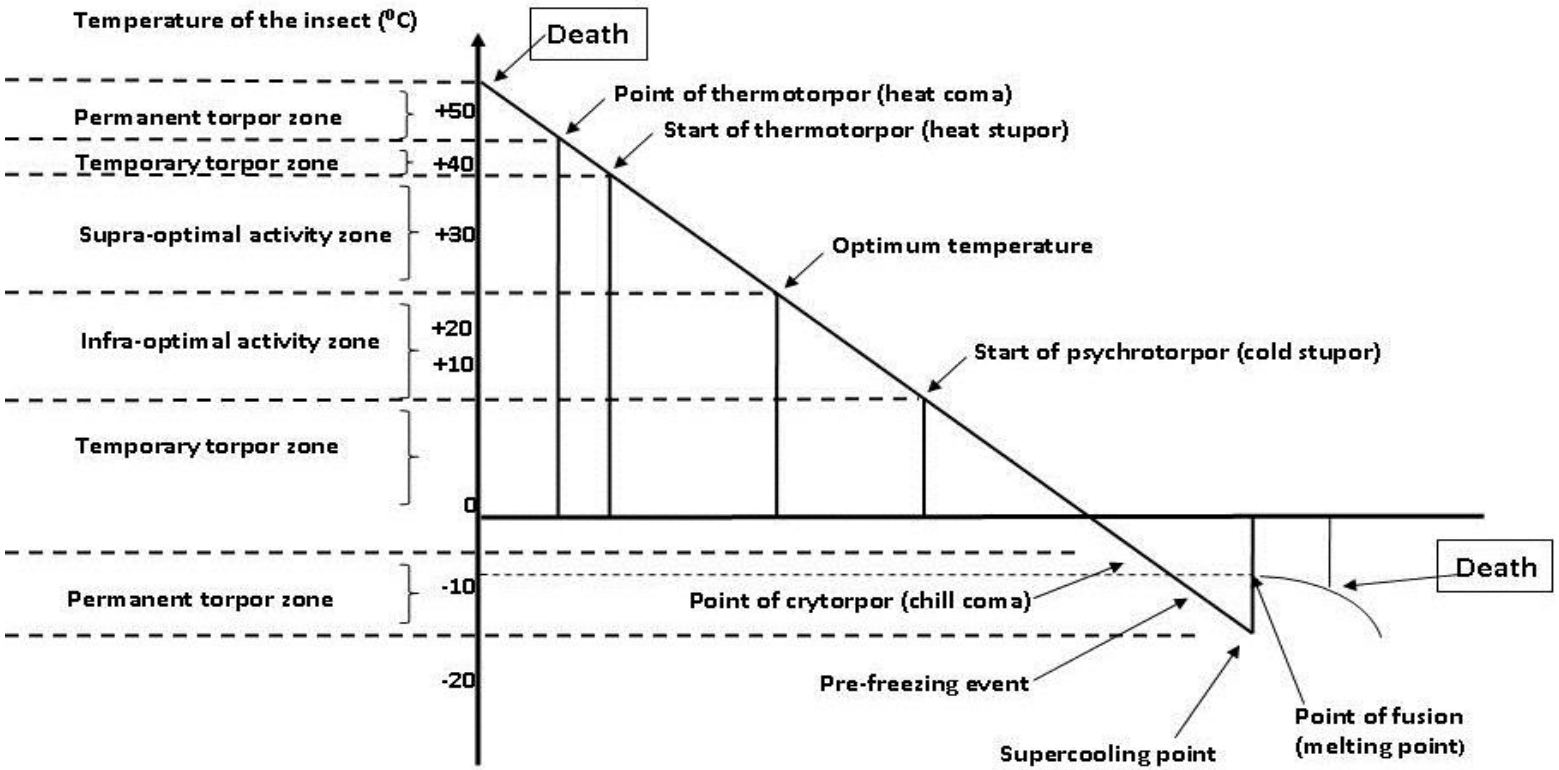


Figure 1.2: The thermobiological scale, redrawn from Vannier (1994).

The temperature at which heat induces injury and/or death in insects varies both spatially and temporally (Chown & Terblanche, 2007). This is because insect thermal tolerance is dynamic and organisms often shift their fitness traits in relation to thermal environment related to timing (seasons) and space. Furthermore, differences in methods of measurement usually assess different traits resulting in dissimilar outcomes hence it is difficult to reach general conclusions regarding upper thermotolerance limits (Chown & Nicolson, 2004). However, in insects they generally do not exceed 53˚C, and are usually not much lower than 30˚C, although these values depend on the trait being measured (Chown & Terblanche, 2007) and methodological context (Chown *et al*., 2009). It has been suggested that heat shock proteins (Hsps) might be responsible for both survival of potentially lethal temperatures and for improved knockdown resistance in insects (Nielsen *et al*., 2005; Chown & Terblanche, 2007). Indeed, expression of Hsps has been observed in response to both heat and cold shock (Chown & Nicolson, 2004), symbolizing some degree of overlap in mechanisms for tolerance to both thermal extremes. Nevertheless, tolerance to low temperatures has been more associated with carbohydrate metabolism (Sinclair *et al*., 2013; Sinclair, 2015).

Knowledge of thermal tolerance is crucial to understanding of ecology, evolution, and physiology of organisms as these traits play an important role in determining species range distributions (Hazell *et al*., 2008) and how they may be altered by climate change (Walther *et al*., 2002). Consequently, thermal tolerance traits are also important determinants of the likelihood that a species will become established upon introduction into a novel environment and likely become invasive (Slabber *et al.*, 2007; Ward & Masters, 2007; Nyamukondiwa *et al*., 2010), and the magnitude of the potential threat due to climate change faced by species with low dispersal ability (Thomas *et al.*, 2004). Thermal tolerance traits include ULT and LLT (Bale *et al*., 1994; Sinclair *et al.*, 2006) and a range of non-lethal measures including the high and low CTLs, knock down and chill coma temperatures (Gaston & Chown, 1999; Hazell *et al*., 2008 reviewed in Chown & Nicholson, 2004) and measures of the time taken to knockdown an organism at high temperature and to recover from cold-induced coma (heat knockdown time [HKDT] and chill coma recovery time [CCRT] respectively) (Ayrinhac *et al*., 2004; Castaneda *et al.,* 2005). Of the non-lethal measures of thermal tolerance described above, the indexes most commonly employed by researchers are the CTLs (CT_{min} and CT_{max}) or coma temperatures (Hazell *et al*., 2008).

The ability of an organism to remain active under extreme conditions is a significant component of fitness (Loeschcke & Hoffmann, 2007). Therefore determining the limits to activity is an important first step in understanding the ways in which environmental variation affects fitness and the dynamics of a given population. Thermal limits have received considerable attention because their investigation provides insight into the manner in which climate shapes variation in the ecology, distribution and evolution of species (Ghalambor *et al*., 2006; Chown & Terblanche, 2007). Furthermore, upper temperature limits are positively related to optimal performance temperatures and these limits are relatively simple to measure (Chown & Nicolson, 2004). In consequence, factors that affect the assessment of thermal tolerance are significant (Chown & Nicolson, 2004). Typically, limits are assessed using either dynamic or static protocols (Hoffmann *et al*., 2003). Briefly, the dynamic method involves changing temperature (either up or down) at a constant rate, starting from a setup standard benign temperature for the experimental organism and assessing individual knockdown. One dynamic technique that is widely used to resolve both high and low temperature thresholds is the determination of CTLs (Lutterschmidt & Hutchison, 1997; Beitinger *et al*., 2000; Chown & Nicolson, 2004). Critical thermal limits are considered ecologically relevant because they provide an indication of the activity range for a population under acute exposure conditions (Somero 2005; Terblanche *et al*., 2007), although animals may mediate effects of environmental temperature variation through behavioral adjustments (Huey *et al*., 2003). Critical thermal limits are defined as the lowest (CT_{min}) and maximum (CT_{max}) temperatures that allow organismal activity (Chown & Nicholson, 2004) and are manifested in insects as the temperature at which each individual organism loses coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding). In the case of CT_{max}, this loss of muscle function coincides with death such that recovery will not be possible, while in the case of CT_{min}, recovery occurs, and hence, it's not immediately lethal.

HKDT is the time required for an individual to lose motor function during exposure to a high temperature (Huey *et al*., 1992; Angilletta, 2009) while CCRT is the time required for an individual to recover from loss of motor function following exposure to a low temperature (Castaneda *et al*., 2005; Angilletta, 2009; MacMillan *et al*., 2016). Many different methods have been used to test for heat resistance, the time it takes an insect to be knocked down by higher temperatures and knockdown temperature, the upper temperature at which insects lose mobility

or are unable to remain upright (Gilchrist & Huey, 1999; Milton & Partridge, 2008). Heat knockdown is induced when insects gradually become paralysed and unresponsive to stimulus during heat stress exposure. Changes in low temperature tolerance have often been measured by survival rates after exposure to low temperatures or CCRT (Gibert *et al*., 2001a; Milton & Partridge, 2008). A chill coma is induced when insects become immobilised upon exposure to low, non-freezing temperatures (approximately 0˚C). Similar to heat knockdown, the insects lose motor activity and remain paralysed until they are returned to normal temperatures where they progressively regain mobility and activity (David *et al*., 1998; Milton & Partridge, 2008). Such indices represent vulnerable temperatures ecologically as they represent thermal windows where insects are vulnerable to predation and compromise different life history fitness traits.

1.2 Insect freeze strategies and supercooling points (SCPs)

SCP is the temperature at which ice begins to form within the body fluids after exposure to low temperature (also called the temperature of crystallization) (Hance *et al*., 2007). At this point water and aqueous solutions remain unfrozen at temperatures below their melting point. In laboratory experiments, SCP for each individual organism is determined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (Nyamukondiwa *et al*., 2013). Consequently, following determination of the SCP of an insect, it is possible to establish whether the insect is freeze tolerant, freeze intolerant or chill susceptible. Insect freeze tolerance refers to the ability of some insect species surviving ice formation within their tissues (Lee & Costanzo, 1998). Freeze-intolerant species which cannot survive ice formation within their tissues are killed once they reach their SCP hence can also sustain lethal damage at temperatures above that point (Hance *et al*., 2007). Freeze intolerant insects avoid ice formation in body fluids through developing a high supercooling capacity, whereas some dehydrate, thereby reducing their melting point to the ambient temperature (Denlinger & Lee, 2010). In that respect, insects that have evolved freeze-tolerance strategies avoid tissue damage through controlling where, when and to what extent ice forms in their body fluids (Lee & Costanzo, 1998). In contrast to freeze intolerant insects that are able to exist in cold conditions by supercooling, freeze tolerant insects tolerate the formation of ice in their body tissues and fluids (Sinclair *et al*., 2015). Physiologically this is accomplished through inoculate freezing, the production of ice nucleating proteins, crystalloid proteins, crystalloid compounds

and microbes (Lee & Constanzo, 1998). An exposure to low temperature can result in physical damage caused by either ice crystals or metabolic injuries such as osmotic stress or anoxia (Turnock & Fields, 2005; Hance *et al*., 2007), both of which can be lethal to insect pests and associated parasitoids. Chill susceptible insects are killed by cold in the absence of internal ice formation (Sinclair *et al*., 2015). Generally, non-freezing injury is cumulative and can be lethal if the intensity or duration of that injury surpasses a certain threshold (Turnock & Fields, 2005; Hance *et al*., 2007). Such damage can be permanent, leading to death, or it can be repaired if the insect is temporarily exposed to higher temperature. As an insect is cooled to sub-zero temperatures ice does not form at 0°C until the temperature falls below the melting point of the insect's body fluids (Figure 1.3) Insects only begin to supercool when they are cooled to temperatures below their melting point (Denlinger & Lee, 2010). Determination of SCPs is critical under global change, since it is expected that climate variability will increase (Deutsch *et al.,* 2008), with consequent increase in likely freezing temperatures. Thus, SCPs may provide a clue on how insects may cope upon exposure to sub-zero temperatures.

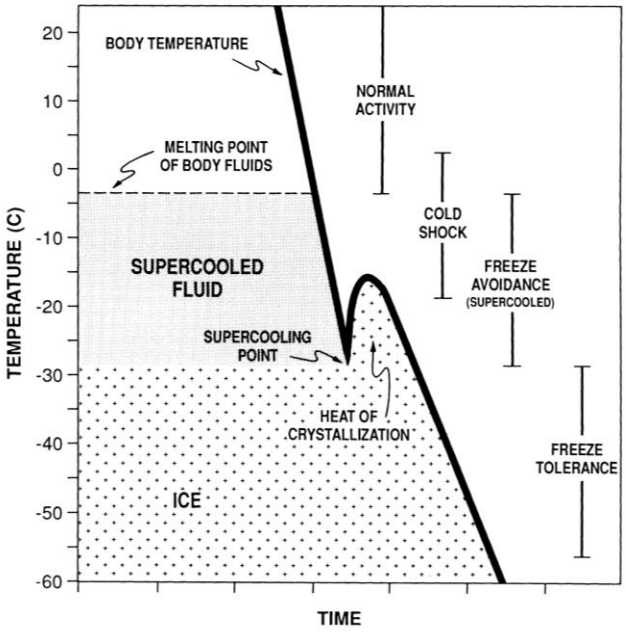


Figure 1.3: Responses of insects cooled to low temperature, redrawn from (Denlinger & Lee, 2010)

1.3 Phenotypic plasticity in insects as a response to changes in temperature

Phenotypic plasticity is the range of phenotypic traits (physiological, morphological, behavioural, and life-history traits) expressed by a single genotype in response to variable environmental conditions (Schlichting & Smith, 2002; Whitman & Agrawal, 2009; Nicotra *et al*., 2010). Insects are able to survive extreme temperature variation at daily to weekly time scales using a host of physiological and biochemical mechanisms. One such mechanism is rapid cold hardening (RCH), defined as a rapid improvement in survival at a low lethal temperature after brief pre-treatment to a prior sub-lethal temperature shock (Stotter & Terblanche, 2009). In certain life-stages of a particular species as much as 80-100% improvement in survival has been reported, for example *Bactrocera oleae* (Koveos, 2001), *Drosophila melanogaster* (Jensen *et al*., 2007; Rajamohan & Sinclair, 2008), in Tephritids, *Ceratitis capitata* and *C. rosa* (Nyamukondiwa *et al*., 2010), *Zaprionus vittiger* (Nyamukondiwa & Terblanche, 2010a) and an array of other drosophilids (Nyamukondiwa *et al*., 2011). In addition, both *C. rosa* and *C. capitata* show the ability to alter thermal tolerance to new thermal conditions (e.g. simulated summer or winter conditions in the laboratory) within a single generation (Nyamukondiwa *et al*., 2013). Low temperature responses in Lepidoptera, *Thaumatotibia leucotreta* (Stotter & Terblanche, 2009) proved its ability to adjust thermal tolerance at longer time scales. Furthermore, of the insect species that show acute physiological protection mechanisms such as RCH, considerable variation in the magnitude of the protection afforded exists both within (Jensen *et al*., 2007) and among species (Chown & Nicolson, 2004; Nyamukondiwa & Terblanche, 2010a) and the time course of responses (Weldon *et al*., 2011). Similarly, at warm temperatures, brief exposure to sub-lethal high temperatures can result in greater survival at a lethal high temperature (Stotter & Terblanche, 2009) a phenomenon called rapid heat hardening (RHH). Indeed, this mechanism has been shown to improve survival in several insects including *Trichogramma carverae* (Scott *et al*., 1997), *Ceratitis capitata* (Kalosaka *et al*., 2009; Nyamukondiwa *et al*., 2010), *Cydia pomonella* (Chidawanyika & Terblanche, 2011a), and several *Drosophila* species (Nyamukondiwa *et al*., 2011). Another way that insects can use phenotypic plasticity to adjust their physiology to their current environment is through acclimatization (Angilletta, 2009; Austin, 2013). Acclimatization is the physiological change that is associated with a chronic change in the natural environment of an organism, where many environmental factors are changing at once (Hochachka & Somero, 2002; Tattersall *et al*., 2012).

The effect of an individual component of the environment (such as temperature) on an organism is called acclimation (Austin, 2013). Acclimation can be observed in a controlled laboratory environment where various environmental factors are held constant to isolate the physiological effects of a single factor of interest on performance and survival of an organism (Hochachka & Somero, 2002; Tattersall *et al*., 2012). There has been increasing interest by physiological and behavioural ecologists in acclimation effects on ectotherms to varying temperature regimes (Jumbam *et al*., 2008; Gray, 2013; Hemmati *et al*., 2014). It is thought that these plasticity mechanisms are employed by insects to quickly alter their physiological tolerances at short timescales, for example over the course of a day to optimise key features like mating and feeding (Shreve *et al*., 2004) consequently critical to the survival of sudden, unpredictable temperature extremes typical in the wild (Chown & Nicolson, 2004).

Many researchers assume phenotypic plasticity has evolved as a mechanism to improve fitness in an environment in which the organism has been acclimated. This is known as the "beneficial acclimation hypothesis" (BAH) (Kristensen *et al*., 2008; Angilletta, 2009; Cooper *et al*., 2010). However, this BAH has been debated in literature, as organisms often adjust incorrectly to their environment and as a result, suffer fitness consequences (Wilson & Franklin, 2002; Angilletta, 2009; Cooper *et al*., 2010). For example, *D. melanogaster* reared at a high temperature did not have better fitness traits compared to flies reared at intermediate and low temperatures (Gibert *et al*., 2001a; Austin, 2013). Therefore, phenotypic plasticity may in some instances, not necessarily always increase the fitness of organisms in their natural environment (Gibert *et al*., 2001b; Austin, 2013). Indeed, the optimal developmental temperature hypothesis suggests otherwise, that organisms developed optimally have a fitness advantage at both thermal extremes (Bahrndorff *et al*., 2016; Slotsbo *et al*., 2016), and the phenomenon has been demonstrated in *Drosophila*.

1.4 Economic Importance of Cereal Stemborers

The cereal stemborer species present in sub-Saharan Africa belong to the Noctuidae, Crambidae and Pyralidae families. From these families, the major cereal stemborer species include the indigenous pyralid *Eldana saccharina* Walker, the crambids *Chilo orichalcociliellus* (Strand) and exotic *C. partellus* Swinhoe and the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Kfir *et al*., 2002; Obonyo *et al*., 2010; Addo-Bediako and Thanguane,

2012). In most cases, these borers occur as a complex of species with overlapping spatial distributions (Chabi-Olaye *et al*., 2001). In sub-Saharan Africa, cereal crops are mainly grown at subsistence level by resource-poor farmers and these stemborers are the major biotic constraints to cereal production, causing losses ranging between 5% and 75% of potential crop yield (De Groote, 2002; Kipkoech *et al*., 2006). Severity and nature of stemborer damage is dependent on the season, variety (Overholt *et al*., 1994; Kfir *et al*., 2002; Tefera *et al*., 2010), borer species, the plant growth stage, the number of larvae feeding on the plant, and the plant's reaction to borer feeding (DeGroote, 2002). Almost all cereal plant parts, leaves, stems, tassels and ears are attacked. Infestations by these stemborers increase the incidence and severity of stalk rots and may increase the contamination of the grains with toxin-producing fungi such as *Aspergillus flavus* (Kfir *et al*., 2002).

1.4.1 Biology and Ecology of *Busseola fusca*

The African maize stemborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is indigenous to Africa and is a common pest in many sub-Saharan African countries (Tefera *et al*., 2010). Its distribution and pest status varies with region. In eastern and southern Africa, it is a pest at higher altitudes (above 600 m), but in central Africa it occurs from sea level to over 2000 m, while in west Africa, it is primarily in the dry savannah zone (Kfir *et al*., 2002; Tefera, 2004). The life cycle of *B. fusca* takes 7 to 8 weeks (Polaszek, 1998) and an adult moth lays about 400-500 eggs (Sharma & Nwanze, 1997) between the leaf sheaths and stalk and on the outer ear husks (Tefera *et al*., 2010). The eggs hatch after 10 days (at benign temperatures) and larvae move up the plant and start feeding on the tender leaves in the funnel creating 'window' like structures (Tefera *et al*., 2010). The latter stages of larvae bore into the stem and feed on central tissue. Larval stage takes ≥ 35 days undergoing 6 instars before pupating. The pupal stage takes 9 to 14 days (at benign temperatures) before adult eclosion (Tefera *et al*., 2010).

1.4.2 Biology and Ecology of *Sesamia calamistis*

The pink stemborer, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) is one of the indigenous stemborer pests associated with maize and sorghum in Africa (Ong'amo *et al*., 2008). However, its economic importance varies across the continent. In West Africa, it is a

major cereal insect pest, but is a minor pest in eastern and southern Africa (Bosque-Pérez & Schulthess, 1998; Ong'amo *et al*., 2008). Differences in its pest status may be attributed to variations in host range and ecological preferences among populations (Seshu Reddy, 1998). *Sesamia calamistis* is reported to have originally colonised non-cultivated hosts belonging to the Poaceae and Cyperaceae (Ong'amo *et al*., 2008) and presumably switched to cultivated crops after the domestication of sorghum and the introduction of maize in Africa (Polaszek & Khan, 1998; Ong'amo *et al*., 2008). Adult female can lay up to 400 eggs between the lower leaf sheaths and stems (Sharma & Nwanze, 1997). Eggs hatch in 5 to 7 days and first instar larvae penetrate the stems where they start feeding. Larval stage also undergoes 6 instars in 4 to 6 weeks before pupation. Pupation takes between 9 and 13 days before adult eclosion (Sharma & Nwanze, 1997).

1.4.3 Biology and Ecology of *Chilo partellus*

The spotted stemborer, *C. partellus,* exotic to Africa, is one of the most important stemborers in East and Southern Africa (Tefera *et al*., 2010). Since its invasion of the African continent, it was limited to warm, low-altitude regions of eastern and southern Africa (Kfir, 1997) but recent studies have reported it extending its geographical distribution to cooler and higher altitude areas (Khadioli *et al*., 2014; Mutamiswa *et al*., 2017a). The life cycle of spotted stemborer takes about 3 to 4 weeks, sometimes longer at lower temperatures, and shorter in warm climates. The emerging larvae move to the whorls where they start feeding on tender leaves of the plants and later extending into the stem. Some may move to nearby host plants (Berger, 1992). Severely attacked plants usually dry up partly or entirely, resulting in 'dead heart' symptom' (Tefera *et al*., 2010). Early attacked plants show stunted growth and poor ear development. Stem tunneling by older larvae has been reported to interfere with nutrients transfer to the ear (Polaszek, 1998; Kfir *et al*., 2002; Tefera *et al*., 2010). Larvae spin silken threads which are launched by wind into the air to infest neighboring plants (Sithole, 1989). This is an instinctive dispersal mechanism which serves in reducing competition between larvae that hatch from the same egg batch thereby increasing the chances of their survival. Up to six generations of *C. partellus* can occur per year (Chinwada, 2002).

Chilo partellus moths sexually mature in 3 days at benign temperatures (Lwande *et al*., 1993) after which they lay flat and oval, creamy white eggs approximately 0.8mm long. Females

are capable of laying between 600-800 eggs in their entire lifetime (Sharma & Davies, 1988). These eggs hatch into larvae within 2-5 days (Sharma & Davies, 1988). Larvae are up to 25 mm long when fully grown, with a prominent reddish-brown head (Tefera *et al*., 2010). The body is creamy-white to yellowish-brown, with four purple-brown longitudinal stripes and usually with very conspicuous dark-brown spots along the back, which give them a spotted appearance. Young larvae initially feed in the leaf whorl whereas older larvae tunnel into stems. In warm conditions larval development is usually completed in about 15 to 20 days after which they pupate in damaged stems (Tefera *et al*., 2010). Pupae are up to 15mm long, slender, shiny and light yellow-brown to dark red-brown in colour. Adults eclose within 7 to 10 days depending on temperature (Ofomata *et al*., 2000; Kfir *et al*., 2002; Muhammad & Underwood, 2004; Tefera *et al*., 2010). Adults are relatively small moths with wing lengths ranging from 7 to 17mm and a wingspan of 20 to 25mm (Tefera *et al*., 2010). Adults emerge from pupae in the late afternoon or early evening. They are active at night and rest on plants and plant debris during the day (Tefera *et al*., 2010).

1.5 Control of Cereal Stemborers

1.5.1 Chemical Control

Under severe infestation, chemical control can provide an effective means of managing stemborers. However, chemical application is only effective economically, if applied based on economic threshold levels, thus pest scouting and monitoring may be successful prior to crop damage (Bosque-Perez, 1995). All stemborer control strategies involving the use of chemicals generally target the larval stage. Dusts (Malathion and Carbaryl) and sprays are applied down the funnel of young plants to kill the emerging and feeding first instar larva and are effective against these developmental stages (Van den Berg & Nur, 1998). Deltamethrin also gives an effective control against stemborers in maize and sorghum when applied 10-14 days after crop emergence. In commercial farming systems, foliar sprays of Monocrotophos and Pyrethroids are common for the control of stemborers. Granular application of insecticides in the whorls of maize and sorghum plants is the most effective and economic way of controlling stemborer species which feed in the whorls (Van den Berg & Nur 1998). In the smallholder sector, whorl-applied granular insecticides such as Trichloforn (Dipterex) are the most widely used because of their ease of

application and lack of the requirement for special application equipment (Chinwada, 2002). They are easy and safe to handle, can be applied by hand, and can be applied accurately in a controlled manner (Van den Berg & Nur 1998). Soil applied granular systemic pesticides such as Carbofuran applied at planting have also been shown to be effective in controlling stemborers (Van den Berg & Nur, 1998).

Although chemicals are effective in controlling stemborers, they have a danger of resistance development (Hill, 1987). In addition, they may not be available or affordable to smallholder resource poor subsistence farmers and may come at a cost to environmental biodiversity. The boring characteristic of the larvae protects them against the sprays and hence regular sprays may be required which subsistence farmers in developing countries cannot afford (Sithole, 1989). Because of the low profit margin of maize and sorghum, smallholder farmers cannot afford the cost of chemical control against stemborers (Kfir, 1998). Against this background, it is necessary to involve as many ecologically sound and sustainable control methods as possible in integrated pest management (IPM) systems.

1.5.2 Cultural Control

Cultural control is a long-established method of modifying the habitat to make the environment unfavourable for the survival and reproduction of pests (Calatayud *et al*., 2014). Indeed, it is the most relevant and economic method of stemborer control available for resource-poor farmers in Africa (Van den Berg *et al.,* 1998). This management strategy is considered the first line of defense against pests and among the oldest traditional practices.

Practicing good crop hygiene, which includes the destruction of crop residues (stems and stubbles), removing volunteer crop plants and/or alternative hosts can be used as a cultural control strategy against stemborer infestations. This reduces carryover of stemborers from one growing season to the next, and will help limit the most damaging attacks on young crops early in the growing season. Burning of crop debris breaks the life cycle by destroying larvae that hibernate inside stalks and plant material. It has been suggested that the partial burning of stalks will result in the killing of 95% of larvae (Adesiyum & Ajay, 1980; Plant Health Australia, 2009). Damage avoidance by manipulation of sowing dates may also be used to avoid synchronisation of the pest and the most vulnerable plant stages. However, this is not practical in situations where lack of water is a major constraint as farmers usually plant after first rains

(Adesiyum & Ajay, 1980). Studies on several stemborers in Africa showed that soil nutrient levels, such as nitrogen, greatly influenced nutritional status of the plant and the plant's tolerance to stemborer attack (Mgoo *et al*., 2006). Although an increase in nitrogen is related to higher pest level loads and tunnel damage, there is also an increase in plant vigour with a net benefit to the plant as reflected in lower yield losses (Sétamou *et al*., 1995; Mgoo *et al*., 2006). Intercropping maize with cowpea is an effective way of reducing damage by the spotted stemborer larvae migrating from neighbouring plants, where the cowpeas serve as a physical barrier to the movement of borers across plants.

1.5.3 Push - Pull Technology

The push-pull technology is a strategy for controlling agricultural pests by using repellant "push"- and attractant "trap" plants (Cook *et al*., 2007). The pull approach relies on a combination of companion crops to be planted around and among maize and sorghum crops. Both domestic and wild grasses can help to protect the crops by attracting and trapping stemborers. The grasses are planted in the border around the maize and sorghum fields where invading adult moths become attracted to chemicals emitted by the grasses. Instead of landing on the maize or sorghum plants, the insects head to the attractive trap plant (Cook *et al*., 2007). These grasses also serve as a haven for the borers' natural enemies which ultimately attack stemborers species on cultivated cereal crops. Napier grass (*Pennisetum purpureum*) and Sudan grass (*Sorghum vulgare sudanese*) act as trap crops with Napier more attractive to stemborers than maize. It 'pulls' the moths to lay eggs on it but does sustain stemborer larvae to develop on it due to the sticky substance it secretes which physically traps the insect pest and limits its damage (Cook *et al*., 2007). In addition, Sudan grass attracts maize female stemborers for oviposition resulting in maize yield increase (Khan *et al*., 1997). The "push" approach is provided by the plants that emit chemicals (kairomones) which repel stemborer moths and drive them away from the main crop (maize or sorghum). *Desmodium uncinatum* planted between rows of maize or sorghum is well known for producing a smell or odour that repels stemborer moths (Khan *et al*., 1997). Since it is a legume, it has the advantage of improving soil fertility through enhanced soil organic matter content and nitrogen fixation (Khan *et al*., 1997). In addition, it can also serve as an animal feed and can also effectively suppress deleterious weeds, for example *Striga asiatica* (Teka, 2014). Intercropping maize with molasses grass (*Melinis*

minutiflora), a non-host for stemborers, will also reduce stemborer infestation on maize. This grass produces volatile agents which repel stemborers but attract the parasitic wasp *Cotesia sesamiae* (Khan *et al*., 2005).

1.5.4 Biological control

Biological control is a method of managing pests that relies on predation, parasitism, herbivory and other natural mechanisms and can be an important component of IPM programs (Hoy, 1994; Chidawanyika *et al*., 2012). The main attraction of biological control is that it lowers the need for using chemicals and there is therefore no environmental pollution, which affect non-targeted flora and fauna. If pests reach economically damaging levels, biological control agents may not imminently eliminate pest pressure (Linker *et al*., 2009). Thus, under these scenarios, biological controls should be integrated with other control measures because different methods are effective at different times and locations under varying conditions (Anchal *et al*., 2013)

There are a number of known natural enemies of cereal stemborers in Southern Africa including parasitoids of eggs, larvae and pupae (see Mutamiswa *et al*., 2017b). Predators like ants, spiders, earwigs, nematodes and microbial pathogens have been reported to attack different life stages under various natural conditions. In general, evidence has shown that indigenous natural enemies are unable to keep stemborer populations below economic injury levels (Bonhof *et al*., 1997). Natural enemies successful in controlling *C. partellus* include the pathogen *Bacillus thuringiensis kurstaki*, egg parasitoids *Trichogramma* spp. pupal parasitoids *Xanthopimpla stemmator*, *Dentichasmias busseolae*, *Pediobus furvus*, *Lepidoscelio* sp. (Sithole, 1990) and larval parasitoids *Cotesia sesamiae*, *Cotesia flavipes*. *Bacillus thuringiensis kurstaki* attacks stemborer larvae and *Cotesia flavipes* has been successful in attacking stemborer larvae in Kenya, Pakistan, South Africa, Uganda and Tanzania (Kfir *et al*., 2002).

1.6 Biology and ecology of *Cotesia flavipes* and *Cotesia sesamiae*

Cotesia flavipes and *C. sesamiae* have similar biology and ecology (Overholt *et al*., 1997; Niyibigira, 2003). *Cotesia flavipes*, native to Indo-Australian region, is a gregarious koinobiont, larval endo-parasitoid of lepidopteran stemborers (Emana, 2007). *Cotesia sesamiae* (Cameron)

(Hymenoptera: Braconidae), indigenous to Africa belongs to the *C. flavipes* monophyletic complex (Kimani-Njogu & Overholt, 1997; Muirhead *et al*., 2012) and is also a gregarious endoparasitoid of lepidopteran stemborers of Crambidae, Pyralidae, and Noctuidae families.

Cotesia flavipes was imported, mass reared in the 90's, and subsequently released in East and Southern Africa. Adult females of *C. flavipes* and *C. sesamiae* parasitise medium and large sized (4-6th instar) stemborer larvae while the stemborers are feeding inside the plant stems (Overholt *et al*., 1994; Ngi-Song *et al.*, 1997). Each female wasp lays about 40-60 eggs in a host larva (Mochiah *et al.*, 2001). At 25˚C, 50-80% RH, and 12L:12D photocycle, the egg/larval period lasts 9-13 days, after which the larvae exit the host and spin cocoons (Overholt *et al*., 1994). Adults emerge from cocoons 5-6 days later (Overholt *et al*., 1994) and the sex ratio is usually female biased (60-70%) (Poting, 1996). Adult longevity ranges from < 24 hours if the parasitoid is unfed (Wiedenmann *et al*., 1992) to as long as 10 days if honey and water are provided (Overholt *et al*., 1994). Following field releases, *C. flavipes* is now established in several countries (Kenya, Tanzania, Mozambique, Uganda, Ethiopia, Zambia, Zimbabwe, Zanzibar, Malawi, and Somalia) (Kfir *et al*., 2002). When *C. partellus* is the host, *C. flavipes* has a higher host searching capacity than *C. sesamiae* (Sallam *et al*., 1999). The distribution of these two *Cotesia* species is influenced by climate, with *C. sesamiae* common in wetter regions while *C. flavipes* is common in warm and dry regions (Songa *et al*., 2001; Niyibigira, 2003).

1.7 Problem statement and justification of the study

Abiotic factors such as temperature and relative humidity are critical in insect pest management and successful control with temperature being the most predominant (Aneni *et al*., 2013). Most importantly, temperature determines the likelihood of reproductive sterility or mortality, especially at extremes and is therefore of critical importance to insect population dynamics (Wang *et al*., 2014). However, temperature tolerance of an insect is not a fixed but rather plastic trait (Nyamukondiwa & Terblanche, 2009; Stotter & Terblanche, 2009). Temperature tolerance may vary significantly depending on the ecological and evolutionary history of a species (e.g. temperate vs. tropical environment) (Kleynhans *et al*., 2014), or its life-stage (e.g. adult, larvae) or age and sex (Nyamukondiwa & Terblanche, 2009). Ultimately, it is these factors, combined with complex interactions between duration and severity of exposure (longer or more severe exposures are typically more lethal) that determine an insect's thermal

tolerance. Therefore, a comprehensive understanding of the effects of temperature, rainfall and relative humidity on interactions between insect pests and parasitoids is important in developing improved pest management strategies (Aneni *et al*., 2013). However, this information is currently unknown for lepidopteran stemborers and their antagonistic parasitoids.

Some insect pests have been reported to increase their invasion potential in relation to their ability to deal with changing climates through phenotypic plasticity and variation in basal tolerance (Nyamukondiwa *et al*., 2010; Chidawanyika *et al*., 2012). In addition, diapause which is a form of suspended growth and development (Alford, 2010), behavioural adaptation such as habitat selection, basking intensity, restriction of activity periods and selective exploitation of environmental thermal fluxes (Yang *et al*., 2008; Piyaphongkul, 2013) also increase invasion potential. A classic example is the invasive spotted stemborer *C. partellus*, which was first introduced in Africa accidentally in Malawi around 1930 (Tams, 1932) but managed to establish itself in several African countries, becoming more destructive than the major indigenous species namely *B. fusca*, *S. calamistis* and *E. saccharina* (Sithole, 1990; Chidawanyika *et al*, 2012; Mutamiswa *et al*., 2017a). In addition, this stemborer species has been reported extending geographical distribution from lowland areas to high elevation tropical areas and humid transitional regions (Khadioli *et al*., 2014) thereby displacing the indigenous species (Mutamiswa *et al*., 2017a). Whilst the first introduction of *C. partellus* into Africa may have not been due to climate change, it has become apparent that its further establishment in several African regions follows a distinct pattern, which may be partly influenced by both climate and altitude (Ong'amo *et al*., 2006; Chidawanyika *et al*., 2012) as well as repeated propagule pressure (Hayes & Barry, 2008; Mainali *et al*., 2015). However, information on plasticity of thermal tolerance for *C. partellus*, *B. fusca*, *S. calamistis* and their larval parasitoids *C. sesamiae* and *C. flavipes* is currently unknown.

In this regard, the ability of insects to undergo such dramatic and rapid changes in temperature tolerance deserves thorough investigation for several reasons of which four are most significant to biological control. First, since the thermal tolerance of a species forms an integral component of bioclimatic and phenology modelling (e.g. Kuhrt *et al*., 2006), without knowledge of a species' temperature tolerance any prediction of a pest's geographic distribution and abundance under future climate change scenarios is severely limited. This has great implications on pest/parasitoid interactions and consequently biological control. Second, the ability of an

549 insect species to undergo rapid thermal hardening, or improve its temperature tolerance quickly,
550 could severely affect interactions between pest and its natural enemy at the short and long
551 timescales. This is especially important since terrestrial organisms are likely to experience
552 increasing frequency and magnitude of extreme temperatures with predicted climate change
553 (Easterling *et al*., 2000; Tomozeiu *et al*. 2006; Im *et al*., 2011). Third, *C. flavipes* and *C.*
554 *sesamiae* have overlapping ecological niches, and often have synergistic effects on biological
555 control efficacy of stemborers. However, how climate change will affect the two congeners, and
556 its effects on biological control is not well understood. Fourth, insects encounter heterogeneous
557 stressors periodically and simultaneously under natural environments (Bubliy *et al*., 2012) and
558 these have a bearing on their thermal tolerance ultimately influencing trophic level interactions
559 in the agroecosystem, biogeography and biological control efficacy in the face of climate change.
560 Against this background, an understanding of cereal stemborers (*B. fusca*, *S. calamistis*
561 and *C. partellus*) and their larval endoparasitoids (*C. flavipes* and *C. sesamiae*) population
562 dynamics under variable climate conditions is essential for predicting seasonal phenology and
563 accurate forecasting of moth abundance which has downstream impacts on basic applications of
564 any biological control programme (timing and parasitoid). Fluctuating patterns of stemborer
565 abundance suggest that DD models are perhaps inadequate or incomplete for predicting pest
566 numbers (Worner, 1992; Bonhomme, 2000) because insect microhabitats temperatures are
567 different from the environmental temperature data obtained from weather stations used in DD
568 calculations (Herms, 2004). Moreover, many insects employ physiological and behavioral
569 mechanisms to counter extreme conditions. For example, insects may move to dark surfaces in
570 the sun when they are too cool, and to light surfaces in the shade when they are too warm
571 (Herms, 2004). Key aspects of variation in population dynamics are still poorly predicted and
572 problematic, resulting at least in some cases repeated and unpredicted outbreaks or infestations
573 even in areas under strict pest control. The reason for this mismatch between predictions and
574 observed moth catches are unclear but could include a lack of trait variability incorporated into
575 DD models or that abiotic factors interact in a non-linear fashion to affect population dynamics
576 and performance.
577 While previous studies have assayed thermal tolerance in *Ceratitis* sp. *Bactrocera* sp.
578 *Drosophila* sp. plant hoppers and codling moths (e.g. Koveos, 2001; Grout & Stoltz, 2007;
579 Rajamohan & Sinclair, 2008; Kalosaka *et al*., 2009; Nyamukondiwa & Terblanche, 2009;

Chidawanyika & Terblanche, 2011a; Piyaphongkul, 2013), little has been done for stemborers and their parasitoids, especially in the context of climate change. Indeed, dramatic changes in high and low temperature tolerance can occur between life-stages of various insect species (Bowler & Terblanche, 2008) but this has not been investigated for *B. fusca*, *S. calamistis C. partellus* and their parasitoids *C. flavipes* and *C. sesamiae*. Moreover, to my knowledge, no study to date, has looked at trophic level thermal biology, with a view of predicting how changing thermal regimes may offset trophic level coevolved relationships (though see Schreven *et al*., 2017). Consequently, the kind of data that is required to understand how natural diurnal temperature and humidity fluctuations impact population dynamics through direct temperature and moisture-dependent mortality is currently not available. To better elucidate the link between cereal stemborers (*B. fusca*, *S. calamistis* and *C. partellus*) and their parasitoids (*C. flavipes* and *C. sesamiae*) thermal biology, population persistence and species expansion, this study aims at developing a mechanistic physiological model approach (and one which considers *B. fusca*, *S.calamistis*, *C. partellus*, *C. flavipes* and *C. sesamiae* developmental stages) to understanding thermal biology and population dynamics of these insect species. This specifically incorporates the thermal parameters such as lower and upper lethal temperatures, critical limits to activity, SCPs, CCRT and HKDT measured from laboratory experiments. The data generated from this study will fill a substantial knowledge gap for a range of environmental conditions with significant management implications. Importantly, these results will directly improve the predictive ability of which environmental factors determine population fluctuations in *B. fusca*, *S. calamistis*, *C. partellus*, *C. flavipes* and *C. sesamiae* with consequent improvement in the efficacy of biological control programmes.

1.8 Objectives

1.8.1 Overall Objective

The broad objective of this project was to improve understanding of climate variability on stemborers-natural enemies trophic level interaction and its implications on biological control under global change

1.8.2 Specific Objectives

1. To investigate the dominance of exotic *C. partellus* relative to indigenous stemborer species (Chapter 2)
2. To determine basal thermal tolerance for *C. partellus* and *C. sesamiae* (Chapter 3)
3. To assess phenotypic plasticity of temperature tolerance for *C. partellus* and *C. flavipes* (Chapter 4)
4. To determine thermal tolerance for larval endoparasitoids *C. sesamiae* and *C. flavipes* (Chapter 5)
5. To assess the effects of environmental heterogeneity on *B. fusca, S. calamistis* and *C. partellus* thermal fitness (Chapter 6)

1.8.3 Hypotheses

1. Exotic *C. partellus* and indigenous stemborer species are equally codominant
2. Thermal tolerance of *C. partellus* and *C. sesamiae* are in synchrony and their interaction may evolve together with thermal variability
3. *Chilo partellus* is equally thermally plastic compared to its biological control agent, *C. flavipes*
4. *Cotesia sesamiae* and *C. flavipes* have similar basal thermal tolerance.
5. Heterogeneous environmental stressors have no effects on *B. fusca*, *S. calamistis* and *C. partellus* thermal fitness

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CHAPTER 2

Dominance of spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) over indigenous stemborer species in Africa's changing climates: ecological and thermal biology perspectives*

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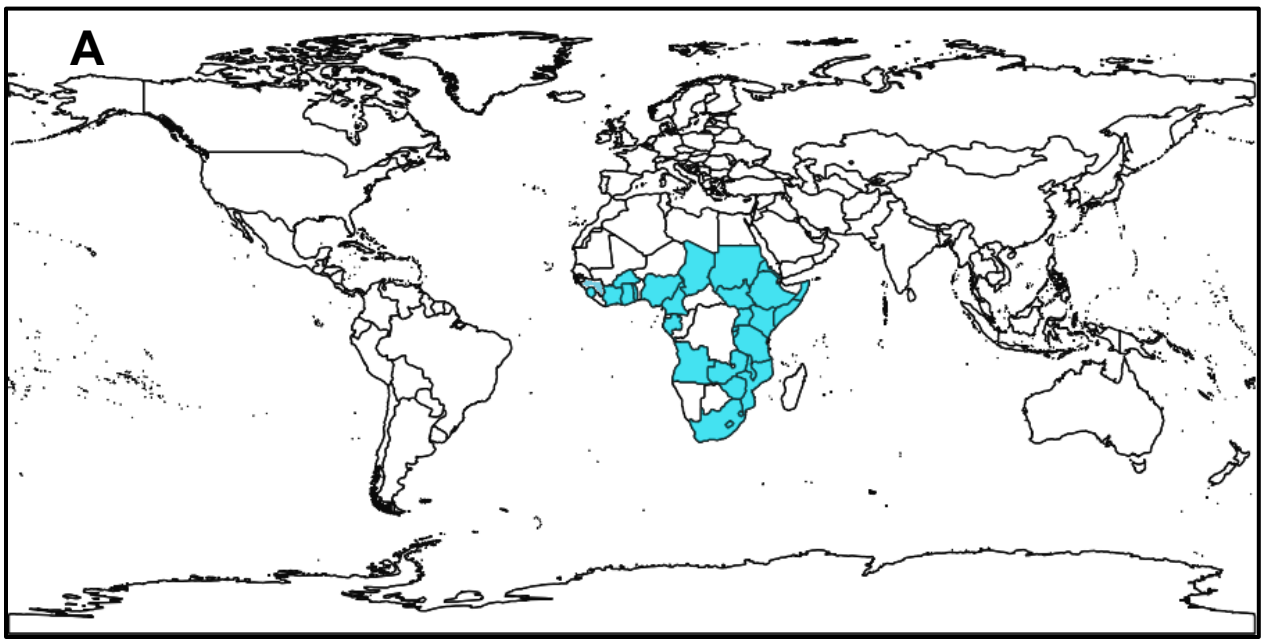
2.1 Introduction

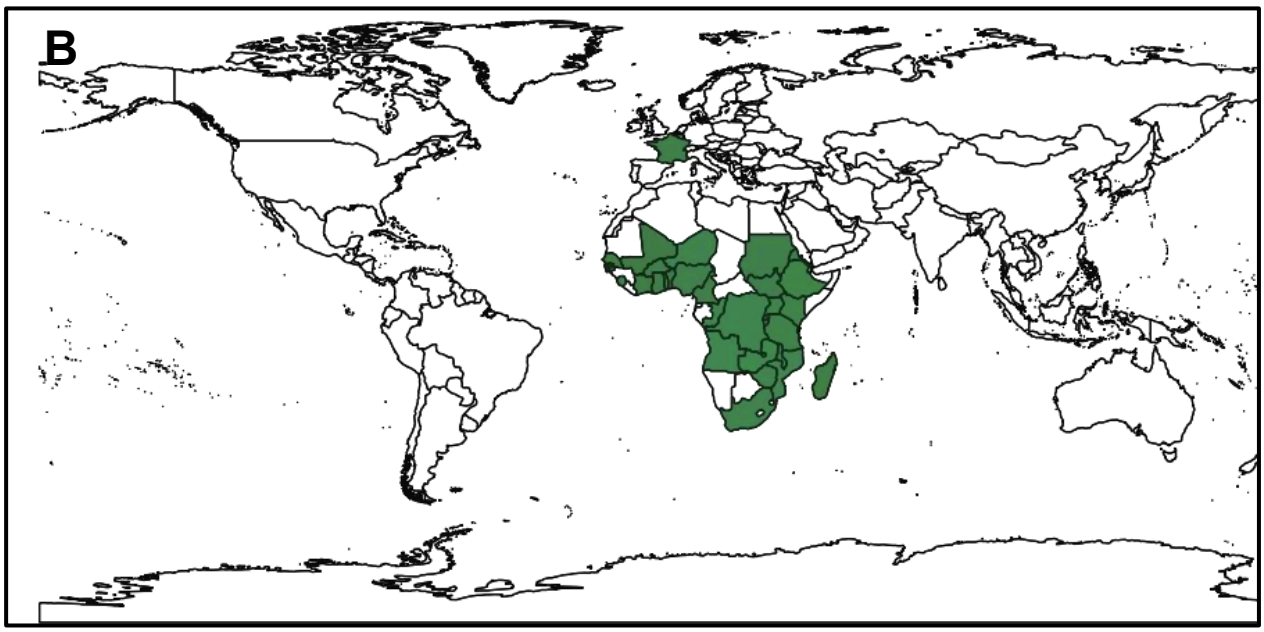
The evidence of anthropogenic global climate change is increasingly becoming apparent (IPCC, 2014). Climatic changes such as shifts in rainfall and seasonal thermal patterns are already being witnessed through extreme weather events such as droughts and floods, heat waves and cold snaps at increased magnitude and frequency (IPCC, 2014). With global mean surface temperature predicted to increase by 1.4 to 5.8°C by 2100 (Karuppaiah & Sujayanad, 2012), much concern has been duly placed on the potential impact of such changes on biodiversity including the potential invasion pathways of invasive fauna and flora (Sutherst *et al*., 2007; Ward & Masters, 2007). For example, several works have shown that different plant and animal species are vulnerable to climate change (Parmesan & Yohe, 2003; Thomas *et al*., 2004; Hance *et al*., 2007).

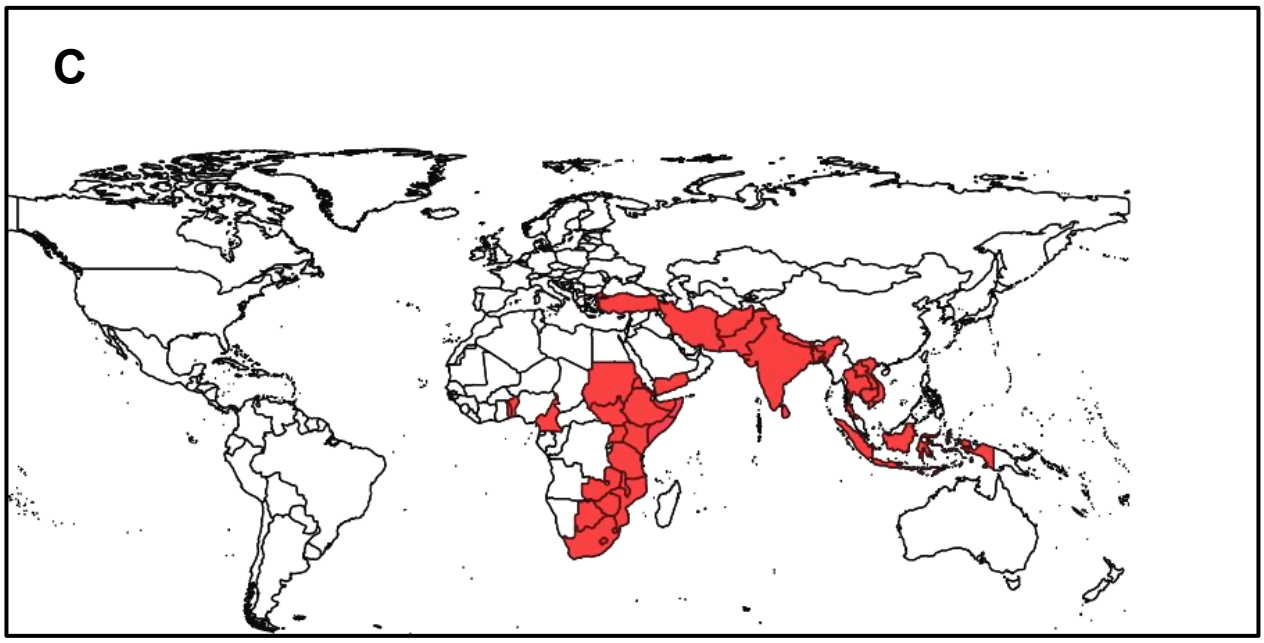
In Southern Africa, agriculture is very important for food security and livelihoods, yet vulnerability to climate risks is considered very high because of heavy reliance on natural resources, particularly in rain-fed traditional agricultural systems (Kutywayo *et al*., 2013). For crop production systems, the threat of undesirable biota such as weeds, insect pests and disease pathogens is projected to increase under climate change scenarios (Juroszek & von Tiedmann, 2013). For example, it has been postulated that rising temperatures may increase geographical ranges of insects (Parmesan *et al*., 1999) resulting in exotic insects, including destructive crop pests realizing new hosts among indigenous plants and crops. In addition, temperature changes may alter the phenology of indigenous pests resulting in decreased duration of over-wintering period and rapid population growth, thereby increasing the pest pressure on the production system (Jaramillo *et al*., 2011). The potential reduction in diversity of beneficial insects including natural enemies of pests and crop pollinators coupled with phenological asynchrony between insect pests and their natural enemies will further compound problems already bedeviling agriculture (Mwalusepo *et al*., 2015). The minimization of crop losses and reduction of pesticide use to meet regulatory environmental obligations are two key areas in agriculture which will become more challenging (Chidawanyika *et al*., 2012). The magnitude of climate change is expected to be greater in the tropics, whose countries are mostly developing and hence have low capacity for mitigation and adaptive measures (IPCC, 2014; Mwalusepo *et al*., 2015). The understanding of potential impacts of climate change on this pivotal sector is therefore important towards building resilience.

Cereal crops, in particular maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench), are strategic crops in achieving food security. Despite significant strides in maize varietal development to suit diverse conditions, many problems still impede optimal production. Among them, a complex of lepidopteran stemborer insect pests damage the crop at different developmental stages (Addo-Bediako & Thanguane, 2012) with losses ranging between 5% and 73% of potential crop yield (De Groote, 2002; Mailafiya *et al*., 2009). The major cereal stemborers of economic importance present in Africa include spotted stemborer (*Chilo partellus* Swinhoe), African maize stemborer (*Busseola fusca*) (Fuller), pink stemborer (*Sesamia calamistis* Hampson), sugarcane stemborer (*Eldana saccharina* Walker) and coastal stemborer (*Chilo orichalcociliellus*) (Strand) (Kfir *et al*., 2002; Addo-Bediako & Thanguane, 2012). Distribution of these stemborers species in Africa varies spatially (Figure 2.1A-D), with most of them occurring as a complex of species with overlapping spatial and temporal distributions (Chabi-Olaye *et al*., 2001).

Several questions using diverse approaches have aptly been asked in an attempt to understand future insect pest-maize interactions under climate change scenarios (e.g. Aragòn *et al*., 2010; Mwalusepo *et al*., 2015). Bioclimatic models based on species distribution records and phenology data have successfully explained current distribution of the stemborers with their predictive power justifiably accepted with high degree of confidence (see e.g. Mwalusepo *et al*., 2015). These correlative habitat models describe the relationship between the distributions of a species across the landscape with physical or biotic factors (Herborg *et al*., 2007). These models have therefore been useful in identifying potential hotspots for biodiversity in general (Herborg *et al*., 2007; Mika *et al*., 2008) and specifically maize pests distribution (Aragòn *et al*., 2010; Mwalusepo *et al*., 2015). The distribution may be as a result of not only the abiotic factors, but also the consequence of other historical, unique and contingent factors such as certain dispersal constraints, biotic interactions, anthropogenic effects and stochastic events (Lo´ pez-Darias *et al*., 2008; Aragòn *et al*., 2010).


A


B



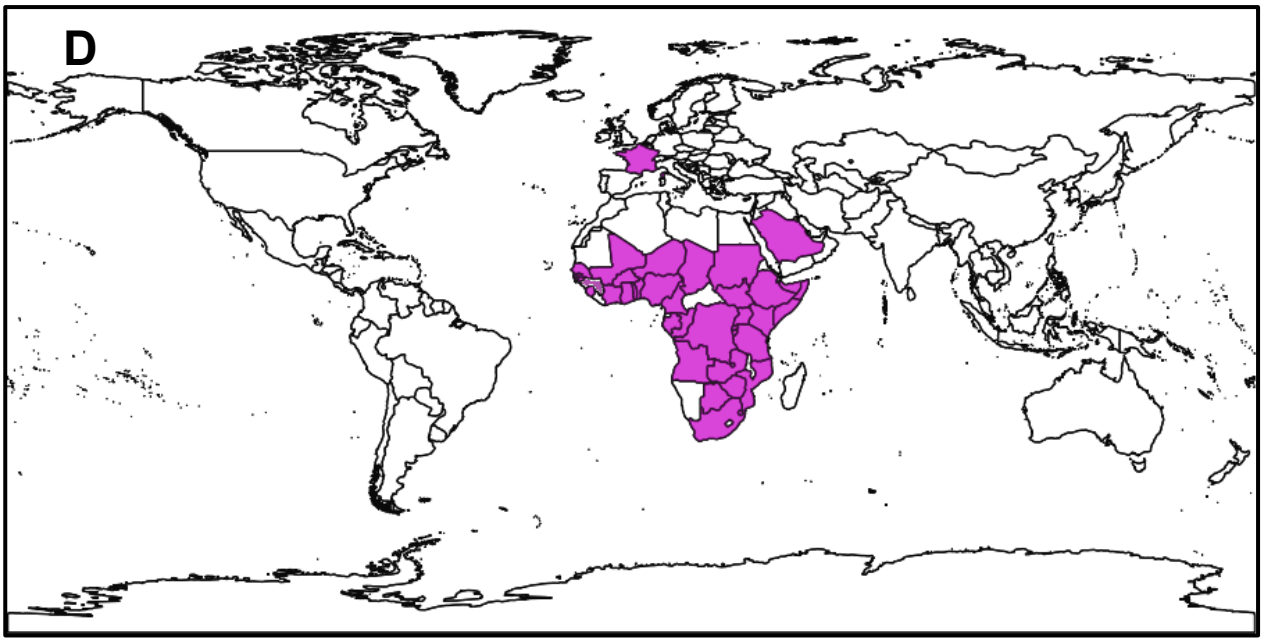


Figure 2.1: Global distribution of cereal stemborers (A) *Busseola fusca* (B) *Sesamia calamistis* (C) *Chilo partellus* (D) *Eldana saccharina* (redrawn from Plantwise Technical Factsheet 2013; Yonow *et al*., 2016**).** Colours indicate presence of stemborer species.

Insects are ectotherms by nature and therefore their body temperature is always in equilibrium with environmental temperature (Chown & Nicolson, 2004). Temperature thus plays a key role in defining survival ranges together with performance of biological activities such as locomotion (Nyamukondiwa *et al*., 2010), reproduction, feeding and developmental rates (Nyamukondiwa *et al*., 2013). As such, insects have evolved a diverse range of morphological, physiological, and behavioral adaptations to counter adverse climate conditions (Chown & Terblanche, 2007; Nyamukondiwa *et al*., 2013). Several studies have already demonstrated how insect distribution, in particular Lepidoptera, can be influenced by temperature (Parmesan *et al*., 1999; Thomson *et al*., 2010). Temperature effects on life history traits have been investigated in *C. partellus* and *B. fusca* in Africa (Khadioli *et al*., 2014a; Mwalusepo *et al*., 2015; Glatz *et al*., 2016). Other studies have also looked at the impact of drought on the performance (crop damage, fecundity, development) of maize pests or their natural enemies (Chidawanyika *et al*., 2014). While lethal/sub-lethal temperature effects largely affect insect phenology, several physiological mechanisms have been reported to be key for survival and preservation of crucial life history traits during typical thermal stress. For example, rapid heat hardening through accumulation of heat shock proteins has been shown to improve survival in otherwise lethal temperatures (Chown & Nicolson, 2004; Nyamukondiwa *et al*., 2010).

Dominance is the degree of ecological influence of a particular species within a community (Freedman, 1995). It can be manifested in the form of individual numerical advantage, a greater degree of damage (for pest insects), and higher growth and reproductive rates relative to competitors. Dominance can lead to success of a species and coupled with ecological plasticity, these may be the most demographic traits related to invasion and dominance success of some species. In addition, climate matching between the new and native environment of the invasive species coupled with the absence of natural enemies are the ecological characteristics aiding invasion success (Farji-Brener & Coley, 1998). Lepidopterans are known to respond to climate change through changes in phenology i.e. the timing of annually recurring events (e.g. emergence from overwintering, voltinism) and also changes in geographical distribution, such as altitudinal range expansion (Parmesan, 2007). The distribution and abundance of the stemborers may be influenced by altitude and environmental conditions especially temperature, precipitation, relative humidity (Getu *et al*., 2002) and biotic factors such as natural enemies and alternative host plants (Addo-Bediako & Thanguane, 2012). *Chilo*

partellus, which is indigenous to Asia was first recorded in Malawi around 1930 (Tams, 1932). In 1953 it was recorded in Tanzania (Duerdon, 1953) and in South Africa in 1958 (Van Hamburg, 1979). It later spread to most of the lowland areas of the countries in eastern and southern Africa dominating the indigenous stemborer species (Kfir, 1997). Recent studies have reported *C. partellus* in cooler and higher altitude areas, conversely to its known optimal ecological conditions (Kfir, 1997; Ong'amo *et al*., 2006; Mwalusepo *et al*., 2015) indicating an expansion of its geographic ranges. Indeed, C. *partellus* has been observed to displace indigenous stemborers in some of the locations it has invaded (Kfir, 1997; Ofomata *et al*., 1999a). The reasons behind *C. partellus* competitive advantage and hence dominance are largely unexplained. In addition, it is largely unknown how temperature tolerance, its interactions with relative humidity and plasticity thereof are likely to shape *C. partellus* and its larval parasitoid *Cotesia flavipes*, especially in the face of climate change.

This paper therefore reviewed the factors that may contribute to the dominance of *C. partellus* over indigenous stemborer species in Africa, with emphasis on thermal physiology under changing African climates. I hypothesized that, *C. partellus* may possess a high basal thermal tolerance and plasticity of temperature tolerance, allowing it to invade and thrive in new environments.

2.2 Africa's Changing Climate

The climatic zones in Africa are shaped by sea surface temperature changes, land use and vegetation patterns, which play significant roles in both intra-seasonal variability and onset of rain season (Stathers *et al*., 2013). The average temperatures in Africa are projected to increase by 1.5 to 3°C, by 2050 and will gradually continue upwards beyond this time (IPCC, 2014). Climate change in Africa will especially be associated with a rise in temperatures and its variability thereof, erratic rainfall patterns, increased extreme weather events (flooding, droughts, heat waves and cold snaps) (Stathers *et al*., 2013; IPCC, 2014). Temperatures are projected to rise by about 1°C in East, Central, Southern and West Africa and slightly less in the Sahel by 2030 (Lobell *et al*., 2008; Stathers *et al*., 2013). Furthermore, for the southern African region during the period 2036 – 2065, small areas along western and central Zimbabwe, eastern Zambia and north-eastern Mozambique, might experience a decline in rainfall. By contrast, western and northern Angola, northern Mozambique, and eastern South Africa (including

Lesotho) are simulated to experience higher annual rainfall totals than at present (IPCC, 2014). During the same period, an increase in minimum and maximum temperature of between 0.8 and 3.6˚C per annum is expected across the region with the interior regions expected to experience more intense warming than the coastal areas. Spring is expected to experience the greatest increase in temperature compared to the other seasons (IPCC, 2014). The fourth Intergovernmental Panel on Climate Change (IPCC) assessment suggests that warming of the African continent (~3.4˚C by 2080-2099) is most likely to be higher than the global mean annual warming all over the continent and in all seasons, with drier subtropical regions warming more than the humid tropics (Wingqvist & Dahlberg, 2008; Stathers *et al*., 2013). Therefore, during the same period southern Africa is likely to experience a 10% rainfall decrease and more frequent droughts, while east Africa is likely to experience increased rainfall (Lobell *et al*., 2008; Stathers *et al*., 2013 but see Williams & Funk, 2011; Mwalusepo *et al*., 2015). In recent decades, eastern Africa has experienced an increased rainfall over the northern sector and declining amounts over the southern sector (Schreck & Semazzi, 2004; Stathers *et al*., 2013). It is predicted that in equatorial and subtropical eastern Africa, warm sea surface temperatures will lead to increased droughts as the number of rain events during the dry season will decrease as well as overall annual precipitation (Masters & Norgrove, 2010). Such changes in precipitation are likely to reduce the annual flow of rivers. For African agroecosystems, shifts in climate are significant as they generate novel structures in original insect pest abundance, emergence of secondary to primary pests (Newton *et al*., 2011), colonization of novel habitats previously unfavorable (Parmesan et al. 1999) and, importantly, modifications of habitats (Webb *et al*., 2005), which may compromise the maintenance of biodiversity and efficacy of biological control through natural enemies (Chidawanyika *et al*., 2012). By 2055, such complex changes in climate are predicted to treble cereal yield losses, especially associated with stemborers. And these losses will be more pronounced in higher than lower altitudes (Mwalusepo *et al*., 2015).

2.3 Competitive Displacement of Indigenous Stemborer Species

Competitive displacement is the removal of a formerly established species from a habitat through superior use, acquisition, or defense of resources by another species (Reitz & Trumble, 2002). Most displacements that have been identified were triggered by the introduction or invasion of an exotic species although other environmental factors may predispose a species to

being displaced (Reitz & Trumble, 2002). Temperature has been reported as one of the factors influencing intra and interspecific interactions as well as limiting the geographical distribution of insects. For instance the interaction between *Nicrophorus orbicollis* Say and *N. defodiens* Mannerheim (Coleoptera: Silphidae) when feeding on the same carcass has been reported to be dependent on temperature (Wilson *et al*., 1984). The displacement of one insect species by another on crops of economic importance is of great significance to pest management (Kfir, 1997). Intra and inter-specific competition among exotic and native stemborer species can influence their performance as well as spatio-temporal population dynamics. As for *C. partellus*, crawling and ballooning were reported as the mechanisms employed by larvae in dispersing (Berger, 1992). The process of ballooning includes the spinning of thin silk-like threads which are launched by wind into the air to infest neighboring plants (Sithole, 1990; Berger, 1992). This mechanism serves to reduce intraspecific competition amongst larvae that hatch from the same egg batch thereby increasing survival through resource use optimization (Chapman *et al*., 1983; Berger, 1992). However, this survival mechanism is not very common in indigenous stemborer species and may confer a relative survival advantage to *C. partellus*. *Chilo partellus* has a high colonization potential and this may facilitate its invasiveness and interspecific competition success. In an experiment involving *C. partellus*, *B. fusca* and *S. calamistis*, interspecific competition was more apparent than intraspecific competition with *C. partellus* being highly competitive in resource utilization indicating its high fitness potential as compared to *S. calamistis* and *B. fusca* (Ntriri *et al*., 2016). Therefore, in favorable environments where *C. partellus* coexists with either of these noctuid species, the crambid will have high survival advantage (Ntiri *et al*., 2016). In most of the low-lying (lowveld) areas of eastern and southern Africa, *C. partellus* has become the most significant and dominant borer species (Kfir, 1997; Mwalusepo *et al*., 2015). Similarly, in other African landscapes, *C. partellus* has managed to successfully displace other indigenous stemborer species (Kfir, 1997; Kfir *et al*., 2002; Yonow *et al*., 2016). For example, *C. partellus* was first detected in Madagascar in 1972, and three years thereafter became an economically significant insect pest of maize and sorghum compared to the native stemborer *Chilo orichalcociliellus* (Delobel, 1975). The displacement of *C. orichalcociliellus* by *C. partellus* was reported in Kenya (Ofomata *et al*., 1999a; Dejen *et al*., 2014), with *C. partellus* abundance increasing from 28.5% to 87% while *C. orichalcociliellus* reduced from 66% to 14% (Zhou *et al*., 2001; Jiang *et al*., 2006). In Kenya's eastern province, *B.*

fusca was more abundant than *C. partellus* around 1980 (Seshu, 1983; Dejen *et al*., 2014). However, between 1996 and 1998, *B. fusca* became less pronounced with *C. partellus* dominating (Dejen *et al*., 2014). Similarly, there was a partial displacement of *B. fusca* by *C. partellus* over a seven year period in the South African eastern highveld region (Kfir, 1997; Sylvain *et al*., 2015). The displacement was more pronounced in sorghum where *C. partellus* proportion increased 664 fold between 1986 and 1992 (Kfir, 1997; Dejen *et al*., 2014). Due to its superior invasion potential to indigenous stemborer species following introduction to novel habitats, (Kfir, 1997; Ofomata *et al*., 2000; Niyibigira *et al*., 2001), *C. partellus* occupies appropriate feeding habitats earlier than the native borer species, thereby outcompeting the population of competitive stemborers that successfully colonize the same habitats (Niyibigira *et al*., 2001). *Busseola fusca* has been reported to avoid ovipositing on host plants with previous *C. partellus* infestation (Kfir, 1997). However, *C. partellus* can oviposit on host plants which are infested with and/or have previous *B. fusca* infestation (Kfir, 1997). This may be because *C. partellus* females, upon oviposition emit chemical odours that deter *B. fusca* females from ovipositing on the same plants or that plant volatiles produced by the host deter *B. fusca* oviposition (Kfir, 1997). Moreover, neonate *C. partellus* larvae can disperse longer distances as compared to *C. orichalcociliellus*, and this may enable *C. partellus* to utilise more host plants than the relatively less mobile indigenous stemborer (Ofomata, 1997; Overholt *et al*., 2000). Limited studies have been conducted in assessing the effects of global temperature increases on the competitive interactions of insect species utilising the same resource and habitat (Ntiri *et al*., 2016). Although competitive displacement of indigenous stemborers by *C. partellus* has been reported in Africa, mechanisms behind the borer species displacement remain relatively unknown (Ntiri *et al*., 2016).

2.3.1 Extension of C. partellus geographical range

As global climatic temperatures increase, threats from invasive tropical and subtropical insect species may increase as they expand their geographical distribution to temperate regions (Chown *et al*., 2007; Weldon *et al*., 2016). Therefore, the ability of these insect species to endure dry conditions may contribute to their invasion potential since migration from the moist subtropical and tropical regions exposes them to low rainfall and increasingly drier environments (Chown *et al*. 2007; Weldon *et al*., 2016). Following its first record in Africa (Malawi) (Tams,

1932), *C. partellus* has become a widespread pest for cereal crops in eastern and southern Africa, becoming more destructive than the indigenous stemborer species including *C. orichalcociliellus* (Sithole, 1990; Kfir, 1997; Ofomata *et al*., 1999a; Overholt *et al*., 2000; Chidawanyika *et al*., 2012; Dejen *et al*., 2014). The interaction between elevation and temperature was thought to be the most important factor determining the distribution of *C. partellus* (Overholt *et al*., 1997; Tamiru *et al*., 2007). However, some researchers documented a combination of climatic factors such as temperature, rainfall and humidity influencing its distribution, with temperature being the most important (Getu *et al*., 2002; Tamiru *et al*. 2007). Since *C. partellus* is predominantly found in lowvelds where average temperatures are high, I therefore hypothesize that, it may possess an enhanced high temperature tolerance and a compromised low temperature tolerance. The former allows it to thrive in warmer environments while the latter limits its colonization to new high altitude areas. However, high basal tolerance may indicate greater plasticity for high temperature tolerance for *C. partellus* (see Nyamukondiwa *et al*., 2011), which may confer a survival advantage for the stemborer under novel environments. Furthermore, insects also trade-off their basal low temperature tolerance for plasticity (Nyamukondiwa *et al*., 2011), a scenario which may mean that *C. partellus* may also be able to cope with low temperature variations typical of climate change through phenotypic plasticity. Since the appearance of *C. partellus* in African continent, it has continuously expanded its distribution to nearly all countries in the warm, low-altitude regions of eastern and southern Africa (Kfir, 1997; Dejen *et al*., 2014; Mwalusepo *et al*., 2015). Previous research in Ethiopia, reported that *C. partellus* was a predominant species at lower altitude of less than 1700 m a.s.l. and *B. fusca* was dominant at high altitude of 1160 - 2600 m a.s.l. and in cooler areas (Assefa, 1985). Until the year 1990, the distribution of *C. partellus* was limited to low and mid-altitude areas (<1500 m a.s.l.), and was attributed to favorable abiotic factors, mainly temperature and humidity (Zhou *et al*., 2001; Khadioli *et al*., 2014a). From 1999 to 2000, a survey in Ethiopia reported that *C. partellus* had widened its distribution from 500-1700 to between 500 and 1900 m a.s.l. whereas *B. fusca* was reported 1030-2320 m a.s.l. (Emana *et al*., 2001; Dejen *et al*., 2014). This work revealed the extension of *C. partellus* into landscapes where only *B. fusca* and *S. calamistis* would 'naturally inhabit'. Similarly, Dejen *et al*. (2014) reported *C. partellus* extending its distribution from 1400 to 2084 m a.s.l. This therefore shows that *C. partellus* distribution may not solely be limited by elevation, but rather a complex of factors

which likely include relative humidity/rainfall, temperature and topographic features (Dejen *et al*. 2014; Mwalusepo *et al*., 2015). In South Africa the only stemborer that used to survive at an elevation of ≥1600 m a.s.l. was *B. fusca*. Following *C. partellus* invasion in the region, it rapidly increased its share of the total stemborer population every year. Within two years, it became the predominant stemborer, constituting 90% of the total population (Dejen *et al*., 2014). In addition, Tamiru *et al*. (2007) recorded *C. partellus* at an elevation of 2088 m a.s.l. in Ethiopia suggesting gradual expansion of the insect to new ecological niches. Furthermore, in Kenya, Ong'amo *et al*. (2006) reported the presence of *C. partellus* in relatively high altitude zones where the insect is least expected (Tamiru *et al*., 2012). Therefore, considering its rate of expansion, *C. partellus* is most likely to continue expanding to high altitude areas and occupying new niches. The establishment of *C. partellus* in several African regions follows a distinct pattern, which may be partly influenced by the close interaction across temperature, relative humidity and altitude (Ong'amo *et al*., 2006; reviewed in Chidawanyika *et al*., 2012). This therefore means any experiments which sought to predict the likely distribution and abundance of *C. partellus* in future should incorporate all these parameters as factors likely dictating its population phenology/dynamics and hence geographic distribution. Similarly, models predicting the likely effects of climate change on stemborer population dynamics should incorporate all factors of temperature, humidity and the ability of organisms to compensate for such changes in environmental conditions through plasticity (within generation) or evolutionary potential (across generations).

2.3.2 Increase in numbers of C. partellus generations

For multivoltine insects, the number of generations per year is significantly affected by climatic conditions, chief amongst them, temperature (Bale, 2010). Higher temperatures associated with climate change result in faster accumulation of degree days hence shorter generation times, milder winters thus longer growing seasons with probable shifts from univoltine to bivoltine/multivoltine life cycles in some insect species (Bale, 2010). It has been estimated that with a 2°C temperature increase, insects might experience one to five additional life cycles per season (Yamamura & Kiritani, 1998). Model predictions indicate a potential increase in the number of *C. partellus* generations in the year 2050, which indicates that higher insect pest infestations and yield losses may occur (Kroschel *et al*., 2013). This success may be

attributed to differences in its thermal biology as compared to that of other related stemborers, *C. orichalcociliellus*, *B. fusca*, *S. calamistis* and *E. saccharina*. The life cycle of *C. partellus* is highly temperature dependent (Ofomata *et al*., 2000; Mbapila *et al*., 2002), taking about 3 to 4 weeks at an optimum temperature of 30°C (Kfir, 1997; Khadioli *et al*., 2014a) (Table 2.1). However, for some indigenous stemborers, life cycles range from 6 to 12 weeks with *E. saccharina* having the longest life cycle of 8-12 weeks at 27±2°C (see Table 2.1).

Table 2.1: A compilation of generation time of four major cereal stemborer life cycles under optimum environmental conditions. Generation time was measured as the amount of time needed to complete development of one stemborer life cycle (from egg to adult).

Stemborer Species	**Average Life Cycle (Weeks)**	**Optimum Environmental Conditions**			**Reference**
		T°C	RH(%)	(L:H)	
B. fusca	7-8	25	65± 5	12:12	Harris & Nwanze, 1992; Polaszek 1998; Khadioli *et al*., 2014b
S. calamistis	6-8	28	65± 5	12:12	Khadioli *et al*., 2014b
E. saccharina	8-12	27±2	75± 5	10:14	Sharma & Nwanze, 1997; Polaszek, 1998
C. partellus	3-4	30	65± 5	12:12	Kfir, 1997; Khadioli *et al*., 2014a

T°C: Temperature; RH: Relative Humidity; L:H: Photoperiod

Due to its short life cycle, *C. partellus* may have more generations per year as well as rapid population growth, suggesting its likely dominance over other stemborer species. Furthermore, *C. partellus'* short generation time may confer the species a superior competitive advantage over its competitors, through its higher potential rate of increase (Kfir, 1997; Mbapila *et al*., 2002; Dejen *et al*., 2014) and rapid development (Reitz & Trumble, 2002) which may possibly give it a numerical advantage (Sujay *et al*., 2010). Up to six generations of *C. partellus* can occur per year

(Panwar, 1995) and this may likely be higher given the increase in mean temperatures with global change. In addition, it is known that when equal numbers of *C. partellus* and *C. orichalcociliellus* infest the same maize, sorghum, or wild-sorghum host plant, *C. partellus* can successfully complete development, suggesting superiority during interspecific competition (Ofomata, 1997).

The average fecundity of stemborers varies in relation to species (see Table 2.2). *Chilo partellus* has the highest fecundity per unit time compared to the other indigenous stemborer species. In an experiment involving *C. partellus* and *C. orichalcociliellus*, a comparison of life traits revealed that the invasive *C. partellus* laid more viable eggs, with its larvae consuming more food and had a higher survival and shorter developmental time as compared to *C. orichalcociliellus* (Ofomata *et al*., 2000; Ntiri *et al*., 2016). Therefore, with its short life cycle coupled with high fecundity, *C. partellus* may have a numerical advantage thereby dominating other stemborer species which are associated with longer life cycles and low fecundity. Similarly, with climate change coupled with shorter generation times, Khadioli *et al*. (2014a) predicted an increase in the number of *C. partellus* generations in most countries in east and southern Africa suggesting that economic losses might increase and also encroach to new agro-ecosystems/habitats. Hence I suggest both intrinsic factors (e.g. rapid generation times, polyphagy and interspecific competitiveness), and extrinsic factors (e.g. increased reproductive output), likely caused by high fecundity may be contributory factors to *C. partellus* dominance when introduced into novel environments. This may explain why it has actively displaced indigenous stemborers upon introduction to new habitats. Nevertheless, in addition to temperature, many other interacting ecological factors need to be considered. For example, selection of a good host plant permitted multivoltism while selection of poorer quality hosts enhanced 'escape' from natural enemies in *Papilio* spp. larvae (Scriber, 2002).

Table 2.2: Average fecundity of different species of cereal stemborers (measured as the average total number of eggs produced per life time) under optimum environmental conditions of temperature, humidity and photoperiod.

Stemborer species	Average Fecundity	Duration (Days)	Optimum Environmental Conditions			Reference
			T°C	RH (%)	L:H	
S. calamistis	400	5	28	65± 5	12:12	Sharma & Nwanze, 1997; Khadioli *et al*., 2014
B. fusca	400-500	5-6	26±1	70± 10	12:12/ 14:10	Sharma & Nwanze 1997; Khadioli *et al*., 2014; Glatz *et al*., 2016
E. saccharina	400-600	14	27±2	75± 5	10:14	Sharma & Nwanze, 1997
C. partellus	500-722	4	30	65± 5	12:12	Taneja & Nwanze, 1990; Sharma & Nwanze, 1997; Khadioli *et al*., 2014

T°C : Temperature; RH: Relative Humidity; L:H: Photoperiod

2.3.3 Overwintering of C. partellus

Diapause is a physiological state of arrested growth and development neurohormonally mediated and frequently elicited by environmental cues (Hance *et al*., 2007). It is important in facilitating an insect to survive unfavorable environmental conditions which include, amongst others, extremes of temperature, unavailability of food as well as drought. Initiation of diapause, maintenance and termination are highly characterized by an array of morphological, physiological, biochemical and behavioral features and the process synchronises insect phenologies with seasonal changes in its environment, especially temperature. Towards the end of the cropping season (coincident with winter in southern Africa or the dry season in eastern Africa), most of the cereal stemborers undergo a resting phase in which they spend in crop residues (Kfir *et al*., 2002). In laboratory experiments (26°C; 16:8h daylength), Kfir (1993)

recorded *B. fusca* mean diapause termination time of 34 days (Table 2.3). In addition, Ofomata *et al*. (1999) showed that mean diapause termination time of *C. partellus* and *C. orichalcociliellus* were 9.7 and 14.4 days respectively at 28°C and 12:12 (L:D) photoperiod (Table 2.3).

Table 2.3: Mean diapause termination time for cereal stemborers. Termination time was defined as the amount of time needed to restore activity of a 'sleeping' stemborer following optimum temperature conditions.

Stemborer Species	**Temperature (°C)**	**Photoperiod (L:D)**	**Mean Diapause Termination Time (days)**	**Reference**
Chilo partellus	28±0.5°C	12:12	9.7	Ofomata *et al*., 1999b
Busseola fusca	26°C	16:8	34	Kfir, 1993
Chilo orichalcociliellus	28±0.5°C	12:12	14.4	Ofomata *et al*., 1999b

Therefore, *C. partellus* terminates diapause earlier than *C. orichalcociliellus* and *B. fusca* thus it completes its life cycle earlier and utilizes habitat resources faster hence optimising growth, increasing propagules hence dominance over other species. *Busseola fusca*, *S. calamistis*, and *C. orichalcociliellus* are known to obligatorily diapause for a number of months during the dry season (Kfir, 1991; Ofomata *et al*., 1999b; Kfir *et al*., 2002). However, *C. partellus* only diapause facultatively under unfavorable climatic conditions (Ofomata *et al*., 1999b). It overwinters in the lower and seldom upper parts of dry cereal stems or below the ground where it is protected from environmental extremes and natural enemies (Kfir, 1991). Availability of favorable conditions like humid conditions are known to induce a quick reduction in the juvenile hormone of diapausing larvae and this initiates diapause termination. Overwintering *C. partellus* populations terminate diapause emerging as moths about one month earlier in the season than other indigenous stemborer species (Kfir, 1997; Ofomata *et al*., 1999b). *Chilo partellus* facultative diapause implies that it may survive predictable, unfavorable environmental conditions, such as temperature extremes, drought or reduced food availability consequently having a survival advantage over relatively obligatory diapausing indigenous stemborers.

2.3.4 Impact of Variability in Temperature and Relative Humidity on the Ecology of Stemborers

Temperature is considered as the most important environmental factor that directly influences insect development, survival, reproduction and distribution (Bale, 2010; Khadioli *et al*., 2014b). In a temperature related experiment, lower temperatures conferred a competitive advantage on smaller males of seed beetle *Stator limbatus* (Horn) (Coleoptera: Chrysomelidae), which out-competed larger ones in reaching a potential mate (Moya-Laraño *et al*., 2007). Individual insect populations have specific optimum temperatures at which they develop optimally, and lower and upper threshold temperatures beyond which they cannot develop (Khadioli *et al*., 2014b). Khadioli *et al*. (2014b) reported that *B. fusca* and *S. calamistis* populations develop within the temperature range of 15°C–30°C with an optimum at 25 and 28°C respectively. However, *C. partellus* populations develop within the range of 18 - 35°C (Khadioli *et al*., 2014a). This translates into a greater thermal window (across all developmental stages) for *C. partellus* activity relative to the other two species *B. fusca* and *S. calamistis* (see Table 2.4).

Table 2.4: Lower and upper developmental temperature thresholds, developmental temperature range and mean developmental time at different temperatures for three major stemborers, (*Chilo partellus*, *Sesamia calamistis* and *Busseola fusca*) (modified from Khadioli *et al*., 2014a;b; Glatz *et al*., 2016)

Development Stage	Lower Development Temperature (°C)	Upper Development Temperature (°C)	Thermal activity window (°C)	Mean Development Time (Days)			
				15°C	25°C	30°C	35°C
C. partellus							
Eggs	18	35	17	-	6.4	4.9	4.8
Larvae	18	35	17	-	32.8	21.9	28.3
Pupae	18	35	17	-	9.3	7.0	7.7
Adults	18	35	17	-	10.05	7.6	5.5
B. fusca							
Eggs	15	30	15	22.47	7.2	6.54	-
Larvae	15	30	15	90.2	50.69	58.12	-
Pupae	15	30	15	40.0	14.67	16.6	-
Adults	15	30	15	12.02	9.33	4.57	-
S. calamistis			15				
Eggs	15	30	15	19.7	7.09	6.74	-
Larvae	15	30	15	130.41	46.52	35.46	-
Pupae	15	30	15	35.19	11.2	9.75	-
Adults	15	30	15	8.4	8.89	7.07	-

Greater thermal window optimizes key behaviors/processes such as development, mating, oviposition and dispersal during environmental conditions where the same traits are limited for *S. calamistis* and *B. fusca*. This confers a survival and comparative advantage of *C. partellus* on key behavioral traits relative to other indigenous stemborer species. Previous studies reported mean development time of ~48 days (25°C) for *C. partellus* reared on artificial diet (Mbapila *et al*., 2002; also see Khadioli *et al*., 2014a). However, the total developmental time of *B. fusca* at 25°C and 26-30°C were 72.56 and 52.6 days respectively (Khadioli *et al*., 2014b; Glatz *et al*., 2016) and 64.81 days for *S. calamistis* at 25°C (see table 2.2) (Khadioli *et al*., 2014b). This confers developmental advantage to *C. partellus* relative to *B. fusca* and *S. calamistis*. Moreover, basing on developmental temperatures (see table 2.4), *C. partellus* developmental stages can survive higher basal temperatures as compared to *B. fusca* and *S. calamistis*. However, its developmental stages cannot sustain low temperatures as compared to those of *B. fusca* and *S. calamistis* (Ntiri *et al*., 2016). With global temperatures expected to continue increasing, *C. partellus* is most likely to have a numerical advantage over other species ultimately out competing them. High basal high temperature tolerance and low basal low temperature may mean *C. partellus* can survive both thermal extremes through plasticity, since insects tend to trade-off basal low but not high temperature tolerance for plasticity (see discussions in Nyamukondiwa *et al*., 2011). Hence *C. partellus* may have a thermal tolerance advantage and may likely cope with the imminent climate change effects, relative to other stemborer species. Under future high temperature scenarios, *C. partellus* may expand further from most dry lowland areas to higher elevated areas in east and south eastern African countries (Kroschel *et al*., 2013) since temperatures are gradually increasing extending into high veld areas. It is most likely to expand to higher elevation in eastern Africa (Burundi, Ethiopia, Kenya, Uganda, Tanzania, Rwanda), and to mid elevation in southern Africa (Mozambique, Zambia, Zimbabwe, South Africa) (Khadioli *et al*., 2014a). This may likely increase the level of damage to cereal crops in these high elevation regions, given that *C. partellus* causes more injury than *B. fusca* on maize in some regions (Ong'amo *et al*., 2006). With southern Africa expected to experience a 10% rainfall decrease and more frequent droughts and east Africa expected to receive high rainfalls (Lobell *et al*., 2008; Stathers *et al*., 2013), *C. partellus* may probably enter long periods of diapause in southern Africa thereby surviving harsh climatic conditions. Nevertheless, under favorable conditions, *C. partellus* is known to terminate diapause quicker than the identified

indigenous species (Kfir, 1997; Ofomata *et al*., 1999b; Overholt *et al*., 2000). Laboratory experiments examining the displacement of stemborer *C. orichalcociliellus* showed that *C. partellus* had significantly higher fecundity compared to indigenous *C. orichalcociliellus* (Ofomata *et al*., 2000; Tamiru *et al*., 2012) hence may successfully outcompete the indigenous stemborer. I hypothesize that the expected global change, especially temperature increase and its variability may directly influence *C. partellus* geographical distribution range as well as pest status. Therefore, there is a potential of *C. partellus* invading and establishing in several new habitats through its dominance over other stemborer species (Kfir, 1997).

2.3.5 Physiological Tolerances in C. partellus

Insects generally encounter different stressful environmental extremes and usually survive through morphological and physiological changes that are mediated by a range of behavioral, hormonal, cellular, and biochemical mechanisms (Chown & Nicolson, 2004). Some insect pests may increase their invasion success in relation to their ability to deal with changing climates either through phenotypic plasticity or variation in basal tolerance (Nyamukondiwa *et al*., 2010; 2011; Chidawanyika *et al*., 2012), behavioral adaptation (over short time scales) e.g. habitat selection, basking intensity, restriction of activity periods and selective exploitation of environmental thermal fluxes (Yang *et al*., 2008), diapause and evolution in the longer timescales (reviewed by Bale, 2010). I hypothesize that, the invasion success of *C. partellus* may also stem from physiological adaptations that enable it to have competitive advantage over other indigenous stemborer species and survive in different habitats. The major physiological factors contributing to its invasion success and hence dominance may typically comprise either increased environmental tolerance and/or increased ability to compensate the short and long timescales through plasticity, especially to temperature and humidity. Following introduction to a new habitat, *C. partellus* may be able to thrive in the short term by having higher basal or inherent resistance to prevailing climate, or by mounting short term responses to these extremes thereby avoiding potentially lethal effects. Though not yet reported for *C. partellus*, this form of short-term phenotypic plasticity may be one important mechanism enhancing survival of this stemborer upon introduction to new habitats (reviewed in Whitman & Ananthakrishnan, 2009). Indeed, acclimation, a form of phenotypic plasticity, has been reported to be a crucial determinant of field fitness in insects (Kristensen *et al*., 2008) and may enhance survival of

'alien' species upon introduction to novel thermal habitats (Chown *et al*., 2007). To my knowledge, such physiological mechanisms have not yet been reported for *C. partellus* but I strongly hypothesise it is unlikely the species will invade, establish and be dominant without coping to environmental stressors through plasticity. Following establishment, over the long time-scales, rapid evolutionary adaptation, favouring the selection of novel phenotypes may also aid in the quick establishment and hence dominance of *C. partellus* relative to other stemborer species (Lee *et al*., 2007), unless genetic constraints play a significant role (Gilchrist & Lee 2007).

To determine whether *C. partellus* invasion success over other indigenous stemborer species is aided by basal and plasticity of thermal tolerance, I conducted standard rapid laboratory experiments (see Nyamukondiwa *et al*., 2010; Weldon *et al*., 2011). The initial colonies of *C. partellus*, *B. fusca* and *S. calamistis* were obtained from International Centre for Insect Physiology and Ecology (ICIPE), Kenya. The colonies have been in the laboratory for >20 generations with regular augmentation with wild populations to increase genetic diversity. These stemborers were optimally reared on artificial diet in trays for developing larvae following standardized protocols (Ochieng *et al*., 1985; Onyango & Ochieng'-Odero, 1994; Tefera *et al*., 2010). using 12L:12D photoperiod, 25 ± 1°C and 75 ± 5% relative humidity (RH) for *B. fusca* and 12L:12D, 28 ± 1°C and 75 ± 5% RH for *C. partellus* and *S. calamistis* in Memmert climate chambers (Memmert HPP 260, Memmert GmbH + Co.KG, Germany). Critical thermal limits (CTLs), that is CT_{min} and CT_{max} were measured for adult stemborers (*B. fusca*, *S. calamistis* and *C. partellus*) following rearing at optimum temperatures to test for basal physiological temperature tolerance (see materials and methods). In brief, critical thermal limits started at optimum temperature, ramping up (for CT_{max}) and down (for CT_{min}) at 0.25°C per minute until endpoint. The endpoint or CTLs were regarded as the temperature where insects lost ability to respond to gentle stimuli like mild prodding. Following this, I compared the plasticity of thermal tolerance across the three species using a species specific methodological approach (see e.g. Nyamukondiwa *et al*., 2011) (see materials and methods).

Materials and Methods

Study Animals and Rearing Conditions

Stemborers used in these experiments were obtained from the International Centre for Insect Physiology and Ecology (ICIPE), Kenya as pupae. The insects had been in culture for >150 generations with regular supplementation with wild caught stemborers to reduce inbreeding depression. Upon arrival in Palapye, Botswana, stemborers were reared on artificial diet (see Ochieng *et al*., 1985; Onyango & Ochieng'-Odero, 1994; Tefera *et al*., 2010) in Bugdorm cages ($32.5cm^3$) (Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan), placed in Memmert climate chambers (Memmert HPP 260, Memmert GmbH + Co.KG, Germany), at 25°C; 75% RH; 12L:12D for *B. fusca* and 28°C; 75% RH; 12L:12D photoperiod for *C. partellus* and *S. calamistis*. Upon eclosion, only 'lively' adults of mixed sex were used for thermal biology experiments, since sex seem to play no significant effect in thermal tolerance (see Nyamukondiwa & Terblanche, 2009).

Critical Thermal Limits (CTLs)

To determine temperature tolerance in *C. partellus*, *S. calamistis* and *B. fusca*, critical thermal limits were assessed using species specific methodological approach as outlined by Nyamukondiwa & Terblanche (2009). Adults (n =10) of *C. partellus*, *S. calamistis* and *B. fusca* were individually placed in insulated double-jacketed series of chambers ('organ pipes') connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for subzero temperatures (Chown & Nicholson, 2004). A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the control chamber to record chamber temperature. Both critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) experiments started at a set point temperature of 25°C for *B. fusca* and 28°C for *C. partellus* and *S. calamistis*. Once placed in the 'organ pipes', organisms were given 10 minutes to equilibrate at benign temperature before ramping up or down at a rate of 0.25°C/min until their CT_{max} or CT_{min} were recorded. This was repeated twice to yield sample sizes of n = 20. The CT_{max} and CT_{min} were defined as the temperature at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding) (e.g. Nyamukondiwa & Terblanche, 2009). In the case of CT_{max}, this loss of muscle

function coincided with death such that recovery was not possible, while in the case of CT_{min}, recovery occured.

Acclimation Effects on CTLs

To determine longer-term acclimation effects on thermal tolerance for *C. partellus*, *B. fusca* and *S. calamistis*, CTLs were measured on moths acclimated as adults to low, optimum or high temperature as outlined by Nyamukondiwa & Terblanche (2010b). Low temperature acclimation was defined for each species as 5°C less rearing/benign temperature while high temperature acclimation was defined as +5°C rearing temperature. Here, flies acclimated to optimum temperature were considered as a control group. Two day-old *C. partellus*, *B. fusca* and *S. calamistis* were acclimated for 3 days at (23, 28 and 33°C), (20, 25 and 30°C) and (23, 28 and 33°C) respectively. Acclimation for this duration is suffice to elicit some plastic responses in like invertebrates e.g. *Ceratitis capitata* (see Weldon *et al*., 2011). Ten individual moths from each acclimation were randomly loaded into the 'organ pipes' and were given 10 minutes to equilibrate at benign temperature before ramping up or down at a rate of 0.25°C/min until their CT_{max} or CT_{min} were measured. This was repeated twice to yield sample sizes of n = 20. The CT_{max} and CT_{min} were defined as the temperature at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding) (e.g. Nyamukondiwa & Terblanche, 2010b). Both basal thermal tolerance and acclimation/plasticity data were checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests, respectively, and in all cases assumptions were met. To determine the effect of species on CTLs, and acclimation effects on CTLs, results were analysed using one-way ANOVA and factorial ANOVA respectively followed by Tukey-Kramer's post-hoc tests to identify homogenous groups.

Basal CT_{max} results showed that all species differed significantly with respect to high temperature tolerance. *C. partellus* had significantly higher heat tolerance than *S. calamistis* and *B. fusca* ($F_{(2,177)}$ = 294.32, $p<0.0001$; Table 2.5). *Busseola fusca* and *S. calamistis* had significantly higher cold tolerance than *C. partellus* ($F_{(2,57)}$ = 235.38, $p<0.0001$; Table 2.5). However, there was no significant difference in cold tolerance between *S. calamistis* and *B. fusca* (Table 2.5). We found a significant species x acclimation temperature interaction for both CT_{min} and CT_{max} following low and high temperature acclimation (Table 2.6). Acclimation at high

temperature generally increased CT_{max} for *C. partellus* relative to *B. fusca* and *S. calamistis* (Figure 2.2A). However, acclimation at both low and high temperature compromised high temperature tolerance for both *C. partellus* and *S. calamistis* indicating some compromised potential for plasticity. Similarly, *C. partellus* also showed high plastic responses to low temperature tolerance compared to *S. calamistis* and *B. fusca* (Figure 2.2B). There was no significant difference across all acclimation treatments for *B. fusca* in respect of CT_{min}. Correspondingly, *S. calamistis* acclimated at low temperature performed worse off than the controls indicating some inability to compensate for the cold. Overall, this showed that *C. partellus* has a high basal temperature tolerance and plasticity thereof. In addition, its compromised basal low temperature tolerance may be a cost to its high plasticity to low temperature (Figure 2.2B). These results are in keeping with related research to date, indicating insects may trade off their basal low but not high temperature tolerance for plasticity (as in Nyamukondiwa *et al*., 2011). This may help explain that ecophysiology may aid the current observed invasion potential of *C. partellus* relative to *B. fusca* and *S. calamistis*.

Table 2.5: Results of basal thermal tolerance ±SD in three major stemborers, (*B. fusca*, *S. calamistis* and *C. partellus*) measured as (A) critical thermal maxima and (B) critical thermal minima. Means with the same superscript letter are not significantly different.

Stemborer species	Critical thermal maxima	Critical thermal minima
Busseola fusca	44.69 ± 0.78^{a}	1.47 ± 0.55^{a}
Sesamia calamistis	46.24 ± 1.21^{b}	1.78 ± 0.47^{a}
Chilo partellus	47.81 ± 0.45^{c}	4.83 ± 0.60^{b}

Table 2.6: Summary results from a factorial ANOVA showing the effects of species (*Chilo partellus*, *Busseola fusca* and *Sesamia calamistis*) and temperature acclimation (low, optimum and high) on critical thermal maxima and minima (CT_{max} and CT_{min}, respectively). SS = sums of squares, d.f.=degrees of freedom.

	Effect	**SS**	**DF**	**MS**	**F**	**P**
	Species	416.1	2	208	521,6	0.000
CT_{max}	Acclimation	26.9	2	13.5	33.8	0.000
	Species*Acclimation	30	4	7.5	18.8	<0.0001
	Species	286	2	143	674.3	0.000
CT_{min}	Acclimation	2	2	1	4.6	0.011
	Species*Acclimation	37.6	4	9.4	44.3	<0.0001

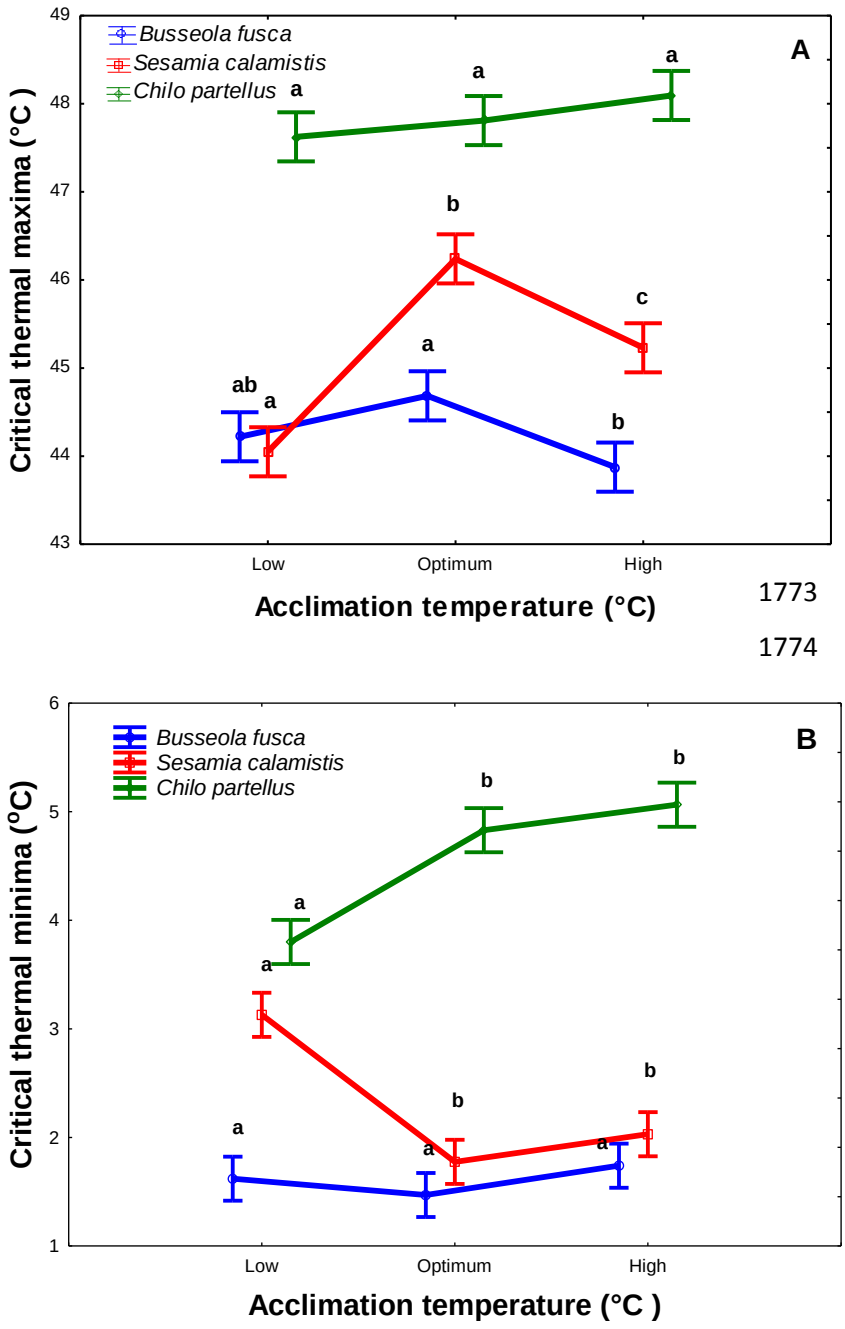


Figure 2.2: Results of plasticity of thermal tolerance in three major stemborers, (*B. fusca*, *S. calamistis* and *C. partellus*) measured as (A) critical thermal maxima and (B) critical thermal minima following acclimation for 3 days at low optimum and high temperature (23; 28 and 33°C respectively). Vertical bars denote ±95% CL. Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each species

Beneficial acclimation hypothesis states that acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not been exposed to that particular environment (Chown & Terblanche, 2007). Basing on this hypothesis, *C. partellus* may be migrating from the relatively warm low to middle altitudes to new, relatively cooler high altitude habitats. Since mean global temperatures are gradually increasing, causing a 'warming' in the rather cool middle and high altitude areas, *C. partellus* may have a performance advantage over other indigenous stemborer species through beneficial acclimation and hence may be fitter than the latter. Furthermore, *C. partellus* may have adopted behavioral mechanisms of coping with high temperature through its thermal history in low altitudes as has been reported in other insects (e.g. Nyamukondiwa *et al*., 2010; 2011). However, indigenous stemborers e.g. *S. calamistis* and *B. fusca* may not be able to sufficiently acclimatize to rapidly changing thermal conditions, hence may have performance and survival disadvantages in the field (see discussions in Khadioli *et al*., 2014b). However, little is known on the thermal requirements for the development of *C. partellus* life stages, which would be useful in the development of pest invasion risk maps (Khadioli *et al*., 2014a) such as those developed by Yonow *et al.* (2016).

2.3.6 Host range of C. partellus

Stemborers are among the phytophagous insects that occurred in wild hosts before domestication of sorghum and introduction of maize in Africa (Polaszek & Khan, 1998; Ristanovic, 2001). They have been associated with a wide range of wild hosts belonging to Poaceae, Cyperaceae and Typhaceae families for millions of years (Le Ru *et al*., 2006). However, their pest status has been elevated by the provision of abundant resources that occur in monocultural ecosystems in which maize and sorghum crops create continuous availability of resources (Overholt *et al*., 1997; Mwalusepo *et al*., 2015). Besides surviving in cultivated habitats, previous studies have also revealed that *C. partellus* can survive in wild grasses more than other indigenous stemborer species (Songa *et al*., 2002 see Ofomata *et al*., 2000). A survey that was conducted in Kenya, Tanzania and Uganda indicated that *B. fusca* was not common in wild hosts, showing its relative preference for cultivated hosts e.g. maize and sorghum crops (Le Ru *et al*., 2006). However, a related study revealed *C. partellus* as the most common borer species in wild host plants (Songa *et al*., 2002). This may be due, in part, to its high potential in

colonizing novel environments (Overholt *et al*., 1997; Kfir, 1997) and ability to utilize diverse range of hosts. It is therefore likely that *C. partellus* adjusts faster to the wild grass hosts and that it colonizes the suitable feeding niches in these habitats much earlier than the other stemborers, hence decreasing the number of other stemborer species that successfully colonize these grasses (Songa *et al*., 2002). In line with this study, the success of *C. partellus* is probably centered on switching from wild to cultivated hosts and *vice versa*. Furthermore, in areas where host plants are abundantly distributed and climate conducive for development, *C. partellus* is known to be active all year round (Khadioli *et al*., 2014a). Therefore, with its short life cycle, host plant switching/polyphagy coupled with quick diapause termination, it can rapidly increase its population thus ensuring numerical advantage over other indigenous stemborer species.

2.3.7 Asynchrony between C. partellus and its natural enemies

Changes in the synchronization of the host and its parasitoid through variations in their thermal preferences, or by changes in the geographical distribution of either species can disrupt the equilibrium between hosts and parasitoids (Hance *et al*., 2007). If a parasitoid species has a lower base temperature than its host, the parasitoid could emerge earlier in the seasonal development of its host. When the parasitoids emerge early, host stemborer population exposed to adult parasitoids is considerably reduced. Mwalusepo *et al*. (2015) predicted future asynchrony between *C. partellus* and its larval parasitoid *Cotesia flavipes* Cameron, with efficacy expected to be better at low than high altitude. Therefore, with *C. partellus* gradually expanding to moist-mid and high altitude areas, there are high chances of disruption of the biological control due to a mismatch between the host and its main larval parasitoid *C. flavipes*. This implies that *C. partellus* may be passing through vulnerable life stages more quickly at higher temperatures, reducing the window of opportunity for parasitism which may give great set back to the survival and multiplication of *C. flavipes*. Future failure of biological control may thus explain the reasons for its population increase and displacement of other indigenous stemborer species. Mailafiya *et al*. (2010) showed that the distribution of *C. flavipes* and *Cotesia sesamiae* Cameron was influenced by geographic range of their respective stemborer hosts. Considering the predicted expansion of *C. partellus* to moist mid-altitude and moist high altitude areas, *C. flavipes* is also expected to shift to these areas. However, predicted climate change indicate 10% rainfall decrease and more frequent droughts in southern Africa (Stathers *et al*.

2013) and a reduction in rainfall during the long rainy season in east Africa (Mwalusepo *et al*., 2015). These dry periods may significantly affect *Cotesia* species (Mwalusepo *et al*., 2015) leading to a disruption in biological control and ultimately promoting a sudden increase in *C. partellus* populations which may eventually displace other indigenous stemborer species, whose biological control may not be compromised as such. The two major parasitoid species of *C. partellus*, *C. flavipes* and *C. sesamiae* are known to have a short generation time (18 days at 25–30˚C) (Mochiah *et al*., 2001) compared to that of their stemborer hosts (30-58 days at 25–30°C) (Polaszek, 1998). These parasitoids' high reproductive rates with a female biased sex ratio (Omwega & Overholt, 1997) demonstrate a high potential for stemborer pest regulation. However, *Cotesia* species lack the ability to locate and parasitize diapause larvae in dried maize stems (Overholt *et al*., 1997). Since the warming of African continent is gradually increasing, synchronization between *C. partellus* and these parasitoids may be affected. This implies that the occurrence of extreme temperatures or higher fluctuations in temperature may strongly affect the host-parasitoid population dynamics through altering the capacity of parasitoids to locate hosts and host habitats. *Chilo partellus* might be escaping parasitism due to asynchrony leading to increase in the number of generations as well as expansion to other regions. Increase in population is known to create intraspecific competition amongst the host species and this may facilitate the migration of *C. partellus* to other regions consequently displacing other indigenous stemborer species.

2.4 Future Directions and Conclusions

Global warming and climate change may have negative consequences on diversity and abundance of stemborers hence increasing the extent of crop losses that will impact negatively profitability of crop production, food security and livelihoods (Gregory *et al*., 2009; Kumar, 2016; Yonow *et al*., 2016). Through a number of inter-related processes, including range extensions and phenological changes, as well as increased rates of population development, growth, migration, and overwintering (Hance *et al*., 2007), most studies have concluded that insect pests generally become more abundant as temperature increases (Cannon, 1998; Yamamura & Kiritani, 1998; Lin, 2011). Few studies have included parasitoids in their models and the effect of these parasitoids in shaping the insect pest. In moist tropical forests, parasitoids may suffer more than their hosts from a decrease in precipitation, which will then cause an

increase in the host population because of a reduced level of parasitism (Coley, 1998; Hance *et al*., 2007).

Understanding stemborers and their parasitoids population dynamics under variable climate conditions is essential for predicting seasonal phenology and accurate forecasting of moth abundance which has downstream impacts on basic applications of any pest management program. Indeed, dramatic changes in high and low temperature tolerance can occur between life-stages of various insect species (Bowler & Terblanche, 2008). Adaptations to extreme temperatures are often different among stemborers and parasitoids developmental stages (Hance *et al*., 2007), but this has not been investigated. Adverse effects of climate change on the activity and effectiveness of stemborer/natural enemy systems need to be considered in future pest management programs. This involves understanding physiological tolerances of these stemborers and their natural enemies in order to devise methods for management. Model predictions indicate a potential increase in the number of spotted stemborer generations in the year 2050, which indicates that higher insect pest infestations and yield losses may occur (Kroschel *et al*., 2013). Most bioclimatic models to date were purely based on temperature and could not take into consideration other climatic factors such as rainfall and relative humidity and, non-climatic factors such as planting patterns and frequency, diapause or other physiological factors that enable insects to cope with climate changes in the short and long timescales e.g. plasticity (see Khadioli *et al*., 2014a; Mwalusepo *et al*., 2015). Despite overwhelming evidence that species are evolving with global change (Bradshaw & Holzapfel, 2006), to my knowledge, no models to date, have incorporated the ability of stemborers to evolve. I suggest future predictions of climate change effects on insect abundance should incorporate evolutionary capacity (e.g. Kearney *et al*., 2009), since it's one of the ways insects use to cope to environmental variation, upon introduction to novel habitats.

Therefore there is need to:

- Predict and map trends of potential changes in geographical distribution, and study how climatic changes will affect development, incidence, performance and population dynamics of stemborer species and associated natural enemies. Furthermore, when predicting direct effects of climate change, on insect hosts, natural enemies and their interactions, species genotypic and phenotypic plasticity should be considered (e.g. Thomson *et al*., 2010).

- It is a major challenge predicting future impacts of climate variability without a comprehensive understanding of the species' thermal tolerance and performance under current climatic conditions. For many insect species, including some economic pests, this information is still lacking. An understanding of stemborers' thermal biology mainly focusing on basal thermal tolerance and plasticity thereof may thus provide valuable information for predictive models for forecasting economic insect pest outbreaks, conducting pest risk assessments, establishing invasive species, biogeographical shifts, and the potential implications of global climate change.
- Evidence from diverse insect taxa suggests that, in a new habitat, species may often undergo rapid and observable evolutionary change (Gilchrist & Lee, 2007). This may mean that genetic heterogeneity in phenotypic traits may be inherently present amongst early invader and propagule generations, following colonization for adaptive evolution to occur in response to novel habitats. In addition, phenotypic plasticity, and particularly cross-generational developmental plasticity may be playing a key role in producing phenotypes capable of competing favorably with native species. With *C. partellus* quickly evolving to new habitats, thriving and dominating other stemborer species, there is need to understand its selection response and genetic variation as well as factors that have facilitated it to evolve and outcompete other indigenous stemborer species.

The invasive stemborer, *C. partellus* is a highly competitive colonizer and with Africa's changing climate, it is most likely to expand and establish in novel habitats. In addition to other biophysical factors likely aiding its abundance, knowledge of thermal tolerance, plasticity and evolutionary potential for *C. partellus* and its larval parasitoid is vital to the understanding of how they may react to potential climate change with implications for the prediction of pest outbreaks. Furthermore, a careful analysis on how stemborers-parasitoids systems react to changes in temperature and relative humidity is needed to assist prediction models for pest management under global change. In ecosystems, the tritrophic interactions between plants, herbivorous insects, and their natural enemies (predators, parasitoids, and pathogens) are affected by climatic changes in diverse ways. Therefore, research should also focus on tritrophic interactions rather than host-parasitoid level dynamics only. The invasiveness of *C. partellus* and its geographic spread are very significant to crop protection. Therefore, a comprehensive total ecology of this pest and other indigenous stemborers should also be studied.

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CHAPTER 3

Comparative assessment of the thermal tolerance of spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and its larval parasitoid, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) *

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3.1 Introduction

Most insect species are ectotherms, having body temperature always in equilibrium with environmental temperature (Chown & Nicolson, 2004). Moreover, most physiological and biochemical processes are maximized at thermal optima and outside this range, insect performance and fitness may be reduced (Martin & Huey, 2008; Angilletta, 2009). At extreme temperatures, the rate of acquisition and consumption of resources is significantly affected, thereby impacting on growth, development, reproduction and mortality (Chown & Nicolson, 2004; Angilletta, 2009). Abiotic factors such as temperature and humidity can affect survival and fitness traits and, over longer timescales, may affect seasonal phenology and population dynamics (Wallner, 1987; Selvaraj *et al*., 2013). Insects consequently employ various physiological adjustments to enhance fitness and survival rates in stressful environments.

Societal concern on the impacts of anthropogenic climate change on biodiversity increases the need to understand how organisms respond to thermal variability, including their potential for geographic range expansion and invasion (Parmesan *et al*., 1999; Andrew, 2013; Andrew & Terblanche, 2013; Karsten *et al*., 2015). Temperature is one key factor that is highly labile under changing climates (IPCC, 2014), and in all likelihood, will result in population shifts in geographic distribution and seasonal phenology of insects over time (Parmesan *et al*., 1999; Mwalusepo *et al*., 2015; Sinclair *et al*., 2015). Under acute adverse thermal exposure, insects employ a range of plastic responses including behavioural avoidance of extreme environments by changes in periods of activity within a day or mere shifts in feeding positions on a plant (Kührt *et al*., 2006; Huey *et al*., 2002; Dillon *et al*., 2009). Other forms of plasticity involve physiological adjustments that are chiefly induced by sub-lethal temperatures (Overgaard & Sorenson, 2008; Chidawanyika & Terblanche, 2011) and sometimes other forms of stress such as starvation (Bubliy *et al*., 2012) and desiccation (Gray & Bradley, 2005; Bazinet *et al*., 2010). However, in the event that a species fails to mount any compensatory response under persistent stressful environments, extinction may likely occur (Chown & Terblanche, 2007). The determination of thermal tolerance is therefore a significant step in understanding the ways in which environmental variation may affect the population dynamics and fitness of an insect species (Ma *et al*., 2014), and its potential establishment in new environments (Nyamukondiwa *et al*., 2010; Buckley & Huey 2016). The thermal tolerance traits commonly measured in ectotherms vary and may include both lethal and sub-lethal tests (see Sinclair *et al*., 2015).

Lethal tests that result in the death of the insects include lethal temperature determination, time to death at set temperatures and supercooling points, dependent on insect freezing strategy (Formby *et al*., 2013; Alford *et al*., 2016). Sub-lethal tests include determination of critical thermal limits (CTLs) (i.e. the onset of extreme conditions, beyond which an insect's activity is compromised), time to heat knock-down and/or recovery after chill coma (Andersen *et al*., 2015; Alford *et al*., 2016). CTLs are considered more ecologically relevant as temperature can be raised or cooled using rates that mimic field conditions (Rezende *et al*., 2011; Alford *et al*., 2016; Verberk *et al*., 2016).

The thermal tolerance of insects is not static. Instead, it is plastic and depends on a range of factors. For example, a prolonged prior exposure to sub-lethal thermal environments commonly referred to as acclimation, can improve performance in otherwise lethal or stressful conditions (Kristensen *et al*., 2008; Nyamukondiwa & Terblanche, 2010; Chidawanyika & Terblanche, 2011; Andrew *et al*., 2013). Under even more acute prior exposure (hardening), similar plastic responses that result in improved survival can also be induced (Yi *et al*., 2007). The rate of heating or cooling has also been shown to influence thermal tolerance. In general, faster rates of temperature change result in poor thermal tolerance due to the limited period for physiological adjustments by insects (Nyamukondiwa & Terblanche, 2010; Chidawanyika & Terblanche 2011). Other studies have also shown population differences in insect thermal tolerance to be influenced by ontogeny (Klockmann *et al*., 2016) and body size (Cavieres *et al*., 2016).

Across species, temperature tolerance may differ, depending on the environments in which they have evolved (Chown & Terblanche, 2007). Even when the species have evolved the same adaptations to the environment, within generation variations in thermal tolerance may occur and in all likelihood shape geographic distributions (reviewed in Sgrò *et al*., 2016). At much finer geographic scales, changes in fitness among interacting species may disrupt co-evolved interactions, be they mutual or antagonistic (Parmesan, 2006). As the climate changes, some species may suffer detrimental effects, while others may emerge as 'winners', at least in the short term. For antagonistic relationships such as host-parasitoid interactions in agriculture, this is of concern as pest abundance may rise due to reductions in parasitoid abundance. Understanding the thermal tolerance of pest-parasitoid complexes is therefore important in order

to understand and better predict agricultural pest pressure under changing climates (Kleynhans *et al*., 2014).

The spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most important insect pests of cereal crops in eastern and southern Africa (Khadioli *et al*., 2014). It was accidentally introduced to Africa (Malawi) from Asia (Tams, 1932). Its range has expanded to successfully establish in high elevation areas, highland tropical areas and humid transitional regions (Khadioli *et al*., 2014) and threatens to displace indigenous stemborer species (Kfir, 1997). *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) which is native to Africa, is a gregarious larval endoparasitoid that attacks a wide range of stemborer hosts, which include the noctuids *Busseola fusca* Fuller and *Sesamia calamistis* Hampson, and crambids *Chilo orichalcocilielus* Strand, and *C. partellus* (Branca *et al*., 2011). Like other insects, the distribution of *C. sesamiae* is inevitably influenced by changes in climate (Mwalusepo *et al*., 2015). For most studies on thermal tolerance of arthropods of agricultural importance, investigators mainly focus on the insect pests, without considering their natural enemies (e.g. Koveos, 2001; Kalosaka *et al*., 2009; Nyamukondiwa & Terblanche, 2009; Chidawanyika & Terblanche, 2011; Chanthy *et al*., 2012). Determining the thermal biology of *C. partellus* and its endoparasitoid, *C. sesamiae*, is therefore an important step in documenting the potential impacts of a changing climate on host-parasitoid population dynamics. Here, I investigate the thermal tolerance of *C. partellus* and its parasitoid, *C. sesamiae*, and present preliminary host-natural enemy comparisons. To date, thermal tolerance comparisons between insect pests and their biocontrol agents are generally lacking. I hypothesize that thermal tolerance of *C. partellus* and *C. sesamiae* were in synchrony and that this host host-parasitoid interaction may evolve together with thermal variability. Here, I investigated the thermal tolerance of different developmental stages of *C. partellus* and *C. sesamiae* by determining ranges of temperature-time interactions in terms of lower and upper lethal temperatures, critical limits to activity, supercooling points, chill-coma recovery time and heat knock-down time. Information on all these is important in developing pest risk assessments, designing pest management programs and mass-rearing natural enemies for applied biological control programs.

3.2 Materials and Methods

3.2.1 Insect Culture

The initial colonies of *C. partellus* and *C. sesamiae* were obtained from the International Centre for Insect Physiology and Ecology (ICIPE), Kenya and South African Sugar Research Institute (SASRI), South Africa, respectively. *Chilo partellus* was reared on artificial diet (Ochieng *et al*., 1985; Tefera *et al*., 2010), under 12L:12D photoperiod, 28 ± 1°C and 65 ± 10% relative humidity (RH) in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany). Pupae were maintained in open Petri dishes in Bugdorm rearing cages (240mm^3; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under the same rearing conditions as that of larvae until adult eclosion. Upon emergence, adults had access to water-soaked cotton wool. Wax paper was placed in the cages as an oviposition surface for gravid females. To maintain uniformity among test insects, eggs were harvested after every 12 h, and transferred from wax paper to artificial diet and allowed to hatch into larvae, subsequently molting five times before pupation. Pupae were harvested every 24 h and placed in rearing cages for eclosion. Once every month during summer, wild-caught moths were added to the colony to promote heterozygosity among traits of interest (e.g. Nyamukondiwa & Terblanche, 2009). For the *C. sesamiae* colony, parasitised *S. calamistis* from SASRI were maintained in the climate chambers under 12L:12D photoperiod, 28 ± 1°C and 65 ± 10% RH until parasitoid emergence. Emerged parasitoids had access to food *ad libitum* (25% honey water from a cotton wick) until they were used in thermal tolerance assays as 24–48-h-old adults.

3.2.2 Upper and Lower Lethal Temperature Assays

Upper and lower lethal temperatures (ULT and LLT) were assayed as outlined by Chidawanyika & Terblanche (2011), using a direct plunge protocol (e.g. Terblanche *et al*., 2008) in programmable water baths (Systronix, Scientific Engineering (Pty) Ltd, South Africa). ULT assays ranged 37-48°C and 41-49°C for *C. partellus* eggs and larvae respectively (for durations 0.5; 1; 2 and 4 h) while that for *C. sesamiae* adults ranged 36-39°C (for 2 h duration) until 0-100% mortality was determined. A mixture of propylene glycol and water (1:1 ratio) was used to enable water baths to operate at sub-zero temperatures without freezing. Age was strictly

controlled in all assays as it has been shown to significantly affect thermal tolerance (Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009; Mudavanhu *et al*., 2014). Ten *C. partellus* eggs (black head stage) or larvae (6th instar), as well as *C. sesamiae* adults (24–48 h old), were placed in 60 ml polypropylene vials (n = 50) and placed in the water bath for each temperature/time treatment. Relative humidity in the ULT experiments was maintained using a wet cotton wick in the vials to avoid desiccation-related mortality. Water bath temperatures were verified using digital thermometers (Fluke 53/54IIB, Fluke Cooperation, USA) for the duration of each treatment. Following ULT treatment, vials containing the assayed *C. partellus* eggs, larvae and *C. sesamiae* adults were placed in a climate chamber (28 ± 1°C, 65% ± 10% RH and 12:12 L:D photoperiod) until survival was recorded. *Chilo partellus* larvae and *C. sesamiae* adults had access to food during the 'recovery' period. For the purposes of this study, survival was defined as hatching success (for eggs) and ability to pupate (for larvae). For adults, a coordinated muscle response to stimuli such as gentle prodding, or normal behaviors such as feeding, flying or mating (24 h post treatment) symbolized survival. For LLT assays, the same plunge protocols were followed, as ULTs, with low temperature x time treatment interactions, before recording survival. Assays ranged -9 to 6°C and -14 to -2°C for *C. partellus* eggs and larvae respectively (for durations 0.5; 1; 2 and 4 h) while that for *C. sesamiae* adults ranged -1 to 4°C (for 2 h duration) until 0-100% mortality was determined.

To determine the ecological relevance of LLTs and ULTs for *C partellus* and *C. sesamiae* recorded above, microclimatic temperatures were taken from a cereal field at Veggies For All Farm, Glen Valley, Gaborone, Botswana (S24.61224; E25.97326; 977 m.a.s.l) where both host and parasitoid are present.

3.2.3 Effect of Ramping rate on Critical Thermal Limits (CTLs)

To assess whether heating or cooling rates significantly affected thermal tolerance in *C. partellus* and *C. sesamiae*, CTLs [critical thermal maximum, (CT_{max}) and critical thermal minimum, (CT_{min})] were assessed following standardized protocols (see Nyamukondiwa & Terblanche, 2010; Mudavanhu *et al*., 2014). Only *C. partellus* larvae (6th instar) and adult moths (24-48 h old), and *C. sesamiae* adults (24-48 h old) were used in the experiments. Ten individuals for each species and life-stage were placed in a series of insulated double-jacketed chambers ('organ pipes') connected to a programmable water bath (Lauda Eco Gold, Lauda

DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for subzero temperatures (Chown & Nicholson, 2004) and subjected to different constant heating or cooling rates. In all cases, assays started at a set point 28°C (equivalent to the rearing temperature) for 10 mins (to allow for insects temperature equilibration) before ramping the temperature up (CT_{max}) or down (CT_{min}) using 3 independent rates (0.12, 0.25 and 0.5°C min^{-1}). This was repeated twice to yield sample sizes of n = 20 per ramping rate treatment (20 replications) for each life-stage and species. A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into a control chamber to record chamber temperature. It was assumed that individual organism body temperature was in equilibrium with the chamber temperature under the experimental conditions employed, as has been observed for other similar insect species (Terblanche *et al*., 2007a; Mudavanhu *et al*., 2014). CTLs were defined as the temperature at which each individual insect lost coordinated muscle function which was regarded as a lack of response to mild prodding (e.g. Nyamukondiwa & Terblanche, 2010).

3.2.4 Supercooling points (SCPs)

The supercooling points of three *C. partellus* life-stages stages, larvae (6th instar), pupae and adults (24-48 h old) were assayed as outlined by Nyamukondiwa *et al*. (2013). To determine SCPs, sixteen individuals of each developmental stage were individually loaded into 0.65 ml microcentrifuge tubes. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762-1121, Cambridge, UK), inserted through the lid of the tube and both the insect and thermocouple were held in place using cotton wool. Thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperatures were recorded at 1s intervals using PicoLog software for windows (Pico Technology, Cambridge, UK). Experiments began by holding individual insects at 15°C for 10 mins (to allow equilibration of insect body temperatures) before ramping down at 0.5°C min^{-1} until SCPs were recorded. SCP for each individual was determined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (Nyamukondiwa *et al*., 2013).

3.2.5 Heat knock-down time (HKDT) and Chill-coma recovery time (CCRT)

HKDT and CCRT were assayed on *C. partellus* larvae (6th instar) and adults (24-48 h old) as outlined in Weldon *et al*. (2011). For HKDT, 10 replicate organisms were individually placed in 0.65 ml microcentrifuge tubes and were placed in a climate chamber connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer. The organisms were then exposed to a test temperature of 49.0±0.3°C and 65% RH in the climate chamber. This knockdown temperature (49.0°C) was chosen based on preliminary investigations of upper critical temperatures to activity, which ranged 47.88±0.76°C for adults and 48.5±0.74°C for larvae. This was repeated three times to yield sample sizes of n = 30 for each developmental stage. All observations were recorded from the climate chamber video recording. HKDT was defined as the time (in minutes) at which activity was lost by the organisms following exposure to 49.0°C in a climate chamber. For CCRT, 10 replicate insects (6th instar larvae) and (24-48 h old moths) were individually placed in 0.65 ml microcentrifuge tubes and then loaded into a large zip-lock bag which was submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol set at 0°C for 1 hour. This temperature x time treatment has been shown to elicit chill-coma in other insect taxa (see Weldon *et al*., 2011; Sinclair *et al*., 2015). Following 1 h at chill-coma temperature, the tubes were removed from the water bath and immediately placed in a climate chamber (Memmert HPP 260, Memmert GmbH + Co.KG, Germany) set at 28°C, 65% RH for recovery. The chamber was connected to a camera (as in HKDT) that was linked to a computer where observations of CCRT were recorded. This was also repeated three times to yield sample sizes of n = 30 per treatment. CCRT was defined as the time (in minutes) required to regain coordinated movement ability (in larva) to stand upright on its legs (for adult moths) (Milton & Partridge, 2008).

3.2.6 Statistical analyses

Data analyses were carried out in STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0 (R Development Core Team, 2016). Lethal temperature assays (upper and lower lethal limits), SCPs, HKDT and CCRT were analysed using generalized linear models (GLZ) assuming a binomial (for lethal temperature assays) and Gaussian (SCPs, HKDT and CCRT) distribution and a logit link function in R statistical software. CTLs met the linear

model assumptions of constant variance and normal errors, so the effects of ramping rate on CTLs were analysed using one-way ANOVA in STATISTICA. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups.

3.3 Results

3.3.1 Upper and Lower Lethal Temperature Assays

An increase in the severity of temperature exposure at both low and high temperatures resulted in increased egg (Table 3.1; Figure 3.1) and larval (Table 3.2; Figure 3.1) mortalities. In addition, an increase in duration of exposure at any lethal temperature resulted in increased mortality of the eggs (Figure 3.1A; 3.1D) and larvae (Figure 3.1B; 3.1D). The interaction of temperature and duration of exposure for *C. partellus* eggs was highly significant at both low and high temperature (Table 3.1). Similarly, temperature by duration of exposure interaction effects for C. *partellus* larvae were highly significant for LLT and marginally non-significant for ULT (Table 3.2).

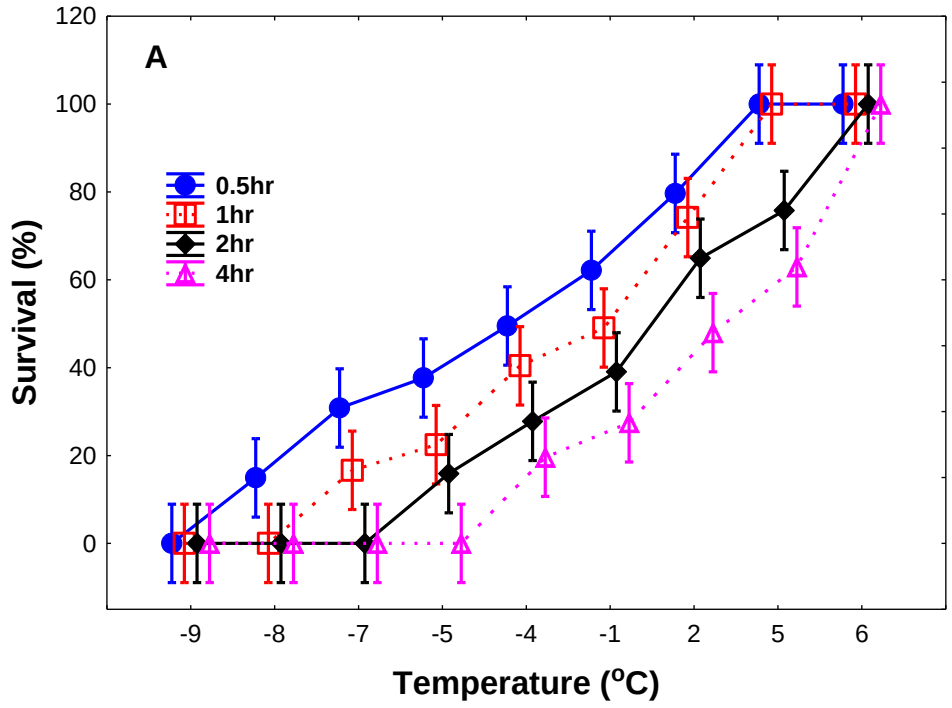
Temperature significantly affected the survival of *C. sesamiae* adults at both low and high temperature (LLT: χ^2 =180.05, d.f =4, $P < 0.0001$; ULT: χ^2 =162.78, d.f =3, $P < 0.0001$). An increase in severity and duration of both low and high thermal exposure generally resulted in increased *C. sesamiae* adult mortality (Figure 3.2). By comparison of the 2 h stress exposure, host *C. partellus* LLTs varied from -9 to 3°C (Figure 3.1A; B) while ULTs ranged 37 to 46°C (Figure 3.1C; D), compared with *C. sesamiae* LLT range of -1 to 4°C (Figure 3.2A) and ULT range of 36 to 39°C (Figure 3.2B).

Table 3.1: Summary statistical results of the effects of temperature, duration of exposure and their interactions on the survival of *Chilo partellus* eggs following lower and upper lethal temperature treatments. Analysis were done using generalized linear models (GLZ) assuming binomial distribution with a logit link function in R version 3.3.0.

Parameter	**d.f**	χ^2	**P-Value**
Lower lethal temperature			
Duration	3	154.57	<0.0001
Temperature	8	1348.59	<0.0001
Temperature * Duration	24	74.27	<0.0001
Upper lethal temperature			
Duration	3	143.79	<0.0001
Temperature	7	1252.86	<0.0001
Temperature * Duration	21	58.69	<0.0001

Table 3.2: Summary statistical results of the effects of temperature, duration of exposure and their interactions on the survival of *Chilo partellus* larvae following lower and upper lethal temperature treatments. Analysis were done using generalized linear models (GLZ) assuming binomial distribution with a logit link function in R version 3.3.0.

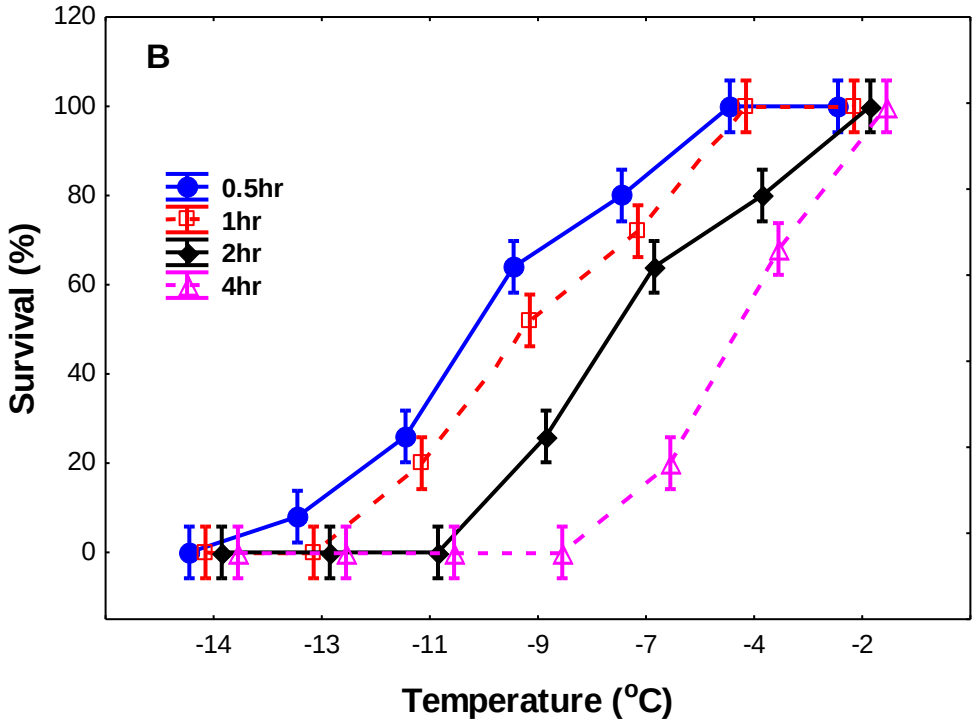
Parameter	**d.f**	χ^2	**P-Value**
Lower lethal temperature			
Duration	3	169.16	<0.0001
Temperature	6	1143.82	<0.0001
Temperature * Duration	18	33.73	0.0135
Upper lethal temperature			
Duration	3	361.18	<0.001
Temperature	6	1217.68	<0.001
Temperature * Duration	18	28.10	0.0605



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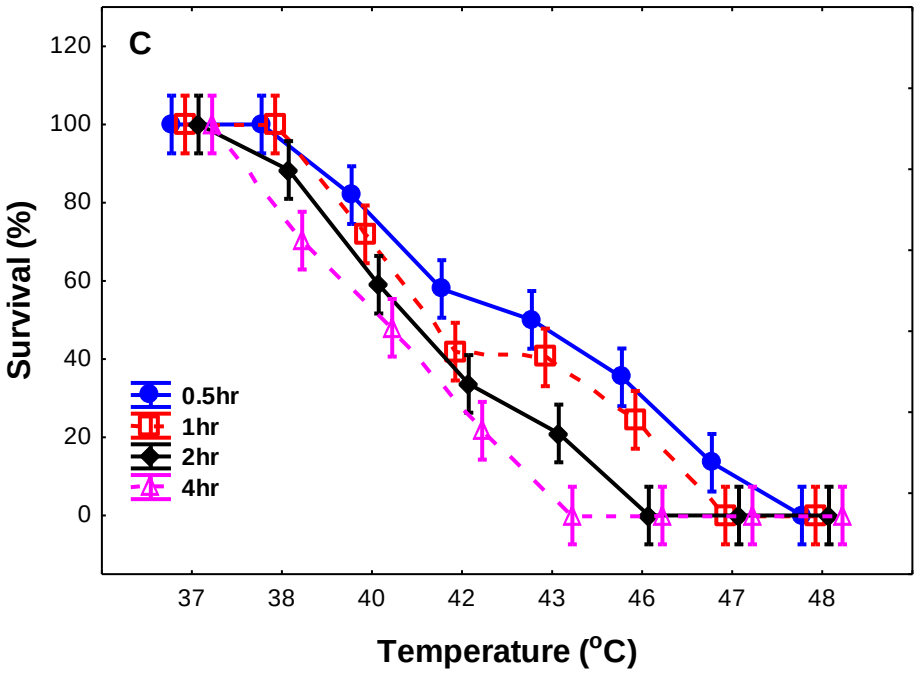
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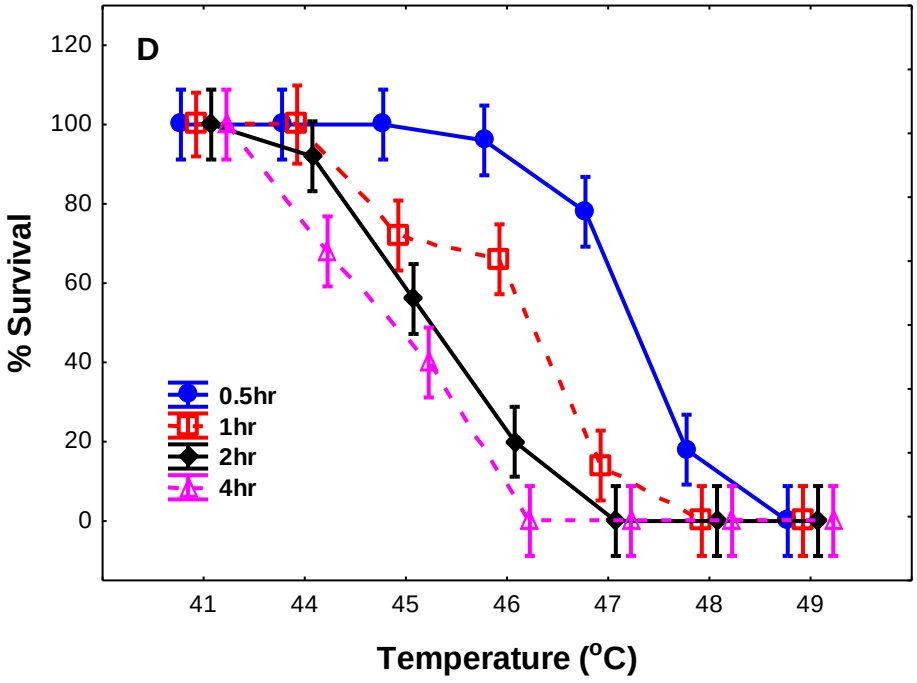


Figure 3.1: Mean (±95% confidence interval) survival of *Chilo partellus* (A) eggs and (B) larvae at different low temperatures, and (C) eggs and (D) larvae at different high temperatures each applied over four different exposure times

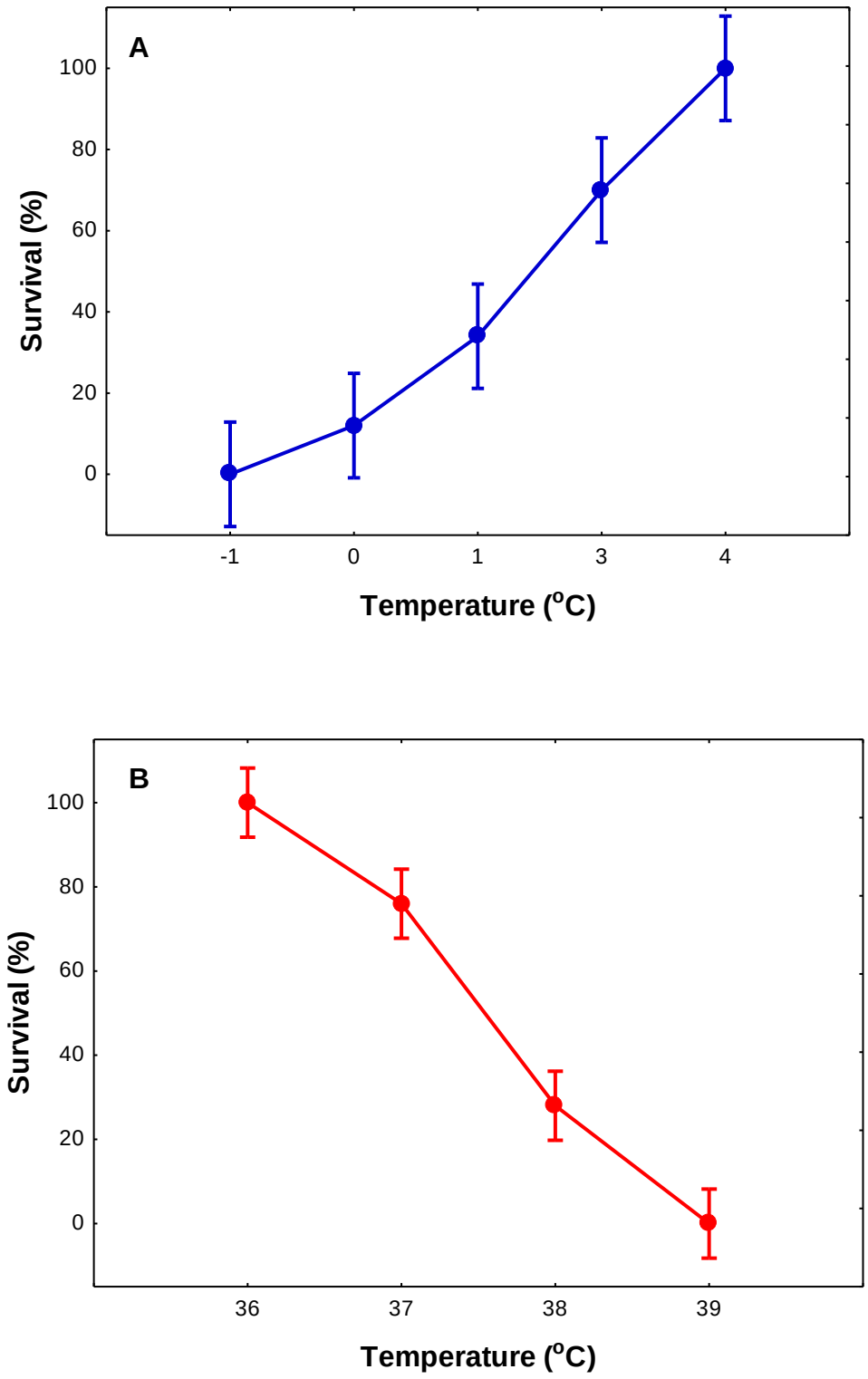


Figure 3.2: Mean (±95% confidence interval) survival of *Cotesia sesamiae* adults at different low (A) and high (B) temperatures applied for two hours.

3.3.2 Effect of Ramping rate on Critical Thermal Limits (CTLs)

Ramping rate significantly affected CT_{min} in *C. partellus* adults ($F_{2.57}$ =45.79, $P < 0.0001$) (Figure 3.3B), larvae ($F_{2.57}$ =95.72, $P < 0.0001$) (Figure 3.3D) and *C. sesamiae* adults ($F_{2.57}$ =232.81, $P < 0.0001$) (Figure 3.3F). Faster cooling rates compromised adult *C. partellus* and *C. sesamiae* CT_{min} (Figure 3.3A; F) but enhanced CT_{min} in *C. partellus* larvae (Figure 3.3D). Similarly, ramping rate also affected CT_{max}. Faster ramping rates significantly improved CT_{max} in *C. partellus* adults ($F_{2.57}$ =61.7, $P < 0.0001$) (Figure 3.3A) and larvae ($F_{2.57}$ =59.73 $P < 0.0001$) (Figure 3.3C), and adult *C. sesamiae* ($F_{2.57}$ =533.04, $P < 0.0001$) (Figure 3.3E).

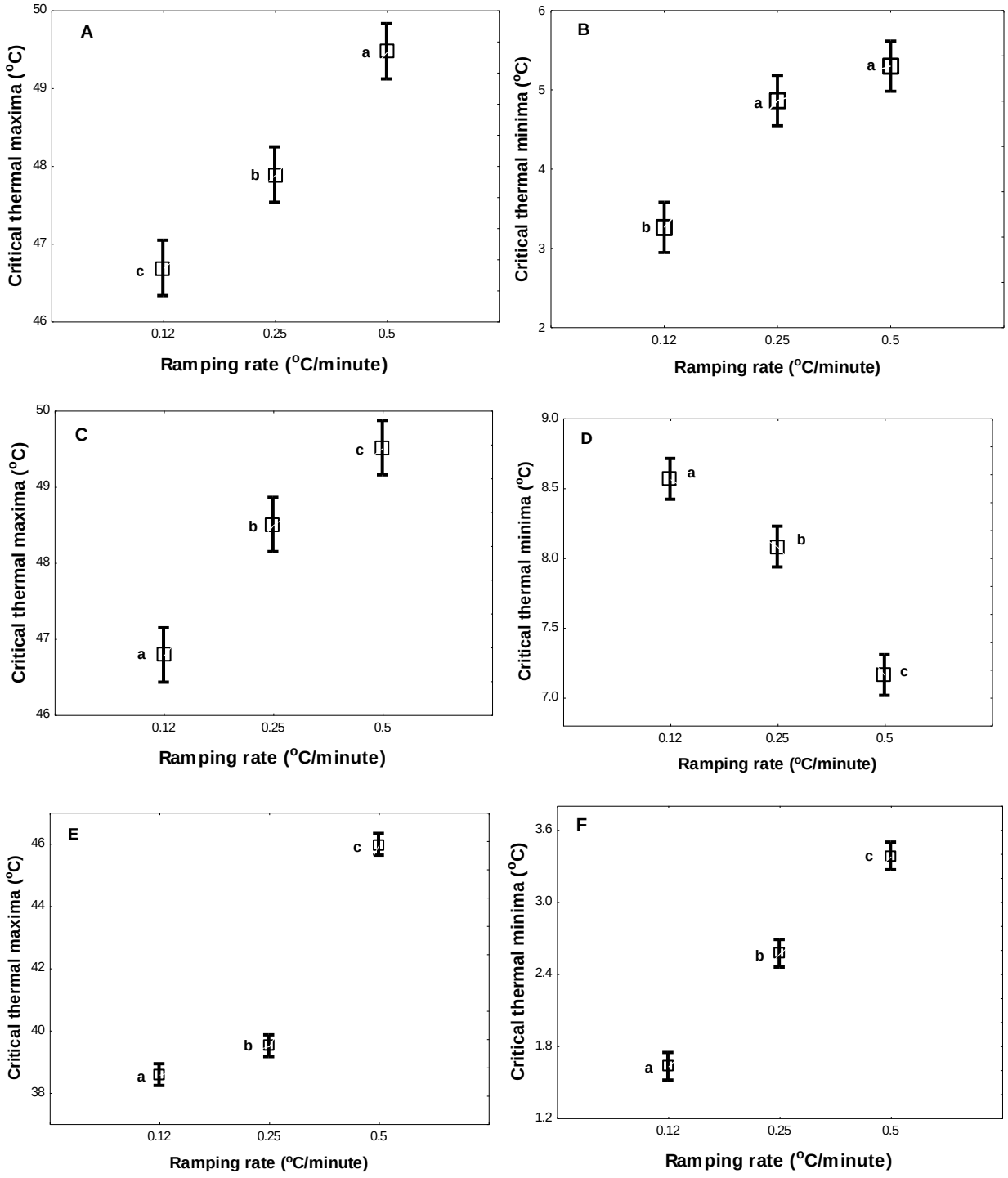


Figure 3.3: Effects of experimental heating and cooling rates on *Chilo partellus* adult (A) CT_{max} and (B) CT_{min}, larval (C) CT_{max} and (D) $CT_{min,}$ and *Cotesia sesamiae* adult (E) CT_{max} and (F) $CT_{min.}$ (mean± 95% confidence limits). *n* = 20 per group. Means with the same letter are not significantly different from each other.

3.3.3 Supercooling Points (SCPs)

The mean supercooling points for larvae, pupae and adults were -11.82±1.78, -10.43±1.73 and -15.75±2.47 respectively. There was no significant difference in *C. partellus* SCPs for larvae and pupae (χ^2 =59.34, d.f =2, $P > 0.05$) (Figure 3.4) However, adult *C. partellus* had significantly enhanced supercooling points compared with its larvae and pupae (χ^2 =59.34, d.f =2, $P < 0.0001$) (Figure 3.4).

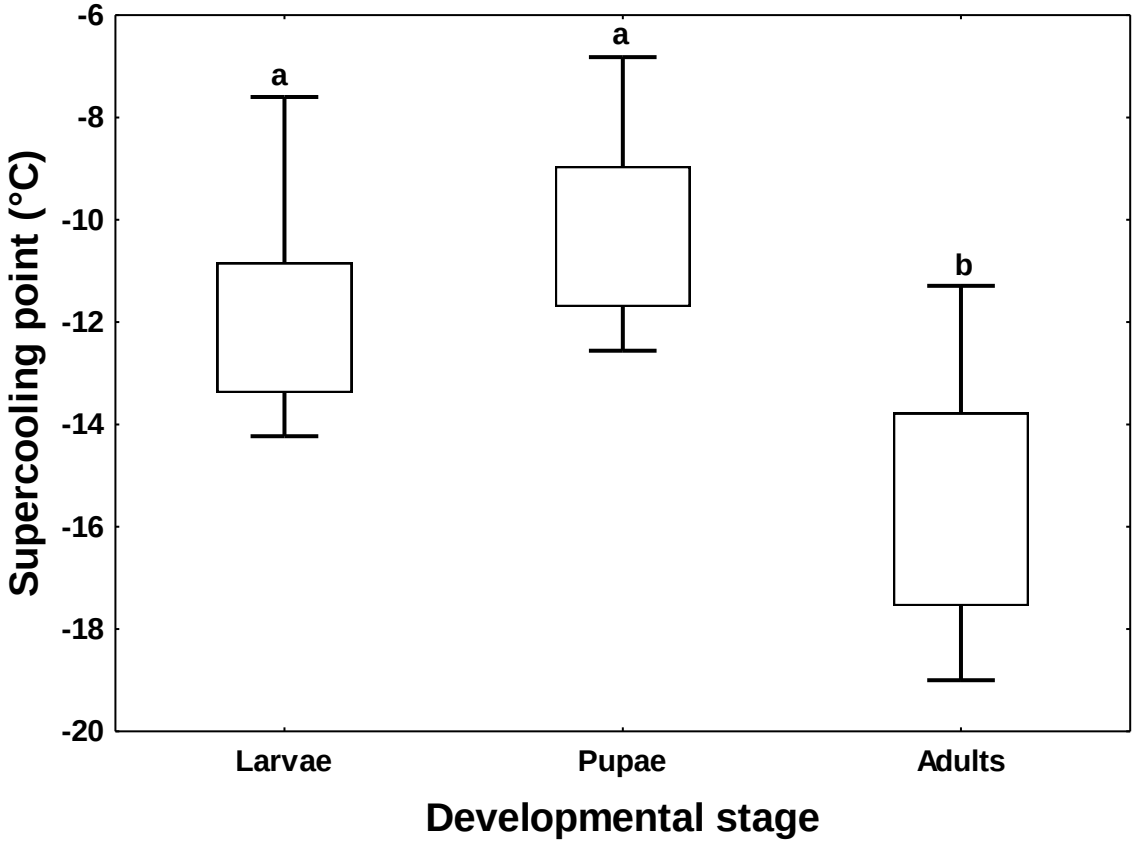
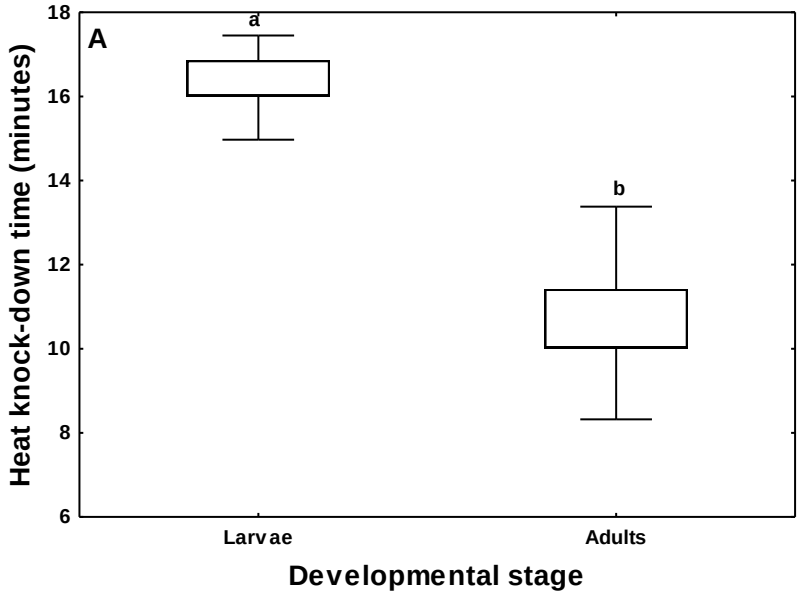


Figure 3.4: The effect of developmental stage on supercooling points in *Chilo partellus* larvae, pupae and adults. Error bars represent 95% CLs (N = 16 per group). Means with the same letter are not significantly different from each other.

3.3.4 Heat knock-down time and Chill-coma recovery time

Heat knock-down time varied significantly between *C. partellus* larvae and adults (χ^2 =352.67, d.f =1, $P < 0.0001$) (Figure 3.5A). Larvae showed a significantly longer time to knock-down compared to the adults (Figure 3.5A). Similarly, there was a significant difference in

CCRT between *C. partellus* larvae and adults (χ^2 =303.02, d.f =1, $P < 0.0001$) (Figure 3.5B). Contrary to HKDT, adults recovered faster than larvae following chill-coma (0°C; 1 h) (Figure 3.5B).



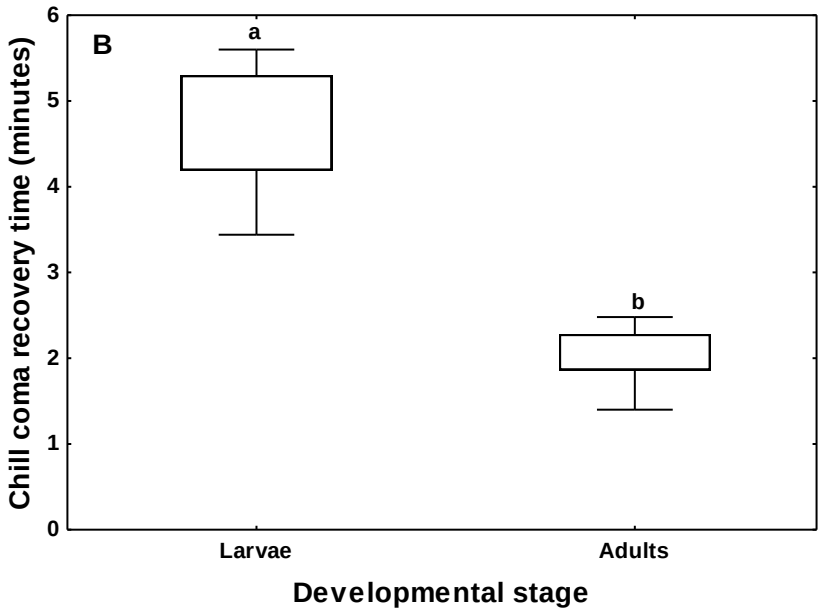


Figure 3.5: The effect of developmental stage (larvae vs adults) on *Chilo* partellus (A) heat knock-down time and (B) chill-coma recovery time. Error bars represent 95% CLs (N = 30). Means with the same letter are not significantly different from each other.

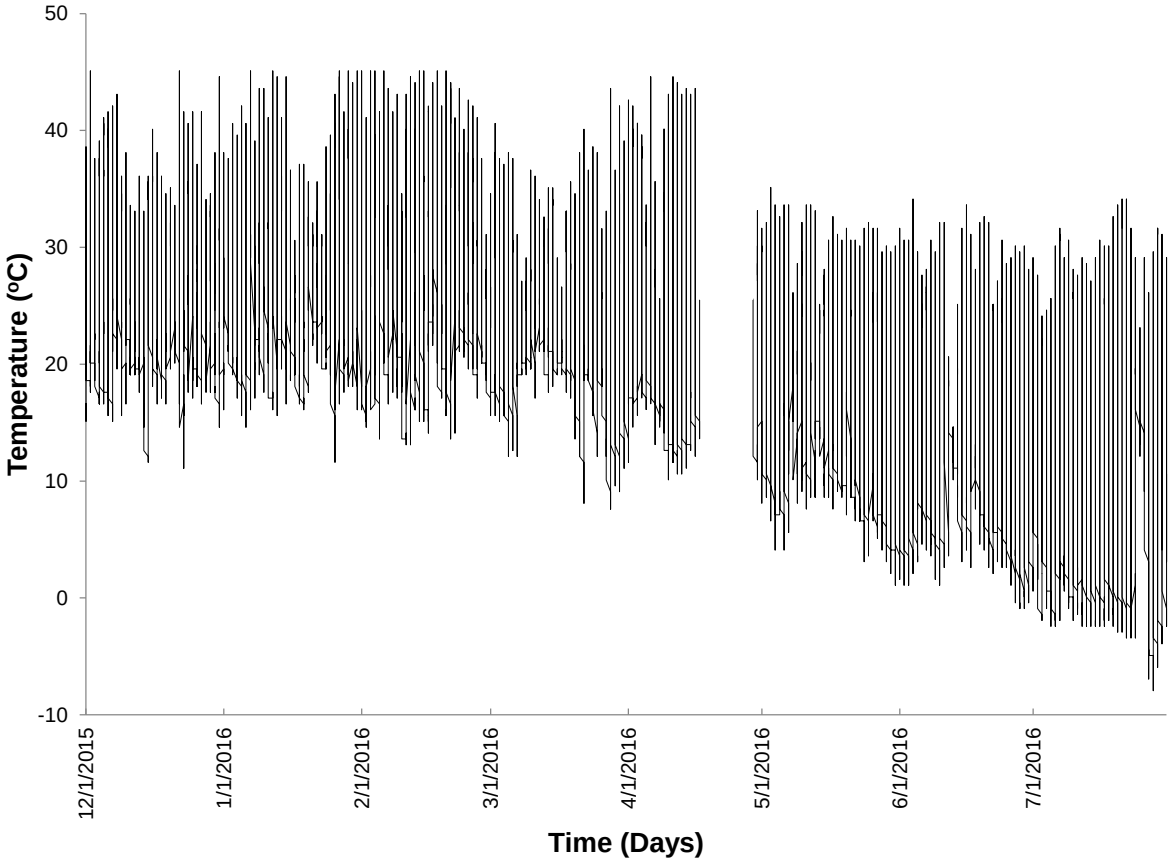


Figure 3.6: Microclimatic temperature data in austral summer (December 2015-April 2016) and winter (May-July 2016) were recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5°C accuracy; 1 h sampling frequency) from a maize field, Veggies For All Farm, Glen Valley, Gaborone, Botswana (S24.61224; E25.97326; 977 m.a.s.l), where both *Chilo partellus* and *C. sesamiae* are found.

3.4 Discussion

Enhanced basal and plastic responses by invasive species to abiotic stressors are key physiological factors contributing to their invasion success (Slabber *et al*., 2007; Nyamukondiwa & Terblanche, 2010). The interactions between temperature and RH tolerance are likely to shape the survival of insects, and indeed *C. partellus* and its larval parasitoid, *C. sesamiae*. To my knowledge, this is the first report detailing comparative thermal tolerance of *C. partellus* and *C. sesamiae*, and how this may shape future biological control in the face of climate change.

Consequently, the thermal tolerance of *C. partellus* and *C. sesamiae* are in asynchrony revealing that the host and the parasitoid may not evolve together with thermal variability.

As expected, the current results showed that the severity and duration of exposure were important in determining the survival of *C. partellus* and *C. sesamiae*, in keeping with previous studies (Chown & Terblanche, 2007; Chidawanyika & Terblanche, 2011). LLTs and ULTs for *C. partellus* eggs and larvae ranged from -9 to 6°C; 37 to 48°C and -14 to -2°C; 41 to 49°C respectively for 0.5–4 h treatments, revealing that larvae were more temperature tolerant than the eggs. This is contrary to previous studies, highlighting that mobile insect developmental stages may require less physiological protection against environmental stressors, as their mobility, may employ behavioral compensatory mechanisms in avoiding extreme conditions (Jensen *et al*., 2007; Blanckenhorn *et al*., 2014; Abbar *et al*., 2016). Nevertheless, the current results are also in keeping with other previous studies documenting developmental stage related differences in environmental stress tolerance (Cavieres *et al*., 2016; Klockmann *et al*., 2016). Indeed, Zhang *et al*. (2015) showed that diamondback moth (*Plutella xylostella*) eggs had compromised high temperature tolerance, relative to its larval stages. For *C. sesamiae*, LLTs ranged from -1 to 4°C while ULTs ranged from 36 to 39°C (2 h treatments). A comparison of LLTs and ULTs for *C. partellus* and *C. sesamiae* (for the 2 h duration) indicated that the parasitoid is more vulnerable to temperature stress at both temperature extremes (see Figure 3.1; 3.2). Larval *C. partellus* is known to be the economically destructive stage, and is also the target by endoparasitoid *C. sesamiae*. Differential high and low temperature tolerance therefore suggests a mismatch between the host and its parasitoid in the face of climate change. LLT and ULT data gathered here is important in determining thermal windows for the performance, fitness and survival of these species. High and low temperatures are known to limit flight, mating, reproduction, development (measured as accumulated degree days) and other biological processes that affect insect phenology (Chidawanyika & Terblanche, 2011; Nyamukondiwa *et al*., 2013). With African temperatures expected to increase by 1.5 - 3°C by 2050 (Gemeda & Sima, 2015), coupled with increased likelihood of prolonged heat waves, and cold snaps (IPCC, 2014), it may be expected that host stemborer *C. partellus* may likely survive both prolonged high and low temperatures better than its parasitoid. In Africa, this represents a potential risk in *C. partellus* pest outbreaks, which may negatively affect cereal crop productivity, food and nutrition security, and ultimately household incomes. Microclimate data recorded here showed that both low and

high temperatures causing *C. partellus* and *C. sesamiae* mortality are frequently encountered under field conditions (Figure 3.6).

This may imply that, in the absence of compensatory mechanisms, temperature associated mortality may be a major factor shaping the population dynamics of the pest-parasitoid complex in nature.

The present study also demonstrates the significant effects of ramping rates on CTLs. This is in agreement with Terblanche *et al*. (2007b) who indicated that CTLs highly depend on methodological context. CTLs significantly vary with the experimental protocol *vis-à-vis* starting temperature, ramping rate and experimental duration. Although CTLs are widely accepted as important indices of thermal tolerance (Wang *et al*., 2002; Nyamukondiwa & Terblanche, 2010), few studies specifically examined these responses in an attempt to gain insight into ecologically relevant estimates of thermal tolerance under laboratory conditions (Slabber, 2007; Nyamukondiwa & Terblanche, 2010). Here, I demonstrate that slower ramping rates significantly reduced CT_{max} in *C. partellus* adults, larvae and *C. sesamiae* adults. This suggests that a prolonged heating period, even under dynamic thermal variability, may compromise fitness of organisms due to the longer period under stress. In line with the notion that mortality at a stressful temperature depends on temperature severity and the duration of exposure (Rezende *et al*., 2011; 2014), my results suggest that *C. partellus* may not be able to adjust its phenotypic plasticity of high temperature tolerance in the short term (see discussions in Nyamukondiwa & Terblanche, 2010; Sgrò *et al*., 2016). Furthermore, slower cooling rates significantly improved CT_{min} in adult *C. partellus* and *C. sesamiae* but not in *C. partellus* larvae. This suggests that larval *C. partellus* may not have the capacity to adjust its lower temperature tolerance through phenotypic plasticity, similar to the rapid cold hardening (RCH) response in other insects (see Bale *et al*., 1989; Kelty & Lee, 1999). However, improved CT_{min} following slower ramping rates in adult *C. partellus* and *C. sesamiae* suggests that prolonged duration at low temperatures may allow the insects to develop some low temperature protection, which suggest RCH. Such ecologically relevant cooling rates have been observed to improve CT_{min} in many Diptera [*Bactrocera tryoni* (Meats, 1973), *Musca domestica* (Coulson & Bale, 1990), *Eurosta solidaginis* (Lee *et al*., 1993), *D. melanogaster* (Kelty & Lee, 1999; Jensen *et al*., 2007), *Sarcophaga crassipalpis* (Rinehart *et al*., 2000), *Bactrocera oleae* (Koveos, 2001), *Ceratitis* (Nyamukondiwa & Terblanche, 2010)] and the lepidopteran, *Cydia pomonella* (Chidawanyika & Terblanche,

2011). A comparison of upper temperature limits to *C. partellus* and *C. sesamiae* activities (Figure 3.3A; C; E) suggest that *C. sesamiae* is likely to cease activity at mild temperatures relative to the host. This disadvantage may compromise the efficacy of the parasitoid as a biological control agent in the face of climate change. However, the current comparative assessment of thermal tolerance in *C. partellus* vs *C. sesamiae* is based on evaluation of LLTs, ULTs and CTLs. While these are reliable means of measuring thermal tolerance (Chown & Nicolson, 2004; Andersen *et al.*, 2015), I suggest further investigations on other thermal biology life history traits including whether the parasitoid exhibits improved performance under stressful conditions through phenotypic plasticity and laboratory adaptation. In addition, an understanding of diurnal thermal activity of the parasitoid in relation to host should also be investigated in the face of climate change.

The mean supercooling points for larvae, pupae and adults of *C. partellus* were -11.82±1.78, -10.43±1.73 and -15.75±2.47 respectively. Previous studies on *Ceratitis* sp revealed immobile pupae had the lowest SCP as compared to the mobile developmental stages, larvae and adults (Nyamukondiwa *et al*., 2013). Indeed, from an evolutionary perspective, 'immobile' developmental stages (eggs, and pupae) are almost always confined to a specific habitat, and are less likely capable of compensating to environmental stress using behaviour, e.g. escape. As a result, these 'immobile' stages often have evolved enhanced inherent basal temperature tolerance (see Bowler & Terblanche, 2008; Lansing *et al*., 2000). The SCP results are, however, in disagreement with that since adult *C partellus* moths had the lowest SCPs (most enhanced). Overwintering insects are divided into two main categories basing on their ability to survive the freezing of their body water. Insects that can survive ice formation within their tissues are called freeze tolerant whereas those that cannot survive ice formation within their tissues are called freeze susceptible or freeze intolerant (Andreadis *et al*., 2005). By contrast, chill-susceptible insects are killed by the cold in the absence of internal ice formation (Sinclair *et al*., 2015). In my study, there were no insects that recovered from chill-coma following SCP measurement, regardless of developmental stage. This therefore suggests that *C. partellus* may be chill-susceptible. Lower SCPs of adult *C. partellus* may thus be ecologically not that significant since the species may not be freeze tolerant, and moreover, LLTs were much higher than the freezing temperature (Figure 3.1A; B) (Sinclair *et al*., 2015). Furthermore, microclimate data recorded here were above the SCPs recorded for the species (Figure 3.6). Generally, for many insects,

cold tolerance strategy is derived from separate measurement of SCP and LLT; where LT_{50} < LLT = freeze tolerant, LT_{50} = LLT = freeze intolerant/avoidant and LT_{50} > LLT = chill-susceptible (Bemani *et al*., 2012; Sinclair *et al*., 2015). This study therefore shows that all *C. partellus* developmental stages are likely killed by a prolonged low temperature exposure and thus chill susceptible.

HKDT and CCRT measures the time required for enzyme activity and/or membrane permeability to be lost or return to levels sufficient for neuromuscular function, respectively (MacMillan & Sinclair, 2011a). Since chill-coma is reversible and locomotion easy to detect visually in mobile insects, chill-coma recovery is often used as an ecologically sound measure of cold tolerance (Sinclair *et al*., 2015). As in previous studies (Cavieres *et al*., 2016; Klockmann *et al*., 2016), the current results show that *C. partellus* HKDT and CCRT were significantly affected by developmental stage. Larvae took more time to be knocked down by high temperature relative to adults, suggesting an enhanced temperature tolerance, for the trait of HKDT. The reason why larvae were more heat tolerant in this case is unknown but may be related to body water content. Larvae generally have higher water content than adults and thus may have high dehydration tolerance and low rates of water loss (Yoder *et al*., 2003). This is in agreement with comparison of water loss rate for larvae and adults of *Belgica antarctica* in dry air which revealed that larvae had a higher dehydration tolerance than adults (Benoit *et al*., 2007). These suggest that organisms with less body water may be more susceptible to temperature and desiccation related mortality than organisms with high body water content (Gray & Bradley, 2005). In most insect species, high levels of internal body water usually extend the time that is required for dehydration to levels that elicit mortality. In chill-susceptible insects, exposure to chill-coma temperature causes the bulk movement of hemolymph sodium, magnesium, calcium and water into the alimentary canal (MacMillan & Sinclair, 2011b) and chill-coma recovery usually involves the re-establishment of hemolymph ion homeostasis (MacMillan *et al*., 2012). *Chilo partellus* larvae took more time to recover from chill-coma as compared to the adults indicating a higher cold tolerance for the adults for traits of CCRT. This suggests developmental stage related differences in maintenance and re-establishment of ion homeostasis, essential for chill-coma recovery. Indeed, similar studies have indicated interspecific variation in maintenance of water and ion homeostasis accounts for differences in *Drosophila* species cold tolerance in a laboratory (MacMillan *et al*., 2015) and field setting

(MacMillan *et al*., 2016). This therefore shows that the ability to maintain ion homeostasis is significant for insects in the wild to cope with the diurnal temperature fluctuations involving chilling (see e.g. Figure 3.6). Nevertheless, developmental stage related differences in *C. partellus* CCRT and ion homeostasis recorded here still warrants further investigation.

In conclusion, results of the present study document thermal tolerance in *C. partellus* and its larval endoparasitoid, *C. sesamiae*, and implications for biological control in the face of climate change. To my knowledge, this is the first comparative study detailing the thermal tolerance between this host and parasitoid. The current results show that *C. partellus* has a high basal thermal tolerance as compared to *C. sesamiae*. This implies that for traits of thermal tolerance, the two species may not be capable of co-evolving, which is an undesirable characteristic for interacting species. With global temperatures expected to gradually increase, this suggests that *C. partellus* can inhabit slightly warmer, and cooler climates where *C. sesamiae* may not thrive. This represents significant potential burden to biological control programmes of the economic pest, with implications on food security. Furthermore, I also document developmental stage differences in high and low temperature tolerance for *C. partellus*. Overall, this work provides insight into how thermal biology affects the population dynamics of pest-parasitoid interactions and is thus useful for model-based predictions of pest distribution and population dynamics, pest risk analysis and pest management.

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CHAPTER 4

Thermal plasticity mediates the interaction between host *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) under rapidly changing environments*

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4.1 Introduction

Temperature is arguably the most important abiotic factor influencing insect physiology, ecology and consequently overall environmental fitness (Dillon *et al*., 2009). As ectotherms, insect exposure to extreme temperatures may cause irreversible loss of fitness and ultimately mortality (Dillon *et al*., 2009). Thus, temperature influences seasonality and evolutionary responses over short and long time scales in the wild (Nguyen *et al*., 2014). Due to global change, resistance to temperature stress in insects may be compromised (Fischer *et al*., 2010), with some organisms emerging 'winners' while others suffer the harmful effects (Mutamiswa *et al*., 2017a). Indeed, this is true for different trophic levels, and more especially herbivore parasitoid systems that show differential thermal sensitivity at different trophic levels (Romo & Tylianakis, 2013; Schreven *et al*., 2017). In consequence, insects employ a diverse range of morphological, physiological, and behavioral adaptations, or a combination of the mechanisms to counter adverse climate conditions (Chown & Terblanche, 2007; Nyamukondiwa *et al*., 2013). Physiologically, insects are known to survive extreme temperature variation through phenotypic plasticity, the ability of a same genotype to produce variable phenotypes under heterogeneous environments (Whitman & Ananthakrishnan, 2009). This may be in the form of either hardening (in the short-term) or acclimation under controlled laboratory conditions (e.g. Chidawanyika & Terblanche 2011a) and acclimatization in the field (longer-term) (Whitman & Ananthakrishnan, 2009; Chidawanyika & Terblanche 2011b; Colinet & Hoffman, 2012; Nyamukondiwa *et al*., 2013; Sgrò *et al*., 2016). At short time scales, hardening allows individuals to adjust their phenotype temporarily to the environment in which they currently inhabit (David *et al*., 2004; Austin & Moehring, 2013), and this may be significant in preserving key life-history traits (see Shreve *et al*., 2004). One such mechanism is rapid cold hardening (RCH), defined as a rapid improvement in survival at a low lethal temperature after brief pre-treatment to a prior sub-lethal temperature shock (Lee *et al*., 1987). Indeed, RCH has been reported to improve survival in several insect taxa including Lepidoptera, *Thaumatotibia leucotreta* (Stotter & Terblanche, 2009; Chidawanyika & Terblanche 2011a), Tephritids, *Ceratitis capitata* and *Ceratitis rosa* (Nyamukondiwa *et al*., 2010), *Zaprionus vittiger* (Nyamukondiwa & Terblanche, 2010a) and several *Drosophila* species (Kelty & Lee, 2001; Overgaard & Sørensen, 2008; Nyamukondiwa *et al*., 2011; Overgaard *et al*., 2014a). Similarly, at high temperatures, brief exposure to sub-lethal high temperatures can result in improved survival at a lethal high temperature (Chen *et al*.,

1991), a phenomenon called rapid heat hardening (RHH). This mechanism has been shown to improve survival in several insects including *C. capitata* (Kalosaka *et al*., 2009; Nyamukondiwa *et al*., 2010), *Cydia pomonella* (Chidawanyika & Terblanche 2011a), *Drosophila* species (Nyamukondiwa *et al*., 2011) and *Zygogramma bicolorata* (Chidawanyika *et al*., 2017). Acclimation, on the other hand, improves survival through a rapid reversible change in phenotype in response to a relatively chronic pre-exposure period (days, seasons) to conditions similar to future potentially stressful novel thermal conditions (Woods & Harrison, 2002; Terblanche *et al*., 2006). This is sometimes referred to as beneficial acclimation (Woods & Harrison, 2002) and only a match in pre-exposure and future stressful conditions will result in improved survival whilst mismatches may result in reduced fitness (Kristensen *et al*., 2008; Chidawanyika & Terblanche, 2011b). These plastic responses are the first in a suite of mechanisms that an organism may use to survive when faced with novel environments (Nyamukondiwa *et al*., 2010), and are significant for maintaining fitness under field conditions (Kristensen *et al*., 2008; Chidawanyika & Terblanche, 2011b). Indeed, similar beneficial acclimatory responses have been reported in *D. melanogaster* (Colinet & Hoffman, 2012), *C. capitata* (Basson *et al*., 2012), *Nezara viridula* (Chanthy *et al*., 2012) and *Phthorimaea operculella* (Hemmati *et al*., 2014).

Temperature tolerance across species has been reported to vary, depending on ecological and evolutionary history (Chown & Terblanche, 2007; Stotter & Terblanche, 2009; Nyamukondiwa & Terblanche, 2010b), developmental stage or age and sex (Nyamukondiwa & Terblanche, 2009). Hence even for species with related evolutionary adaptation strategies, variations in thermal tolerance may occur ultimately shaping species abundance and distributions (Chen *et al*., 2011; Sorte *et al*., 2011; Hoffmann *et al*., 2012; Nguyen *et al*., 2014; Overgaard *et al*., 2014b; Mutamiswa *et al*., 2017a; reviewed in Sgrò *et al*., 2016). For interacting species, differential changes in fitness may disrupt long evolved mutual or antagonistic ecological interactions (Parmesan, 2006; Yang & Rudolf, 2010), ultimately changing ecosystem community structures and function (Grimm *et al*., 2013) For example, some species may have limited compensatory abilities and thus are highly vulnerable to heterogeneous thermal regimes while others may compensate through plasticity thereby avoiding potentially lethal effects (Sgrò *et al*., 2016). In agriculture, the efficacy of biological control programs will be poor if important interactions between crop pests and their natural enemies are disrupted due to asynchrony

between the host-natural enemy complex, should there be differential thermal sensitivity at the two trophic levels (Dyer *et al*., 2013; Romo & Tylianakis, 2013). Consequently, understanding the physiological responses of pest-parasitoid complexes to thermal stress is essential for predicting how climate change may affect their distribution, population phenology and ultimately the efficacy of biological control thereof (Hance *et al*., 2007; Bale, 2010; Amarasekare & Savage, 2010; Schreven *et al*., 2017; Kleynhans *et al*., 2014).

The spotted stemborer, *C. partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most damaging lepidopteran stemborers of sorghum and maize in eastern and southern Africa (Khadioli *et al*., 2014). It is native to Asia and was first recorded in Africa (Malawi) around 1930 (Tams, 1932). *Chilo partellus* has a history of invasion success, expanding to high elevation areas, highland tropical areas and humid transitional areas of east and southern African regions (Khadioli *et al*., 2014; Mutamiswa *et al*., 2017b). The successful establishment of *C. partellus* in novel environments and expansion to highland areas (Khadioli *et al*., 2014) probably stems from basal physiological tolerances (Mutamiswa *et al*., 2017b), morphological and behavioural adaptations that enabled them to displace indigenous stemborer species (Kfir, 1997). *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), native to Asia is a gregarious koinobiont larval endoparasitoid of *C. partellus* (Dejen *et al*., 2013). The parasitoid prefers warm and dry areas (Songa *et al*., 2001) and, like other insects, variation from this optimum may affect fitness. While previous research investigating phenotypic plasticity of arthropods of agricultural importance mainly focused on insect pests (e.g Rajamohan & Sinclair, 2008; Nyamukondiwa *et al*., 2010; Nyamukondiwa & Terblanche, 2010a; Chidawanyika & Terblanche, 2011a) but see (Schreven *et al*., 2017), to my knowledge, no studies to date have focused on plastic thermal responses among host-parasitoid complexes, and their implications on pest management. Hence, investigating phenotypic plasticity of insect pest and its parasitoid gives insights into how host-parasitoid population dynamics will be affected by changes in climate.

Here, I investigate the phenotypic plasticity of thermal tolerance of *C. partellus* developmental stages (larvae, pupae and adults) and its larval parasitoid *C. flavipes* (adults) and present implications on biological control efficacy in the face of climate change. Specifically, I determined short (RCH and RHH) and long (acclimation) term plastic responses of survival at extreme temperatures for these two species. I hypothesize that *C. partellus* is equally thermally plastic compared to its biological control agent, *C. flavipes*, as set by their long host-parasitoid

co-evolutionary history. The results of plastic responses for the two interacting species will be essential in developing pest risk assessments and designing pest management programs under global change.

4.2 Material and Methods

4.2.1 Study insects and rearing conditions

Chilo partellus and *C. flavipes* colonies were obtained from International Centre for Insect Physiology and Ecology (ICIPE), Kenya. These colonies had been in the laboratory for more than 20 generations with regular augmentation with wild populations to maintain genetic diversity. *Chilo partellus* larvae were reared in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) on artificial diet (Ochieng *et al*., 1985; Tefera *et al*., 2010), in trays at 28 ± 1°C, 65 ± 10% relative humidity (RH) and 12L: 12D photocycle. Pupae were held in open Petri dishes in Bugdorm rearing cages (240mm^3; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under the same larval rearing conditions until adult eclosion. Upon emergence, all adults had access to water *ad libtum* from soaked cotton wool while gravid females had access to wax paper placed in the cages as an oviposition surface. For uniformity among test insects, eggs were harvested after every 12 h and transferred from wax paper to artificial diet where they hatched into larvae. The larvae then went through five instar stages before pupation. Thereafter, pupae were collected daily and placed in Petri dishes in rearing cages for adult eclosion. To maintain genetic similarity to wild populations, wild-caught moths were added to the colony once every month during summer (e.g. Nyamukondiwa & Terblanche, 2009). For *C. flavipes* colony, parasitised *C. partellus* larvae from ICIPE were maintained in the climate chambers under 12L: 12D photocycle, 28 ± 1°C and 65 ± 10% RH until adult parasitoid eclosion. Emerged parasitoids had access to food *ad libitum* (25% honey: water mixture from a cotton wick) until they were used in thermal tolerance assays. In the hardening and acclimation experiments, *C. partellus* larvae (6th instar), pupae, adults (24-48 h old) and *C. flavipes* adults (24-48 h old) were used.

4.2.2 Phenotypic Plasticity of Low Temperature Tolerance

Rapid cold hardening experiments were performed using established protocols. (e.g. Terblanche *et al*., 2008; Nyamukondiwa *et al*., 2010; Chidawanyika & Terblanche, 2011a). I first determined in the preliminary trials the lower discriminating temperatures i.e. temperatures causing 80-100% mortality upon 2h exposure to a stressful low temperature. A pre-treatment of 10°C above the lower discriminating temperature is generally suffice to elicit a RCH response. (reviewed in Lee & Denlinger, 2010). Since discriminating temperatures differed across species and developmental stage, I thus used a standardized, species-developmental stage-specific approach for eliciting hardening responses (see e.g. Nyamukondiwa *et* al., 2011) (Table 4.1).

Table 4.1: Summary table showing species and developmental stage specific hardening and discriminating temperatures (2 h duration) for host, *C. partellus* and parasitoid, *C. flavipes*.

Species	**Developmental stage**	**Hardening low temperature (°C)**	**Hardening high temperature (°C)**	**Discriminating low temperature (°C)**	**Discriminating high temperature (°C)**
Chilo partellus	Larvae	2	41	-8	46
	Pupae	2	42	-8	47
	Adults	1	39	-9	44
Cotesia flavipes	Adults	3	35	-7	40

Five replicates of 60 ml vials consisting 10 stemborers of each life-stage (larvae, pupae and adults) and *C. flavipes* (adults) were placed in a growth chamber at 28°C, 65 ± 10% RH for 30 min to allow for equilibration. These insects were later hardened for 2h at a non-lethal stressful low temperature and then immediately subjected to the discriminating temperature (Table 4.1). Control organisms were held at optimum temperature before subjecting them directly into the

discriminating temperature so that any difference in mortality was a result of the hardening treatment. Following treatment, insects were returned to 28°C, 65% RH and 12L: 12D in climate chambers before scoring survival. Treatment and control insects had access to food and water *ad libtum* during the recovery period. Survival was defined as ability to pupate (for larva), eclose (for pupae) and coordinated muscle response to stimuli such as gentle prodding, or normal behaviours such as feeding, flying or mating for adults (as in e.g. Nyamukondiwa *et al*., 2010).

Because of the close interaction between insect physiology and ecology, CTLs ramping assays remain ecologically relevant measures of understanding how species respond to heterogeneous stressful environments (Terblanche *et al*., 2011). Longer-term acclimation effects on thermal tolerance for *C. partellus* and *C. flavipes* were assayed as outlined by Nyamukondiwa & Terblanche (2010b). High temperature acclimation was defined for each species as 5°C above rearing/benign temperature while low temperature acclimation was defined as 5°C less rearing temperature. The organisms acclimated to optimum temperature were considered as a control group (e.g. Terblanche *et al*., 2006). Critical thermal minima (CT_{min}) were measured following acclimation of insects to 23 (low), 28 (optimum) and 33°C (high) for duration of 3 days (Figure 4.1).

Acclimation for this duration is generally suffice to elicit some plastic responses in other similar insect taxa eg. *Bicyclus anynana*, *C. capitata* (Fischer *et al*., 2010; Weldon *et al*., 2011). Ten individual organisms (for each species and developmental stage) from each acclimation were randomly placed in the 'organ pipes' connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for subzero temperatures (Chown & Nicolson, 2004). Assays started at a set point of benign/rearing temperature of 28°C, 65 ± 10% RH for 10 mins (to allow equilibration of insect body temperatures) before ramping the temperature down at a rate of 0.25°C/min until their CT_{min} were recorded. A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into a control chamber to record organ pipe chamber temperature. This was repeated twice to yield sample sizes of n = 20. Critical thermal minima was defined as the temperature at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding) (e.g. Nyamukondiwa & Terblanche, 2010b).

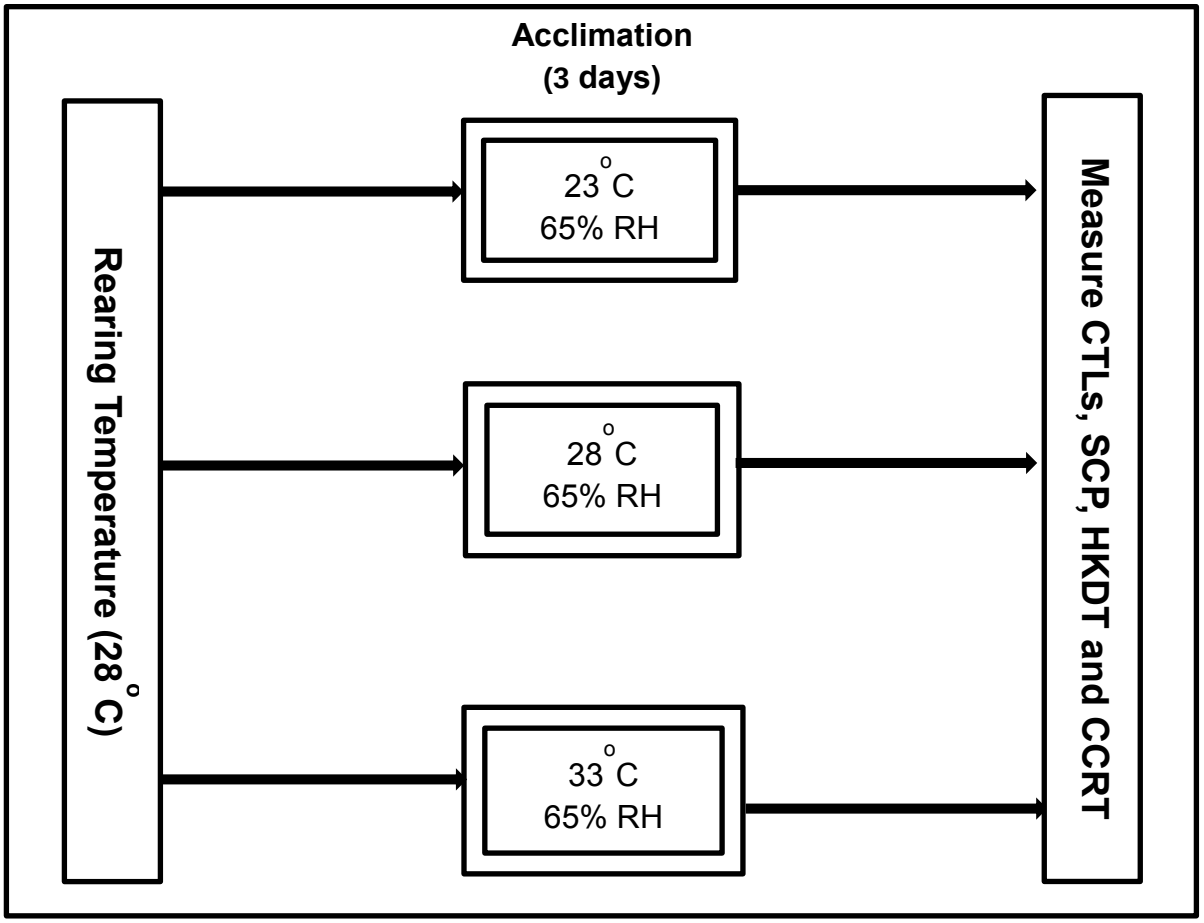


Figure 4.1: Schematic diagram for acclimation experimental protocols. For full description see sections 4.2.2 and 4.2.3

CCRT experiments were performed following 3 days acclimation (as in e.g. Weldon *et al*., 2011) (Figure 4.1). To determine CCRT, 10 replicate organisms from each developmental stage were individually placed in 0.65ml microcentrifuge tubes and then loaded into a large zip-lock bag that was submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol set at 0°C for 1 hour. This temperature by time treatment has also been reported to elicit chill-coma in other insect taxa (see Weldon *et al*., 2011; Sinclair *et al*., 2013). Following 1 h at this chill-coma temperature, the tubes were immediately transferred from the water bath to the climate chamber (Memmert HPP 260, Memmert GmbH + Co.KG, Germany) set at 28°C, 65% RH for recovery. This was repeated thrice to yield sample sizes of *n*

= 30 (30 replications) per each treatment. The chamber was connected to a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) which was linked to a computer. All observations and data were recorded from this climate chamber video recording system. CCRT was defined as the time (in minutes) required to regain coordinated movement ability (in larva) to stand upright on its legs (for adults) (Milton & Partridge, 2008; Chown & Nicolson, 2004).

SCPs were assayed as outlined by Nyamukondiwa *et al*. (2013) following acclimation as previously outlined. A total of 16 acclimated individuals of each life stage were individually placed in 0.65 ml microcentrifuge tubes. Each insect was attached to a tip of a type-T copper-constantan thermocouple (762-1121, Cambridge, UK) that was inserted through the lid of the tube and both the insect and thermocouple were secured in contact using a cotton wool. The thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperatures were recorded at 1s intervals using PicoLog software for windows (Pico Technology, Cambridge, UK). Experiments started at a set point temperature of 15°C for 10 mins (for insects' temperature equilibration) before ramping down at a rate of 0.5°C min^{-1} until the observation of SCPs. In this study, SCP for each individual was defined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (e.g. Nyamukondiwa *et al*., 2013).

4.2.3 Phenotypic Plasticity of High Temperature Tolerance

Rapid heat hardening experiments were also performed using established protocols (e.g. Terblanche *et al*., 2008; Nyamukondiwa *et al*., 2010; Chidawanyika & Terblanche, 2011a). Like in RCH experiments, discriminating high temperatures were first determined during preliminary trials. A pre-exposure of 5°C below the discriminating upper temperature is generally suffice to elicit a RHH response (reviewed in Hoffmann *et al*., 2003; Nyamukondiwa *et al*., 2011). Organisms were allowed to equilibrate in a growth chamber at 28°C, 65 ± 10% RH for 30 min before being hardened for 2h at a stressful high temperature (see Table 4.1). Following hardening, organisms were given 30 minutes recovery time to allow the *de novo* synthesis of heat shock proteins (HSPs) (see Nyamukondiwa *et al*., 2010) before subjecting them for 2h at discriminating high temperature (Table 4.1). Control organisms were also held at optimum temperature before subjecting them directly into the discriminating temperature so that any

difference in mortality was a result of the hardening treatment. Following treatment, insects were later returned to 28°C, 65% RH and 12L: 12D in climate chambers before scoring survival.

Following high temperature acclimation CT_{max} was measured following generally the same methodology as in CT_{min} above. However, for CT_{max}, temperature was ramped up at a rate of 0.25°C/min until the temperature at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding) (e.g. Nyamukondiwa & Terblanche, 2010b).

For HKDT following acclimation, 10 replicate insects were loaded individually into 0.65ml microcentrifuge tubes and placed in a climate chamber that was connected to a camera linked to a computer. In the climate chamber, C. *partellus* larvae and adults were subjected to a test temperature of 49.0±0.3°C, 65% RH whist *C. flavipes* adults were exposed to 45.0±0.3°C, 65% RH. These knockdown temperatures (45.0 and 49.0°C) were selected based on preliminary trials of upper critical temperatures to activity, which were 44.6±0.37°C; 47.88±0.76°C and 48.5±0.74°C for *C. flavipes* adults, *C. partellus* adults and larvae, respectively. This was repeated thrice to yield sample sizes of n = 30 (30 replications) per each experiment and developmental stage. All observations were recorded from the climate chamber video recording system. HKDT was defined as the time (in minutes) at which activity was lost by the organisms following exposure to heat knockdown temperature.

4.2.4 Microclimate data

To determine the thermal environment experienced by *C. partellus* and *C. flavipes* in the field, shaded microclimatic temperatures were recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5°C accuracy; 1 h sampling frequency) from a maize field, BCA Farm, Gaborone, Botswana (S24.56449; E25.94883; 994 m.a.s.l) which hosts these two species. Recordings were done during the period January 2016 to February 2017.

4.2.5 Statistical analyses

Data analyses were carried out in STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma). Before analysing the results for hardening and acclimation responses, data were checked for normality and equality of variances using the Shapiro-Wilk test and Hartley-Bartlett

tests, respectively. In the hardening assays, key assumptions of ANOVA were not met, while for the acclimation assays (and subsequent CTLs, CCRT, HKDT and SCPs), key assumptions of ANOVA were satisfied. To investigate the effects of hardening on the survival of *C. partellus* and *C. flavipes*, treatment groups were analysed using Generalised linear models (GLZ) assuming a binomial distribution and a logit link function. Acclimation effects on CTLs, SCPs, HKDT and CCRT were assessed using full factorial analysis of variance (ANOVA). Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups. Overlap in 95% confidence limits was used to identify statistically homogeneous groups.

Using microclimatic data, I calculated the number of likely natural RCH and RHH events occurring during the time I recorded my microclimate data using Matlab software, R2017a (MathWorks). The RCH events were taken from the following range of temperatures: $0.5 \leq 1 < 1.5$; $1.5 \leq 2 < 2.5$ and $2.5 \leq 3 < 3.5$ whilst RHH events were generated from the temperature ranges: $34.5 \leq 35 < 35$; $38.5 \leq 39 < 39.5$; $40.5 \leq 41 < 41.5$ and $41.5 \leq 42 < 42.5$ for a period ≥2 h, enough to elicit hardening responses.

4.3 Results

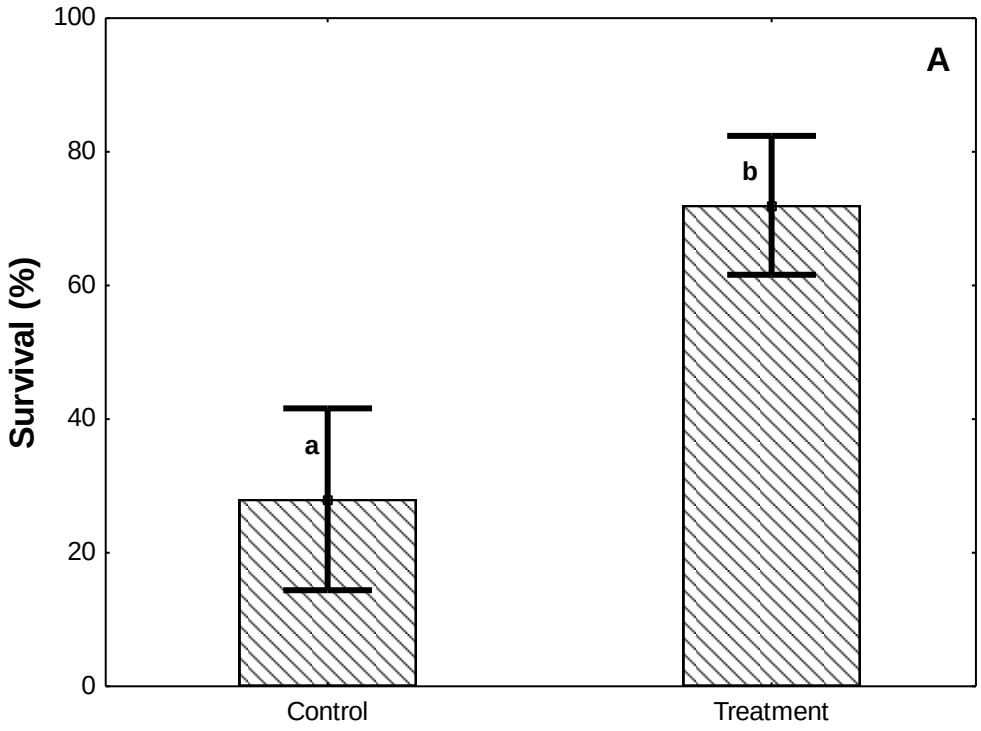
4.3.1 Phenotypic Plasticity of Low Temperature Tolerance

A 2 h low temperature pretreatment (RCH) of 2 and 3°C significantly enhanced survival during a 2 h exposure at discriminating low temperature of -8 and -7°C for *C. partellus* larvae (χ^2 =39.97, d.f=1, $P < 0.0001$) (Figure 4.2A), and *C. flavipes* adults (χ^2 =60.01, d.f=1, $P < 0.0001$) respectively (Figure 4.2B). Similarly, RCH (1 and 2°C) significantly improved low temperature survival (-9 and -8°C) for *C. partellus* pupae (χ^2 =6.91, d.f=1, $P = 0.008$ (Figure 4.3A) and adults) (χ^2 =83.72, d.f=1, $P < 0.0001$) respectively (Figure 4.3B).

There was a significant acclimation, species and acclimation by species interaction effect for low temperature tolerance *vis a vis* CT_{min}, CCRT and SCP (Table 4.2). Acclimation to low temperature (23°C for 3 days) significantly improved CT_{min} (Figure 4.2C) and CCRT (Figure 4.2D) for both *C. partellus* larvae and *C. flavipes* adults. Nevertheless, acclimation to high temperature (33°C for 3 days) generally compromised *C. partellus* larvae and adult *C. flavipes* CT_{min} and CCRT (Figure 4.2C; D). Furthermore, high temperature acclimation enhanced CCRT

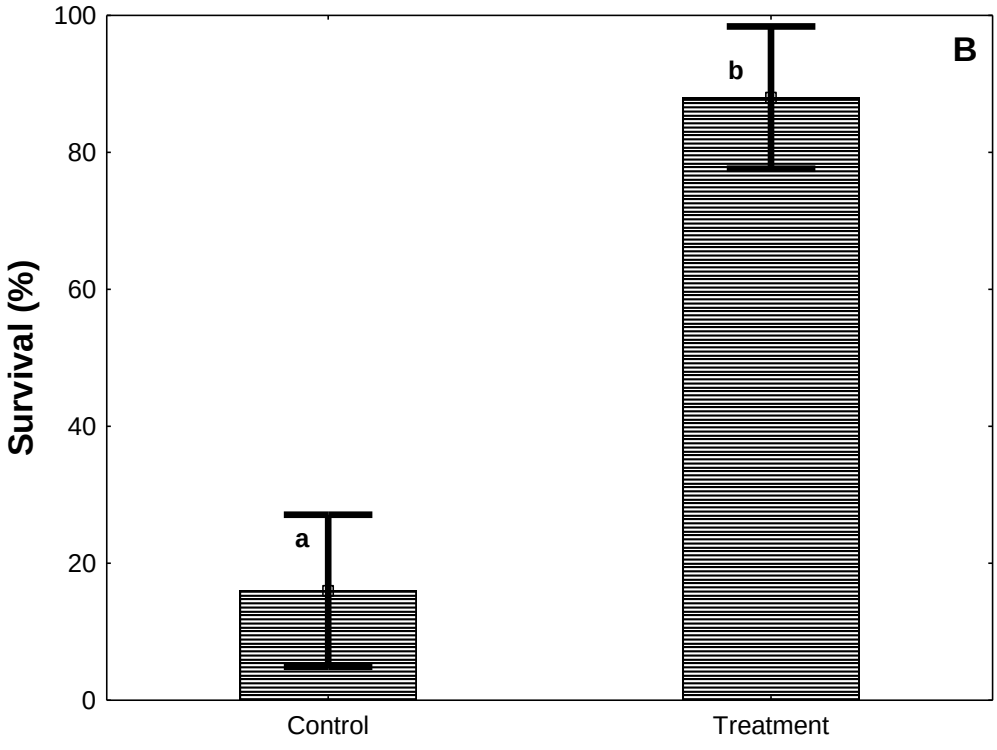
for *partellus* larvae relative to the control (Figure 4.2D). A comparison of *C. partellus* developmental stages for low temperature tolerance shows that low temperature acclimation improved CT_{min} and CCRT for both adults and larvae (Table 4.3; Figure 4.3C; D). The effect of developmental stage by acclimation interaction was highly significant for CT_{min}, CCRT and SCP (Table 4.3).

Acclimation to low temperature (23°C for 3 days) increased SCP (more positive SCP) in *C. partellus* larvae while marginally enhancing it (more negative SCP) in *C. flavipes* adults (Figure 4.2E). However, acclimation to high temperature (33°C for 3 days) enhanced SCP in *C. partellus* while compromising it in *C. flavipes* (Figure 4.2E). There was a significant difference ($P < 0.05$) in SCP for *C. partellus and C. flavipes* acclimated at low temperature while no significant differences ($P > 0.05$) were recorded for the same species acclimated at high temperature (Figure 4.2E). In addition, low temperature acclimation resulted in significantly compromised SCPs for *C. partellus* developmental stages (larvae, pupae and adults) (Figure 4.3E).

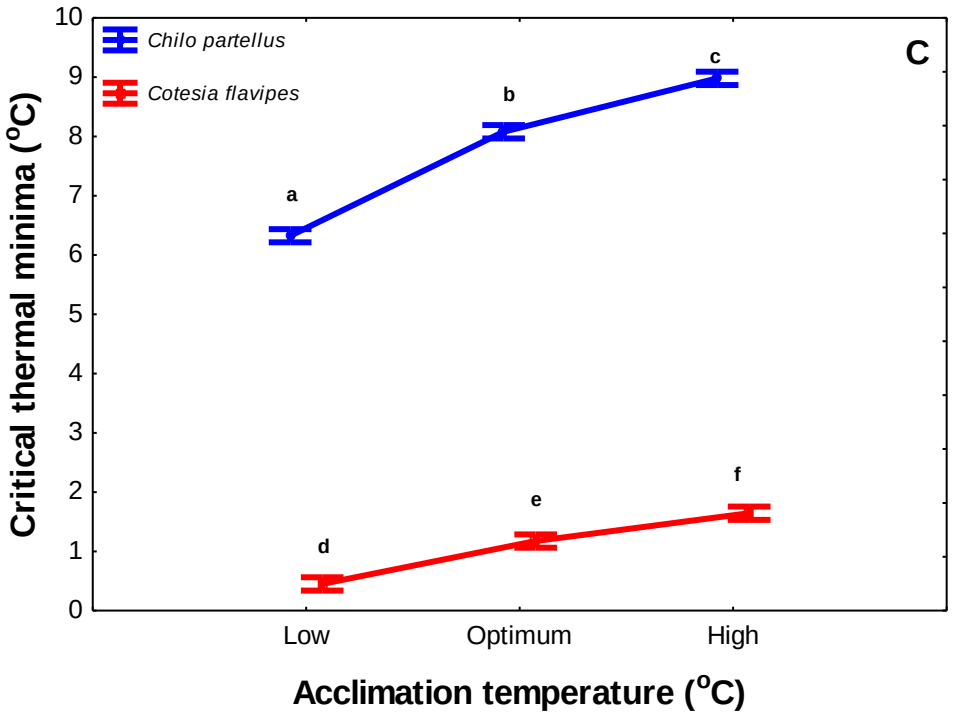


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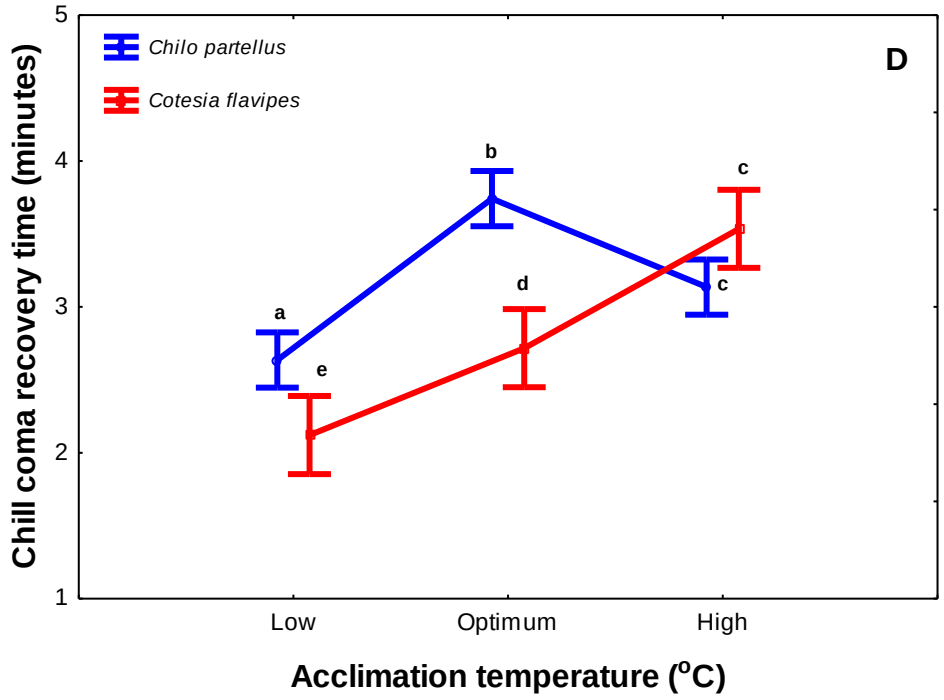


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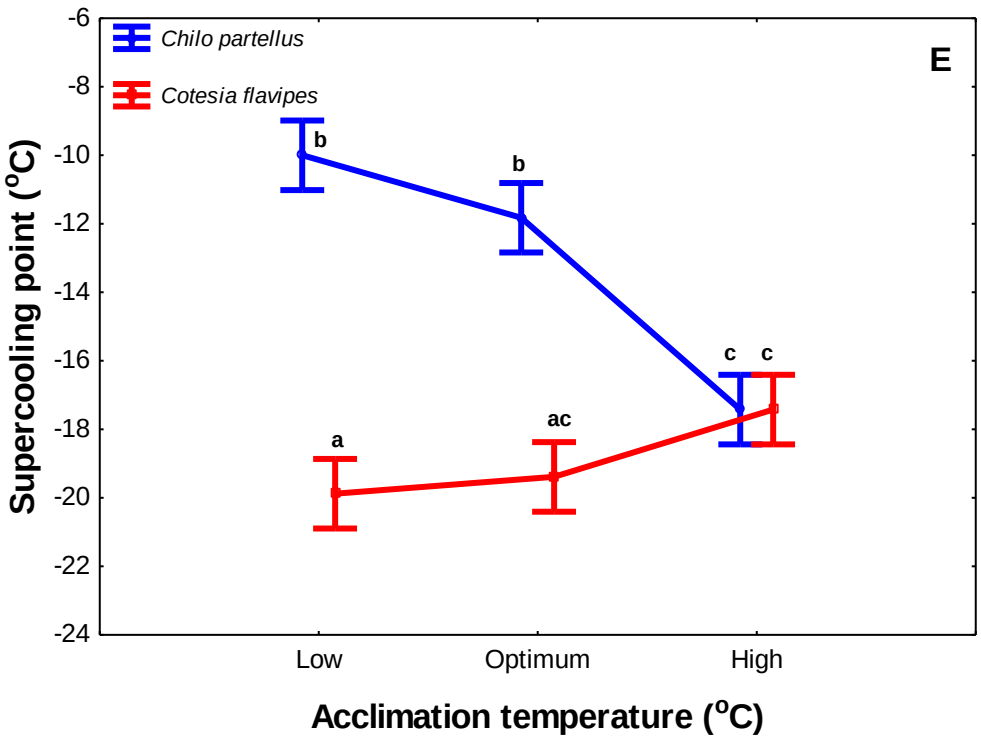
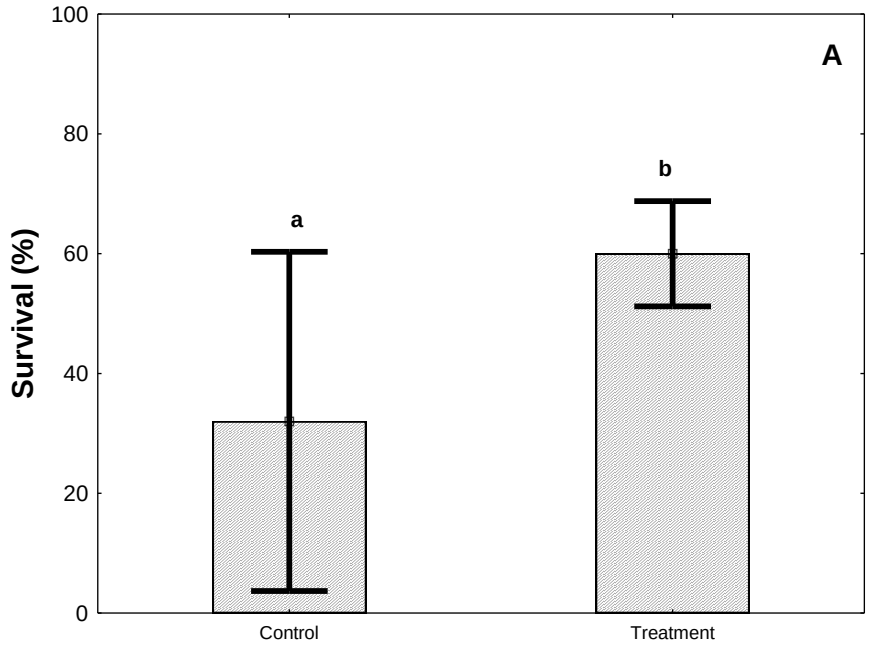
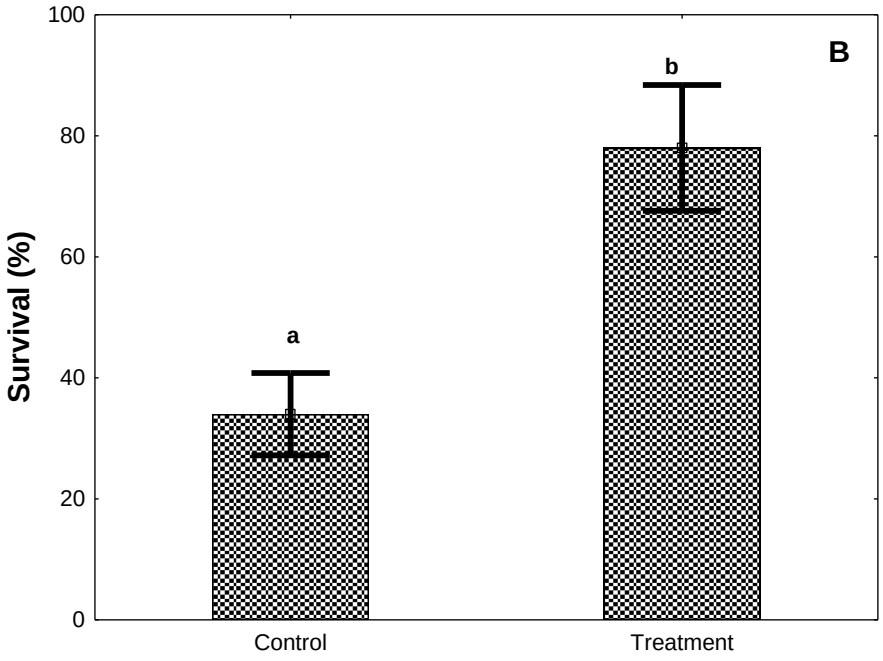


Figure 4.2: Summary low temperature plasticity results showing (A) effects of 2h pretreatments/hardening on survival at discriminating low temperatures of *Chilo partellus* larvae (pretreatment [2°C 2h] on low temperature survival [-8°C 2 h]), (B) effects of (pretreatment [3°C 2 h] on low temperature survival [-7°C 2 h]) on *Cotesia flavipes* adults, (C) acclimation effects to low optimum and high temperature (23; 28 and 33°C respectively) on CT_{min}, (D) CCRT (at 0°C chill coma temperature) and (E) SCP. Error bars represent 95% CLs (N = 50 per group). Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each species

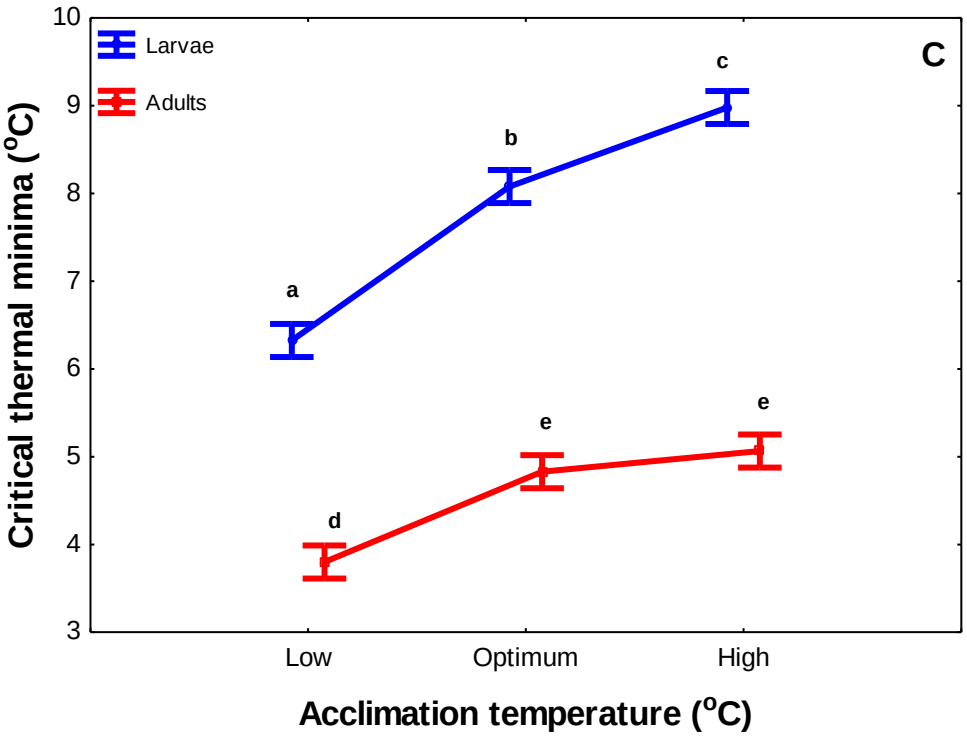


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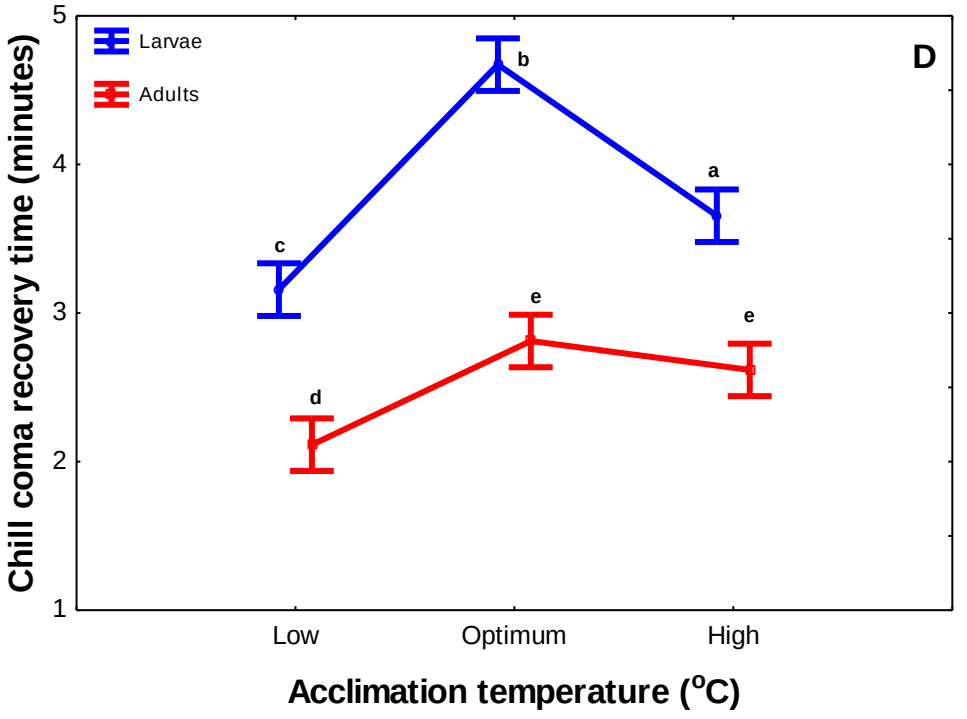


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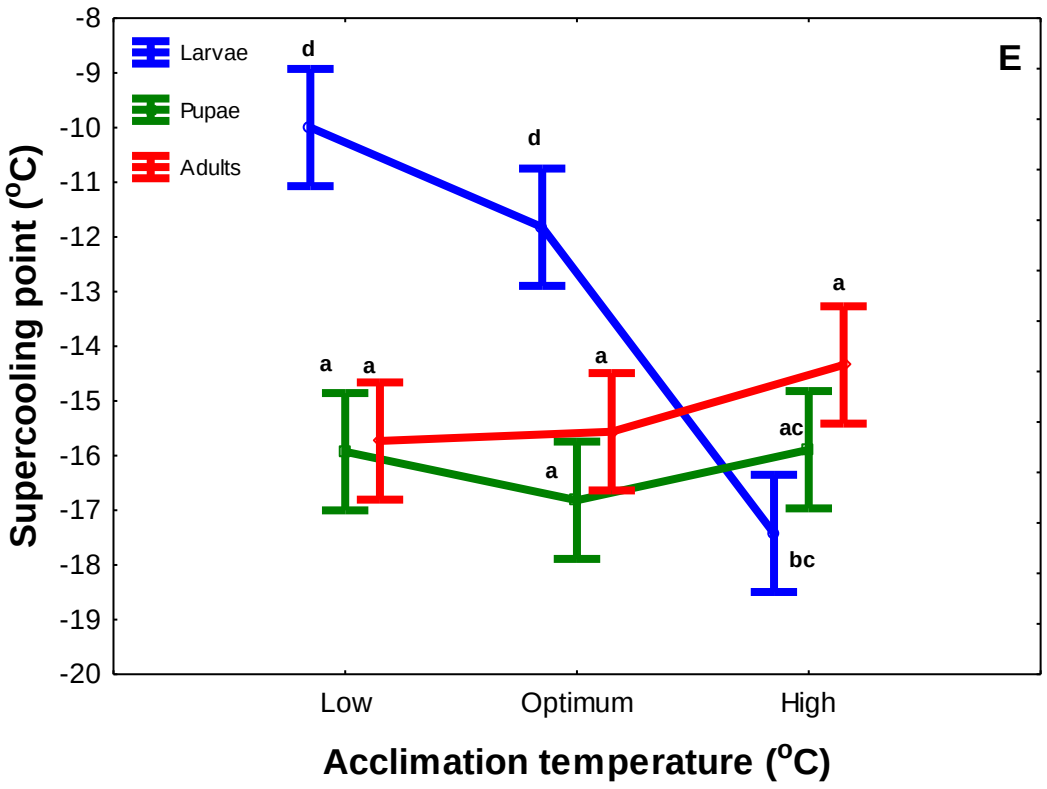


Figure 4.3: Summary low temperature plasticity results showing (A) effects of 2h pretreatments/hardening on survival at discriminating low temperatures of *Chilo partellus* pupae (pretreatment [2°C 2h] on low temperature survival [-8°C 2 h]), (B) effects of (pretreatment [1°C 2 h] on low temperature survival [-9°C 2 h]) on *Chilo partellus* adults, (C) acclimation effects to low optimum and high temperature (23; 28 and 33°C respectively) on CT_{min}, (D) CCRT (at 0°C chill coma temperature) and (E) SCP. Error bars represent 95% CLs (N = 50 per group). Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each developmental stage

Table 4.2: Summary results from a full factorial ANOVA showing the effects of species (*C. partellus* and *C. flavipes*) and temperature acclimation (low, optimum and high) on low temperature activity measured as CT_{min}, CCRT and SCP. SS = sums of squares, d.f.=degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	2368.297	1	2368.297	36991.96	< 0.001
	Acclimation	76.166	2	38.083	594.84	< 0.001
	Species	1348.711	1	1348.711	21066.39	< 0.001
	Species*Acclimation	11.258	2	5.629	87.92	< 0.001
	Error	7.299	114	0.064		
CCRT	Intercept	2133.243	1	2133.243	3850.196	< 0.0001
	Acclimation	44.028	2	22.014	39.732	< 0.0001
	Species	8.667	1	8.667	15.642	< 0.0001
	Species*Acclimation	20.827	2	10.413	18.795	< 0.0001
	Error	146.272	264	0.554		
SCP	Intercept	24541.45	1	24541.45	5883.550	< 0.0001
	Acclimation	105.76	2	52.88	12.677	< 0.0001
	Species	811.54	1	811.54	194.558	< 0.0001
	Species*Acclimation	427.18	2	213.59	51.206	< 0.0001
	Error	375.41	90	4.17		

Table 4.3: Summary results from a full factorial ANOVA showing the effects of *C. partellus* developmental stage (larvae and adults) and temperature acclimation (low, optimum and high) on low temperature activity measured as CT_{min}, CCRT and SCP. SS = sums of squares, d.f.= degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	4583.088	1	4583.088	25327.07	< 0.0001
	Acclimation	81.369	2	40.685	224.83	< 0.0001
	Developmental stage	312.987	1	312.987	1729.63	< 0.0001
	Developmental Stage*Acclimation	9.666	2	4.833	26.71	< 0.0001
	Error	20.629	114	0.181		
CCRT	Intercept	1810.387	1	1810.387	7510.453	< 0.0001
	Acclimation	36.831	2	18.415	76.397	< 0.0001
	Developmental stage	77.710	1	77.710	322.382	< 0.0001
	Developmental Stage*Acclimation	6.713	2	3.357	13.925	< 0.0001
	Error	41.943	174	0.241		
SCP	Intercept	31693.49	1	31693.49	6733.752	< 0.0001
	Acclimation	96.63	2	48.31	10.265	< 0.0001
	Developmental stage	245.59	2	122.79	26.089	< 0.0001
	Developmental Stage*Acclimation	409.42	4	102.36	21.747	< 0.0001
	Error	635.40	135	4.71		

4.3.2 Phenotypic Plasticity of High Temperature Tolerance

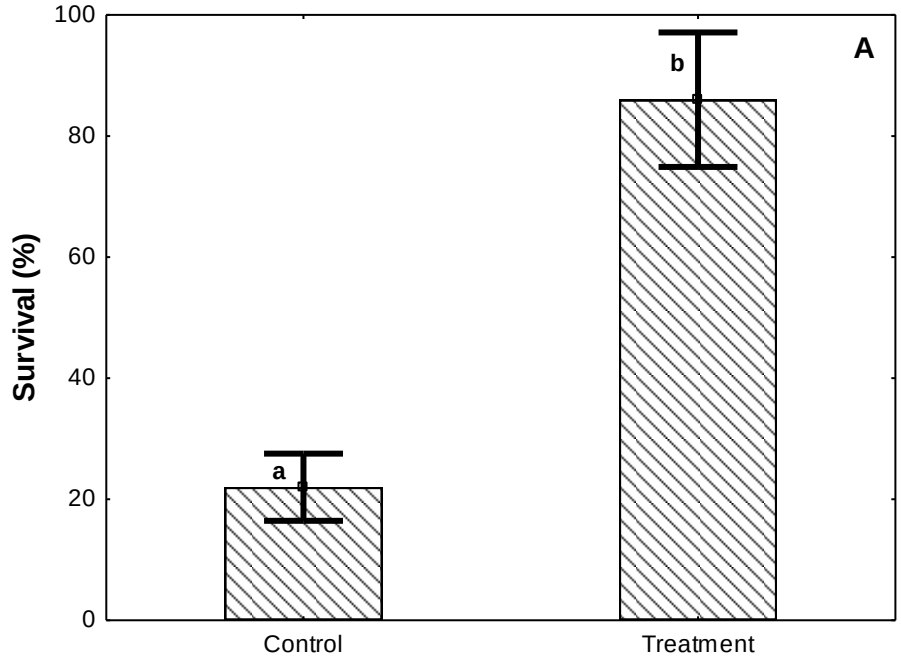
As in low temperature hardening, 2 h high temperature pretreatments (35 and 41°C) significantly improved survival during 2 h exposure at discriminating high temperatures (40 and 46°C) in *C. partellus* larvae (χ^2 =105.54, d.f =1, $P < 0.001$) (Figure 4.4A) and *C. flavipes* adults (χ^2 =96.41, d.f =1, $P < 0.001$) (Figure 4.4B) respectively. Furthermore, RHH at 42 and 39°C significantly improved high temperature survival at 47 and 44°C for *C. partellus* pupae (χ^2 =27.12, d.f =1, $P < 0.0001$) (Figure 4.5A) and adults (χ^2 =23.42, d.f =1, $P < 0.0001$) (Figure 4.5B) respectively.

There was a significant interaction effect among the factors acclimation, species and acclimation by species for high temperature tolerance *vis a vis* CT_{max} and HKDT (Table 4.4). A comparison of *C. partellus* developmental stages for CT_{max} and HKDT showed a significant acclimation and developmental effect for both CT_{max} and HKDT, but interaction effects for the two (developmental stage x acclimation) were only significant for HKDT (Table 4.5).

Acclimation to high temperature (33°C) significantly enhanced CT_{max} for both *C. flavipes* and *C. partellus* larvae (Figure 4.4C). High temperature acclimation marginally increased CT_{max} for *C. partellus* larvae and adults whilst low temperature acclimation showed no significant differences from the control (Figure 4.4C). High temperature (33°C for 3 days) acclimation increased HKDT for *C. flavipes* but had no effect on its larval *C. partellus* host (Figure 4.4D). Similarly, low temperature (23°C for 3 days) acclimation compromised HKDT for larval host *C. partellus* while enhancing the trait for parasitoid *C. flavipes* (Figure 4.4D). A comparison of *C. partellus* developmental stages shows high temperature acclimation increasing CT_{max} for larvae and no effect on adults (Figure 4.5C). Moreover, acclimation to high temperature also increased HKDT for adults, but not for larvae (Figure 4.5D).

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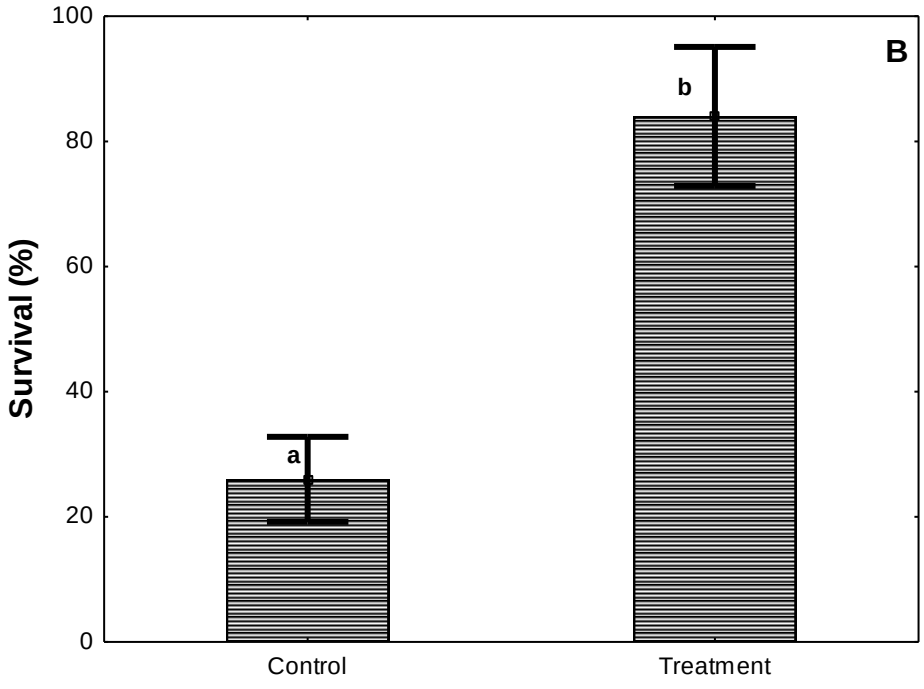
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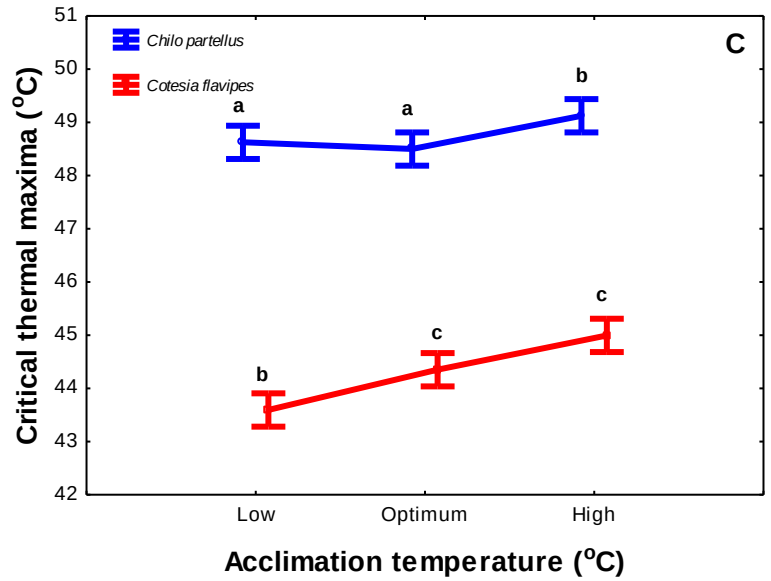
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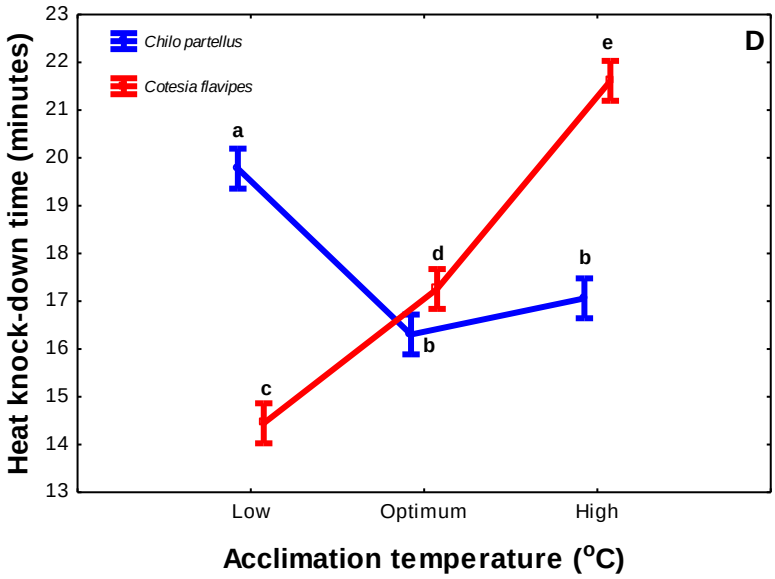
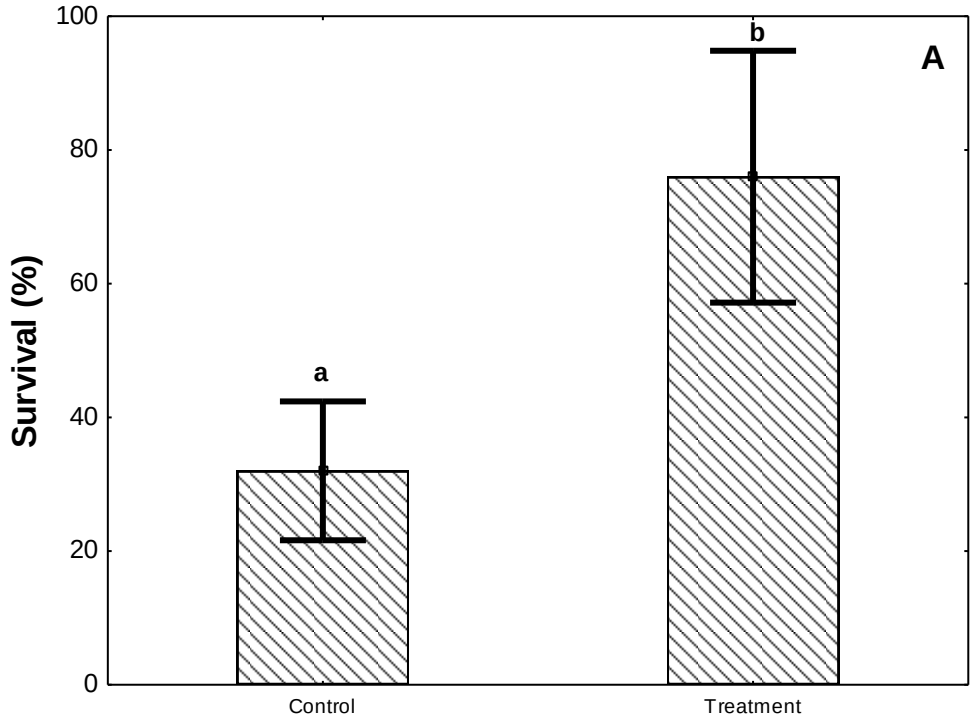
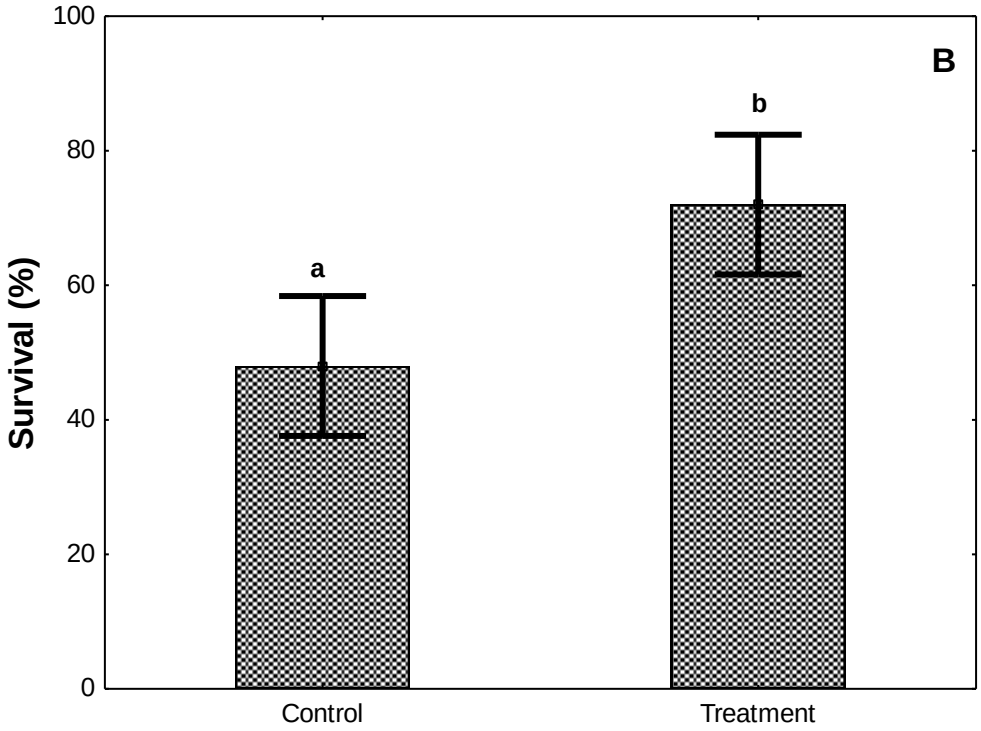


Figure 4.4: Summary low temperature plasticity results showing (A) effects of 2h pretreatments/hardening on survival at discriminating high temperatures of *Chilo partellus* larvae (pretreatment [41°C 2h] on high temperature survival [46°C 2 h]), (B) effects of (pretreatment [35°C 2 h] on high temperature survival [40°C 2 h]) on *Cotesia flavipes* adults, (C) acclimation effects to low optimum and high temperature (23; 28 and 33°C respectively) on CT_{max} and (D) HKDT (at 45 and 49°C knockdown temperature for *C. flavipes* adults and *C. partellus* larvae respectively). Error bars represent 95% CLs (N = 50 per group). Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each species

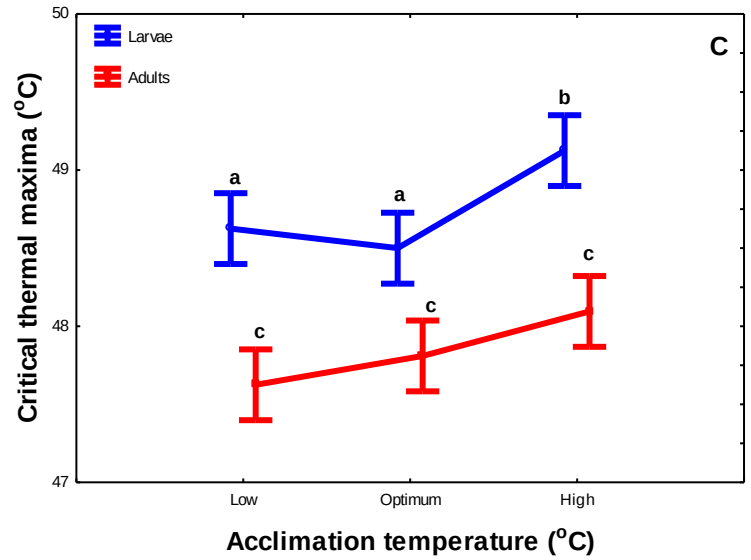


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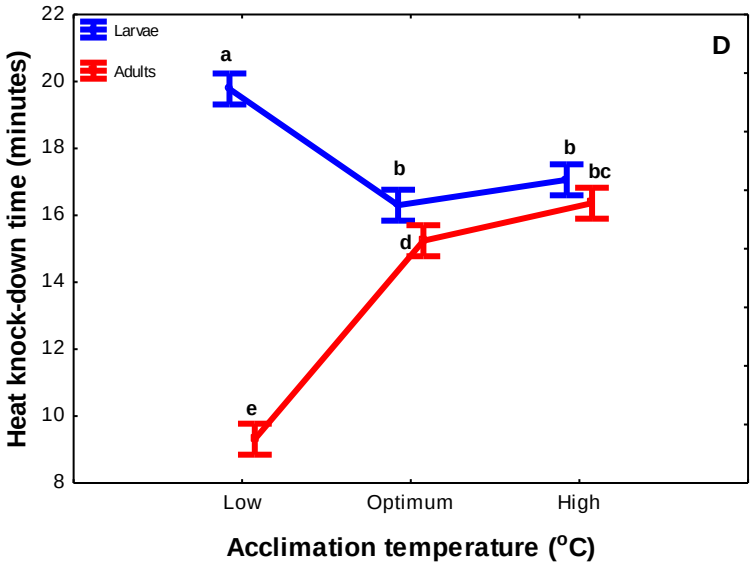


Figure 4.5: Summary low temperature plasticity results showing (A) effects of 2h pretreatments/hardening on survival at discriminating high temperatures of *Chilo partellus* pupae (pretreatment [42°C 2h] on high temperature survival [47°C 2 h]), (B) effects of (pretreatment [39°C 2 h] on high temperature survival [44°C 2 h]) on *Cotesia flavipes* adults, (C) acclimation effects to low optimum and high temperature (23; 28 and 33°C respectively) on CT_{max} and (D) HKDT (at 49°C knockdown temperature). Error bars represent 95% CLs (N = 50 per group). Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each developmental stage

Table 4.4: Summary results from a full factorial ANOVA showing the effects of species (*C. partellus* and *C. flavipes*) and temperature acclimation (low, optimum and high) on high temperature activity measured as CT_{max} and HKDT. SS = sums of squares, d.f.=degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{max}	Intercept	259821.7	1	259821.7	520710.5	< 0.0001
	Acclimation	18.7	2	9.4	18.8	< 0.0001
	Species	590.6	1	590.6	1183.6	< 0.0001
	Species*Acclimation	5.3	2	2.6	5.3	< 0.01
	Error	56.9	114	0.5		
HKDT	Intercept	67121.44	1	67121.44	8642.910	< 0.0001
	Acclimation	876.76	2	438.38	56.448	< 0.0001
	Species	263.38	1	263.38	33.915	< 0.0001
	Species*Acclimation	261.25	2	130.62	16.820	< 0.0001
	Error	2050.24	264	7.77		

Table 4.5: Summary results from a full factorial ANOVA showing the effects of *C. partellus* developmental stage (larvae and adults) and temperature acclimation (low, optimum and high) on high temperature activity measured as CT_{max} and HKDT. SS = sums of squares, d.f.= degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{max}	Intercept	279908.2	1	279908.2	1067923	< 0.0001
	Acclimation	5.9	2	3.0	11	< 0.0001
	Developmental stage	24.7	1	24.7	94	< 0.0001
	Developmental Stage*Acclimation	0.7	2	0.4	1	0.262871
	Error	29.9	114	0.3		
HKDT	Intercept	44231.72	1	44231.72	26834.95	< 0.0001
	Acclimation	141.82	2	70.91	43.02	< 0.0001
	Developmental stage	746.85	1	746.85	453.10	< 0.0001
	Developmental Stage*Acclimation	919.36	2	459.68	278.88	< 0.0001
	Error	286.80	174	1.65		

4.3.3 Microclimate data

Microclimate data revealed several periods where conditions were suitable for eliciting RCH and RHH (Figure 4.6). For *C. partellus*, only one incidence was suitable for RCH whilst the larval and pupal stages had 11. *Cotesia flavipes* had 15 potential RCH events with temperatures and sufficient duration to elicit such responses as determined under laboratory conditions (see table 4.1). In addition, a total of 51, 42, 18 and 3 potential RHH events

corresponding to *C. flavipes* adults, *C. partellus* adults, larvae and pupae respectively were recorded during the same period (Figure 4.6).

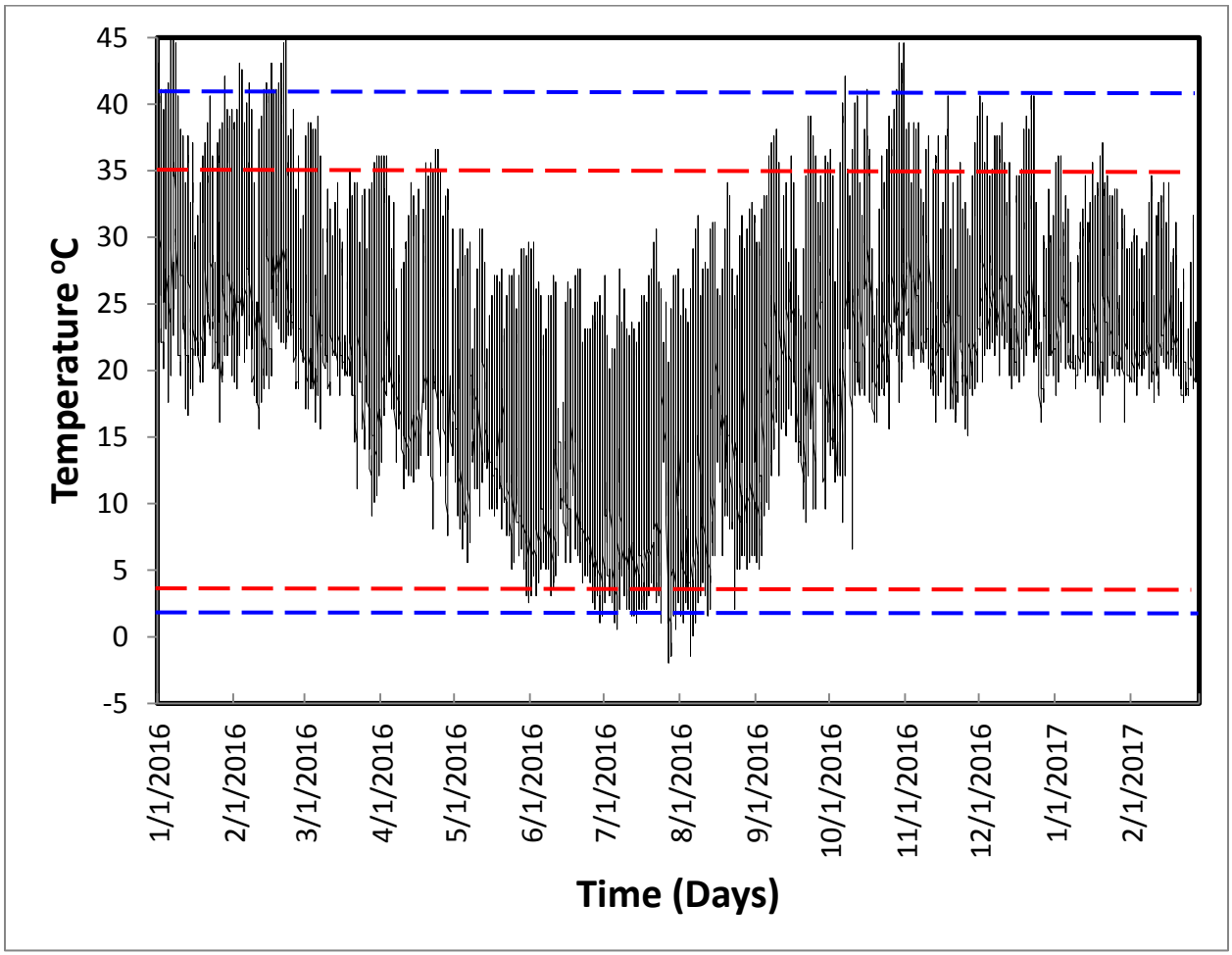


Figure 4.6: Microclimatic temperature data in austral summer and winter (January 2016 – February 2017) from a maize field, BCA Farm, Gaborone, Botswana (S24.56449; E25.94883; 994 m.a.s.l), which hosts both *Chilo partellus* and parasitoid *C. flavipes*. Temperature data was recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5°C accuracy; 1 h sampling frequency). The number of events likely eliciting RCH and RHH within the same timeframe was calculated based on temperature-time interactions. Red and blue dashed lines represents RCH and RHH temperatures for *Cotesia flavipes* and *Chilo partellus* respectively.

4.4 Discussion

Global change exerts a major pressure on insect relative fitness, phenology and demographic patterns with subsequent impact on trophic interactions and ecosystem function (Bale, 2010; Bannerman *et al*., 2011; Ma *et al*., 2014). Indeed, temperature has a critical impact on specialist predator-prey systems such as parasitoid-host relationships that are important in agriculture (Bannerman *et al*., 2011; Romo & Tylianakis, 2013). As a result, thermal limits to activity, symbolizing the physiological range of host versus parasitoid across thermal gradients ought to be known. Parasitoid thermal windows and thresholds have direct consequences on parasitism and hence efficacy of biological control (Romo & Tylianakis, 2013; Schreven *et al*., 2017). A comprehensive understanding of host and parasitoid basal thermal tolerance coupled with plasticity thereof is significant in determining potential net agroecosystem responses among the interacting trophic levels under global climate change (Romo & Tylianakis, 2013; Mutamiswa *et al*., 2017b). To my knowledge, this is the first report detailing plasticity of thermal tolerance of *C. partellus* and *C. flavipes,* two key antagonistic insects in staple cereal production systems of sub-Saharan Africa.

4.4.1 Phenotypic Plasticity of Low Temperature Tolerance

Investigating plasticity of cold tolerance provides a guideline on how natural enemies released for biocontrol will establish under natural conditions (Hatherly *et al*., 2009). The current study reported positive RCH effects in *C. partellus* and *C. flavipes* in keeping with previous studies in *D. melanogaster* (Overgaard & Sørensen, 2008), *Thaumotibia leucotreta* (Stotter & Terblanche, 2009), *C. rosa* (Nyamukondiwa *et al*., 2010), *Z. vittiger* (Nyamukondiwa & Terblanche, 2010a) and *C. pomonella* (Chidawanyika & Terblanche, 2011a). This mechanism improves key fitness traits (Shreve *et al*., 2004) and may likely help both trophic levels in surviving sudden cold snaps, typical under global change. By improving cold tolerance through RCH, *C. partellus* will modify its relationship with parasitoid *C. flavipes*. Hence, for the later to thrive, it should also adaptively respond with phenotypic plasticity matching that of its host in order to keep their phenologies in sync. Indeed, plasticity follows such patterns, among interacting trophic levels, and in all cases where plastic responses offer higher fitness benefits than canalization (Whitman & Ananthakrishnan, 2009). Mechanisms behind RCH responses are not clear but may include the accumulation of carbohydrate cryoprotectants, membrane

restructuring and inhibition of apoptotic cell death (Lee & Denlinger, 2010; reviewed in Teets & Denlinger, 2013). In the ecosystem, RCH response permits insects to adjust their physiological condition so that it matches environmental conditions (Yi *et al*., 2017). Under natural conditions, insects often face concurrent exposure to several stressors. For instance, cold fronts have been reported to reduce relative humidity and temperature (Moeller *et al*., 1993), suggesting that insects may experience brief chilling and desiccation (Yi *et al*., 2017) under these environmental conditions. Given that *C. partellus* and *C. flavipes* can cold harden, it likely explains their survival merit during winter seasons in tropical regions. Furthermore, this implies that RCH may allow these two species to track diurnal or seasonal environmental temperature fluctuations (Terblanche *et al*., 2010) thus enhancing development, courtship behaviours, mating and feeding during their lifetime (Kelty & Lee, 2001; Shreve *et al*., 2004). Nevertheless, plastic responses to low temperature may also be maladaptive (Whitman & Ananthakrishnan, 2009). A comparison of the host and its parasitoid shows that both species have almost similar levels of survival and magnitude of plasticity to acute low temperature exposures following pretreatment to sub-lethal low temperatures. However, these species differ in hardening and discriminating temperatures, with *C. partellus* cold hardening at lower temperatures than *C. flavipes*. Microclimatic temperature data recorded in an agro-ecosystem where both *C. partellus* and *C. flavipes* coexist showed that temperatures eliciting RCH responses at both trophic levels occur naturally (Figure 4.6). This may imply that under current climate variability (IPCC, 2014), RCH may be an important mechanism aiding fitness (Yi *et al*., 2007; Kristensen *et al*., 2008). This has been true for some insects, which can rapidly cold harden under ecological ramping rates (Lee *et al*., 1987; Nyamukondiwa *et al*., 2013).

Similar to RCH responses, I also document significant effects of acclimation on CT_{min}. Acclimation effects on thermal tolerance may depend on severity of stress exposure and has been reported to vary across species (Kellett *et al*., 2005), developmental stages (Terblanche *et al*., 2007: Marais *et al*., 2009) and also thermal history (Nyamukondiwa & Terblanche, 2010b). The current results show that acclimation to low temperature significantly decreased CT_{min} (thus, improved cold tolerance) in both host *C. partellus* and parasitoid *C. flavipes*, in keeping with previous studies to date e.g. *Glossina pallidipes* (Terblanche *et al*., 2006) and *Drosophila* species (Nyamukondiwa *et al*., 2011). In addition, acclimation to high temperature improved CT_{min} for both species, suggesting significant cross-tolerance in corroboration with a previous study on

Linepithema humile (Jumbam *et al*., 2008) and *D. melanogaster* (Bubliy *et al*., 2012). This cross-tolerance suggests the presence of overlapping thermal stress resistance mechanisms (Bubliy *et al*., 2012) or signal pathways ('cross talk') (see Sinclair *et al*., 2013). Species comparison of CT_{min} between the two trophic levels (Figure 4.2C), and across all acclimation treatments showed that *C. flavipes* is more cold tolerant than *C. partellus*. This may have a positive effect on *C. flavipes* survival under variable low temperatures, relative to its herbivore host *C. partellus*. Similarly, a comparison of *C. partellus* pupae and adults showed that indeed they can also adjust their low temperature tolerance at short timescales (Figure 4.3A; B). Critical thermal minima and CCRT also showed the same positive acclimation trends for *C. partellus* pupae and adults (Figure 4.3C; D).

I also recorded an enhanced CCRT in *C. partellus* following both low and high temperature acclimation. However, an improved CCRT in *C flavipes* only occurred following low temperature acclimation (Figure 4.2D). The results corroborate findings in *D. melanogaster* (Hoffmann *et al*., 2005) and *Acheta domesticus* (Lachenicht *et al*., 2010) where significant plastic responses for CCRT were recorded following acclimation to summer and winter conditions. Furthermore, *C. flavipes* generally recovered faster from chill coma than host *C. partellus* suggesting that when faced with chill coma temperatures, the parasitoid has better survival chances than the host. These experimentally investigated chill-coma temperatures were frequently detected in nature (Figure 4.6), implying that chill coma recovery may be a key aspect for the parasitoid to preserve key activities. It has been reported that insects are more prone to attack by predators during chill-coma (see Alford *et al*., 2014). I therefore speculate, with caveats, that the superior chill-coma recovery times prevalent in *C. flavipes* may improve predation rates of *C. partellus* in nature, under variable stressful chill coma temperatures.

The superior enhanced cold tolerance in *C. flavipes* following acclimation to low temperatures was also confirmed by much lower SCPs contrary to what was the case with *C. partellus* (Figure 4.2E). Even though acclimation to high temperatures significantly improved cold tolerance in *C. partellus*, there was no significant difference between the two species after pretreatments at this thermal regime. Insect species with very low SCPs (~-20°C to -30°C) and high cold tolerance levels are regarded as freeze intolerant (Bale, 1996). *Cotesia flavipes* showed SCP of approximately ~-20°C and high levels of cold tolerance following acclimation to low temperatures relative to *C. partellus*. The mechanisms underlying such supercooling capacity

have been reported to include body osmolyte accumulation, clearing of gut contents, specific antifreeze compounds and use of thermal hysteresis proteins (Jones *et al*., 2008; Denlinger & Lee, 2010) suggesting that it may be freeze avoiding. Hence, the parasitoid may survive more acute lower temperatures, a fitness benefit over its host. A comparison of SCPs for *C. partellus* developmental stages showed that pupae had the lowest SCPs relative to larvae and adults (Figure 4.3E). This is consistent with the notion that immobile life-stages with limited capacity to behaviorally thermoregulate, display more phenotypically plasticity with regards to tolerance of thermal extremes (Chown & Nicolson, 2004). Consequently, they have evolved an inherent basal low temperature tolerance (see Lansing *et al*., 2000; Bowler & Terblanche, 2008). This is in agreement with Nyamukondiwa *et al*. (2013), who reported that immobile pupae have inherent low SCPs. Overall, low temperature plasticity results may confer activity and thus fitness advantage for *C. flavipes* relative to its larval host *C. partellus*. In the absence of reciprocal low temperature phenotypic plasticity on the part of the host, this represents a positive trend on biological control under global change (see Agrawal, 2001).

4.4.2 Phenotypic Plasticity of High Temperature Tolerance

Climate scenarios are hypothesized to involve both rising temperatures and frequent rapid changes in diurnal thermal fluctuations as evidenced by the recent occurrence of unusual heat waves in many countries (IPCC, 2014). As such, survival of high temperature variability is critical for survival and interaction of different trophic level species. The current results document a positive RHH response in the host *C. partellus*` larvae and adult *C. flavipes* parasitoids (Figure 4.4A; B). The temperatures eliciting such hardening responses were occasionally recorded in the field. Hence such plasticity, or lack thereof, may mediate the host-parasitoid interactions in tropical agroecosystems in current and future climate change scenarios. Several studies investigating different insect taxa have also reported such RHH and demonstrated its potential to improve survival under rapidly changing thermal environments (Kalosaka *et al*., 2009; Nyamukondiwa *et al*., 2010; Chidawanyika & Terblanche, 2011a; Chidawanyika *et al*., 2017). Similarly, RHH also improved survival of *C. partellus* pupae and adults (Figure 4.5A; B). Mechanisms eliciting RHH responses include heat shock proteins (HSPs) which prevent the accumulation of structurally denatured proteins (Hoffmann *et al*., 2003). In nature, these results imply that these two interacting species may survive variable thermal environments using RHH

responses. This allows them to track diurnal or seasonal environmental temperature fluctuations (Terblanche *et al*., 2010; Nyamukondiwa *et al*., 2013). Although larval *C. partellus* and *C. flavipes* generally have similar magnitude of RHH responses they, nevertheless, vary significantly in hardening and discriminating temperatures (see Table 4.1). *Chilo partellus* discriminating and hardening temperature is higher than that of its endoparasitoid *C. flavipes*. This implies that, while both species may acutely adjust their high temperature plasticity, *C. partellus* does so, at significantly higher basal temperatures than its parasitoid. This means under more severe high temperature fluctuations, plasticity alone may not be able to cushion the parasitoid, since its thermal windows of activity are more benign as compared to those of *C. partellus.* This gives the insect pest (host) a survival advantage over the biocontrol agent (parasitoid), under intense and variable high temperature conditions. Moreover, a look at microclimate data (Figure 4.6) shows that the parasitoid is more likely to survive under stressful high temperature conditions (≥35°C), than its larval host (≥41°C). Hence the parasitoid may suffer more fitness and mortality as a consequence of repeated stressful conditions under natural conditions, relative to its host *C. partellus* (see Chown SL and Nicolson, 2004; Schreven *et al*., 2017).

Acclimation to high temperature generally increased CT_{max} in *C. partellus* and *C. flavipes* in keeping with previous studies on *Ceratitis* species (Nyamukondiwa & Terblanche, 2010b; Weldon *et al*., 2011) Drosophilids (Nyamukondiwa *et al*., 2011) and *Nezara* species (Chanthy *et al*., 2012). Mechanisms for improving this CT_{max} may comprise up-regulation of HSPs consequently minimising aggregation of denatured proteins following heat stress (Edgerly *et al*., 2005). Significant differences in CT_{max} between *C. partellus* and *C. flavipes* across all acclimation treatments indicate that *C. partellus* is more heat tolerant than *C. flavipes* (Figure 4.4D). This means under more drastic thermal variability (e.g. ~45°C), parasitoid *C. flavipes* may be more vulnerable as opposed to the insect pest *C. partellus*, and this has implications on the ecological balance and pest management using the parasitoid. While *C. flavipes* is more cold tolerant than *C. partellus* and African temperatures expected to increase by 1.5 to 3°C by 2050 (IPCC, 2014), the host stemborer pest is highly likely to survive high temperatures compared to its parasitoid, and thus emerge 'winner' relative to its biocontrol parasitoid. Unless the parasitoid mounts constitutive and plastic counter-responses to its host, this may create asynchronous phenologies with negative implications on pest management using the parasitoid.

Acclimation to high temperatures increased HKDT in *C. flavipes* but not in *C. partellus*, suggesting adaptive phenotypic plasticity for the parasitoid and not the larval insect pest for HKDT. This is contrary to CT_{max} results, which documented adaptive phenotypic plasticity for both parasitoid and host, although the host has a merit for having an inherent basal high temperature tolerance. The reason for this trend for HKDT is unknown. However, this may mean that establishing trophic level differences in thermal sensitivity may be complex and most likely trait dependent. Nevertheless, acclimation to low temperature significantly improved HKDT in the insect pest, indicating the presence of cross-tolerance (see Bubliy *et al*., 2012). This was not the case with the parasitoid as low temperature acclimation actually resulted in shorter HKDT.

According to theoretical models (Gabriel *et al*., 2005), insects surviving in more thermal heterogeneous environments are highly likely to have greater magnitude of plasticity compared to those from more homogeneous environments. Since climate change is accompanied by increased climate variability (IPCC, 2014), this implies that acclimation may be a significant physiological mechanism allowing host-parasitoid associations adjust their phenotype and develop compensatory mechanisms to survive thermally heterogeneous environments. Basing on the measurements of CTLs, HKDT and CCRT in larval *C. partellus* host and parasitoid *C. flavipes*, my results generally support the beneficial acclimation hypothesis (Leori *et al*., 1994).

In conclusion, the current results document plasticity of thermal tolerance in *C. partellus* and its larval parasitoid, *C. flavipes*, and implications for pest management using biological control under climate change. My results generally suggest that plasticity may enhance survival of these two species to extreme low and high temperatures. However, parasitoid *C. flavipes* may have fitness advantages at low temperatures than the larval *C. partellus* host, while the latter becomes fitter at high temperatures, (though different for HKDT). In both cases, differential plastic responses may symbolize loss of coevolved relationships between the two trophic levels, and have negative consequences on biological control. These results have two major implications that are of broader importance: 1) with global climate change, *C. partellus* may inhabit slightly warmer environments than its parasitoid *C. flavipes* (performs better in cooler environments), 2) that host-parasitoid relationships are complex and likely trait dependent, 3) host- parasitoid differential plastic responses indicate loss of long coevolved interactions which may offset efficacy of biocontrol under rapidly changing environments.

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CHAPTER 5

Thermal resilience may shape population abundance of two sympatric congeneric *Cotesia* species (Hymenoptera: Braconidae) *

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5.1 Introduction

Abiotic factors such as temperature and relative humidity have a direct effects on development, reproduction, abundance, biogeography (Hoffman *et al*., 2012; Nguyen *et al*., 2014) and survival of ectotherms (Loeschcke & Hoffmann, 2007; Ma *et al*., 2014), including parasitoids (Thomas & Blanford, 2003; Hance *et al*., 2007). Of these, temperature is considered the predominant abiotic factor affecting both herbivorous insects and their antagonistic biological control agents (Bale *et al*., 2002; Romo & Tylianakis, 2013). Given that most insects are ectotherms, and their population growth is temperature driven, extreme temperatures have a bearing on their rate of development as well as biochemical and physiological processes (Nyamukondiwa & Terblanche, 2009). Since most insects have a limited ability to control body temperature, they have developed a range of mechanisms for survival under stressful thermal environments (Bale & Hayward, 2009). Some of the mechanisms involve behavioral avoidance (Bale & Hayward, 2009), morphological adaptations (Nyamukondiwa *et al*., 2010) as well as daily (Overgaard & Sorenson, 2008; Nyamukondiwa *et al*., 2013) and seasonal (Khani & Moharramipour, 2010; Chidawanyika & Terblanche, 2011a) thermal tolerance adjustment. However, failure to employ some compensatory mechanisms to survive these extreme temperatures may offset fitness traits, ultimately leading to limited activity and poor performance of life-history traits leading to population decline and seldom species extinction (Chown & Terblanche, 2007). Insect temperature tolerance is typically not static (Nyamukondiwa & Terblanche, 2009; Stotter & Terblanche, 2009), and may be influenced by a range of factors including age (Nyamukondiwa & Terblanche, 2009), developmental stage (Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009), thermal history (Nyamukondiwa & Terblanche, 2010; Chidawanyika *et al*., 2017) and species ecological and evolutionary history (e.g. temperate vs. tropical environment) (Kleynhans *et al*., 2014). These factors, combined with complex interactions between duration and severity of exposure may determine an insect's thermal tolerance with longer or more severe exposures typically lethal (Chown & Nicholson, 2004; Stotter & Terblanche, 2009). Thermal life history and physiological tolerances vary in insects with some species emerging 'winners' whilst others succumb to detrimental effects (Parmesan, 2006; Mutamiswa *et al*., 2017a) thus disrupting their phenological and biogeographic patterns (Bale *et al*., 2002). Parasitoids such as *Cotesia sesamiae* and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) have different evolutionary and thermal history patterns, even

4031 though they have been reported to coexist occupying the same ecological niche (Overholt *et al*.,
4032 2000; Zhou *et al*., 2003; Mailafiya *et al*., 2011). This competitive interaction has been beneficial
4033 in African agriculture as the combined effect of the two species has led to increased pest
4034 suppression (Zhou *et al*., 2001; Kfir *et al*., 2002). However of concern, is how population
4035 decreases in one species following environmental perturbations e.g. thermal stress may affect
4036 host stemborer abundance outbreaks, and the likely costs of increased pest pressure.

4037 *Cotesia flavipes* is a gregarious larval endoparasitoid of lepidopteran stemborers (Manjoo
4038 & Bajpai, 2011). It is native to Asia and is the closest coevolved larval parasitoid of *Chilo*
4039 *partellus* Swinhoe (Padmaja & Prabhakar, 2004; Divyal *et al*., 2009; Dejen *et al*., 2013).
4040 Following its first release in Kenya in 1993 (Dejen *et al*., 2013), it was subsequently introduced
4041 in various east and southern African countries where it successfully controlled *C. partellus* in
4042 maize and sorghum (Assefa *et al*., 2008). On the other hand, *C. sesamiae* is indigenous to Africa
4043 and endemic to the sub-Saharan region. It belongs to the *C. flavipes* monophyletic complex
4044 (Kimani-Njogu & Overholt 1997; Muirhead *et al*., 2012) and is a generalist gregarious
4045 endoparasitoid of the entire lepidopteran cereal stemborer complex (Mailafiya *et al*., 2010).
4046 *Cotesia flavipes* and *C. sesamiae* have been reported to reduce lepidopteran stemborer densities
4047 between 32 and 55% in agroecosystems and are of importance in cereal farming in sub-Saharan
4048 Africa (Zhou *et al*., 2001; Kfir *et al*., 2002), in particular, maize (*Zea mays* L.) and sorghum
4049 *Sorghum bicolor* (L.) Moench which are the staple crops in large parts of this region. The
4050 distribution of these two *Cotesia* species is influenced by climate, with *C. sesamiae* common in
4051 wetter regions and *C. flavipes* common in warm and dry regions (Songa *et al*., 2001; Niyibigira,
4052 2003).

4053 Due to the discrepancies in both evolutionary origin and habitat preferences among these
4054 parasitoids, I sought to investigate survival and functional activity limits under acute thermal
4055 variability, typical in African agroecosystems. With global mean temperatures projected to
4056 increase by 1.4 to 5.8°C by 2100 (Walther *et al*., 2002; Karuppaiah & Sujayanad, 2012), coupled
4057 with increased frequencies of heat waves and cold snaps (IPCC, 2014), a comprehensive
4058 understanding of the thermal tolerance and performance of parasitoid species (Hance et al. 2002;
4059 Mutamiswa *et al*., 2017b) is of paramount importance for effective prediction of climate change
4060 impacts on insect population dynamics (Ward & Masters, 2007), extinction (Thomas *et al*., 2004;
4061 Amarasekare & Savage, 2012) and efficacy of biological control (Alford *et al*., 2016). Previous

studies reported that climate change may interrupt host-parasitoid relationships due to differences in thermal preferences thus increasing the risk of host outbreaks (Voigt *et al*., 2003; reviewed in Hance *et al*., 2007; de Sassi & Tylianakis, 2012, Mutamiswa *et al*., 2017a). While several studies have assayed thermal tolerance in insect pests (e.g. Koveos, 2001; Grout & Stoltz, 2007; Nyamukondiwa & Terblanche, 2009; Chidawanyika & Terblanche, 2011a; Piyaphongkul, 2013; Nguyen *et al*., 2014; Overgaard *et al*., 2014) and recently *C. partellus* and its parasitoid *C. sesamiae* (Mutamiswa *et al*., 2017a), to my knowledge, no studies have investigated the thermal tolerance of coexisting parasitoids of importance in agriculture. Here, I investigated basal thermal tolerance of indigenous and exotic larval endoparasitoids (*C. sesamiae* and *C. flavipes*, respectively) of lepidopteran cereal stemborers and implications on geographic distribution and biological control under climate change. I investigated ability to tolerate extreme temperatures (lower and upper) following exposure to different ranges of temperature-time interactions, lower and upper critical limits to activity, supercooling points, time to heat knock-down and recovery following chill coma. Since *C. sesamiae* is predominant in warm wetter regions (Songa *et al*., 2001; Niyibigira, 2003), indigenous to Africa and attacks a wide range of stemborer species (Mailafiya *et al*., 2009; 2010), I hypothesised that it has a higher basal thermal tolerance than the exotic *C. flavipes*. An in-depth understanding of the species` basal thermal tolerance is of importance in pest management including site selection for initial parasitoid release (based on thermal preferences), timing of augmentative releases and general designing of efficacious biological control programs for lepidopteran stemborers in changing climate. The study is also significant in explaining how introduced exotic parasitoids in classical biological control may shape ecosystem function and competitive ability of congeneric species occupying the same niche under dynamic environmental conditions.

5.2 Materials and Methods

5.2.1 Study Organisms and Rearing Conditions

Initial colonies of *C. sesamiae* and *C. flavipes* were obtained from South African Sugarcane Research Institute (SASRI), South Africa and International Centre for Insect Physiology and Ecology (ICIPE), Kenya, respectively. These insects had been in the laboratory

for more than 20 generations with regular supplementation with wild populations to maintain heterozygosity. *Cotesia sesamiae* colony was created using parasitized *S. calamistis* larvae whilst parasitised *C. partellus* larvae where used for *C. sesamiae* colony. Both colonies were maintained in the climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) under 12L:12D photoperiod, 28 ± 1°C and 65 ± 10% RH on artificial diet (Ochieng *et al*., 1985; Tefera *et al*., 2010) in 30 ml plastic vials with perforated screw-cap lids. Emerging parasitoid cocoons were separated and held according to species, under similar conditions in open Petri dishes placed in Bugdorm rearing cages (240mm^3; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) until eclosion. Eclosed parasitoids had access to food *ad libitum* (25% honey: water from a cotton wick) until they were used in thermal tolerance assays as 24–48 h-old adults.

5.2.2 Lower and Upper Lethal Temperature Assays

Using a direct plunge protocol in programmable water baths (Systronix, Scientific, South Africa), containing a mixture of propylene glycol and water (1:1 ratio to enable sub-zero temperature operation without freezing), LLT and ULT were assayed using established protocols by (Terblanche *et al*., 2008; Chidawanyika & Terblanche, 2011a; Mutamiswa *et al*., 2017a). In brief, ten 24-48 h old adults from each species (*C. sesamiae* and *C. flavipes*) (n = 50) were placed in 30ml polypropylene vials with perforated screw-cap lids and placed in water tight zip-lock bag which was submerged in a programmable water bath for each temperature/time treatment for either ULT or LLT assays. Digital thermometers (Fluke 53/54IIB, Fluke Cooperation, USA) were used to monitor the temperature of the water bath for the duration of each treatment. Post treatment, propylene vials containing assayed insects were placed in a climate chamber (28 ± 1°C, 65 ± 10% RH and 12:12 L:D photocycle, and supplied with food (25% honey: water from a cotton wick) during the whole recovery period. Survival was then recorded 24 hours post treatment. For the purposes of this study, survival was defined as coordinated muscle response to stimuli such as gentle prodding, or normal behaviors such as feeding, flying or mating (24 h post treatment). ULT assays for both species ranged from 35 to 44°C at 0.5, 1, 2 and 4 h duration of exposure or until 0-100% mortality was recorded. LLT assays also used a range of temperature × time combinations that realized 0-100% mortality from -15 to 5°C for 0.5, 1, 2 and 4 h. In all the assays 24–48 h-old adult parasitoids of mixed sex were

used, since sex seem to play no significant role in thermal tolerance traits (see discussions in Nyamukondiwa & Terblanche, 2009).

5.2.3 Critical Thermal Limits (CTLs)

Critical thermal limits (CT_{min} and CT_{max}) were measured using a dynamic protocol as outlined by Nyamukondiwa & Terblanche (2009). Ten individual adults of *C. sesamiae* or *C. flavipes* (24-48 h old) were randomly placed in a series of insulated double-jacketed chambers ('organ pipes') connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol and subjected to a constant cooling or heating rate. In the 'organ pipes', insects were first given 10 minutes to equilibrate at 28°C (equivalent to the benign rearing temperature) before ramping temperature up (CT_{max}) or down (CT_{min}) at a rate of $0.25^{\circ}C\ min^{-1}$. This was repeated twice to yield sample sizes of $n = 20$ per treatment. A thermocouple (type T 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the control chamber to monitor chamber temperature. Individual insect body temperature was assumed to be in equilibrium with the chamber temperature under experimental conditions as employed in other similar sized insect taxa (Terblanche et al. 2007). The CT_{max} and CT_{min} were defined as the temperature at which each individual insect lost coordinated muscle function, consequently losing the ability to respond to mild prodding (e.g. Nyamukondiwa & Terblanche, 2010).

5.2.4 Supercooling Points (SCPs)

Adult parasitoids (24-48 h old) SCPs were assayed as outlined by Nyamukondiwa *et al*. (2013). For each species, sixteen insects were individually placed in 0.65 ml microcentrifuge tubes where each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762-1121, Cambridge, UK). The thermocouples were inserted through the lid of each tube with both insect and thermocouple secured in place by a cotton wool. All thermocouple readings were taken via two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) that relayed information to a computer equipped with PicoLog software for windows (Pico Technology, Cambridge, UK). Temperatures were continuously monitored and recorded at 1s intervals. In all cases, experiments commenced at a set point temperature of $15^{\circ}C$

for 10 mins (to allow equilibration of insect body temperatures) before ramping down at 0.5°C min^{-1} until SCPs were recorded. In the current study, SCP for each individual was defined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (see Nyamukondiwa *et al*., 2013).

5.2.5 Chill Coma Recovery Time (CCRT)

For both species, CCRTs were assayed as outlined by Weldon *et al*. (2011). A total of 10 replicate adults (24-48 h old) were placed individually in 0.65ml microcentrifuge tubes and then loaded into a large zip-lock bag which was subsequently submerged into a water bath (Systronix, Scientific, South Africa). The water bath, filled with 1:1 water: propylene glycol mixture, was set at 0°C for 1 hour. This temperature × time treatment has been shown to elicit chill-coma in other like insect taxa (see Weldon *et al*., 2011; Sinclair *et al*., 2015). After 1 hour at chill-coma temperature, the tubes were removed from the water bath and transferred to a Memmert climate chamber set at 28°C, 65% RH for recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer where observations were recorded. CCRT was defined as the time (in minutes) required for an adult to stand upright on its legs (Milton & Partridge, 2008). This was repeated three times to yield sample sizes of n = 30 per treatment.

5.2.6 Heat Knock down Time (HKDT)

HKDTs for both species were assayed following Weldon *et al*. (2011). Ten replicate adult parasitoids (24 to 48 hrs) were individually placed in 0.65ml microcentrifuge tubes and placed in a climate chamber connected to a camera linked to a computer as in CCRT. The tubes carrying the parasitoids were then exposed to a test temperature of 45±0.3°C, 65% RH in the climate chamber. This knockdown temperature (45°C) was selected basing on preliminary investigations of upper critical temperatures to activity ranging 39.5±0.99°C and 44.6±0.63°C for *C. sesamiae* and *C. flavipes*, respectively. This was repeated three times to yield sample sizes of n = 30 for each species. All observations were made from the climate chamber video recording system. HKDT was defined as the time (in minutes) at which organisms lost activity following exposure to 45°C in the climate chamber.

5.2.7 Statistical Analyses

Data analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0 (R Development Core Team, 2016). LLT and ULT assays, SCPs, HKDT and CCRT results did not meet the assumptions of ANOVA, thus were analysed using generalized linear models (GLZ) assuming a binomial (LLT and ULT) and Gaussian (SCPs, HKDT and CCRT) distribution and a logit link function in R statistical software. CTLs met the linear model assumptions of constant variance and normal errors, therefore, they were analysed using one-way ANOVA in STATISTICA. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups.

5.3 Results

5.3.1 Lower and Upper Lethal Temperature Assays

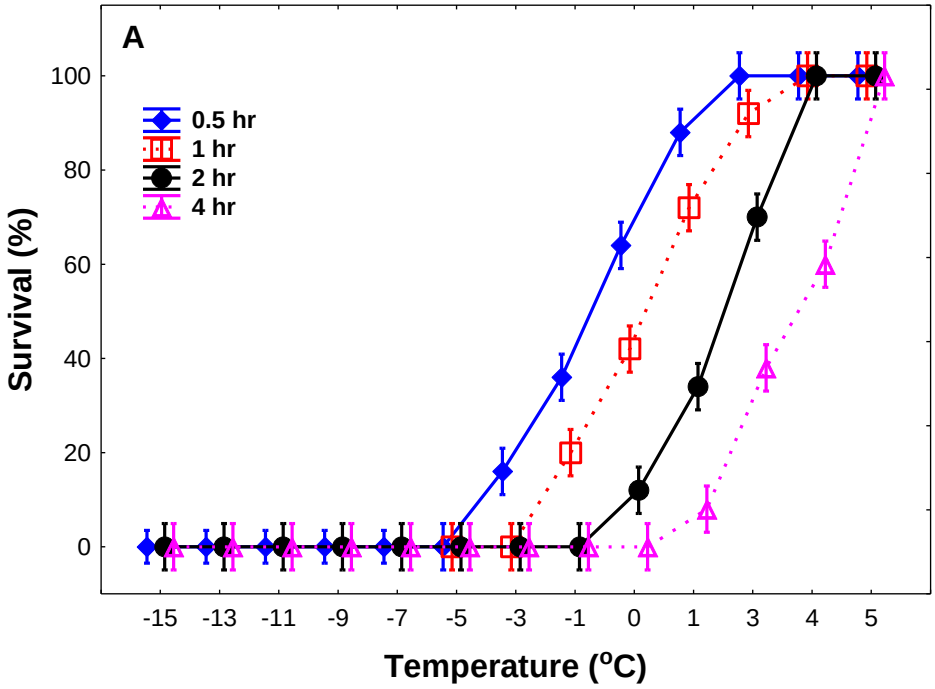
Temperature and duration of exposure significantly influenced survival of *C. sesamiae* and *C. flavipes* adults at both low and high temperatures ($P < 0.0001$) (Table 5.1; 5.2). Duration of exposure × temperature interaction effects were highly significant for *C. flavipes* ULT ($P < 0.001$) (Table 5.2) whilst the same interactions were not significant ($P > 0.05$) for both species' (LLT) and *C. sesamiae* (ULT) (Table 5.1; 5.2). A comparison of 0.5, 1, 2 and 4 h durations for *C. sesamiae* and *C. flavipes* following exposure to lethal low temperatures ranged from -5 to 5°C and -15 to -1°C for 0 – 100% survival respectively (Figure 5.1A; B). Upper lethal temperatures for the same durations of exposure ranged from 35 to 42°C for *C. sesamiae* and 37 to 44°C for *C. flavipes* (Figure 5.1C; D). An increase in severity of low and high temperature exposure resulted in increased mortalities for both species (Figure 5.1A-D). Similarly, an increase in duration of exposure to lethal low and high temperatures generally resulted in survival decrease for both *C. sesamiae* and *C. flavipes* adults (Figure 5.1A-D).

Table 5.1: Summary statistical results of the effects of temperature, duration of exposure and their interactions on the survival of *Cotesia sesamiae* adults following lower and upper lethal temperature treatments. Analysis were done using generalized linear models (GLZ) assuming binomial distribution with a logit link function in R version 3.3.0.

Parameter	**d.f**	χ^2	**P-Value**
Lower lethal temperature			
Duration	3	337.48	<0.0001
Temperature	7	1306.56	<0.0001
Temperature * Duration	21	26.47	0.1892
Upper lethal temperature			
Duration	3	317.98	<0.0001
Temperature	6	1211.61	<0.0001
Temperature * Duration	18	23.15	0.185

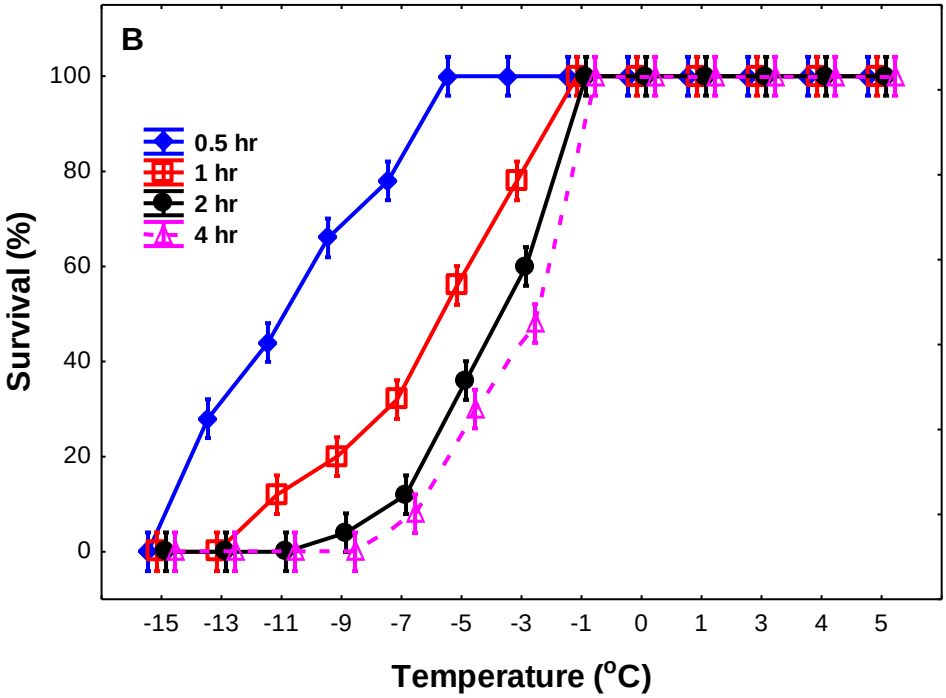
Table 5.2: Summary statistical results of the effects of temperature, duration of exposure and their interactions on the survival of *Cotesia flavipes* adults following lower and upper lethal temperature treatments. Analysis were done using generalized linear models (GLZ) assuming binomial distribution with a logit link function in R version 3.3.0.

Parameter	**d.f**	χ^2	**P-Value**
Lower lethal temperature			
Duration	3	358.91	<0.0001
Temperature	7	1061.1	<0.0001
Temperature * Duration	21	24.19	0.2838
Upper lethal temperature			
Duration	3	296.14	<0.0001
Temperature	5	977.14	<0.0001
Temperature * Duration	15	32.52	<0.001



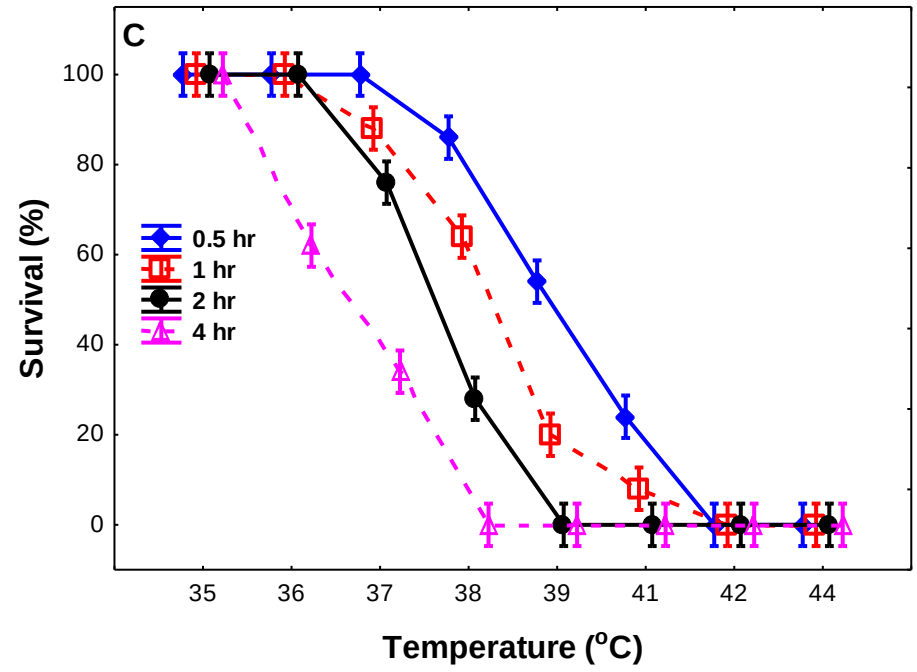
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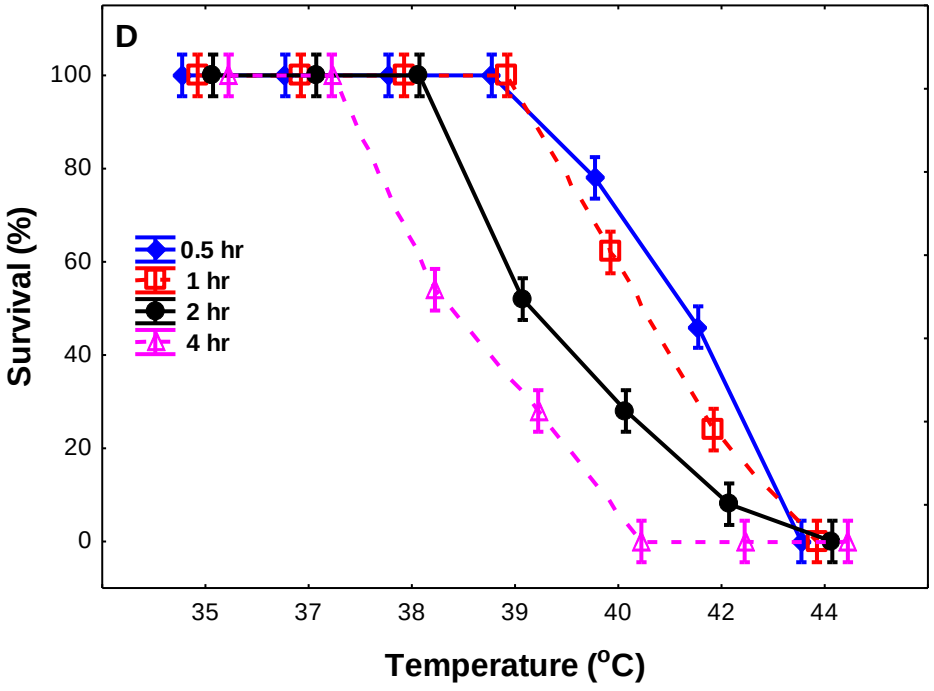
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4255 **Figure 5.1:** Mean (±95% confidence interval) survival of (A) *C. sesamiae* and (B) *C. flavipes* at
4256 different low temperatures, and (C) *C. sesamiae* and (D) *C. flavipes* at different high
4257 temperatures applied over four different durations.

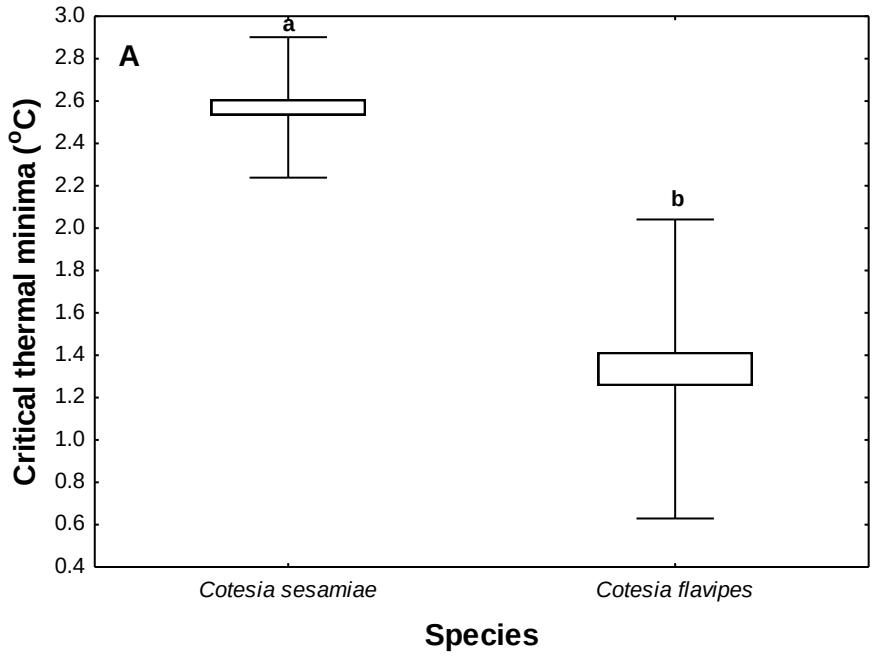
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5.3.2 Critical Thermal Limits (CTLs)

Critical thermal minima and maxima varied significantly between *C. sesamiae* and *C. flavipes* adults (Table 5.3; Figure 5.2A; B). *Cotesia flavipes* recorded a significantly lower ($P < 0.001$) CT_{min} (1.34±0.59°C) than *C. sesamiae* (2.57±0.41°C). Similarly, *C. flavipes* also recorded a significantly higher ($P < 0.001$) CT_{max} (44.63±0.63°C) compared to congeneric *C. sesamiae* (39.51±0.99°C).

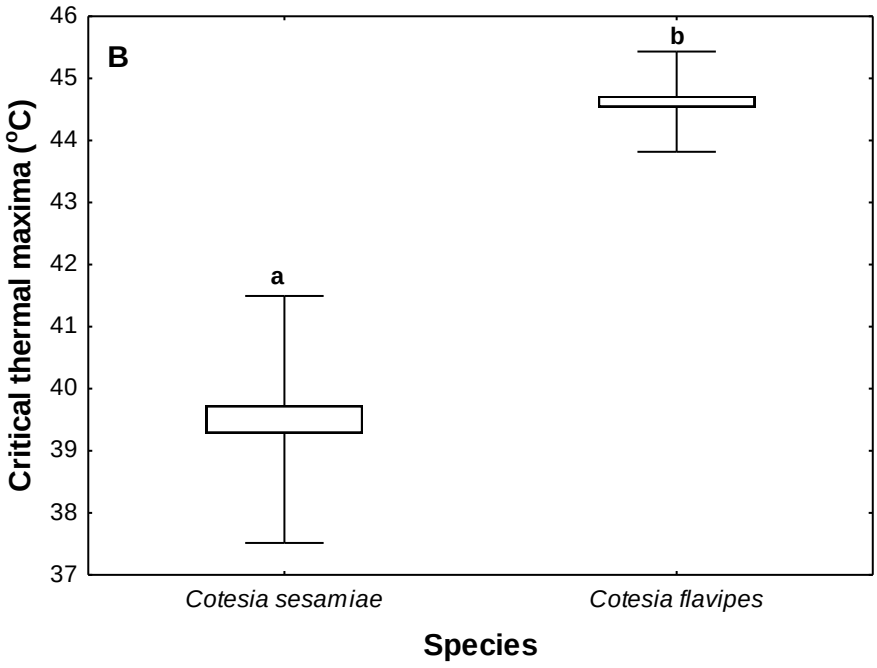
Table 5.3: Summary statistical results from full factorial ANOVA showing effects of species on critical thermal limits (CT_{max} and CT_{min}).

Trait	**Effect**	**SS**	**DF**	**MS**	**F**	**P**
CT_{min}	Intercept	152.49	1	152.49	2006.79	<0.0001
	Species	15.25	1	15.25	200.72	<0.0001
	Error	2.89	38	0.076		
CT_{max}	Intercept	70778.57	1	70778.57	122885.1	< 0.001
	Species	262.14	1	262.14	455.1	< 0.001
	Error	21.89	38	0.58		



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4275 **Figure 5.2:** Effects of species on (A) CT_{min} and (B) CT_{max}. Error bars represent 95% CLs (N =

4276 20). Means with the same letter are not significantly different from each other.

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5.3.3 Supercooling Points (SCPs)

There was no significant difference in the supercooling points (χ^2 =0.0368, d.f =1, P = 0.848) between *C. sesamiae* and *C. flavipes* (Figure 5.3). The mean supercooling points for *C. sesamiae* and *C. flavipes* adults were -20.26±1.07°C and -20.34±1.19°C respectively (Figure 5.3). SCPs were lower (Figure 5.3) than the LLTs (Figure 5.1A; B) for both parasitoid species indicating that mortality temperatures were above those of supercooling.

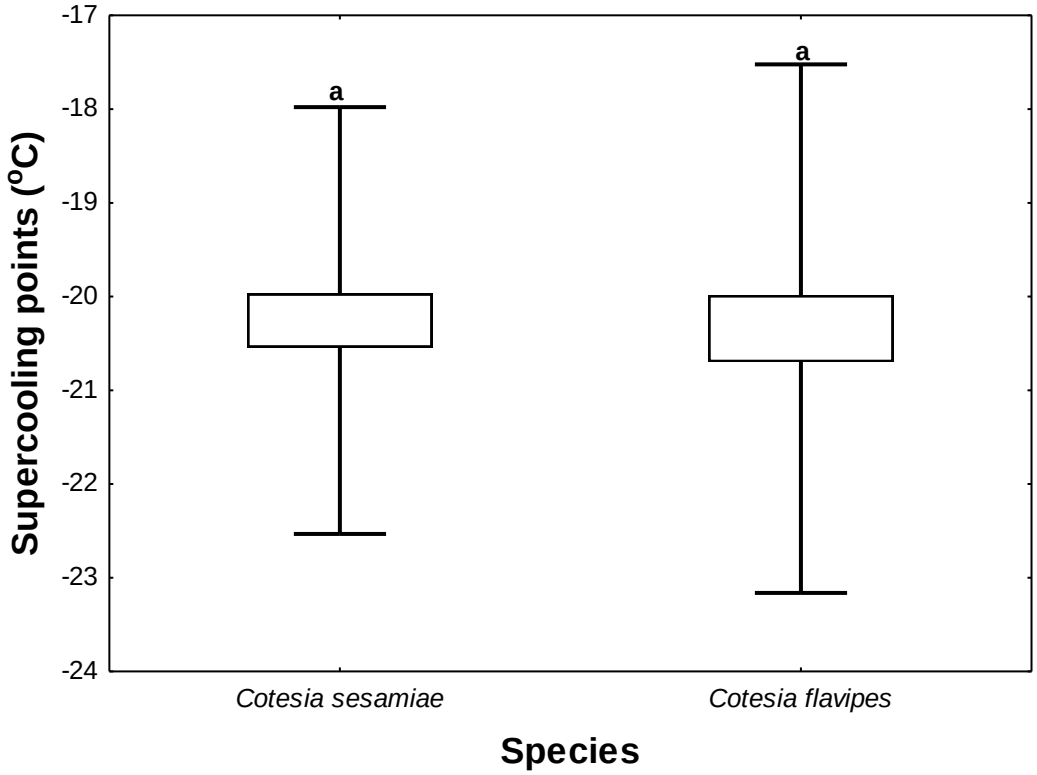


Figure 5.3: The effect of species on supercooling points. Error bars represent 95% CLs (N = 16 per group). Means with the same letter are not significantly different from each other

5.3.4 Chill Coma Recovery Time (CCRT)

There was a significant difference in CCRT between *C. sesamiae* and *C. flavipes* adults (χ^2 =23.41, d.f =1, $P < 0.0001$) (Figure 5.4). The average CCRTs were 3.31±0.69 and 4.01±0.82 minutes for *C. flavipes* and *C. sesamiae* adults respectively (Figure 5.4). *Cotesia flavipes* recovered significantly faster than *C. sesamiae* following exposure to chill-coma temperature

(0°C; 1 h) (Figure 5.4), symbolising a higher tolerance to low temperature for *C. flavipes* relative to *C. sesamiae*.

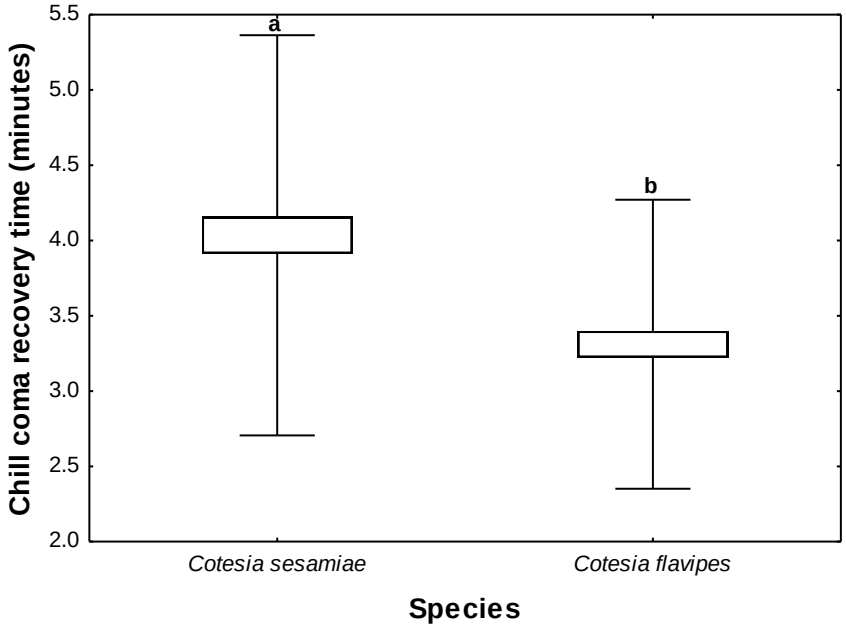


Figure 5.4: Effects of species on chill-coma recovery time. Error bars represent 95% CLs (N = 30). Means with the same letter are not significantly different from each other.

5.3.5 Heat Knockdown Time (HKDT)

Like in CCRT, there was a significant difference in HKDT between *C. sesamiae* and *C. flavipes* (χ^2 =207.77, d.f =1, $P < 0.0001$) (Figure 5.5). The mean HKDTs for *C. sesamiae* and *C. flavipes* adults were 19.91±1.06 and 25.67±1.37 minutes respectively (Figure 5.5). *Cotesia flavipes* showed a significantly longer knock-down time than *C. sesamiae* following exposure to knock-down temperature (45.0±0.3°C) (Figure 5.5), symbolising a significantly higher resistance to heat shock for the exotic *C. flavipes* relative to indigenous *C. sesamiae*.

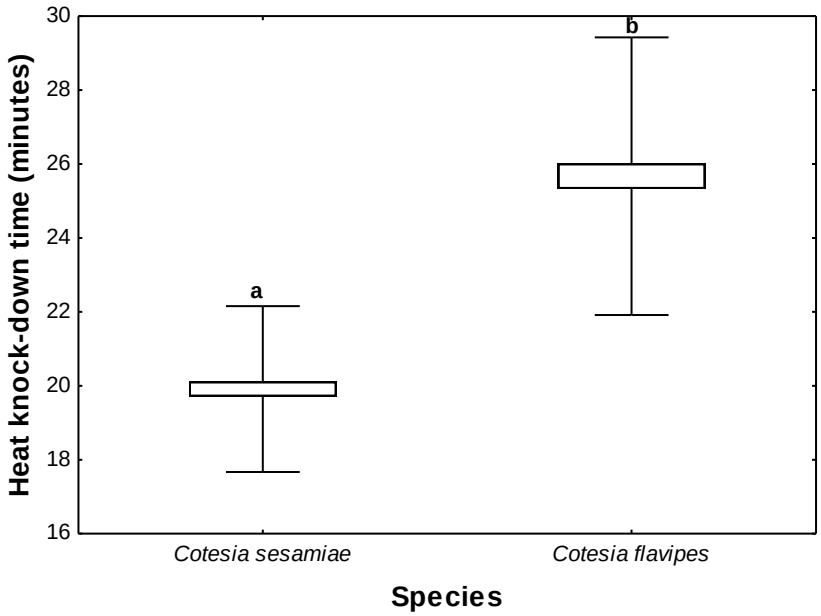


Figure 5.5: Effects of species on heat knock-down time. Error bars represent 95% CLs (N = 30). Means with the same letter are not significantly different from each other.

5.4 Discussion

Evolutionary history, basal thermal tolerance and phenotypic plasticity mediates insect population phenologies and dynamics including biogeographic patterns and fitness traits under climate variability associated with anthropogenic climate change (Helmuth *et al*., 2005; Ghalambor *et al*., 2007; Santos *et al*., 2012). In the current study, survival of *C. sesamiae* and *C. flavipes* was dependent on both severity and duration of temperature exposure as in other insect taxa (Chidawanyika & Terblanche, 2011a; Nguyen *et al*., 2014; Mutamiswa *et al*., 2017a). I report *C. sesamiae* LLTs and ULTs ranging -5 to 5°C and 35 to 42°C respectively (for 0.5-4h exposures), in keeping with related studies (see Mutamiswa *et al*., 2017a). This result affirms the significance of magnitude of temperature and duration of exposure on overall physiological fitness. At more extreme temperatures, duration of exposure was less important than more benign temperatures (see Figure 5.1A-D), perhaps due to the irreversible damage on cell and protein function caused by extreme acute temperatures (Chown & Nicholson, 2004; Harrison *et al*., 2012). For 0.5–4 h treatments, LLTs for *C. sesamiae* and *C. flavipes* ranged from -5 to 5°C and -15 to -1°C whilst ULTs ranged from 35 to 42°C and 37 to 44°C respectively, suggesting that

C. flavipes is more cold and heat tolerant than *C. sesamiae*. Given these responses, an important question will be whether these two species may continue coexisting in agroecosystems efficiently regulating lepidopteran stemborer populations under the current and projected climate change. With African temperatures projected to increase (IPCC, 2007; Gemeda & Sima, 2015), at the same time associated with high frequency of heat waves, and cold snaps (IPCC, 2014), it is highly likely that *C. flavipes* will survive more prolonged low and high temperature extremes than its congener *C. sesamiae*. When *C. partellus* is the host, *C. flavipes* has been reported to have a higher host searching efficiency even at low stemborer host densities than *C. sesamiae* (Wiedenmann & Smith, 1993; Sallam *et al*., 1999). Given these survival advantages, *C. flavipes* may, in all likelihood, have a higher competitive resource utilization and greater fitness than *C. sesamiae* in both cooler and warmer environments. Moreover, ULT and LLT temperatures reported here are often experienced under natural setups (see Mutamiswa *et al*., 2017a), symbolizing the ecological significance of these findings. Furthermore, sudden heat waves and cold snaps are expected to rise with global change (IPCC, 2014) and this likely has a compounding effect on fitness traits and survival of the two congeneric parasitoid species.

The present study also demonstrated how temperature can limit the functional activity of both parasitoid species. CTLs are regarded as ecologically relevant measures of determining the impact of thermal variability on insect functional activities, e.g. mating, host searching, migration, feeding and others (Terblanche *et al*., 2011; Overgaard *et al*., 2012; Andersen *et al*. 2014). These activities represent key fitness traits for insects and in particular parasitoids, whose ecological functions depend on host searching ability (Mailafiya *et al*., 2009), an activity trait highly dependent on temperature. *Cotesia flavipes* had lower CT_{min} and higher CT_{max} compared to *C. sesamiae* (Figure 5.2), indicating a fitness advantage for the exotic *C. flavipes* relative to congeneric *C. sesamiae* at both thermal extremes. This result also translates into a broader thermal activity window for *C. flavipes* relative to its congener. As such, *C. flavipes* may likely optimise key life history activities better off than congeneric *C. sesamiae* under stressful and variable thermal regimes. Hence, under changing climates, biogeographic patterns and abundance of *C. sesamiae* will be more constrained than *C. flavipes* due to differential thermal activity windows. In other insect taxa, such declines in abundance and changes in biogeographic patterns have been attributed to changes in population dynamics due to the influence of temperature (see Terblanche *et al*., 2008; Piyaphongkul *et al*., 2012; Nooten *et al*., 2014). Hence,

thermal tolerance superiority of *C. flavipes* reported here coupled with superior host searching ability (Mailafiya *et al*., 2009), may likely make it a better competitor thus likely dominant over *C. sesamiae* in sub-Saharan Africa, and where the two share the same niche.

I also document, for the first time, SCPs for adult *C. flavipes* and *C. sesamiae.* The average SCPs for *C. sesamiae* and *C. flavipes* were -20.26±1.14 and -20.34±1.41 respectively. Overwintering insects can be regarded as freeze tolerant (surviving ice formation within tissues), freeze intolerant (not surviving ice formation within tissues) (Andreadis *et al*., 2005) and chill susceptible (killed by chilling) (Sinclair *et al*., 2015). Generally, insect species with depressed SCPs ranging between ~ -20°C to -30°C are regarded as freeze intolerant (Bale, 1996). While my findings show both *C. sesamiae* and *C. flavipes* SCPs ~ -20°C, the failure to recover following supercooling suggest both species may be chill susceptible. Moreover, chill susceptibility is further affirmed by LLT results (Figure 5.1A; B) which indicated mortality temperatures for both species were way above those of supercooling. Nevertheless, supercooling ability remains a significant survival strategy for freeze intolerant species, likely facilitated by rapid accumulation of cryoprotectants and extracellular agents such as sugars, polyols and sorbitol, to avoid membrane rupture (Jones *et al*., 2008; Sinclair *et al*., 2015).

Chill coma recovery time is another important indicator of cold stress resistance in terrestrial arthropods (David *et al*., 2003; Findsen *et al*., 2014). In the present study, *C. flavipes* recovered significantly faster than *C. sesamiae* following exposure to chill coma temperature (0°C for 1 h), suggesting cold tolerance and survival advantage over its congener when faced with cold shock. Chill coma recovery has been reported to be mediated by rapid resumption of ion homeostasis (Findsen et al. 2013; MacMillan *et al*., 2016). Therefore, it is likely that the faster chill coma recovery for *C. flavipes* may be through enhanced ability for ion homeostasis relative to *C. sesamiae* suggesting differential time course of ion homeostasis even for closely related taxa. This is despite the two species having shown similar SCPs. My study therefore also demonstrates the complexity of rapid thermal responses among insects and that different metrics of thermal tolerance may suggest divergent conclusions.

Cotesia flavipes took more time (25.67±1.37 minutes) to be knocked down at high temperature (45°C) than *C. sesamiae* (19.91±1.06), further confirming its enhanced fitness advantage when faced with heat shock compared to *C. sesamiae.* These differential responses may be a result of the differences in the ability to rapidly express transient genetic responses

responsible for upregulation of chaperone proteins that enable survival and activity at high temperatures (Feder & Hofmann, 1999). In nature, the ability to rapidly shorten or avoid heat knockdown and recover from chill coma temperatures is of high ecological relevance as any limited locomotion due to temperature stress, attributed to cessation of neuromuscular activity due to disruption of ion homeostasis (Findsen *et al*., 2014; Pujol-Lereis *et al*., 2016), may lead to opportunistic predation by natural enemies. It is therefore highly likely that *C. sesamiae* may be more prone to such predation than *C. flavipes* under stressful thermal environments. Furthermore, prolonged inactivity following chill coma and heat knockdown may result in compromised realization of life history traits including reproductive fitness and ultimately delivery of the ecosystem services like host pest parasitisation. Thus, the current results also report a fitness and survival advantage of exotic *C. flavipes* relative to indigenous *C. sesamiae* following heat shock.

Overall, the current results reveal *C. flavipes* has superior basal thermal tolerance than congeneric *C. sesamiae* at both thermal extremes. Even though *C. sesamiae* and *C. flavipes* can coexist with interactive synergistic effects that maximize biocontrol efficacy against lepidopteran stemborer species (Overholt *et al*., 2000; Mailafiya *et al*., 2011), I conclude that *C. flavipes* will become dominant under climate change scenarios due to its superior thermal physiology. However, I make this conclusion with caveats as I only determined basal responses and not phenotypic plasticity of the various matrices that I determined. Other studies have shown how several factors such as within- and transgenerational -thermal history (Kristensen *et al*., 2008; Nyamukondiwa & Terblanche, 2010; Chidawanyika *et al*., 2011b), age and feeding status (Nyamukondiwa & Terblanche, 2009; Chidawanyika *et al*., 2017) and ontogeny (Andersen *et al*., 2014) can influence responses to thermal exposure with significant potential for mitigating adverse effects of climate change (reviewed in Stillman, 2003; Sgrò *et al*., 2016). Future studies should therefore endeavor to explore such plasticity since the knowledge can aid enhancement of quality of mass-reared parasitoid insects (Chidawanyika *et al*., 2012; Sørensen *et al*., 2012; Terblanche, 2014). For example, developmental and adult acclimation in moths resulted in improved field performance of mass- reared *Cydia pomonella* (measured as flight response to pheromone traps) under conditions similar to acclimation conditions (Chidawanyika & Terblanche, 2011a). This evidence is in addition to earlier work on the Queensland fruit fly where manipulation of thermal plasticity through preconditioning resulted in improved field

performance (Meats, 1973; 1976). Pest managers can therefore utilize such preconditioning to improve the survival and performance of the parasitoids and augment natural populations, in particular for *C. sesamiae* which had poorer basal thermal tolerance. This will not only aid performance of *C. sesamiae*, but also ensure that introduced alien *C. flavipes* may not become over dominant to ensure maintenance of native biodiversity in agroecosystems.

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CHAPTER 6

Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson.

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6.1 Introduction

Global increase in temperature coupled with erratic and limited rainfall exerts potent environmental stressors such as heat, dehydration and starvation that determine insect population dynamics (Bale *et al*., 2002; Clusella-Trullas *et al*., 2011; Bauerfeind & Fischer, 2013; Hoffmann *et al*., 2013). While climate change brings multiple overlapping stressors, temperature tolerance remains a critical attribute for physiological adaptation for invasive species and organisms living in different bioclimatic regions (Nyamukondiwa *et al*., 2010). Temperature stress directly influences diurnal and seasonal activity and the outcome of several life-history traits including, reproduction resource acquisition, dispersal and ultimately survival (Hoffman *et al*., 2012; Ma *et al*., 2014). Under natural environments, insect responses to temperature stress may be influenced by transient events including repeated exposure to sublethal temperatures that can induce plasticity thereby improving performance and survival in otherwise lethal environments (Marshall & Sinclair, 2012; Karl *et al*., 2014; Kingsolver *et al*., 2015). Indeed, thermal suitability of novel environments for invasive insect species is dependent on both basal and phenotypic plasticity to tolerance traits. Failure to acquire such enhanced basal and plasticity may result in even more deleterious consequences under lethal environments (Chidawanyika & Terblanche, 2011).

The mechanisms inducing such thermal plasticity are temperature-time dependent, hence duration and magnitude are key determinants of insect-temperature interactions (Chown & Nicolson, 2004). For example, acute exposure to sub-lethal temperatures may induce hardening which involves physiological adjustments such as expression of heat shock proteins in order to preserve key life history traits (Sinclair & Roberts, 2005). On the other hand, acclimation is employed under relatively chronic exposure ranging from diurnal to seasonal scale (Overgaard & Sørensen, 2008; Khani & Moharramipour, 2010). In nature, however, thermal stress is experienced in concert with other environmental stressors making it difficult to predict outcomes of thermal events. For example, heat stress often coincides with long periods of poor precipitation (drought) as predicted under climate change scenarios (IPCC, 2014). For insect herbivores, such drought events directly influence both quality and availability of host plants (Bale *et al*., 2002; Buerfiend & Fischer, 2013) resulting in additional stressors that mediate thermal responses such as starvation (Chidawanyika *et al*., 2017). Furthermore, periods of water deficit are known to variably exert desiccation stress among different insect life stages with

subsequent impact on thermal sensitivity and ultimately survival (Bubliy *et al*., 2012). Resistance to most of these stressors is energetically costly for most ectotherms (Fusi *et al*., 2016; Verbek *et al*., 2016), hence the quality and availability of host plant material mediates the outcome of environmental stress. For example, in order to reduce desiccation stress, insects convert stored fat to metabolic water, accumulate water (e.g. from water-based food, nectar) in tissues (Hadley, 1994), close their spiracles and increase reabsorption of water in the rectum (Harrison *et al*., 2012). Hence prolonged periods of desiccation stress without replenishment of body fat reserves due to resource unavailability can have dire fitness consequences. To cope with starvation, insects have been reported to improve survival ability through suppression of metabolic rate, seeking cooler habitats and also exploitation of body reserves such as lipids (Rion & Kawecki, 2007; Scharf *et al*., 2016). Hence, resistance to multiple stressors among insects is interlinked and several studies have confirmed the presence of cross-resistance among different stressors (e.g. MacMillan *et al*., 2009, Chidawanyika & Terblanche, 2011; Sinclair *et al*., 2007, 2013; Renault *et al*., 2015; Kalra *et al*., 2017). For example, starvation reduced cold tolerance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Scharf *et al*., 2016) and heat tolerance in *Zygogramma bicolorata* (Coleoptera: Chrysomeliade) (Chidawanyika *et al*., 2017). In addition, desiccation reduced critical thermal maxima in *Drosophila melanogaster* exposed to a range of sub-lethal temperatures (Da Lage *et al*., 1989). Furthermore, in spring-tails (*Folsomia candida*), moderate desiccation improved cold tolerance (Bayley *et al*., 2001). This interplay among different stressors suggests shared physiological mechanisms (cross tolerance) or signal pathways (cross talk) (Sinclair *et al*., 2013) and likely coevolution of resistance mechanisms to related environmental stress traits.

It is also widely accepted that ontogeny (developmental effects) influences sensitivity to environmental stress (Spicer & Gaston, 1999; Chown & Nicolson, 2004; Bowler & Terblanche, 2008). Generally, less mobile life-stages (usually juveniles) are thought to be more tolerant and exhibiting greater phenotypic plasticity to environmental stress as compensation to their limited capacity to maintain optimal conditions through behavioural mechanisms. Several studies have supported this idea in high (Gilchrist *et al*., 1997; Klok & Chown, 2001) and low (Vernon & Vanier, 1996, Klok & Chown, 2001, Jensen *et al*., 2007) temperature stress and desiccation resistance (Woods & Singer, 2001). Given the above background, it is apparent that environmental stress resistance among arthropods is mediated by both biotic and abiotic factors.

For both invasive and indigenous insects, including crop insect pests of economic importance, such basal and phenotypic plasticity (including cross tolerance and cross talk) mediates climate change responses and ultimately invasion potential (Chown *et al*., 2007; Renault *et al*., 2015). Indeed, modeling studies have revealed that, even in scenarios where there is repeated and high propagule pressure, the thermal suitability and adaptation to novel environment remains critical for invasive species establishment (Hayes & Barry, 2008; Mainali *et al*., 2015). It is therefore of importance to elucidate physiological responses of invasive agricultural pests facing multiple stressors as it gives insights into potential population dynamics under climate change.

Here I investigated the effects of multiple environmental stresses (cold and heat hardening, thermal, desiccation and starvation acclimation) on the thermal tolerance of two indigenous stemborer species *Busseola fusca* (Lepidoptera: Noctuidae) (Fuller) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and the exotic *Chilo partellus* Swinhoe (Lepidoptera: Crambidae). The African maize stemborer, *B. fusca* and pink stemborer, *S. calamistis* are regarded as the most destructive maize and sorghum insect pests in many countries of sub-Saharan Africa (Ong'amo *et al*., 2008; Khadioli *et al*., 2014a). The distribution of *B. fusca* varies with region with the pest common at higher altitudes (above 600 m a.s.l) in east and southern Africa, ≥ 2000 m.a.s.l in central Africa and dry lowland savanna areas in west Africa (Ajayi, 1998; Kfir *et al*., 2002). In east Africa, *S. calamistis* is available at all elevations, though in low populations (Ntiri *et al*., 2016a). The spotted stemborer, *C. partellus* is native to Asia and is the predominant insect pest of cereals in east and southern Africa (Kfir, 1997; Overholt *et al*., 2000). It was previously reported limited to low and mid-elevation areas (Zhou *et al*., 2001) and has latter expanded to high altitude and humid transitional areas (Khadioli *et al*., 2014b) displacing indigenous stemborer species (Kfir, 1997; Mutamiswa *et al*., 2017a). Nevertheless, it remains unknown how invasion potential of invasive species is likely shaped in the face of multiple stresses.

Critical thermal limits (CTLs, critical thermal minima [CT_{min}] and maxima [CT_{max}]) have received increasing attention in the study of thermal tolerance of insects because they provide an understanding on species distribution and their response to global change (Chown & Terblanche, 2007; Deutsch *et al*., 2008). They are ecologically relevant (Alford *et al*., 2016), and represent essential functional thermal limits of organisms (e.g. Nyamukondiwa & Terblanche, 2010). Thus, their determination is the first step in understanding the activity range for a given

population (reviewed in Somero, 2005). However, previous studies have indicated that CTLs depend on methodological context (Terblanche *et al*., 2007a), ontogeny (Cavieres *et al*., 2016) and may vary with trait and across species (Kelty & Lee, 1999).

Despite the importance of invasive lepidopteran stemborers to African agriculture, no studies have investigated their physiological responses to multiple stressors. Related studies have specifically focused on non-crop pest insect model organisms (e.g. Sinclair *et al*., 2007; Nyamukondiwa *et al*., 2011; Bubily *et al*., 2013; Chidawanyika *et al*., 2017; Scharf *et al*., 2017). In line with this background, I determined the CTLs to activity for *B. fusca, S. calamistis* and *C. partellus* following rearing at benign temperatures, post thermal, starvation and desiccation acclimation as well as cold and heat hardening. These plastic responses to heterogeneous stressors may help explain invasion potential for invasive species upon facing novel environments.

6.2 Materials and Methods

6.2.1 Insect Culture and Rearing Conditions

The initial colonies of *B. fusca, S. calamistis* and *C. partellus* were obtained as pupae from International Centre for Insect Physiology and Ecology (ICIPE), Kenya. The insects have been in culture for more than 20 generations with regular additions of wild populations to maintain heterozygosity. Pupae were maintained in open Petri dishes in Bugdorm rearing cages ($240mm^3$; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under optimum conditions of 28 ± 1°C, 65 ± 10% relative humidity (RH), 12L: 12D photoperiod for *S. calamistis* and *C. partellus* and 25 ± 1°C, 75 ± 10% RH, 12L: 12D photoperiod for *B. fusca* in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) until adult eclosion. Following emergence, adults had access to water provided as moistened cotton wads. Wax paper was also placed in the cages as an oviposition surface for gravid females. To maintain uniformity among test insects, eggs were harvested after every 12 h, and transferred to artificial diet (Ochieng *et al*., 1985; Tefera *et al*., 2010) where they were allowed to hatch into larvae that were later used in subsequent experiments. In all cases 6th instar larvae (last instar developmental stage) were used in assays for all species.

6.2.2 Thermal Tolerance Assays

Critical thermal limits were assayed using established dynamic protocols (Nyamukondiwa & Terblanche, 2009; Mutamiswa *et al*., 2017b). For each species, ten larvae were individually placed in an insulated double jacketed chamber ('organ pipes') that was connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to accommodate subzero temperatures (Chown & Nicholson, 2004). Critical thermal minima and maxima experiments began at set point rearing temperature of 25°C for *B. fusca* and 28°C for *C. partellus* and *S. calamistis* where 10 mins was given to allow equilibration of insect body and ambient temperatures. Thereafter, temperature was ramped up (CT_{max}) or down (CT_{min}) at a rate of 0.25°C min^{-1} until all the insects reach their CTLs. The CTLs were defined as the temperature at which each individual insect lost coordinated muscle function which was regarded as a lack of response to mild stimuli such as prodding (e.g. Nyamukondiwa & Terblanche, 2010). While this ramping rate is faster than natural diurnal temperature fluctuations, it represents a compromise between experimental throughput and ecological relevance relative to CTLs work undertaken to date (see Chown *et al*., 2009). A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the control chamber to verify chamber temperature. In all cases, insect body temperature was assumed to be in equilibrium with chamber temperature (Terblanche *et al*., 2007b; Mudavanhu *et al*., 2014; Mutamiswa *et al*., 2017b).These experiments were repeated twice to yield sample sizes of $n = 20$ for each species.

6.2.3 Effects of Thermal Acclimation on CTLs

To induce potential plasticity, *B. fusca* larvae (40 per each acclimation temperature) were acclimated in artificial diet in Memmert climate chambers at 20 (low), 25 (optimum) and 30°C (high) for duration of 3 days as outlined by Nyamukondiwa & Terblanche, 2010. Similar aged *S. calamistis* and *C. partellus* were acclimated at 23 (low), 28 (optimum) and 33°C (high) for the same duration as that of *B. fusca*. These acclimation temperatures (5°C above and below optimum) and duration (3 days) are suffice to elicit plastic responses to temperature (Fischer *et al*., 2010; Nyamukondiwa & Terblanche, 2010; Weldon *et al*., 2011). For each species, larvae that remained under their rearing temperature during the acclimation period were treated as the

control groups (e.g. Terblanche *et al*., 2006). Following acclimation, CTLs were measured for each species as outlined in thermal tolerance assays.

6.2.4 Effects of Starvation Acclimation on CTLs

To assess the impact of starvation acclimation on CTLs, a total of 40 larvae from each species were loaded in 8, 30ml plastic vials with perforated screw-cap lids. Larvae were then starved for 4 days in climate chambers under respective rearing conditions, but with access to water *ad libtum*. Control larvae (40 from each species) had access to artificial diet for the same duration at abovementioned conditions. After 4 days, CTLs for both control and starved larvae were measured for each species as previously outlined.

6.2.5 Effects of Hardening on CTLs

To test the impact of hardening (RCH and RHH) on CTLs, I first determined the hardening temperatures for each species. For all species, cold and heat hardening temperatures were derived from CT_{min} and CT_{max} for each stemborer species (Hoffmann *et al*., 2003; Lee & Denlinger, 2010) and these were defined as 6°C below CT_{min} and 7°C below CT_{max} respectively (Table 6.1). A total of 40 larvae from each species were cold and heat hardened for 2 hours. Control larvae (40 from each species) were maintained in climate chambers at respective benign/rearing conditions before measuring their CTLs. Since cold hardening temperatures induce chill coma in test stemborer species, insects were allowed to recover from chill coma for 30 minutes following treatment at respective benign temperature before measuring CTLs. However, for heat hardened insects, CTLs were measured 2 hours post hardening as previously outlined.

Table 6.1: Summary table showing cold and heat hardening temperatures (2 h duration) for *B. fusca*, *S. calamistis* and *C. partellus* larvae.

Species	Low temperature hardening (°C)	High temperature hardening (°C)
Busseola fusca	-3	40
Sesamia calamistis	-2	41
Chilo partellus	2	41

6.2.6 Effects of Desiccation Acclimation on CTLs

Desiccation assays were undertaken using standard established protocols in the laboratory modified from Bauerfeind *et al*. (2014). For all species, five last instar larvae were placed in 8 (N=40) perforated 30ml empty plastic vials with aerated screw-cap lids. The vials were placed on top of wire gauze in a glass sealed desiccator containing 200g silica gel for 24 hours. Preliminary assays had shown this duration eliciting at least 40% body water loss for all species. The desiccator was placed in a climate chamber under rearing conditions for each respective stemborer species. Thermocron iButtons (Dallas Semiconductors, Model DS1920) were used to measure temperature and RH inside the desiccator at 30-min intervals. The average temperature and RH in the desiccators were 25.4 ± 0.38°C and 20.04 ± 2.03% for *B. fusca*, 28 ± 0.44°C and 20.96 ± 1.14 % for *S. calamistis* and 28 ± 0.53°C and 24.11 ± 1.12% for *C. partellus* larvae respectively. After 24 hours, the insects were transferred to artificial diet for a further 24 hours under their respective rearing conditions before measuring CTLs. Controls (40 larvae from each species) were maintained in climate chambers at abovementioned rearing conditions until their CTLs were measured as previously outlined.

6.2.7 Statistical Analyses

All analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma). The Shapiro-Wilk and Hartley-Bartlett tests were used to check data for normality and equality of variances respectively. All data met the linear model assumptions of constant variance and normal errors. Therefore, thermal tolerance assays and thermal acclimation effects on CTLs were analysed using one-way and two-way ANOVA respectively. In addition,

hardening, starvation and desiccation acclimation effects were analysed using full factorial analysis of variance (ANOVA). To separate statistically heterogeneous groups, Tukey-Kramer's *post-hoc* tests were used. Overlap in 95% confidence limits was used to identify statistically homogeneous groups.

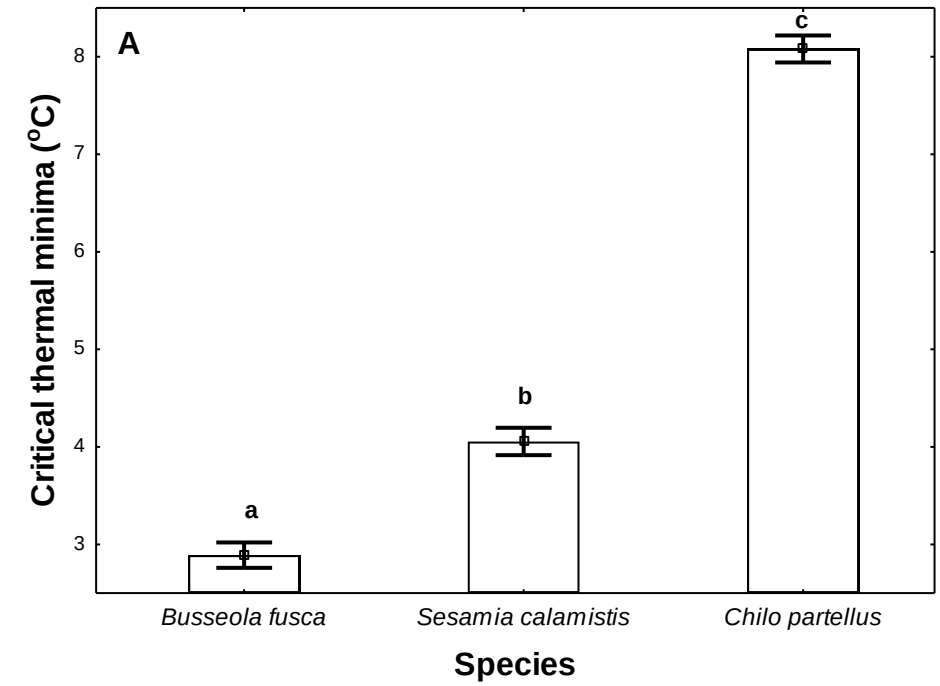
6.3 Results

6.3.1 Thermal Tolerance Assays

Critical thermal limits significantly varied across species of *B. fusca*, *S. calamistis* and *C. partellus* larvae (Table 6.2; Figure 6.1A; B). Critical thermal minima were different for all species, with *B. fusca* having the most enhanced cold tolerance and *C. partellus* the worst (Figure 6.1A). *Busseola fusca* had the lowest CT_{max} compared to *S. calamistis* and *C. partellus* (Figure 6.1B). Nevertheless, there was no significant difference between *S. calamistis* and *C. partellus* CT_{max}.

Table 6.2: Summary statistical results from one-way ANOVA showing effects of species (*B. fusca*, *S. calamistis* and *C. partellus*) on CT_{min} and CT_{max}

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	1505	1	1505	17799.61	< 0.001
	Species	296.63	2	148.31	1754.09	< 0.001
	Error	4.82	57	0.085		
CT_{max}	Intercept	137004.4	1	137004.4	348106.6	< 0.0001
	Species	30.3	2	15.2	38.5	< 0.0001
	Error	22.4	57	0.4		



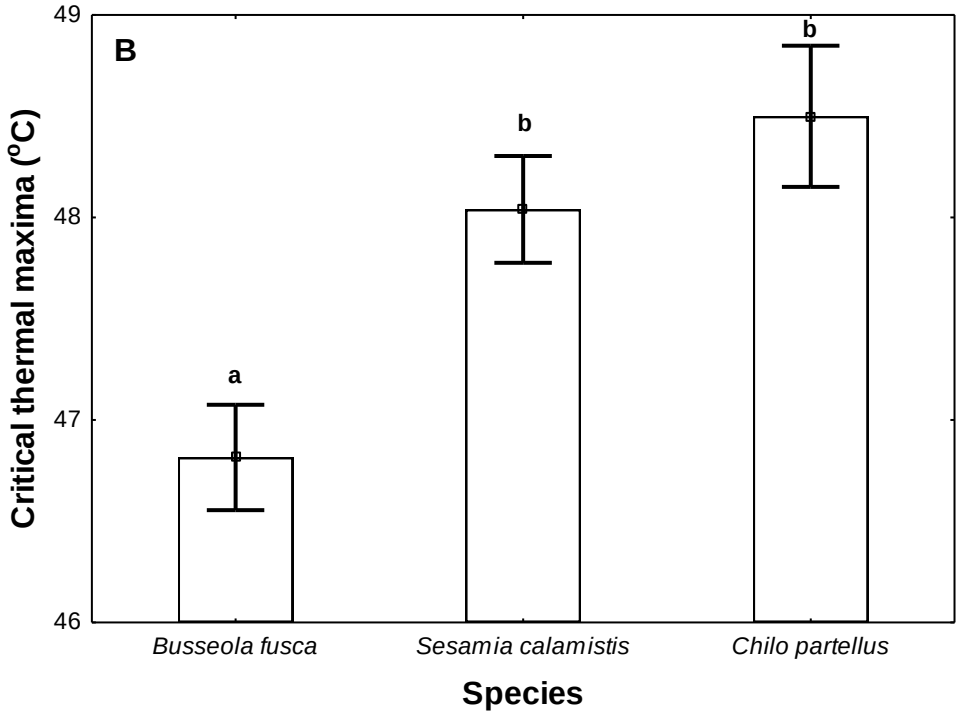


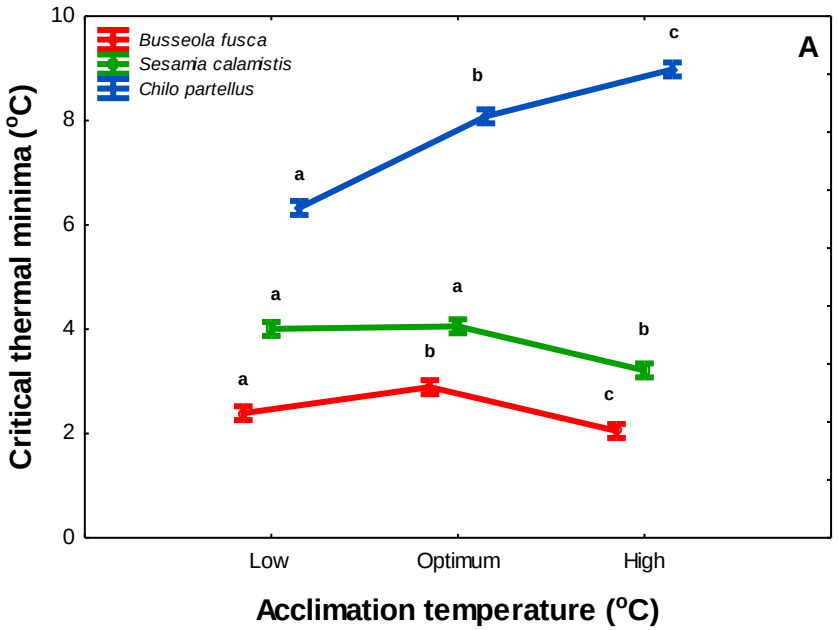
Figure 6.1: Effects of species on (A) CT_{min} and (B) CT_{max}. Error bars represent 95% CLs. Means with the same letter are not significantly different from each other.

6.3.2 Effects of Thermal Acclimation on CTLs

Acclimation and species had highly significant effects on CT_{min}. Furthermore, acclimation * species interaction effects were significant for low temperature tolerance (CT_{min}) (Table 6.3; Figure 6.2A). Acclimation to low temperature enhanced CT_{min} for both *B. fusca* and *C. partellus* larvae while compromising it for *S. calamistis* larvae (Figure 6.2A). In addition, acclimation to high temperature improved CT_{min} for *B. fusca* and *S. calamistis* and reduced it for *C. partellus* larvae (Figure 6.2A). Overall, CT_{min} values were enhanced in *B. fusca*, and worst in *C. partellus.* Acclimation to low temperature marginally increased CT_{max} for *C. partellus* and compromised it for *B. fusca* and *S. calamistis* larvae (Figure 6.2B). Similarly, acclimation to high temperature compromised CT_{max} for both *B. fusca* and *S. calamistis* and enhanced it for *C. partellus* larvae (Figure 6.2B). Generally, *C. partellus* had an enhanced CT_{max} at all acclimation levels, relative to *S. calamistis* and *B. fusca.*

Table 6.3: Summary results from a two-way ANOVA showing the effects of species (*B. fusca, S. calamistis* and *C. partellus*) and thermal acclimation (low, optimum and high) on CT_{min} and CT_{max}. SS = sums of squares, d.f.=degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	3917.2	1	3917.2	42707.21	< 0.001
	Acclimation	18.31	2	9.16	99.81	< 0.001
	Species	933.47	2	466.73	5088.55	< 0.001
	Species*Acclimation	70.75	4	17.69	192.83	< 0.001
	Error	15.68	171	0.092		
CT_{max}	Intercept	411395.5	1	411395.5	1330911	< 0.0001
	Acclimation	1.6	2	0.8	3	0.0743
	Species	112.3	2	56.2	182	< 0.0001
	Species*Acclimation	4	4	1	3	0.0145
	Error	52.9	171	0.3		



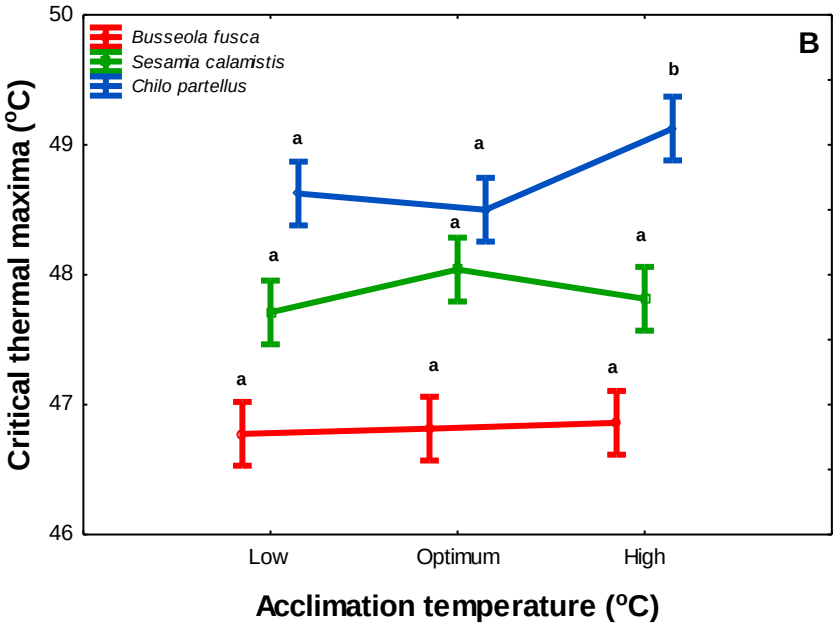


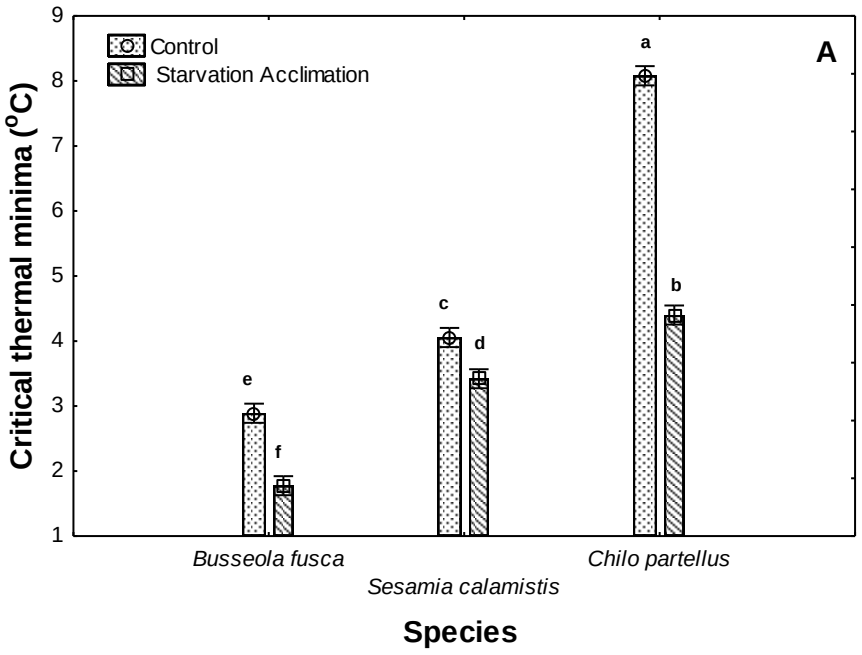
Figure 6.2: Thermal acclimation effects for three major stemborers, (*B. fusca*, *S. calamistis* and *C. partellus*) on (A) CT_{min} and (B) CT_{max} following acclimation for 3 days at low optimum and high temperature. Vertical bars denote ±95% CL. Means with the same letter are not significantly different from each other. Post hoc tests were done separately for each species

6.3.3 Effects of Starvation Acclimation on CTLs

Starvation acclimation significantly improved CT_{min} across all species (Table 6.4; Figure 6.3A), while only improving CT_{max} for *C. partellus* (Figure 6.3B). However, this acclimation compromised CT_{max} for *S. calamistis* and had no significant effect relative to the control in *B. fusca* (Figure 6.3B). *Busseola fusca* recorded the lowest CT_{min} while *C. partellus* recorded the highest CT_{max} (Figure 6.3A; B). The magnitude of CTLs plasticity (difference between control and treatment survival) following starvation acclimation was generally highest in *C. partellus* (Figure 6.3A; B). In addition, there was a significant species * acclimation interaction effect for CT_{min} and non-significant effect for CT_{max} following starvation acclimation (Table 6.4).

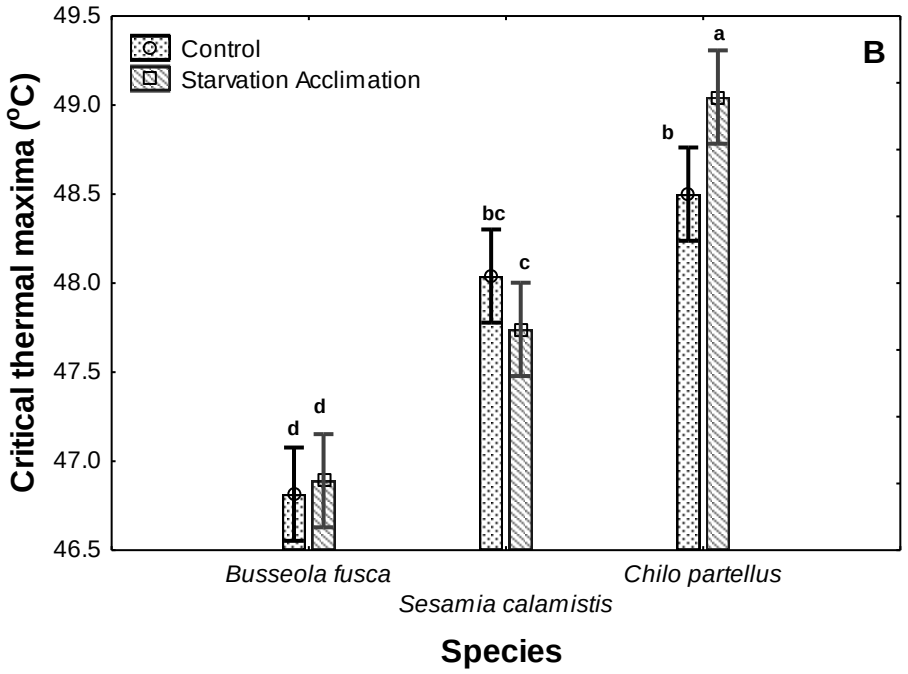
Table 6.4: Summary statistical results from full factorial ANOVA showing effects of starvation acclimation on CT_{min} and CT_{max} for *B. fusca*, *S. calamistis* and *C. partellus*. SS = sums of squares, d.f.= degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	2020.48	1	2020.48	18238.57	< 0.001
	Species	313.4	2	156.7	1414.51	< 0.001
	Starvation Acclimation	98.28	1	98.28	887.19	< 0.001
	Species*Acclimation	53.61	2	26.8	241.94	< 0.001
	Error	12.63	114	0.111		
CT_{max}	Intercept	274620.7	1	274620.7	785240.8	< 0.0001
	Species	73.9	2	36.9	105.6	< 0.0001
	Starvation Acclimation	0.3	1	0.3	1	0.325
	Species*Acclimation	3.6	2	1.8	5.1	0.00739
	Error	39.9	114	0.3		



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4973 **Figure 6.3:** Effects of starvation acclimation on (A) CT_{min} and (B) CT_{max} for *Busseola fusca*,
4974 *Sesamia calamistis* and *Chilo partellus* larvae. Error bars represent 95% CLs. Means with the
4975 same letter are not significantly different from each other. Post hoc tests were done separately for
4976 both treatments and across species

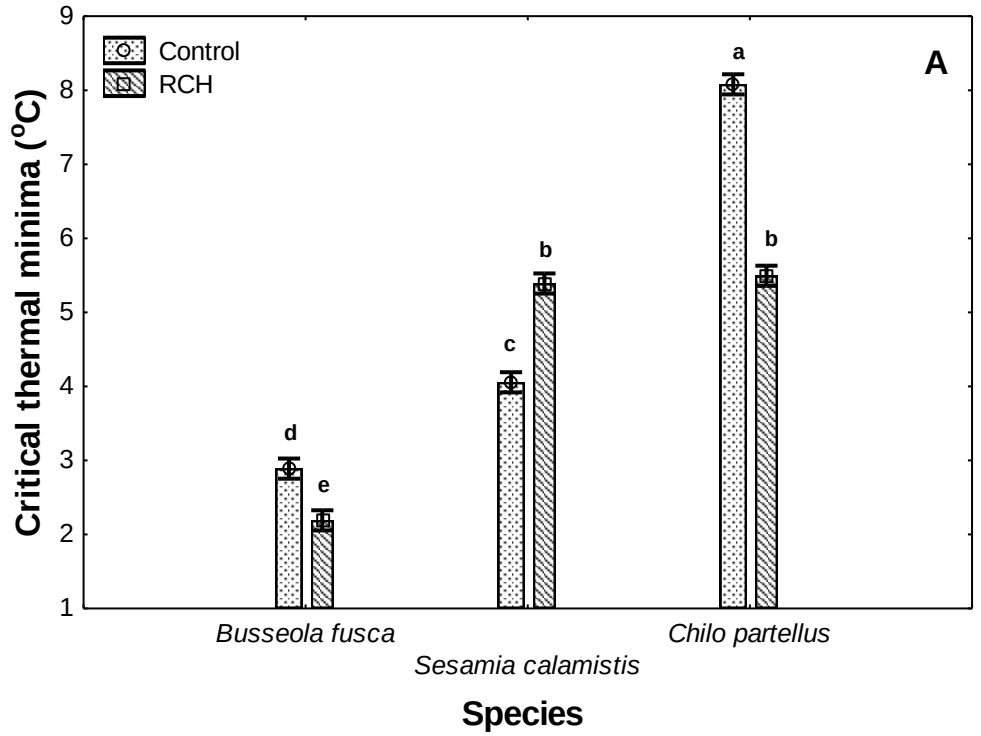
6.3.4 Effects of Hardening on CTLs

RCH significantly affected critical thermal minima and maxima across all species tested (Table 6.5A; Figure 6.4A; B). Short term low temperature pretreatment (RCH) significantly enhanced cold tolerance measured as CT_{min}, for *B. fusca* and *C. partellus* while reducing it for *S. calamistis* (Figure 6.4A). The magnitude of plastic responses for CT_{min} was highest in *C. partellus* relative to *B. fusca* and *S. calamistis.* Similarly, RCH decreased CT_{max} for all species, marginally so, but significant for *B. fusca* and non significant for *S. calamistis* and *C. partellus* (Figure 6.4B). Furthermore, there was a significant species * acclimation interaction effect for CT_{min} and a non-significant interaction effect for CT_{max} (Table 6.5A). On the other hand, RHH significantly affected CT_{min} and not CT_{max} (Table 6.5B). RHH compromised CT_{min} for *B. fusca*, while enhancing it for *C. partellus* (Figure 6.4C). Nevertheless, RHH did not have an effect on *S. calamistis* CT_{min} (Figure 6.4C). Similarly, pretreatment to high temperature (RHH) did not have a significant effect on CT_{max} across all species (Figure 6.4D). Nevertheless, there was a significant species effect for both CT_{min} and CT_{max} following RHH (Table 6.5B). In addition, there was a significant species * acclimation interaction effect for CT_{min} and a non-significant interaction effect for CT_{max} (Table 6.5B).

Table 6.5: Summary statistical results from full factorial ANOVA showing effects of (A) RCH and (B) RHH on CT_{min} and CT_{max} for *B. fusca*, *S. calamistis* and *C. partellus*. SS = sums of squares, d.f.=degrees of freedom.

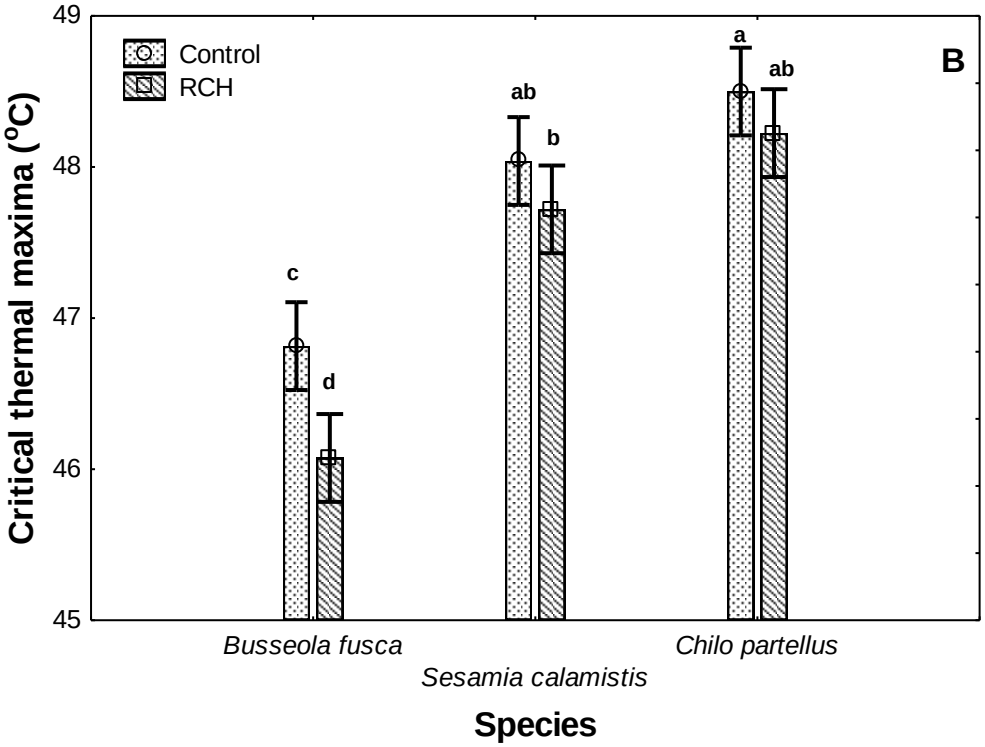
A

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	2632.03	1	2632.03	27924.78	< 0.001
	Species	360.92	2	180.46	1914.59	< 0.001
	RCH	12.68	1	12.68	134.48	< 0.001
	Species*Acclimation	76.87	2	38.45	407.78	< 0.001
	Error	10.75	114	0.094		
CT_{max}	Intercept	271463	1	271463	630788.1	< 0.0001
	Species	79.6	2	39.8	92.5	< 0.0001
	RCH	5.9	1	5.9	13.8	< 0.001
	Species*Acclimation	1.3	2	0.7	1.5	0.222
	Error	49.1	114	0.4		
B						
CT_{min}	Intercept	2836.3	1	2836.3	21842.72	< 0.0001
	Species	204.55	2	102.27	787.62	< 0.0001
	RHH	2.58	1	2.58	19.88	< 0.0001
	Species*Acclimation	113.81	2	56.91	438.25	< 0.0001
	Error	14.8	114	0.13		
CT_{max}	Intercept	273741.2	1	273741.2	895580.4	< 0.0001
	Species	60.5	2	30.2	98.9	< 0.0001
	RHH	0.1	1	0.1	0.2	0.645
	Species*Acclimation	0.1	2	0.1	0.3	0.998
	Error	34.8	114	0.3		

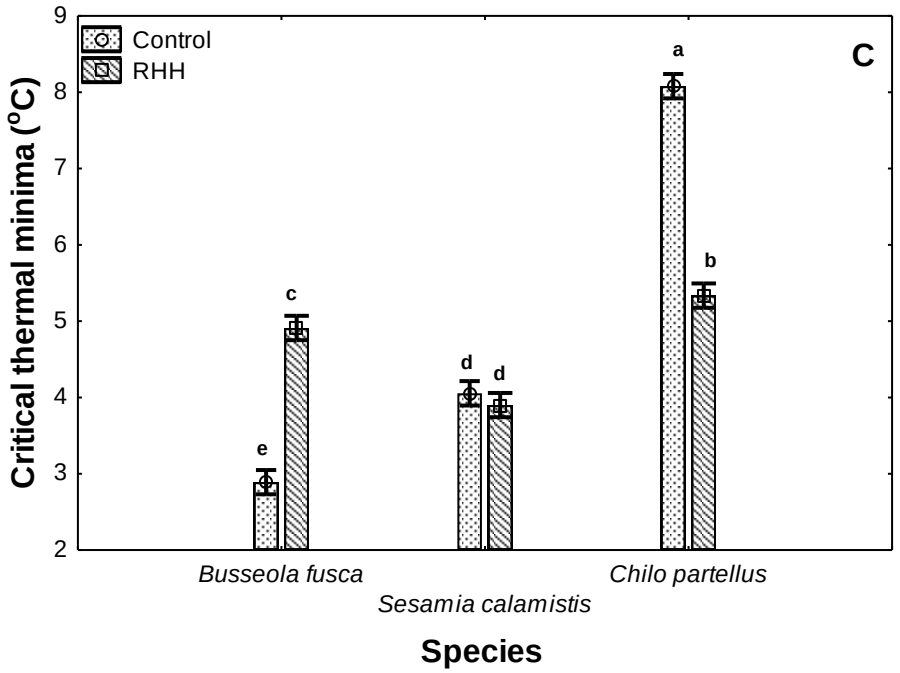


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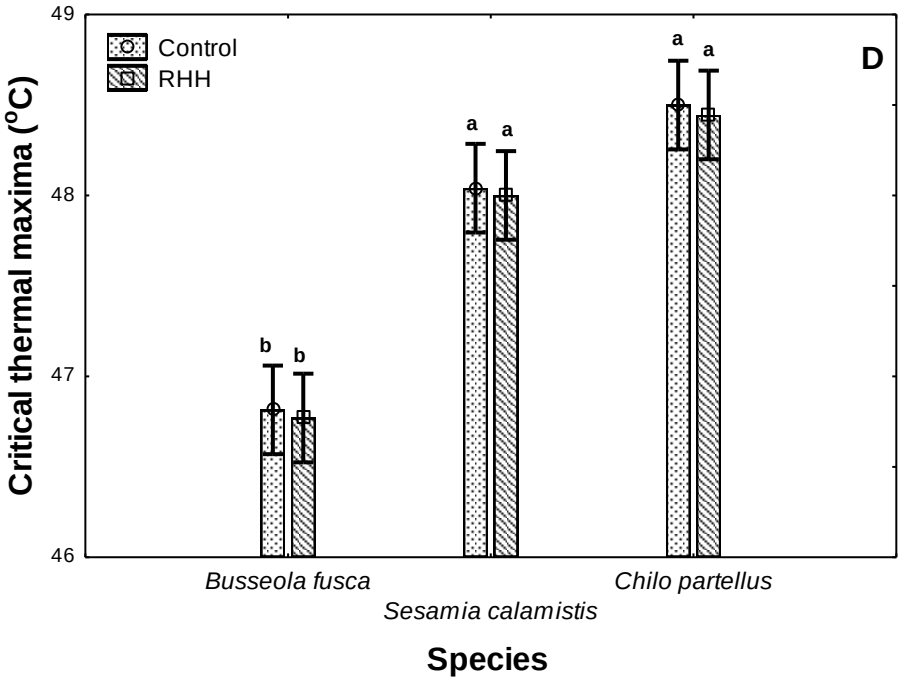
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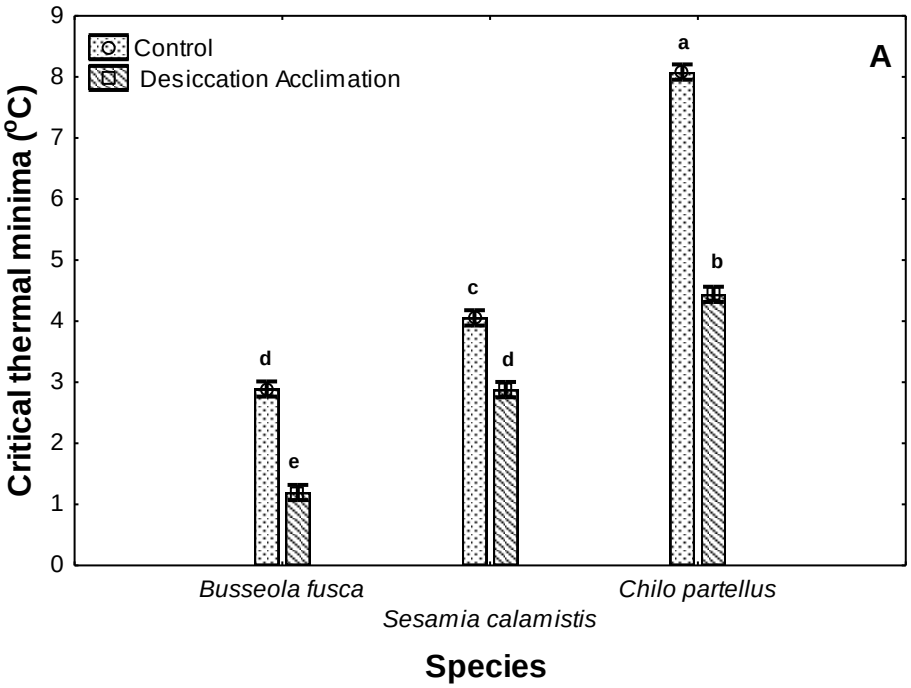
5006 **Figure 6.4:** Effects of RCH on (A) CT_{min} and (B) CT_{max} and RHH on (C) CT_{min} and (D) CT_{max}
5007 for *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* larvae. Error bars represent 95% CLs.
5008 Means with the same letter are not significantly different from each other. Post hoc tests were
5009 done separately for both treatments and across species

6.3.5 Effects of Desiccation Acclimation on CTLs

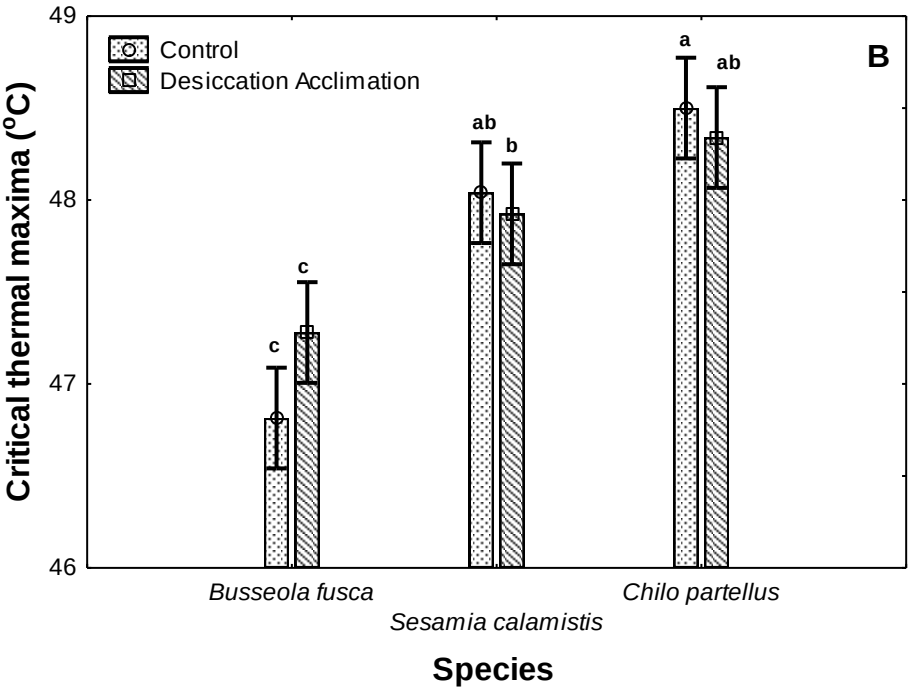
Like in starvation acclimation, desiccation acclimation significantly affected CT_{min} but not CT_{max} across all species (Table 6.6; Figure 6.5A; B). Desiccation acclimation improved CT_{min} across all species relative to their controls, with *C. partellus* recording the highest magnitude of plasticity (Figure 6.5A). Contrary to CT_{min} results, desiccation acclimation increased, marginally so, but not significant for *B. fusca* CT_{max}. Nevertheless, this acclimation did not have any significant effect on *C. partellus* and *S. calamistis* CT_{max} (Figure 6.5B). Furthermore, there was a significant species * acclimation interaction effect for CT_{min} and non-significant interaction effect for CT_{max} following desiccation acclimation (Table 6.6).

Table 6.6: Summary statistical results from full factorial ANOVA showing effects of desiccation acclimation on CT_{min} and CT_{max} for *B. fusca*, *S. calamistis* and *C. partellus*. SS = sums of squares, d.f.=degrees of freedom.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	1847.11	1	1847.11	23167.57	< 0.001
	Species	368.21	2	184.11	2309.18	< 0.001
	Desiccation Acclimation	141.27	1	141.27	1771.86	< 0.001
	Species*Acclimation	33.77	2	16.88	211.75	< 0.001
	Error	9.09	114	0.08		
CT_{max}	Intercept	274372	1	274372	718862.2	< 0.0001
	Species	39.3	2	19.7	51.5	< 0.0001
	Desiccation Acclimation	0.1	1	0.1	0.3	0.576
	Species*Acclimation	2.4	2	1.2	3.2	0.0451
	Error	43.5	114	0.4		



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5027 **Figure 6.5:** Effects of desiccation acclimation on (A) CT_{min} and (B) CT_{max} for *Busseola fusca*,
5028 *Sesamia calamistis* and *Chilo partellus*. Error bars represent 95% CLs. Means with the same
5029 letter are not significantly different from each other. Post hoc tests were done separately for both
5030 treatments and across species

6.4 Discussion

Insect thermal sensitivity is mediated by environmental conditions (Santos *et al*., 2011). The current study revealed that *C. partellus* is generally more heat tolerant compared to *B. fusca* and *S. calamistis*. Furthermore, *B. fusca* was more cold resistant relative to *C. partellus* and *S. calamistis*. While my study reported a higher CT_{max} for *C. partellus* adults and lower CT_{min} for *B. fusca* adults relative to *S. calamistis* adults (Mutamiswa *et al*., 2017b), the current study shows the same trend for larvae across all species (Figure 6.1). Nevertheless, the current study reveals novel differences in magnitude of plasticity of CTLs across the three species following acclimation to heterogeneous stressors. *Chilo partellus* showed a higher magnitude of plasticity of low temperature tolerance (CT_{min}) following thermal, starvation, desiccation, short term low and high temperature acclimation relative to *S. calamistis* and *B. fusca*. The same trend was true for *C. partellus* following acclimation to temperature and starvation for CT_{max}. Such physiological attributes may confer survival merits for *C. partellus* upon introduction to novel habitats and may help explain the increased dominance of the stemborer over indigenous species (Mutamiswa *et al*., 2017a). Similarly, Ntiri *et al*. (2016b) reported that performance of these three stemborer species is dependent on thermal tolerance limits for development. Generally, *B. fusca* thrives under low temperatures while *C. partellus* favors warmer habitats.

Effects of acclimation on insect thermal tolerance have been reported to vary across species (Kellet *et al*., 2005), developmental stages (Terblanche *et al*., 2007a; Marais *et al*., 2009) and also thermal history (Nyamukondiwa & Terblanche, 2010). As for CT_{min}, *B. fusca* and *C. partellus* larvae showed improved cold tolerance compared to *S. calamistis* larvae following low temperature acclimation (Figure 6.2A) indicating some improved potential for plasticity. Similarly, *S. calamistis* and *B. fusca* larvae also showed an enhanced cold tolerance for the same trait following acclimation to high temperature. These results are in keeping with previous study by Mutamiswa *et al*., (2017a) that reported improved performance of *S. calamistis* and *B. fusca* adults following acclimation to high temperature. Acclimation to high temperature improved CT_{max} for *C. partellus* relative to *S. calamistis* and *B. fusca* (Figure 6.2B) suggesting higher level of plasticity for *C. partellus* compared to other stemborer species. In consequence, the results reveal that *C. partellus* has a compromised basal low temperature tolerance and an improved basal high temperature tolerance, which comes with improved plasticity at both ends of temperature relative to other two borer species (see discussions in Nyamukondiwa *et al*., 2011).

This is in agreement with previous study on *Drosophila* species which reported that insects may trade-off basal low but not high temperature tolerance for plasticity (Nyamukondiwa *et al*., 2011). Given that global mean temperatures are projected to increase (Walther *et al*., 2002; Karuppaiah & Sujayanad, 2012), coupled with increased frequencies of heat waves and cold snaps (IPCC, 2014), *C. partellus* is highly likely to have a survival advantage at high temperatures relative to *B. fusca* and *S. calamistis* with lower thermal regimes. However, while *B. fusca* and *S. calamistis* may likely have a survival basal merit at low temperatures, it also appears, *C. partellus* may compensate for this through improved plasticity. This result affirms the notion that thermal trait characteristics among invasive species vary across taxonomic groups (Sakai *et al*., 2001).

Starvation has been reported to influence thermal tolerance in mosquitoes (*Culex fatigans*), head lice (*Pediculus humanus*) and bed bugs (*Cimex lectularius*) (DeVries *et al*., 2016). The current results showed an enhanced high temperature tolerance (CT_{max}) for *C. partellus* relative to other stemborer species following starvation acclimation (Figure 6.3B), in keeping with results on *C. lectularius* (De Vries *et al*., 2016). However, starvation acclimation did not improve CT_{max} for *B. fusca* and *S. calamistis* in agreement with Scharf *et al*. (2016), who reported lack of improved heat tolerance (measured as heat knock down time) for *Tribolium castaneum*. In addition, *B. fusca*, *S. calamistis* and *C. partellus* showed an improved cold tolerance (measured as CT_{min}) following starvation acclimation (Figure 6.3A) in agreement with previous study on *Locusta migratoria* that reported improved cold tolerance (measured as chill coma recovery time) after 24 hour starvation period (Andersen *et al*., 2013). Similarly, starvation also improved cold tolerance for vinegar fly *D. melanogaster* (Le Bourg, 2015). The increased CT_{max} following starvation acclimation likely suggests shared physiological mechanisms for the traits for *C. partellus* and a lack thereof, for *S. calamistis* and *B. fusca*. Moreover, this may likely suggest that mechanisms underlying cross tolerance to environmental stressors are different across different taxonomic groups. Similarly, the improvement in CT_{min} following starvation acclimation indicate cross tolerance for the traits involved, and likely suggest coevolution of resistance mechanisms e.g. through lipid metabolism (Sinclair *et al*., 2015). Differential heat and cold tolerance responses for these stemborer species following starvation gives an insight on the difficulties these insects may face when they encounter multiple but simultaneous interacting environmental stressors in their natural habitats. In the current study, I found supporting

evidence for cross-susceptibility (marginal decrease in tolerance) of upper thermal limits to starvation for *S. calamistis* in keeping with previous study on forest ant, *A. picea* (Nguyen *et al*., 2017). DeVries *et al*. (2016) reported a correlation between lower metabolic rate and lower upper thermal limits for *C. lectularius* starved for 21 days. Low metabolism leads to low respiratory water loss hence the inability to cool through active evaporative cooling thus low CT_{max} (DeVries *et al*., 2016). Given that *B. fusca* recorded the lowest CT_{max} relative to *S. calamistis* and *C. partellus*, it's highly likely that its metabolic rate may have been lowered during starvation.

The current study also demonstrates insects' response to CTLs following short term pretreatment (RCH and RHH). *Busseola fusca* and *C. partellus* recorded an improved cold stress resistance (CT_{min}) following RCH, in agreement with previous studies on *D. melanogaster* and *F. candida* which reported a significant cold stress resistance following cold hardening (Sejerkilde *et al*., 2003; Waagner *et al*., 2013). Nevertheless, magnitude of this improvement was asymmetrical, being higher for *C. partellus* relative to other two stemborer species (Figure 6.4A). This cold tolerance may give survival advantage for these two borer species *B. fusca* and *C. partellus* when they experience cold winters in sub Saharan Africa, albeit *C. partellus* may have a fitness edge over *B. fusca* through greater plasticity magnitude. The results are also in keeping with previous studies to date (Assefa, 1985; Mwalusepo *et al*., 2015) that showed *B. fusca* preferring cooler climates compared to *S. calamistis* and *C. partellus*. However, cold hardening did not improve heat tolerance for all the borer species (Figure 6.4B) indicating evidence of marginal cross susceptibility of CT_{max} to RCH. The results also showed that RHH did not improve cold tolerance (CT_{min}) for *S. calamistis* while significantly compromising for *B. fusca.* However, RHH significantly improved CT_{min} for *C. partellus* (Figure 6.4C). This result suggests a survival demerit for *S. calamistis* and *B. fusca* when faced with these multiple stressors relative to *C. partellus.* Such cross tolerance has been reported in other studies (e.g. Bublily *et al*., 2012), and may be linked with overlap in mechanisms e.g. heat shock proteins. Although, the stemborer species were hardened at temperatures ranging from 40-41°C, RHH results showed negligible and non significant change in CT_{max} (Figure 6.4D) in keeping with Chidawanyika *et al*. (2017) who reported poor heat tolerance (measured as CT_{max}) using dynamic protocol for *Z. bicolorata* following 2 h hardening at 35°C. However, the reason for this trend is unknown. The beneficial acclimation hypothesis nevertheless states that acclimation response to changing environmental conditions would be beneficial for survival under the new

set of conditions (Leori *et al*., 1994). Heat shock proteins have been reported to enhance heat resistance in insects (Sørensen *et al*., 2003). For example, Krebs (1999) reported that short term exposure of *D. simulans* adults to temperatures between 33 and 37°C resulted in an increased expression of Hsp70. Similarly, Tungjitwitayakul *et al*. (2015) reported production of heat shock proteins (Hsp70 and Hsp90) by *Sitophilus zeamais* following heat shock at 30–50°C for 1 h. However, in the current study *S. calamistis* and *C. partellus* were exposed to a sub-lethal temperature of 41°C for 2 hours, thus it is highly likely that enhanced heat tolerance for these species may have been facilitated by the production of heat shock proteins and other metabolic regulation pathways following RHH. However, the mechanisms behind cold and heat tolerance following RCH and RHH in these stemborer species still warrants further investigation.

Like in starvation acclimation, desiccation acclimation also resulted in improved CT_{min} for all stemborer species (Figure 6.5A) indicating cross tolerance effects against cold stress. Insects have been reported to upregulate their heat shock proteins synthesis following desiccation (e.g Feder & Hofmann, 1999) and this response may have led to improved cold tolerance for the three borer species. This improved CT_{min} is in agreement with previous study on the soil collembolan, *F. candida* which reported improved cold tolerance following desiccation stress (Bayley *et al*., 2001). Ring & Danks (1994) reported that there is a linkage between desiccation tolerance and cold tolerance in insects with some species utilising desiccation as a mechanism for cold tolerance strategy (Holmstrup *et al*., 2002). Contrary to the relationship between starvation acclimation and CT_{min}, I found no significant association between starvation acclimation and CT_{max}. This result suggests no cross tolerance between traits of starvation and high temperature tolerance for the three stemborer species. A previous study on *D. melanogaster* has shown negligible impact of desiccation and starvation stress on CT_{max} (Kellermann *et al*., 2012), in agreement with the current study. The current results also show marginal negative impacts of desiccation stress on high temperature tolerance (CT_{max}) for *S. calamistis* and *C. partellus*. Similarly, Tammarielo *et al*. (1999) reported a decrease in high temperature tolerance for flesh fly, *Sarcophaga crassipalpis* following 24 h desiccation stress. In tropical regions the frequencies and severity of droughts are expected to increase under climate change (IPCC, 2014), hence small insects will be at risk of desiccation stress due to their high surface area to volume ratio (Bujan *et al*., 2016). Moreover, in southern African future climate change scenarios, extreme heat is expected to occur simultaneously with reduced rainfall (Lobell *et al*., 2008;

Stathers *et al*., 2013) resulting in insects experiencing simultaneous heat and desiccation stress (Mueller & Seneviratne, 2012; Nguyen *et al*., 2016). This result suggests vulnerability of the three borer species when faced with multiple stressors of desiccation and high temperature.

The current study demonstrates significant impacts of heterogeneous environmental stressors on stemborer species thermal tolerance and how that likely shapes their fitness traits under climate change. I document significant improvement in CT_{min} following acclimation to high temperature for *B. fusca* and *S. calamistis* and improvement in CT_{max} following high temperature acclimation for *C. partellus*. Similarly, I record costs of CT_{min} following acclimation to high temperature for *C. partellus*. Nevertheless, one thing is apparent for my study, that *C. partellus* has a higher magnitude of plasticity for CTLs following acclimation to temperature, starvation, RCH, RHH and desiccation. This increased plasticity may likely aid its invasion potential and establishment when faced with novel heterogeneous stressors ultimately displacing indigenous stemborer species in sub Saharan Africa. Indeed, invasive species should possess inherent high potential for rapid evolutionary response to changing environmental conditions (Lee, 2002; Whitney & Gabler, 2008). The results of this study have major implications on mechanisms allowing insects survival under diverse heterogeneous stressors and may likely explain invasive potential of economic insects.

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CHAPTER 7

General Discussion

Climate change effects on agro-ecosystems are becoming apparent with seasonal and long term variations influencing insect pests population dynamics, abundance and activities of natural enemies and efficacy of pest management (Selvaraj *et al*., 2013). In the agroecosystem, higher trophic levels, usually associated with natural enemies are highly sensitive to climate variability compared to the hosts (Voigt *et al*., 2003). In this respect, divergences in the thermal tolerances of the pest and its natural enemy may ultimately lead to a disruption of their temporal or geographical synchronization, increasing the risk of host outbreaks (Hance *et al*., 2007). Although there have been extensive studies on thermal tolerance of various insect pests (e.g. Koveos, 2001; Grout & Stoltz, 2007; Rajamohan & Sinclair, 2008; Kalosaka *et al*., 2009; Nyamukondiwa & Terblanche, 2009; Chidawanyika & Terblanche, 2011; Piyaphongkul, 2013), to the best of my knowledge, this is the first study detailing climate variability effects on stemborers-natural enemies trophic level interaction and how this may impact on biological control under global change.

The present study reviewed the ecological and thermal physiological factors contributing towards *C. partellus* invasion potential and dominance over indigenous stemborer species in Africa (Chapter 2; Mutamiswa *et al*., 2017a). Laboratory experiments showed that the exotic *C. partellus* had a higher basal thermal tolerance and plasticity thereof, compared to indigenous *B. fusca* and *S. calamistis*. This indicated that *C. partellus* trades off its basal low but not high temperature tolerance for plasticity as in like insect taxa (e.g Nyamukondiwa *et al*., 2011), further explaining that ecophysiology may aid its invasion potential relative to *B. fusca* and *S. calamistis*. Apart from its improved physiological tolerances, the study also showed that short generation time, overwintering physiology, temperature and relative humidity resilience, wide host preferences and asynchrony with biocontrol agents may have facilitated *C. partellus* invasion potential and dominance over indigenous stemborer species. These attributes revealed that *C. partellus* is highly competitive and with Africa's changing climate, it has a high likelihood of expanding and establishing in novel habitats.

Basal and plasticity of thermal tolerance of stemborers and their coexisting stemborer parasitoids determine their abundance, biogeographical patterns and activity over spatial and temporal scales ultimatly impacting biocontrol programs under climate variability. The current study also explored the comparative thermal tolerance of different developmental stages of *Chilo partellus* and its larval parasitoid, *Cotesia sesamiae* (Chapter 3; Mutamiswa *et al*., 2017b) and

coexisting parasitoid species (*C. sesamiae* and *C. flavipes*) (Chapter 5) through measuring their lower and upper lethal temperatures, critical limits to activity, supercooling points, chill-coma recovery time and heat knock-down time. The severity and duration of exposure influenced the survival of *C. partellus*, *C. sesamiae* and *C. flavipes* in agreement with previous studies (Chown & Terblanche, 2007; Chidawanyika & Terblanche, 2011). LLTs and ULTs comparisons for *C. partellus* and *C. sesamiae* (2 h duration) showed the parasitoid being less cold and heat tolerant relative to the host (Figure 3.1B; 3.1D; 3.2A; 3.2B; Chapter 3). On the other hand, for 0.5–4 h treatments, LLTs for *C. sesamiae* and *C. flavipes* ranged from -5 to 5°C and -15 to -1°C whereas ULTs ranged from 35 to 42°C and 37 to 44°C respectively (Figure 5.1A-D; Chapter 5), suggesting that *C. flavipes* is more cold and heat tolerant than *C. sesamiae*. Critical thermal limits are dependent on methodological context (Terblanche *et al*. (2007) and experiments conducted using ecologically relevant ramping rates showed slower ramping rates significantly reducing CT_{max} in *C. partellus* adults, larvae and *C. sesamiae* adults (Figure 3.3A; C; E; Chapter 3) indicating that in the short term these species may not be able to adjust their phenotypic plasticities of high temperature tolerance (see discussions in Nyamukondiwa & Terblanche, 2010; Sgrò *et al*., 2016). Similarly, slower cooling rates significantly improved CT_{min} in adult *C. partellus* and *C. sesamiae* indicating that a prolonged low temperature exposure may allow the insects to develop some low temperature protection, suggesting RCH. *Cotesia flavipes* had lower CT_{min} and higher CT_{max} than *C. sesamiae* (Figure 5.2; Chapter 5), indicating a fitness advantage for the exotic *C. flavipes* relative to indigenous *C. sesamiae* at both thermal extremes. This result translates into a broader thermal safety margin for *C. flavipes* which ultimately confers better optimization of key life history activities such as host searching ability (Mailafiya *et al*., 2009) more than congeneric *C. sesamiae* under stressful and variable thermal regimes. *Cotesia flavipes* recovered faster from chill coma and took more time to be knockdown by high temperature than *C. sesamiae* (Figure 5.4; 5.5; Chapter 5) signifying its survival and fitness advantage to cold and heat exposure relative to its congener. Furthermore, SCPs were recorded for the first time for these two *Cotesia* species and ranged ~ -20°C. In the current study the two *Cotesia* species failed to recover after supecooling suggesting that they were chill susceptible (killed by chilling) (Sinclair *et al*., 2015). With the host larvae being the most targeted developmental stage by the parasitoids, the differential thermal tolerance responses indicate a mismatch between *C. partellus* and *C. sesamiae* in the face of climate change. Consequently, this may negatively affect future

biological control efficacy. Furthermore, with African temperatures projected to increase (Gemeda & Sima, 2015), coupled with increased likelihood of prolonged heat waves, and cold snaps (IPCC, 2014), it is highly likely that *C. partellus* may survive both prolonged high and low temperatures better than *C. sesamiae*. This shows potential risks associated with *C. partellus* outbreaks, which may negatively affect cereal crop productivity, household and national food security. Similarly, *C. flavipes* may survive more prolonged low and high temperature extremes than its congener *C. sesamiae* due to its superior thermal physiology ultimately displacing the generalist indigenous parasitoid thus more oubreaks of other stemborer species.

An understanding of plasticity of cold and heat tolerance provides an insight for survival and interaction of different trophic level species and how natural enemies released for biocontrol will establish under natural conditions (Chapter 4). The current study showed evidence of RCH and RHH responses in *C. partellus* and *C. flavipes* in corroboration with previous studies (e.g Overgaard & Sorenson, 2008; Kalosaka *et al*., 2009; Stotter & Terblanche, 2009; Nyamukondiwa *et al*., 2010; Nyamukondiwa & Terblanche, 2010; Chidawanyika & Terblanche, 2011; Chidawanyika *et al*., 2017). Microclimatic temperature data recorded in an agro-ecosystem where both *C. partellus* and *C. flavipes* coexist showed that temperatures eliciting RCH and RHH responses at both trophic levels occur naturally (Figure 4.4; Chapter 4). This may imply that under projected climate change (IPCC, 2014), RCH and RHH may enhance fitness for both species albeit *C. partellus* recorded a lower cold hardening and discriminating temperature and higher heat hardening and discriminating temperature relative to *C. flavipes* (Table 4.1; Chapter 4). Species comparison of CCRT and SCP (following acclimation to low and optimum temperatures) and CT_{min} (across all acclimation treatments) between the two trophic levels (Figure 4.2C; 4.2D; 4.2E; Chapter 4), showed that *C. flavipes* is more cold tolerant than *C. partellus*. In consequence, this may have a fitness and survival advantage for *C. flavipes* relative to *C.partellus* under variable low temperatures hence positive trend on biological control under global change (see Agrawal, 2001). On the other hand, *C. partellus* showed a higher CT_{max} than *C. flavipes* across all acclimation treatments indicating that the host is more heat tolerant than the parasitoid. While *C. flavipes* was more cold tolerant than *C. partellus* and Africa projected to experience rise in temperatures, erratic rainfall patterns and increased extreme weather events (Stathers *et al*., 2013; IPCC, 2014), the host is highly likely to survive high temperatures

compared to its parasitoid, and this may create asynchronous phenologies with negative implications on biological control efficacy.

In nature, insects are often exposed to episodic multiple environmental stressors that may influence their activity, survival abundance and geographic range expansion. In this study, I investigated the effects of acclimation to temperature, starvation and desiccation on thermal tolerance, measured as critical thermal limits on laboratory reared *B. fusca*, *S. calamistis* and *C.partellus* using dynamic (ramping) protocols (Chapter 6). *Chilo partellus* had a higher magnitude of plasticity of low temperature tolerance following thermal, starvation, desiccation, short term low and high temperature acclimation relative to *B. fusca* and *S. calamistis*. In addition, C. *partellus* also showed the same trend following thermal and starvation acclimation for high temperature tolerance. This indicates fitness and survival advantage for *C. partellus* upon introduction to novel habitats and may reflect its increased dominance over indigenous species (Mutamiswa *et al*., 2017a). *Busseola fusca* and *C. partellus* larvae showed improved cold tolerance following low temperature acclimation whereas *S. calamistis* and *B. fusca* larvae also showed an enhanced cold tolerance following acclimation to high temperature in keeping with previous study (Mutamiswa *et al*., 2017a). On the other hand, high temperature acclimation improved CT_{max} for *C. partellus* relative to *S. calamistis* and *B. fusca* suggesting higher plasticity levels for *C. partellus* relative to other stemborer species. Overall, the results show that *C. partellus* has a compromised basal low temperature tolerance and an improved basal high temperature tolerance, which is generally associated with improved plasticity relative to other two borer species (see discussions in Nyamukondiwa *et al*. 2011). Starvation acclimation improved high temperature tolerance for *C. partellus* relative to other stemborer species (Figure 6.3B; Chapter 6), in keeping with *C. lectularius* results (De Vries *et al*., 2016). Furthermore, starvation also improved cold tolerance (Figure 6.3A; Chapter 6) for *B. fusca*, *S. calamistis* and *C. partellus* in agreement with previous study on *Locusta migratoria* (Andersen *et al*., 2013) and *D. melanogaster* (Le Bourg, 2015). Overall, this improvement in cold and heat tolerance following starvation acclimation indicates cross tolerance effects for the involved traits. *Busseola fusca* and *C. partellus* recorded an improved cold stress resistance following RCH, in agreement with previous studies on *D. melanogaster* and *F. candida* (Sejerkilde *et al*., 2003; Waagner *et al*., 2013) although *C. partellus* had a higher magnitude of improvement (Figure 6.4A; Chapter 6). This cold tolerance may give these two borer species survival advantage when they encounter

cold winters in sub Saharan Africa, albeit *C. partellus* may have fitness superiority over *B. fusca* through greater magnitude of plasticity. On the other hand, RHH significantly improved cold tolerance for *C. partellus* (Figure 6.4C; Chapter 6) indicating cross tolerance as reported in previous studies (e.g. Bublily *et al*., 2012). This implies that *S. calamistis* and *B. fusca* may have survival disadvantage when faced with these multiple stressors in their natural habitats relative to *C. partellus.* Desiccation acclimation also resulted in improved cold tolerance for all stemborer species (Figure 6.5A; Chapter 6) indicating cross tolerance effects against cold stress. Insects have been reported to upregulate their heat shock proteins synthesis following desiccation (e.g. Feder & Hofmann, 1999) and this response may have led to improved cold tolerance for the three borer species. Desiccation stress marginally impaired high temperature tolerance (CT_{max}) for *S. calamistis* and *C. partellus* in keeping with previous study by Tammarielo *et al*. (1999). Given these heat and cold tolerance variabilities for these stemborer species following hardening, thermal, starvation and desiccation acclimation, it therefore gives an insight on the difficulties these insects may face when they experience multiple environmental stressors in their natural habitats.

While the current study investigated basal thermal tolerance and plasticity for laboratory reared stemborer and parasitoid species, future studies should consider comparative assessment of laboratory and wild populations for the same traits to determine their fitness and survival under climate change. Although the current study explored the effects of environmental stressors on thermal tolerance (measured as CTLs) on stemborer species only, future studies should focus on determining how these stressors may influence thermal tolerance (incorporating more life history traits) of these stemborer species and their biological control agents under climate change. The current study revealed evidence of hardening (RCH and RHH) in *C. partellus* and *C. flavipes* which led to improved cold and heat tolerance in these species, thus future studies should investigate mechanisms behind these hardening responses. Furthermore, a combined temperature and relative humidity variability effect on life history traits of stemborers and their parasitoids should also be considered in future studies.

In conclusion, the results of this thesis indicate that climate variability may have dire consequences on stemborers-natural enemies trophic level interactions in sub Saharan Africa. Variations in thermal preferences for stemborer species and their associated parasitoid species recorded in this project may lead to changes in temporal and geographical synchronization

ultimately increasing the risks of host outbreaks. Because of its superior basal thermal tolerance and plasticity thereof, exotic *C. partellus* has a high likelihood of displacing indigenous stemborer species, *B. fusca* and *S. calamistis* as well as escaping parasitism from *C. sesamiae* and *C. flavipes* with low thermal regimes under global change. This may negatively influence future classical biological control programs since the host may have a comparative advantage following exposure to extreme conditions relative to the parasitoid. Overall the results of this project provide valuable information for predictive models for forecasting economic insect pest outbreaks, conducting physosanitary risk assessments, establishing invasive species, biogeographical shifts, and the potential implications of global climate change.

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APPENDIX 1

Diversity and abundance of lepidopteran stem borer natural enemies in natural and cultivated habitats in Botswana*

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1.1 Introduction

Cereal crops such as maize, sorghum and millet are mostly grown by small-scale farmers at subsistence level in sub-Saharan Africa, with the majority of cereal fields usually surrounded by patches of natural habitats harbouring stemborer wild host plants (e.g. grasses and sedges) (Mailafiya *et al*., 2009). These natural habitats often serve as refugia for stemborers and their parasitoids, sustaining a diversity of stemborer natural enemies within the agroecosystem (Mailafiya *et al*., 2009; Moolman *et al*., 2013). Lepidopteran stem borers are among the most destructive insect pests of cereal crops in sub-Saharan Africa, accounting for 5-75% of yield losses (De Groote, 2002; Kfir *et al*., 2002; Kipkoech *et al*., 2006; Moolman *et al*., 2013). In Africa, this represents a significant household food insecurity burden since ~70-80% of the population depends on subsistence agriculture (FAO, 2002). The major lepidopteran cereal stemborer species accounting for damage in Africa include the indigenous pyralid *Eldana saccharina* Walker, the crambid *Chilo orichalcociliellus* (Strand), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the exotic crambid *Chilo partellus* Swinhoe (Kfir *et al*., 2002; Obonyo *et al*., 2010; Addo-Bediako & Thanguane, 2012).

The abundance and distribution of cereal stemborers may be influenced by abiotic factors such as temperature and precipitation, and biotic factors such as natural enemies and alternative host plants (Mailafiya *et al*., 2009; Addo-Bediako & Thanguane, 2012). Both parasitoid diversity and parasitism levels have been reported as higher in arable fields intermixed with natural habitats than in arable fields only (Thies *et al*., 2003), indicating the role of natural habitats in hosting parasitoid diversity. Although natural habitats have been reported to provide refuges for some parasitoid species, low levels of stemborer parasitism (<8%) have been recorded in East and Austral Africa in wild host plants (Mailafiya *et al*., 2011; Moolman *et al*., 2013). Gramineous plants are known to produce secondary metabolites that recruit stemborer parasitoids (Potting *et al*., 1995; Arab & Bento, 2006). Indeed, this is the case for maize and sorghum plants infested with *B. fusca* and *C. partellus* (Mutyambai *et al*., 2015). Isolated volatiles from these plants are effective in recruiting the braconid parasitoids *Cotesia sesamiae* Cameron and *Cotesia flavipes* Cameron, and the ichneumonid *Dentichasmias busseolae* Heinrich (Arab & Bento, 2006).

An understanding of the diversity, spatial distribution, abundance and ecological contributions of stemborer natural enemies in both natural and managed ecosystems is required

to implement an integrated approach to stemborer pest management (Conlong & Rutherford, 2009). Natural enemies play an important role in the control of lepidopteran stemborers (Sylvain *et al*., 2015; Midingoyi *et al*., 2016) and help prevent pest outbreaks (Muyekho *et al*., 2005; Mailafiya *et al*., 2010a, 2013; Kodjo *et al*., 2013). In eastern and southern Africa, cereal stemborer populations are regulated by a wide range of native and exotic parasitoid species (Conlong, 2000; Zhou *et al*., 2003; Moolman *et al*., 2013). Most of these parasitoids belong to the orders Hymenoptera and Diptera (Polaszek, 1998; Conlong, 2000; Zhou *et al*., 2003; Moolman *et al*., 2013). There have been numerous attempts over the past decades to introduce exotic parasitoids into Africa for biological control of indigenous and exotic stemborers, but only a few species were established successfully (Conlong, 1994, 1997, 2001; Kfir *et al*., 2002). Nevertheless, *C. flavipes*, a *C. partellus* larval endoparasitoid, was introduced from Pakistan into Kenya in 1993 where it became established (Overholt *et al*., 1997; Kfir *et al*., 2002; Kipkoech *et al*., 2006; Dejen *et al*., 2013), and releases have subsequently been successful in nine other countries in east and southern Africa (Kipkoech *et al*., 2006; Omwega *et al*., 2006; Chinwada *et al*., 2008). Different life stages of stemborers are attacked by predators such as ants, spiders, earwigs, nematodes and microbial pathogens [*Beauveria bassiana* (Balsamo), *Bacillus thuringiensis* Berliner, *Serratia marcescens* Bizio] in various landscapes (Oloo, 1989; Bonhof *et al*., 1997; Assefa *et al*., 2006). However, data also have shown that indigenous natural enemies may be unable to keep stemborer populations below economic levels (Overholt *et al*., 1994; Bonhof *et al*., 1997), unless their potential efficacy can be improved through conservation.

The diversity and abundance of stemborer natural enemies both vary across the landscape and the distribution of some may be threatened by global climate change (Batalden *et al*., 2007; Kodjo *et al*., 2013). Previous studies have documented lepidopteran stemborers, their parasitoids, and associated host plants in many African countries, including Ethiopia (Assefa *et al*., 2006), Uganda (Conlong and Mugalula, 2001; Matama-Kauma *et al*., 2007; 2008), Kenya (Mailafiya *et al*., 2009), DR Congo (Kankonda *et al*., 2017), Cameroon (Ndemah *et al*., 2007), Togo (Kodjo *et al*., 2013), Zimbabwe (Chinwada & Overholt, 2001; Mazodze & Conlong, 2003), Mozambique (Conlong & Goebel, 2002, 2006; Moolman *et al*., 2013) and South Africa (Moolman *et al*., 2013). Apart from a survey of *Cyperus papyrus* L. for parasitoids of *E. saccharina* (Conlong *et al*., 1988), similar information has been lacking for Botswana. Through extensive surveys, I assessed the diversity and abundance of stemborer natural enemies and their associated hosts in

both cultivated and natural habitats in Botswana. Such information is crucial in understanding the roles of natural enemies in regulating insect pest populations, and may help develop improved pest management systems. Following the results of Mailafiya *et al*. (2009), I hypothesized that natural enemy species diversity would be higher in natural compared to cultivated habitats in Botswana.

1.2 Materials and methods

1.2.1. Field surveys

Field surveys in cereal crops and wild host plants were conducted in all ten districts of Botswana during the austral summers of 2014/15 and 2015/16 (Figure 1.1). Sampling was conducted once in each district during both seasons, for a total of 84 sites sampled.

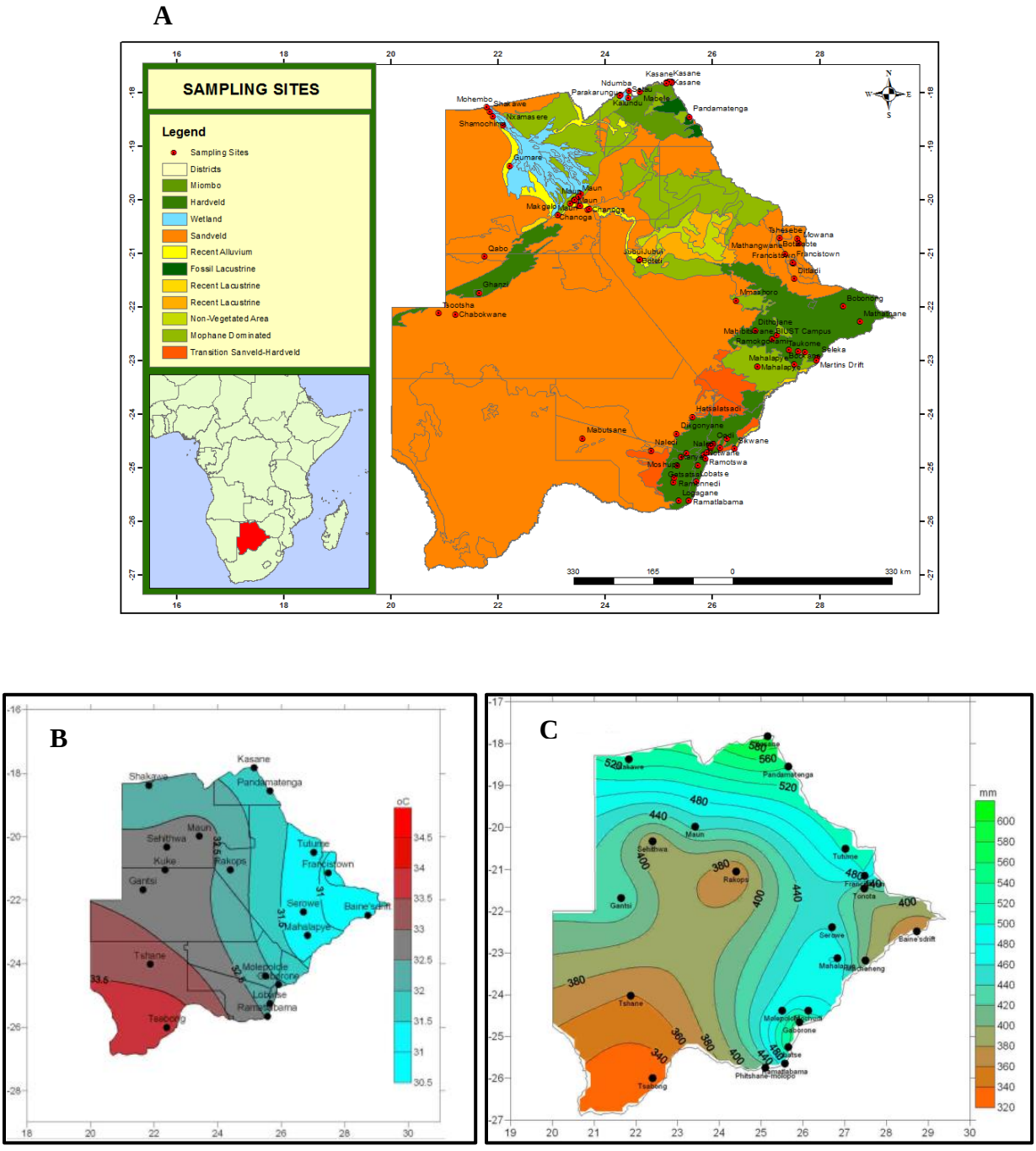


Figure 1.1: Botswana maps showing (A) areas surveyed for stemborers in cultivated and natural habitats during 2014/15 and 2015/16 austral summer (B) average annual temperatures and (C) average annual rainfall for the sampled districts.

1.2.1.1. Sampling in natural habitats

Wild lepidopteran stemborer host plants belonging to the families Poaceae, Cyperaceae and Typhaceae were sampled (i) around cereal crops, (ii) in open patches along forest roads, (iii) on banks of streams or rivers (iv) in swamps and, (v) in national parks. A biased sampling procedure was employed to increase the chances of finding stemborers, as previous studies reported considerably lower densities of borers in wild host plants than in cultivated crops (Gounou & Schulthess, 2004; Le Ru *et al*., 2006; Mailafiya *et al*., 2009; Moolman *et al*., 2013; 2014). Potential host plants were inspected for stemborer damage symptoms such as dry leaves and shoots (dead hearts), scarified leaves (window panes and pin/shot holes), larval entry and exit holes in stems, and the presence of frass. Stems that exhibited damage symptoms were dissected in the field and any borer life stages found within were placed individually in 30 ml plastic vials (South African Sugarcane Research Institute, South Africa) containing artificial diet and sealed with aerated screw-caps. The diet for noctuids was that formulated by Onyango and Ochieng'-Odero (1994), and that for pyralids and crambids, that of Walton & Conlong (2016). Predatory insects, parasitoid cocoons or dead larvae were placed individually in labelled plastic vials without diet, while pupae were placed in the same vials with a piece of wet cotton wool to prevent desiccation.

1.2.1.2. Sampling in cultivated habitats

To survey cereal crops, fields of sweet sorghum, maize, millet and sorghum were selected every 10–15 km along minor and major roads in each district 10-15 fields per village in each district. Each field was divided into four quadrats and 10 plants per quadrat were randomly sampled along a transect. Each plant was dissected and searched for stemborer and parasitoid life stages which were collected as described above for wild host plants.

For each vial, I recorded (1) the date of collection, (2) name of host plant (if it could be identified in the field), (3) stemborer developmental stage and (4) borer and/or parasitoid species (if it could be identified in the field). Latitude, longitude and altitude were also recorded for each field using a portable Geographic Positioning System (GPS) (Garmin GPSMAP 62st). Following collection, all vials were placed in coolers and taken to the laboratory for subsequent incubation.

1.2.2. Laboratory incubation

In the laboratory, all insects were incubated in climate-controlled chambers (HPP 260, Memmert GmbH + Co.KG, Germany) at 25 ± 1 ^{o}C, 65 ± 10% RH, and a 12:12 (L:D) photoperiod until adult moth or parasitoid emergence. Predatory insects and emerged adult parasitoids were preserved in 70% alcohol for subsequent identification. Adult stemborers and parasitoids were identified to family by gross morphology, and to genus or species level by the third author in the biosystematics unit of African Insect Science for Food and Health (ICIPE), Kenya. This was done by examining external morphology and genitalia as described in Polaszek (1998). When identification to species level was not possible, genus and family levels were used. Wild host plant species were identified at the department of National Museum and Monuments, Herbarium unit, Botswana.

1.2.3. Data analysis

Host plant - parasitoid and stemborer - parasitoid interaction networks were analyzed in R using 'BIPARTITE' package (Dormann et al., 2009). In all interaction network analyses, the number of parasitized hosts against host plants and stemborer species in all habitats were used. Parasitoid species diversity in natural and cultivated habitats was computed using Simpson's diversity index (Simpson, 1949):

$$D = \frac{\sum \mathrm{n}(\mathrm{n}-1)}{\mathrm{N}(\mathrm{N}-1)}$$

Where D is the Simpson diversity index, N is the total number of organisms found and n is the number of individuals of each species. Percentage parasitism of (1) larvae and (2) pupae and, (3) the relative abundance of each parasitoid species were calculated as outlined by Zhou *et al* (2003), Dejen *et al*. (2013), Rahaman et al. (2014) and Baskaran *et al*. (2017) as follows:

(1) $Larval\ parasitism = \frac{\text{Number of parasitized larvae X 100}}{\text{Number of healthy larvae + Number of parasitized larvae}}$

(2) $Pupal\ parasitism = \frac{\text{Number of parasitized pupae X 100}}{\text{Number of healthy pupae + Number of parasitized pupae}}$

(3) $Relative\ abundance\ of\ each\ parasitoid\ sp = \frac{\text{Total of individuals of each sp X 100}}{\text{Total number of all parasitoids}}$

1.3 Results

1.3.1. Natural enemy diversity, stemborer hosts and host plants

A total of 1497 stemborer larvae and 228 pupae were collected from three different cultivated plants and 11 different wild host plants belonging to the families Poaceae, Cyperaceae and Typhaceae (Table 1.2; 1.3; Figure 1.2B). From these collections, 30 natural enemy species (27 parasitoids and 3 predators) belonging to 13 insect families and two orders were recovered. Parasitoid diversity was generally higher in cultivated than in natural habitats (D = 0.23 versus 0.14; Table 1.1). Nineteen of the 30 parasitoid species (16 larval, three pupal) were recovered from three stemborer species in cultivated hosts, whereas 11 species (nine larval, two pupal) were recovered from seven stemborer species in wild hosts. Furthermore, three predator species were recovered from both cultivated and natural habitats. The hymenopteran parasitoids appeared dominant to the dipteran parasitoids in all the sampled districts, with Braconidae and Tachinidae the most common families represented in the two orders, respectively. The stemborer – parasitoid interaction network showed a moderate Shannon interaction diversity (H' = 2.56) and a low generality (G_{qw} =1.23). Similarly, the host plant – parasitoid interaction showed moderate Shannon interaction diversity (H' = 2.93) and generality (G_{qw}= 1.81). A total of 19 different parasitoid species were recovered from *C. partellus* in both cultivated and natural habitats (Figure 1.2A). Eleven and 10 parasitoid species were recovered from maize and sweet sorghum, respectively, with four species parasitizing stemborers on *S. corymbosus* in natural habitats (Figure 1.2B).

The most common larval parasitoids were the gregarious species *C. flavipes* and *C. sesamiae*, plus the solitary *C. curvimaculatus*, all recovered from *C. partellus* (Figure 1.2A). The main pupal parasitoids included the gregarious *P. furvus* and the solitary *Gambroides nimbipennis* Seyrig (Hymenoptera: Ichneumonidae), also recovered from *C. partellus*. The larval parasitoids *G. indicus, C. curvimaculatus* and the pupal parasitoid *G. nimbipennis* were recovered from both cultivated and wild host plants infested with *C. partellus, Sesamia*

nonagrioides Lefèbvre, and *S. jansei* (Table 1.1). Nevertheless, the exotic *C. flavipes* and the indigenous *C. sesamiae* were more specific to *C. partellus* in cultivated habitats (Table 1.1; Figure 1.2A). The stemborers, *C. partellus* and *S. calamistis* were most common on the cultivated host plants, whereas *E. saccharina, S. jansei* and *S. nonagrioides* were more common on wild host plants. As for predators, *L. humile*, *C. peringueyi* and *Aenictus* sp were common on both cultivated and wild host plants where they fed on larvae and pupae of *E. saccharina, C. partellus S. jansei* and *S. calamistis* within their larval galleries (Table 1.1). Hymenopteran parasitoids were recovered from borers on Poaceae, Cyperaceae and Typhaceae, whereas Dipterans were recovered from borers on plants of the Poaceae and Cyperaceae only (Table 1.2; 1.3). In cultivated habitats, maize and sorghum were the most common host plants, whereas *S. corymbosus* was the most common stemborer host in natural habitats (Table 1.2; 1.3; Figure 1.2B).

5993 **Table 1.1:** Details of natural enemy species recovered from stemborers on cultivated and wild host plants in Botswana.

5994

Location	Coordinates	Parasitoid species	Family	Habitat	Host	Host stage	Parasitism Strategy
Hymenoptera							
Chanoga	S20.17703; E23.67496; 939m	*Pediobius furvus* Gahan	Eulophidae	C	Cp	P	G
Mogobane	S24.94659; E25.73672; 1048m						
Modipane	S24.61832; E26.15096; 1020m						
Tsootsha	S22.11460; E20.88564; 1190m						
Oodi	S24.54710; E26.00949; 966m	*Euvipio rufa* Szepligeti	Braconidae	C	Cp	L	S
Mabele	S17.98124; E24.64453; 935m			C	Con	L	S
Maun	S19.99686; E23.43182; 937m	*Gambroides nimbipennis* Seyrig	Ichneumonidae	N	Tot	P	G
Mabele	S17.98124; E24.64453; 935m			C	Cp	P	G
		Goniozus indicus Ashmead	Bethylidae	C	Cp	L	G
Maun	S19.99686; E23.43182; 937m			N	Sj	L	G
Kazungula	S17.80556; E25.24944; 937m			N	Sn	L	G
Oodi	S24.54710; E26.00949; 966m	*Cotesia flavipes* Cameron	Braconidae	C	Cp	L	G
Seleka	S22.95112; E27.96191; 783m						
Bobonong	S21.98344; E28.44528; 707m						
Ramatlabama	S25.60480; E25.56388; 1301m						
Mabele	S17.98124; E24.64453; 935m						
Glen valley	S24.59256; E25.97819; 960m						
Ramotswa	S24.82314; E25.86776; 1003m	*Cotesia sesamiae* Cameron	Braconidae	C	Cp	L	G

Tlokweng	S24.62219; E25.97400; 945m						
Hatsalatladi	S24.04468; E25.62869; 1107m						
Dithojane	S22.45031; E26.76353; 1076m	*Chelonus curvimaculatus* Cameron	Braconidae	C	Cp	L	S
Moshupa	S24.78787; E25.41477; 1096m			N	Cp	L	S
Maun	S20.00484; E23.42598; 924m			N	Sj	L	S
Gaborone Dam	S24.70752; E25.90632; 992m			N	Sj, Cp	L	S
Glen valley	S24.59256; E25.97819; 960m	*Dolichogenidea polaszeki* Walker	Braconidae	C	Cp	L	S
Qabo	S21.05622; E21.74792; 1082m	*Stenobracon rufus* Szepligeti	Braconidae	C	Cp	P	S
Lobatse	S25.25092; E25.70840; 1200m	*Temelucha picta* Holmgren	Ichneumonidae	C	Cp	L	S
Chanoga	S20.17411; E23.69703; 935m	*Chalcidoidea* sp.	Encrytidae	C	Cp	L	S
Bookane	S23.06528; E27.53493; 881m	*Ceraphronidae* sp.	Ceraphronidae	C	Cp	L	S
Ditladi	S21.47007; E27.52998; 952m	*Eichneumonidea* sp.	Braconidae	C	Sc, Cp	L	S
Mabutsane	S24.44993; E23.58053; 1091m	*Rhaconotus* sp.	Braconidae	C	Cp	L	G
		Norbanus sp.	Pteromalidae	C	Sc, Cp	L	G
Kumakwane	S24.72081; E25.51619; 1059m	*Linepithema humile* Mayr	Formicidae	C	Cp	L	G
Ndumba	S17.97402; E24.44734; 932m						
Parakarungu	S18.03961; E24.29545; 938m			N	Es	P	G
Maun	S20.00484; E23.42598; 924m			N	Es	L	G
Nxamaseri	S18.60587; E22.08834; 1008m			N	Es	L	G
Kasane,	S24.61832; E26.15096; 1020m	*Aenictus* sp.	Formicidae	C	Sc	L	G
Mochudi	S17.98124; E24.64452; 935m			C	Cp	P	G

Matlapana	S 19.94073; E 23.50739; 937m						
Martins Drift	S 22.99681; E 27.94028; 783m			N	Es	L	G
Hatsalatladi	S24.04468; E25.62869; 1107m	*Crematogaster peringueyi* Emery	Formicidae	C	Sc	L	G
Ramotswa	S24.82314; E25.86776; 1003m						
Maun	S20.00484; E23.42598; 924m			N	Sj	L	G
Kasane	S17.793325; E25.18863; 944m	*Bracon testaceorufatus* Granger	Braconidae	N	Sc	L	G
Kanye	S24.94807; E25.34476; 1239m	*Dentichasmias busseolae* Heinrich	Ichneumonidae	N	Tot	P	S
Kasane	S17.79203; E25.19751; 930m	*Cremastinae* sp.	Ichneumonidae	N	Sc	L	S
Maun	S20.00484; E23.42598; 924m	*Cercerinae* sp.	Philantenidae	N	Sj	L	S
Nxamaseri	S18.60587; E22.08834; 1008m	*Formicidae* sp.	Formicidae	N	Es	L	S
Samedupi	S 20.11207; E 23.52979; 935m	*Syzeuctus* sp.	Ichneumonidae	N	Tot, Sj	L	S
		Diptera					
Hatsalatladi	S24.04468; E25.62869; 1107m	*Descampsina sesamiae* Mesnil	Tachinidae	C	Cp	L	G
Qabo	S21.05622; E21.74792; 1082m						
Mabutsane	S24.44993; E23.58053; 1091m	*Sturmiopsis parasitica* Curran	Tachinidae	C	Cp	L	S/G
Oodi	S24.54710; E26.00949: 966m	*Sepsidae* sp.	Sepsidae	C	Cp	L	G
Hatsalatladi	S24.04468; E25.62869; 1107m	*Chloropidae* sp.	Chloropidae	C	Cp	L	S
Gatsatsa	S25.26881; E25.27763; 1254m						
Satau	S18.09805; E24.43839; 944m	*Siphona murina* Mesnil	Tachinidae	N	Sn, Sp	L	G

Shakawe	S 18.35927; E 21.84229; 956m	*Phoridae* sp	Phoridae	N	Es	L	G

C, cultivated; N, natural; Cp, *Chilo partellus*; Con, *Concofrontia* sp.; Sj, *Sesamia jansei*; Sn, *Sesamia nonagrioides*, Tot, *Totricidae* sp.; Sc, *Sesamia calamistis*; Es, *Eldana saccharina*; Sp, *Sesamia perplexa*; L, larva; P, pupa; G, gregarious; S, solitary

Table 1.2: Natural enemies and their associated cultivated host plants in Botswana

Natural Enemy Species	Crop plant (Poaceae)		
	Maize	Sorghum	Sweet sorghum
Bethylidae			
Goniozus indicus			X
Braconidae			
Euvipio rufa		X	X
Cotesia flavipes	X	X	X
Cotesia sesamiae	X	X	X
Chelonus curvimaculatus			X
Dolichogenidea polaszeki	X		
Stenobracon rufus	X		
Eichneumonidea sp.			X
Bracon testaceorufatus			
Rhaconotus sp.			X
Ceraphronidae			
Ceraphronidae		X	
Chloropidae			
Chloropidae sp.		X	
Encrytidae			
Chalcidoidea sp.	X		
Eulophidae			
Pediobius furvus	X	X	

Formicidae			
Aenictus sp.	X	X	
Linepithema humile	X		X
Crematogaster peringueyi	X	X	
Formicidae sp.			
Ichneumonidae			
Telemucha picta	X		
Gambroides nimbipennis		X	
Cremastinae sp.			
Dentichasmias busseolae			
Syzeuctus sp.			
Philantenidae			
Cercerinae sp.			
Phoridae			
Phoridae sp.			
Pteromalidae			
Norbanus sp.			X
Sepsidae			
Sepsidae sp.	X		
Tachnidae			
Descampsina sesamiae	X	X	
Sturmiopsis parasitica		X	
Siphona murina			

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6003 **Table 1.3:** Natural enemies and their associated wild host plants in Botswana

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Natural Enemy Species	**Poaceae**								**Cyperaceae**					**Typhaceae**
	Sorghum bicolor subsp *arundinaceum*	*Echinochloa pyramidalis*	*Hyparrhenia* sp	*Vossia cuspidata*	*Oryza longistaminata*	*Chrysopogon nigritanus*	*Cyperus dives*	*Schoenoplectus corymbosus*	*Cyperus papyrus*	*Cyperus digitatus* subsp *auricomus*	*Typha latifolia*
Bethylidae											
Goniozus indicus					X			X			
Braconidae											
Euvipio rufa											
Cotesia flavipes											
Cotesia sesamiae											
Chelonus curvimaculatus							X				
Dolichogenidea polaszeki											
Stenobracon rufus											
Eichneumonidea sp											
Bracon testaceorufatus		X									
Rhaconotus sp.											
Ceraphronidae											
Ceraphronidae											
Chloropidae											
Chloropidae sp.											

Encrytidae								
Chalcidoidea sp.								
Eulophidae								
Pediobius furvus								
Formicidae								
Aenictus sp.							X	
Linepithema humile					X	X		
Crematogaster peringueyi		X			X			
Formicidae sp.						X		
Ichneumonidae								
Telemucha picta								
Gambroides nimbipennis				X				X
Cremastinae sp.			X					
Dentichasmias busseolae					X			
Syzeuctus sp.	X							X
Philantenidae								
Cercerinae sp.					X			
Phoridae								
Phoridae sp.						X		
Pteromalidae								
Norbanus sp.								
Sepsidae								
Sepsidae sp.								

Tachnidae		
Descampsina sesamiae		
Sturmiopsis parasitica		
Siphona murina	X	X

6005

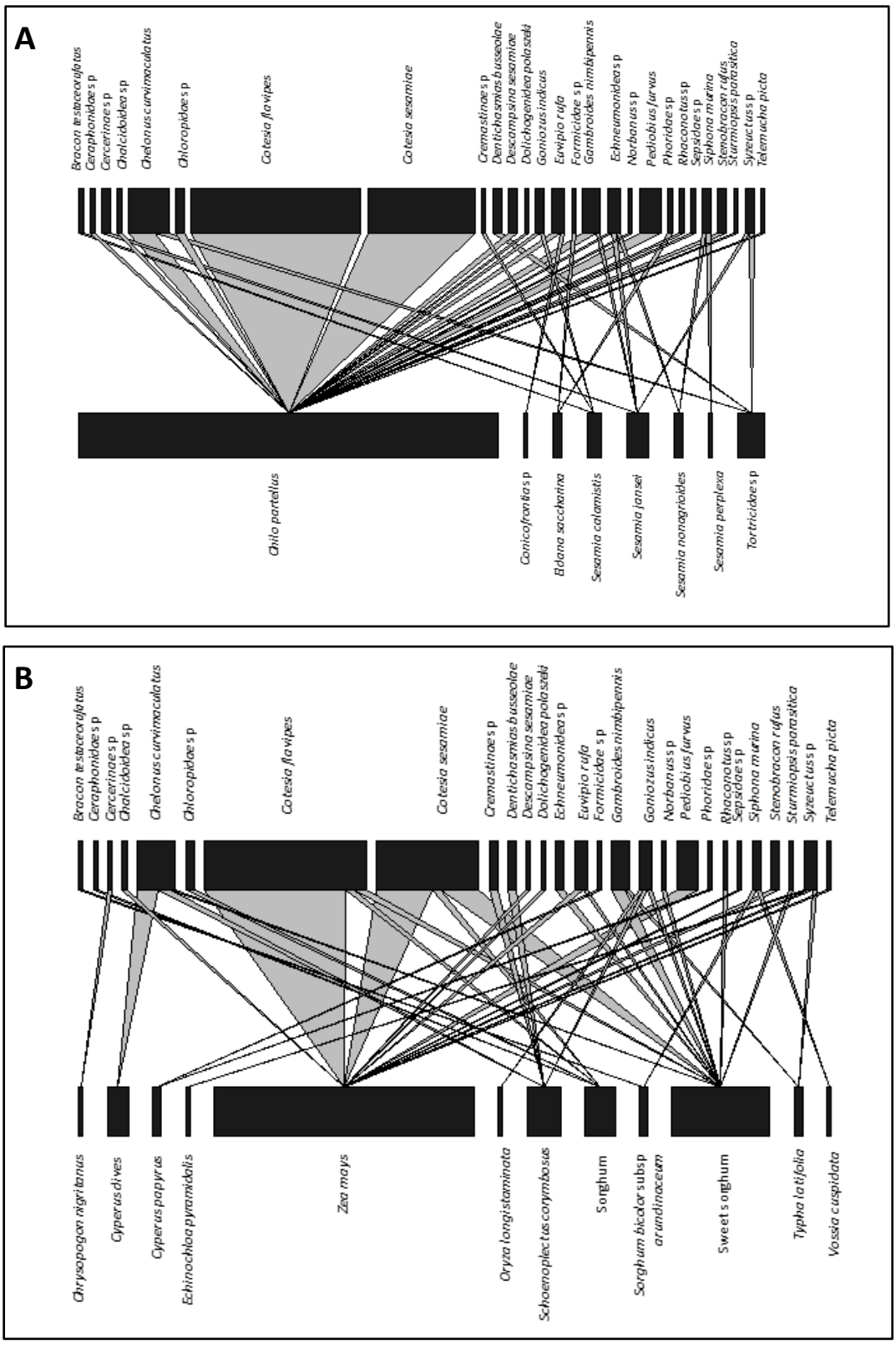


Figure 1.2: Quantitative bipartite interaction networks for (A) stemborer - parasitoid and (B) host plant - parasitoid in cultivated and natural habitats in Botswana

1.3.2. Stemborer parasitism in cultivated and wild host plants

A higher level of parasitism was recorded in cultivated habitats compared to natural habitats (Table 1.4; 1.5). In cultivated habitats, the parasitoids *C. flavipes* and *C. sesamiae* had the highest levels of larval parasitism, whereas *G. nimbipennis* had the highest levels of pupal parasitism. In natural habitats, *C. curvimaculatus* had the highest larval parasitism, whereas *D. busseolae* had highest pupal parasitism.

Table 1.4: Larval and pupal parasitism in cultivated host plants in Botswana.

Host Plant Species	Host species	Parasitoid species	% Parasitism
Maize	*Chilo partellus*	*Cotesia flavipes*	34.7 (33)
		Cotesia sesamiae	18.8 (13)
		Euvipio rufa	4.3 (2)
		Sepsidae sp.	2.1 (1)
		Pediobius furvus	8.5 (5)
		Descampsina sesamiae	5.9 (1)
		Chalcidoidea sp.	7.7 (1)
		Telemucha picta	2.9 (1)
		Dolichogenidea polaszeki	6.3 (1)
		Stenobracon rufus	6.1 (2)
Sorghum	*Chilo partellus*	*Cotesia flavipes*	5.7 (2)
		Cotesia sesamiae	12.5 (2)
		Chloropidae sp.	4.5 (2)
		Descampsina sesamiae	6.3 (1)
		Ceraphonidae sp.	7.1 (1)
Sweet sorghum	*Concofrontia* sp	*Euvipio rufa*	5.9 (1)
	Sesamia calamistis	*Echneumonidea* sp.	2.8 (1)
	Chilo partellus	*Cotesia flavipes*	17.6 (3)
		Cotesia sesamiae	29 (9)
		Goniozus indicus	5.9 (1)
		Rhaconotus sp.	4.3 (1)
		Norbanus sp.	4.3 (1)
		Sturmiopsis parasitica	4.3 (1)
		Chelonus curvimaculatus	9.1 (1)
		Echneumonidea sp.	3.2 (1)
		Gambroides nimbipennis	10.6 (3)

( ) number of parasitized hosts

Table 1.5: Larval and pupal parasitism in wild host plants in Botswana.

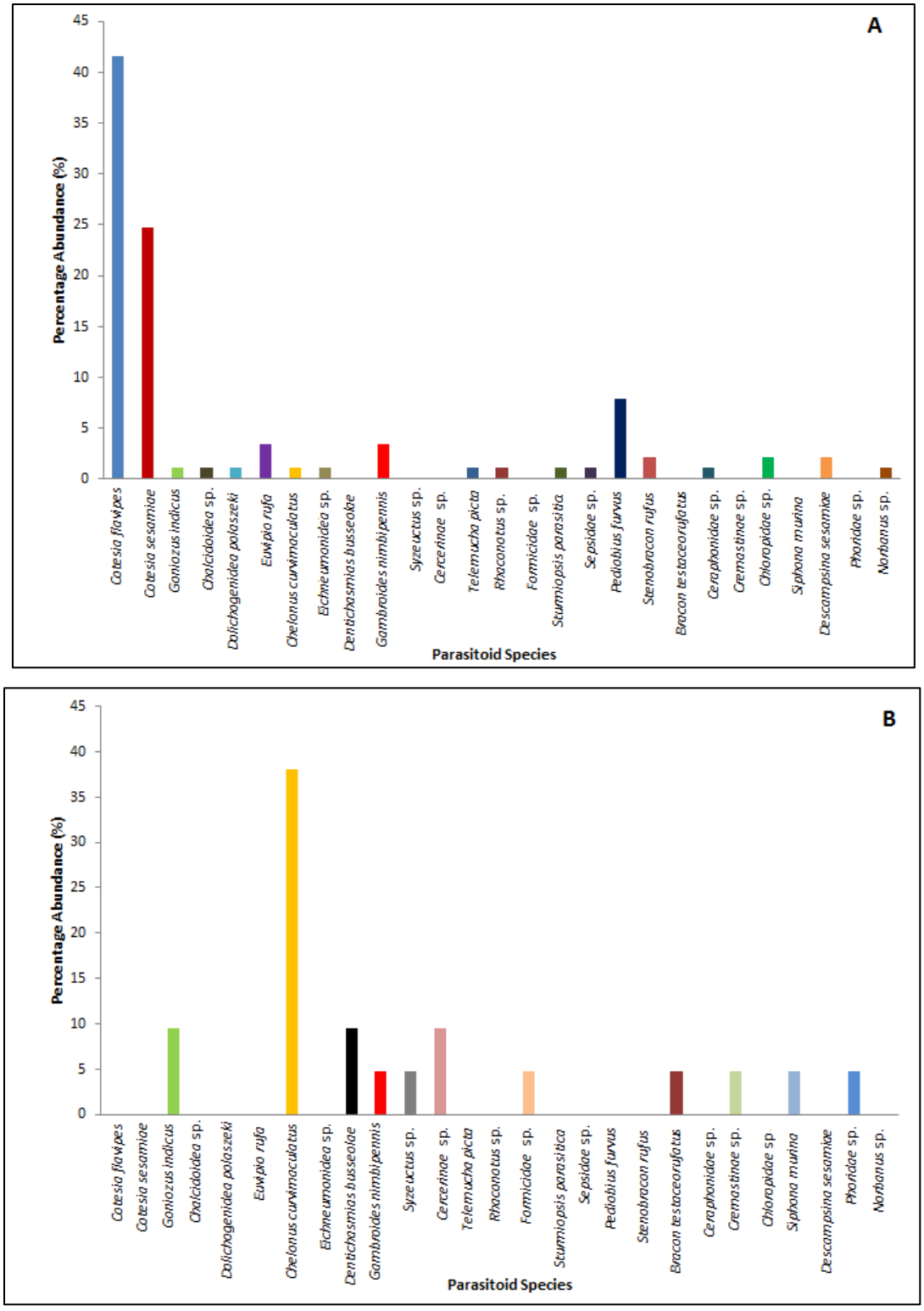
Host Plant Species	Host species	Parasitoid species	% Parasitism
Sorghum bicolor subsp *arundinaceum*	*Sesamia calamistis*	*Bracon testaceorufatus*	4 (1)
	Sesamia nonagrioides	*Siphona murina*	3.3 (1)
Schoenoplectus corymbosus	*Sesamia jansei*	*Goniozus indicus*	4.8 (1)
		Cercerinae sp.	9.5 (2)
	Tortricidae sp	*Chelonus curvimaculatus*	14.3 (3)
		Dentichasmias busseolae	9.1 (2)
Cyperus dives	*Chilo partellus*	*Chelonus curvimaculatus*	7.8 (5)
Oryza longistaminata	*Sesamia nonagrioides*	*Goniozus indicus*	3.5 (1)
Typha latifolia	*Sesamia jansei*	*Gambroides nimbipennis*	6.7 (1)
	Tortricidae sp	*Syzeuctus* sp.	4.2 (1)
Chrysopogon nigritanus	*Sesamia calamistis*	*Cremastinae* sp.	4 (1)
Cyperus papyrus	*Eldana saccharina*	*Formicidae* sp.	3.8 (1)
Vossia cuspidata	*Sesamia perplexa*	*Siphona murina*	4.5 (1)
Echinochloa pyramidalis	*Sesamia jansei*	*Syzeuctus* sp.	5.2 (1)
Cyperus papyrus	*Eldana saccharina*	*Phoridae* sp.	3.7 (1)

( ) number of parasitized hosts

1.3.3 Relative abundance of parasitoid species

Hymenopteran parasitoids were more abundant than Dipteran parasitoids in both kinds of habitats (Figure 1.3A; B). The larval parasitoids *C. flavipes* and *C. sesamiae* were the most abundant in cultivated habitats (Figure 1.3A), whereas *C. curvimaculatus* dominated in natural habitats (Figure 1.3B). Similarly, among pupal parasitoids, the hymenopteran *P. furvus* was the most abundant in cultivated habitats, whereas *D. busseolae* dominated in natural habitats.

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6037 **Figure 1.3:** Relative abundance of cereal stemborer parasitoids in (A) cultivated and (B) natural

6038 habitats in Botswana.

1.4 Discussion

1.4.1. Natural enemy diversity, stemborer hosts and host plants

I collected 30 natural enemy species attacking immature stages of lepidopteran stemborers (27 parasitoids and three predators) in both cultivated and natural habitats in Botswana. Previously, only nine parasitoid species had been reported on sorghum, maize and *Cyperus papyrus* L. (Conlong *et al*., 1988; Obopile & Mosinkie, 2003) and no predatory species had been reported attacking stemborers in the country. In the current study, the predators *L. humile*, *C. peringueyi* and *Aenictus* sp. were all observed on both cultivated and wild host plants. Conlong *et al*. (1988) reported the presence of *Goniozus natalensis* Gordh (= *G. indicus*) on *C. papyrus* along the Chobe river in Botswana, which is consistent with my collection of this parasitoid in natural habitats. Similarly, Obopile & Mosinkie (2003) reported the presence of *C. sesamiae*, *P. furvus* and *S. rufus*, which is in agreement with my observations (Table 1.1).

My results showed a higher parasitoid diversity in cultivated than natural habitats, which is contrary to previous surveys in Cameroon (Ndemah *et al*., 2007), Uganda (Matama-Kauma *et al*., 2008) and Kenya (Mailafiya *et al*., 2009) that all reported a higher parasitoid diversity in natural habitats. However, my results are consistent with a previous study by Zhou *et al*. (2003) that reported a higher parasitoid diversity in cultivated habitats in the coastal region of Kenya. The current study also recorded a moderate Shannon interaction diversity and generality for both stemborer–parasitoid and host plant– parasitoid interaction, indicating that, on average, each stemborer species was attacked by one to two parasitoid species (Helena et al., 2016). This also implies that every host plant hosted stemborers that were attacked by one to two parasitoid species. This niche overlap among parasitoids may serve to improve biological control efficacy, assuming that increased interspecific competition does not compromise the impact of key species.

Chilo partellus was the stemborer most commonly attacked by parasitoids and predators in cultivated habitats, whereas *Sesamia* species were most commonly attacked in natural habitats, which may reflect the high abundance of these species in these respective habitats. The sugarcane stemborer, *E. saccharina*, was the least attacked by natural enemies in natural habitats, possibly because the larvae spin a lot of silk around themselves and the walls of their galleries and secrete exudates upon disturbance and may be repellent to natural enemies (Sampson &

Kumar, 1986; Hordzi & Botchey, 2012). The larval parasitoids *C. flavipes* and *C. sesamiae* and pupal parasitoid *P. furvus* were the most prevalent species, in keeping with previous results from eastern Africa (Zhou *et al*., 2003; Mailafiya *et al*., 2009). Whereas *C. sesamiae* is considered a generalist parasitoid (Branca *et al*., 2011), *C. flavipes* is more specific to *C. partellus* (Mailafiya *et al*., 2010b), but the abundance of these two parasitoids on *C. partellus* in cultivated habitats may reflect the high availability of *C. partellus* as a host. Although *C. partellus* was recovered from *Cyperus dives*, *C. flavipes* was not recorded in natural habitats in the current study, similar to the results by Moolman *et al*. (2013). The gregarious larval parasitoid *Bracon testaceorufatus* Granger was recorded parasitizing *S. calamistis* in the natural habitats and is known to attack rice stemborers (van Achterberg & Walker, 1998). In South Africa, Moolman *et al*. (2013) recovered this parasitoid species from *S. jansei* suggesting that the parasitoid may have a broader stemborer host range. The Cyperaceous host plant *S. corymbosus* had the highest parasitoid diversity in natural habitats, in keeping with results by Moolman *et al*. (2013), whereas maize and sorghum had the highest parasitoid diversity in cultivated habitats. This may be because cultivated plants have higher levels of nutrients for stemborers, and reduced allelochemical toxicity, relative to wild host plants (Ofomata *et al*., 2000; Mailafiya *et al*., 2009). These attributes could promote higher stemborer survival and thus greater parasitoid richness in cultivated relative to wild hosts. To my knowledge, this is the first report of *C. curvimaculatus* parasitizing C. *partellus* on *C. dives*.

1.4.2 Parasitism levels in cultivated and wild host plants

Larval and pupal parasitism in cultivated habitats were in keeping with previous reports from East Africa for both life stages (Matama-Kauma *et al*., 2008; Mailafiya *et al*., 2009; Zhou *et al*., 2003), and parasitism levels were higher in cultivated than in natural habitats, in agreement with the results of Mailafiya (2011). I did not measure stemborer densities across habitats, but low levels of parasitism in natural habitats probably reflect lower densities of stemborers relative to cultivated habitats (Mailafiya *et al*., 2009; Moolman *et al*., 2014) which would have negative effects on parasitoid searching efficiency (Udayagiri & Welter, 2000). Indeed, the observed parasitoid diversity may depend on complex interactions between the host plants and related stemborer species. Obopile & Mosinkie (2003) reported *C. partellus* parasitism levels reaching 13.3% for *C. sesamiae*, 10.3% for *P. furvus* and 3.2% for *S. rufus* on

maize and sorghum in Botswana, whereas the current study found generally higher *C. partellus* parasitism levels in cultivated habitats. Again, this may be due to higher densities of *C. partellus* relative to other stemborer species in these habitats. *Cotesia flavipes* showed the highest levels of larval parasitism in cultivated habitats, which corroborated the results of Sétamou *et al*. (2005). The gregarious *C. flavipes* has demonstrated high searching capacity that permits it to exploit habitats with low stemborer densities (Wiedenmann & Smith, 1993; Mailafiya *et al*., 2009). Although the exotic *C. flavipes* has not been released in Botswana, it has been released in South Africa, (Skoroszewski & Van Hamburg, 1987), Mozambique, (Cugala & Omwega, 2001), Malawi, Zambia (Sohati *et al*., 2001) and Zimbabwe (Chinwada *et al*., 2001). Since these countries border Botswana (except Malawi), releases in these neighboring countries probably account for the presence of this exotic species in Botswana. The parasitoid has been reported to disperse >2000km away from release sites (Assefa *et al*., 2008), suggesting high potential for migration, spread and establishment in novel environments. *Gambroides nimbipennis* showed higher pupal parasitism levels in cultivated habitats relative to natural habitats, which is probably also indicative of high host density and resource availability in cultivated habitats.

1.4.3. Relative abundance of parasitoid species

Compared to other parasitoid species, the larval parasitoids *C. flavipes* and *C. sesamiae* and the pupal parasitoid *P. furvus* were higher in relative abundance in cultivated habitats, whereas the larval parasitoid *C. curvimaculatus* and the pupal parasitoid *D. busseolae* had higher relative abundance in natural habitats. Previous studies have reported ecological similarities between *C. sesamiae* and *C. flavipes* that suggest niche overlaps between these two parasitoids. Indeed, competition between the two congeners has resulted in the displacement of *C. sesamiae* in some regions (see Ngi-song & Overholt, 1997; Sallam *et al*., 1999; Zhou *et al*., 2003). However, in the current study, the two parasitoid species dominated in both abundance and parasitism of the same host, *C. partellus*. This may indicate that *C. flavipes* and *C. sesamiae* tend to share the same habitat, regardless of whether they attack the same host or not (Overholt *et al*., 2000). Consistent with this inference, Mailafiya *et al*. (2011) reported a significant interaction between *C. flavipes* and *C. sesamiae* in regulating cereal stemborer populations in cultivated habitats. The presence and dominance of *C. partellus* in most districts in Botswana makes *C. flavipes* a suitable exotic larval parasitoid for augmentative field releases (Overholt *et al*., 2000).

Chelonus curvimaculatus was recovered in both habitat types, suggesting that this species is a generalist that can switch habitats, and could potentially be used in augmentative biological control with *C. flavipes* and *C. sesamiae*, assuming they have a synergistic effect collectively. Since *P. furvus* is indigenous, and also a dominant species in Botswana, this pupal parasitoid may supplement the actions of larval parasitoids in managing cereal stemborers (Conlong and Cugala, 2008). Although some studies have reported the presence of hyperparasitoids in the stemborer food web (Polaszek & LaSalle, 1995; Rwomushana *et al*., 2005; Wale *et al*., 2006; Moolman *et al*., 2013), their absence in the present study suggests they will not be an interfering factor in Botswana.

In conclusion, the current study documented the diversity of stem borer natural enemies in Botswana, and their associations with stemborers and particular host plants in both natural and cultivated habitats. The 30 species of natural enemies collected included 24 species of parasitoids and three species of predators that were recorded for the first time in Botswana. Natural enemy diversity and stemborer parasitism were both higher in cultivated than natural habitats, probably due to higher host availability in the former. Overall, Botswana landscapes host a diverse community of natural enemies that contribute to biological control of lepidopteran stemborers, and an equally diverse flora that hosts stemborers and their guild of parasitoids.

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APPENDIX 2

First Report of Tomato Leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Botswana*

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2.1 Introduction

Insect pest invasions have been rapidly increasing worldwide, and with increased movement of people and goods from one country to another, there are high chances for increased numbers of invasive species conquering many regions (Pimentel *et al*., 2001). Furthermore, climate is changing (IPCC, 2014), and in consequence, impact on the rate of biological invasions, insect distribution, abundance, and impacts of such invasions on a global scale (Hill *et al*., 2016). In Africa, the risk and rate of invasion has dramatically increased (Pimentel *et al*., 2001), with destructive insect pests like *Chilo partellus* Swinhoe (Tams, 1932), *Prostephanus truncatus* (Horn) (Dunstan *et al*., 1981), *Phenacoccus manihoti* Matile-Ferrero (Neuenschwander, 2001) and *Bactrocera dorsalis* (Hendel) (Lux *et al*., 2003) invading the continent over the past decades. These invasions pose a significant risk in a continent where ~70-80% of the population depends on agriculture for household food security and sustenance (FAO, 2002). Exports of vegetables from developing countries have immensely increased in the past decades and horticultural exports have been reported to contribute to improvement in food security and livelihoods in these developing countries (Van den Broecke & Maertens, 2016). Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) is highly ranked as a food and cash crop (Varela *et al*., 2003; Tumuhaise *et al*., 2016) in Africa and indeed Botswana (Madisa *et al*., 2012). However, biotic factors such as insect pests hamper production (Tumuhaise *et al*., 2016), which may compromise household and national food security.

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) also known as South American tomato pinworm is one of the key insect pests of tomato (Brévault *et al*., 2014). This may be because of the extensive damage it elicits, lack of ecologically relevant methods for management (Ferrara *et al*., 2001) and pesticide resistance (Roditakis *et al*., 2015). It is very destructive to tomato plants and fruits causing between 80-100% yield losses if left uncontrolled (Desneux *et al*., 2010). Furthermore, it has a wide host range, and has been reported on cultivated solanaceous plants such as eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), tobacco (*Nicotiana tabacum* L.), wild solanaceous weeds like black nightshade *Solanum nigrum, Solanum elaeagnifolium, Solanum puberulum, Datura stramonium, Datura ferox, Nicotiana glauca* and garden bean (*Phaseolus vulgaris* L.) (Ferracini *et al*., 2012). It is of South American origin, Andes region, Peru, and was first detected in eastern Spain in 2006 (Desneux *et al*., 2010; Brévault *et al*., 2014). Thereafter, it

invaded the Mediterranean Basin, Europe, Middle East, South Asia (India), north, east and west Africa (Desneux *et al*., 2010; Desneux *et al*., 2011; Brévault *et al*., 2014). In north Africa, *T. absoluta* was detected north of the Sahel, Tunisia and Morocco in 2008 (Desneux *et al*., 2010; Abbes *et al*., 2012; Ouardi *et al*., 2012), west Africa; Niger and Nigeria in 2010, Senegal in 2012 (Pfeiffer *et al*., 2013) east Africa; Sudan and Ethiopia in 2011 (Anon, 2012; Pfeiffer *et al*., 2013), Kenya in 2013 (Tumuhaise *et al*., 2016), Tanzania in 2014 (Chidege *et al*., 2016) and Uganda in 2015 (Tumuhaise *et al*., 2016), southern Africa; Zambia (IPPC, 2016a) and South Africa in 2016 (IPPC, 2016b; Visser *et al*., 2017) (see distribution map, Figure 2.1).

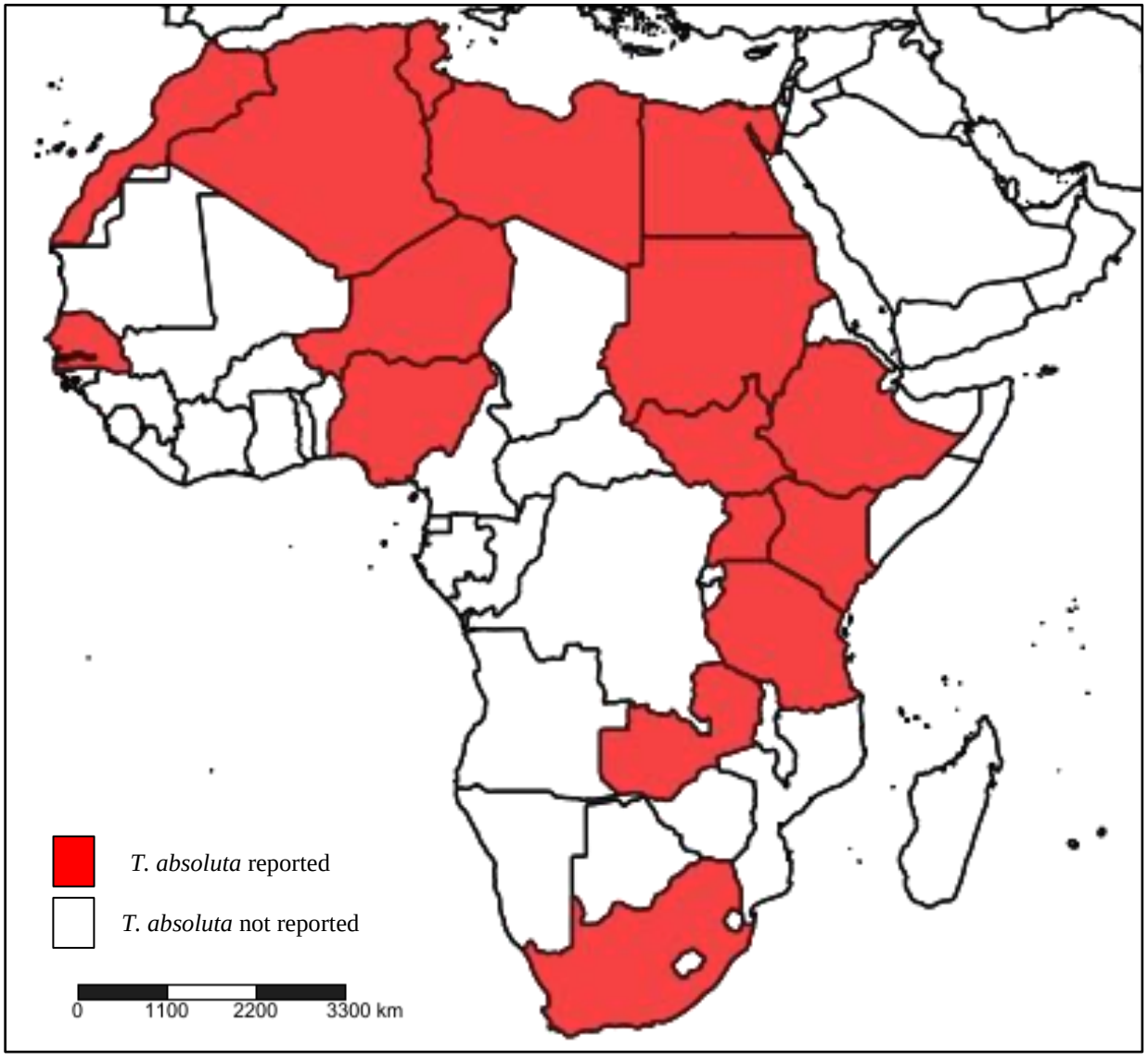


Figure 2.1: Current distribution of *Tuta absoluta* in Africa, redrawn from EPPO, 2016).

Tuta absoluta is nocturnal and adults spend the day hiding between leaves (Desneux *et al*., 2010). It is multivoltine, producing between 10-12 generations per year (Barrientos *et al*., 1998; EPPO, 2005). It is highly tolerant to high temperature, with a developmental optimum of 30ºC and a wide developmental thermal window (Krechemer *et al*., 2015; Martins *et al*., 2016). This implies that during its geographic range expansion, *T. absoluta* has a high probability of thriving in novel variable thermal environments, and even with global climate change (IPCC, 2014). The biological cycle takes an average of 24-38 days at 27 ºC and females can lay 250-300 cylindrical creamy yellow eggs, mostly singly on aerial parts and young fruits of the host plant (Barrientos *et al*., 1998; EPPO, 2005; Desneux *et al*., 2010). After 4-6 days, the eggs develop into 0.5mm yellow or green larvae (EPPO, 2005; Krechemer *et al*., 2015). The larval stage takes 12-15 days and goes through four developmental instars (EPPO, 2005; Harizanova *et al*., 2009). The first 2 instars have been reported to mine between the epidermal layers of the leaf leading to a reduction in the photosynthetic area and premature senescence (Pfeiffer *et al*., 2013). Thereafter, larvae leave the mines as 3rd and 4th instars, boring into stalks, apical buds and fruits (Ferracini *et al*., 2012). Larval damage also promotes the entry of secondary pathogens causing fruit rot (EPPO, 2005) and forms the most destructive developmental stage (Harizanova *et al*., 2009; Desneux *et al*., 2010; Retta & Berhe, 2015). Pupation takes place in the soil, within the mines, on the leaves or in packaging materials (Pfeiffer *et al*., 2013; Retta & Berhe, 2015) and can last 9-11 days at benign climatic conditions (EPPO, 2005). With the rapid north to south movement and high invasion potential of *T. absoluta* over wide geographic areas in Africa (Fig 1), it still poses a biosecurity threat to countries where it has not yet been detected. Here, I make the first report of *T. absoluta* in the north eastern part of Botswana, and recommend management strategies following detection. Detection and description of new species upon introduction in novel environments is of paramount importance in crafting management plans for invasive species (Saccaggi *et al*., 2016). I then explore preventative and control measures to be undertaken by various stakeholders to reduce its spread into pest free areas or further invasions into neighboring countries.

2.2 Materials and Methods

The first economic damage of *T. absoluta* was reported at Genesis farm (S21.14776; E27.64744), Matshelagabedi village in North East District of Botswana. A report was made to

the Department of Plant Protection in the Botswana Ministry of Agriculture. The second detection point was Noka Farm (S21.12860; E27.48830), Francistown, in the same district. Following detection, a physical assessment of crop damage symptoms and presents of the pest was conducted and infested tomato fruit samples were collected and incubated in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) at 24 ± 1 °C, 70±5% RH (Bawin *et al*., 2014) in insect cages (35cm^3) until adult eclosion. Emerged adult moths were collected and examined for confirmation.

2.2.1 Morphological identification

Morphological characters were confirmed at Botswana International University of Science and Technology (BIUST), Botswana and Stellenbosch University, Cape Town, South Africa. Adult moths were collected and knocked down in killing jars containing Chloroform absorbed in cotton wool. Using a stereo microscope, Bestscope (model BS3060BT) connected to a computer, morphological features were examined and photographed using a microscope mounted 5.0 MP digital camera (DCM-510) (Hangzhou Scopetek® Opto Electric Co, Hangzhou, China). *Tuta absoluta* can be reliably identified morphologically using male genital features, by examining the valvae and gnathos (Hayden *et al*., 2013). Males have broad, horseshoe-shaped gnathos and a digitate valva, with a medial hump and constriction (Hejazi *et al*., 2016).

2.2.2 Plant damage symptoms

Damaged tomato plants, plant parts and fruits from tunnels, open fields and net shades were collected and examined visually using illuminated bench magnascopes (RBM 101 model, Radical Instruments, India). Tunnelling and feeding behaviour in fruits, stems and leaves was observed.

2.2.3 Sex Pheromone Trapping

Yellow delta traps (Chempac- Progressive Agricare®) equipped with sticky pads were placed in tomato tunnels, open fields on and around the core detection point (Figure 2.2). A synthetic sex pheromone, *Tuta absoluta*-optima PH-937-OPTI (Russel IPM) was used as the lure (see Figure 2.3). The traps were set following a survey protocol developed by (Manrakhan *et al*., 2012) with necessary modifications (Table 2.1)



Figure 2.2: Yellow delta pheromone trap placed at (A) Genesis farm (S21.14776; E27.64744; 987 m.a.s.l) (tunnel agroecosystem) and (B) open forest (S21.16684; E27.57120; 1013 m.a.s.l) wild habitat) with predominant *Colophospermum mopane*, North East District of Botswana. *Photos by H. Machekano and R. Mutamiswa*



Figure 2.3: *Tuta absoluta* PH-937-OPTI female sex pheromone (Russell IPM®) used in the delta traps.

Table 2.1: Delimited detection zones for *Tuta absoluta* detection in Matshelagabedi village, Northern District of Botswana. Data was collected 7 days (03 December to 10 December 2016) and trapped moths were counted using dyed pointers and tally counters.

Location	Distance from the core detection site (m)	Number of traps/zone
Core detection point (Genesis farm)	0	6
1st (Open forest)	500	6
2nd (Open forest)	8000	2
3rd (Open forest)	16000	2
4th (Tomato fields)	24000	2

2.2.4. Molecular analysis

I extracted total genomic DNA from moths and larvae from the field collected and incubated samples. DNA extractions were performed using the QIAmp®DNA Micro Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Extracted DNA concentrations were measured using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Inc.). One nanogram of DNA was used in subsequent PCR amplifications. PCR products of ~685 bp in length were amplified for the COI gene using the primer set LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), which amplifies a 710 bp fragment of the COI gene in a wide range of invertebrate taxa (Folmet *et al*., 1994). The TopTaq MasterMix kit (QIAGEN) was used in all reactions. Thermocycling conditions consisted of denaturation at 95 °C for 1 minute, followed by 35 cycles of 95 °C for 45 seconds, 51 °C for 45 seconds and 72 °C for 1 minute; with a final extension at 72 °C for 3 minutes. The PCR product was visualized on a 1.2 % agarose gel and purified using the Wizard® Genomic DNA Purification Kit (Promega Corporation). Purified products were sequenced both ways using BigDye® Terminator v3.1 chemistry (Applied Biosystems) with the same primer pair used for the PCR reactions. Sequences were edited and aligned using the CLC main workbench 6.9.

2.2.5. Data analysis

Insect morphological features were identified under microscope (1:10 zoom ratio, 0.8 × ~ 0.8 obj. mag, 50~75mm binocular head) (BestScope®, China). Qualitative data on crop damage symptoms were collected by observation and sample incubation. Moth trap count data were collected from the sticky pads and counted. Detected adult moth counts were presented as graphs. The COI sequence data from molecular analysis was compared to the standards for confirmation. Sequence data was compared with existing sequences in the Genebank (KX443111, KX443108, KP793741, KP814057 and JQ749676) and (KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872, KP324753 and an outgroup KX862248) in MEGA6 (Tamura *et al*. 2013), the latter of which were used to draw the phylogenetic tree.

2.3. Results

2.3.1 Morphological features

Morphological features on the collected moths show head vertex covered with appressed scales that appear flattened against the head. The labial palps were also scally and had the same colour as the head with a distinguishingly forward projecting, up-curved shape with a relatively long pointed apical segment. Other general features like body size and colour also concurred with those of *T. absoluta*. Observed male genitalia conformed to that of *T. absoluta* as described by (Hayden *et al*., 2013) and also in agreement with (Visser *et al*., 2017) (Figure 2.4). The aedygus/ phallus was characterised by a broader, basal prominent caecum. The uncus was hood-shaped and quite broad at the apex. The uncus was attached to a tegument basally broadened with an ovate gnathos. The valvae were digitate within inner margin covex shape and each was covered with lightly dense setae. The vinculum was broad and well developed, with an elongated and broad saccus.

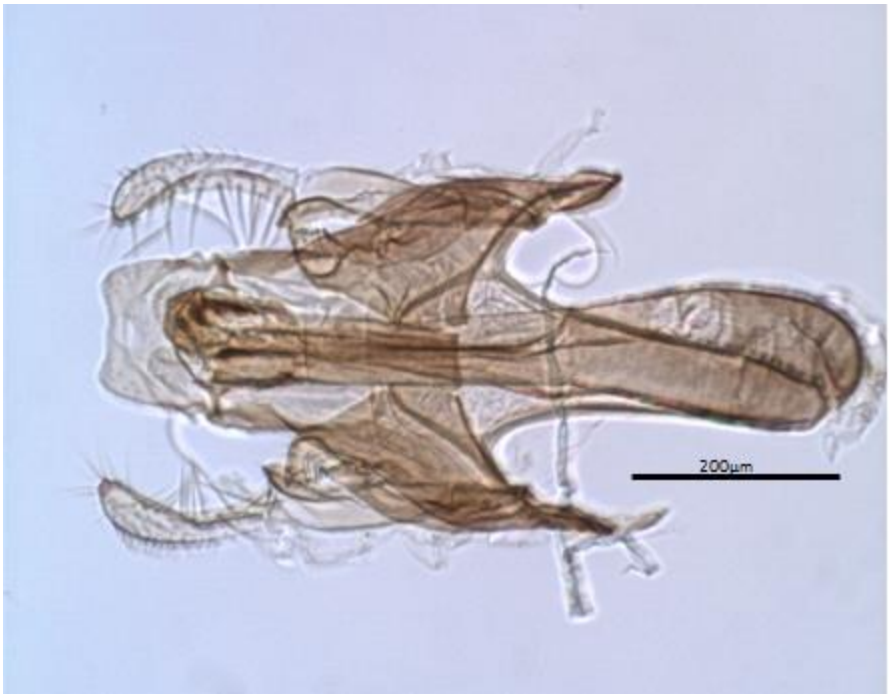


Figure 2.4: *Tuta absoluta*: male genitalia. *Photograph by R. Ramukhesa*

2.3.2 Crop damage symptoms

Survey of the tomato fields and surrounding habitats showed extensive damage inflicted by *T. absoluta*. The damage was characterised by extensive wilting of whole plants associated with severe leaf and stem. The shoots had distorted shoots with signs of die back, dead hearts and wilting. The leaves showed lesions of different sizes, large necrotic areas, wilting and chlorosis (Figure 2.5A). Frass was largely visible on all damaged parts of the plants. *Tuta absoluta* damage was also observed on wild hosts e.g. *Solanum lichtensteinii* (Willd), a wild solanaceous plant native to Southern Africa (Figure 2.5B). The damage was also characterised by lesions and necrotic damage. However, the damage on *S. lichtensteinii* was less severe than that on tomato plants (see Figure 2.5A; B). Tomato fruit damage symptoms were characterised by internal feeding with distinct exit holes (Figure 2.5C; D) and substantial frass. Fruits attacked in their early developmental stages had distorted shapes and relatively smaller size (Figure 2.5C). Most damaged mature fruits showed signs of secondary infection, subsequent decomposition and loss of internal fruit contents (Figure 2.5D).



Figure 2.5: Typical *Tuta absoluta* damage symptoms on (A) tomato plants (B) wild host *Solanum lichtensteinii* (C) distorted and damaged fruit following larval fruit infestation, and (D) advanced damage signs with exit holes, secondary infection, hanging skins with consumed internal contents and accelerated senescence at Genesis farm (S21.14776; E27.64744; 987 m.a.s.l). *Photos by H. Machekano and R. Mutamiswa*

2.3.3 Pheromone trapping

Sex pheromone traps caught varying numbers of *T. absoluta* male moths (Figure 2.6) depending on location and whether the traps were in the open tomato fields, in tunnels or open natural/forest habitat. I recorded *T. absoluta* male moths on all pheromone baited traps and sites in the north eastern part of Botswana (see Figure 2.7 for detection sites). The core detection site however, showed generally higher mean number of moths per trap than the rest of the sites. More moths were caught inside the tunnels than outside (see Figure 2.8). The capture of male moths

using a species synthetic equivalent of female emitted species specific sex pheromone (Ferrara *et al*., 2001) confirms the moths reported here were indeed *T. absoluta*.

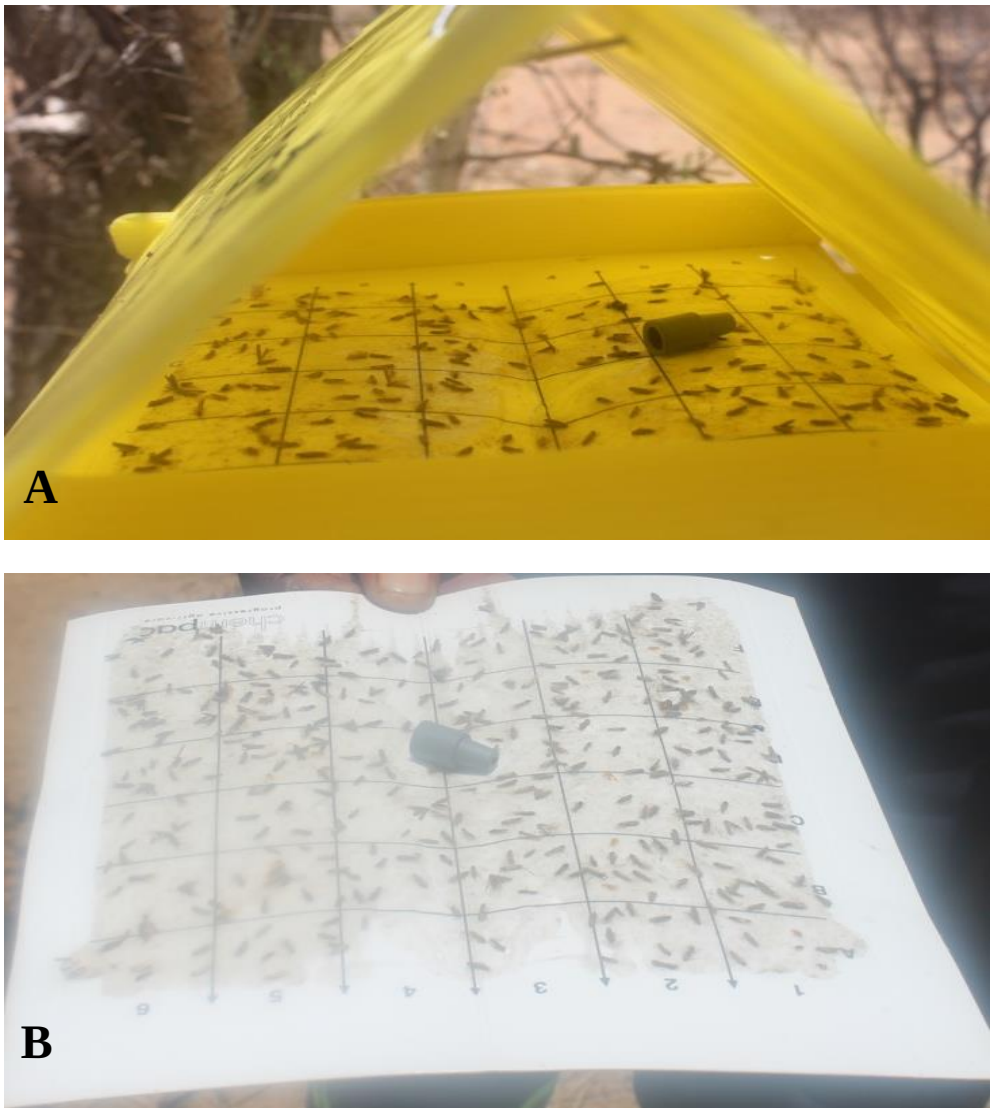


Figure 2.6: Sex pheromone trap catches for *T. absoluta* male moths (A) set up inside the yellow delta trap and (B) sticky pad retrieved from the yellow delta trap following one week trapping period at Genesis Farm (S21.14776; E27.64744; 987 m.a.s.l). *Photos by H. Machekano and R. Mutamiswa*

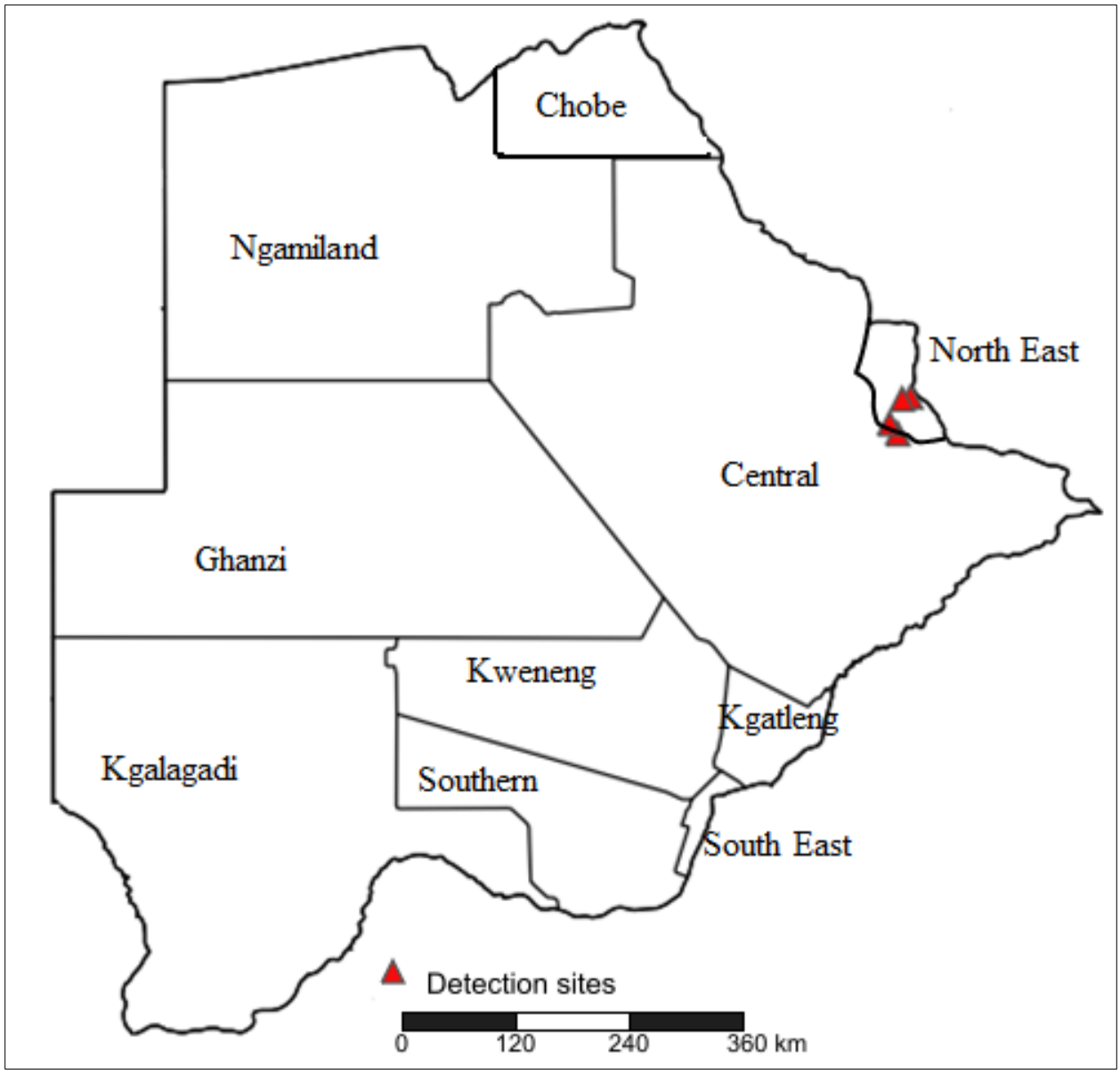


Figure 2.7: A map of Botswana showing detection sites for *Tuta absoluta*

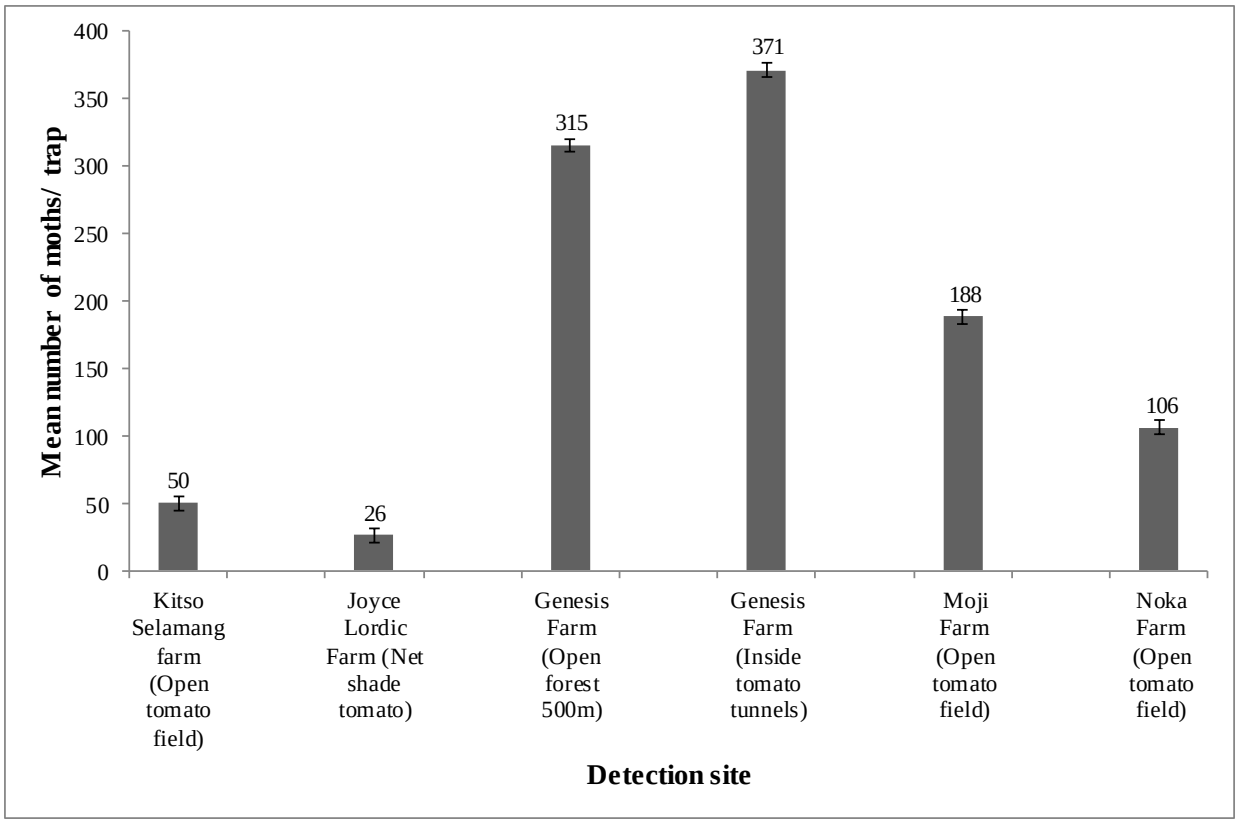


Figure 2.8: Mean number of *Tuta absoluta* male moths/trap over a period of seven days from different sites distant from core detection point in north east Botswana. Traps were baited using *Tuta absoluta* PH-937-OPTI (Russel IPM).

2.3.4 Molecular analysis

The consensus sequence was used in a BLASTN (basic local alignment search tool) search (Altschul *et al*., 1990) to find matching sequences (Zhang *et al*., 2000). The COI sequence and phylogenetic tree matched the *T. absoluta* (100% identity) sequences KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872 (see Figure 2.9), as well as KX443111 and KX443108 (Sint *et al*., 2016); KP793741 (first report from India, Asokan et al., 2015, unpublished data) KP814057 (Shashank *et al*., 2015) and JQ749676 (Bettaïbi *et al*., 2012). Phylogenetic analysis showed genetic distances between 0.00-0.59 between the Botswana *T. absoluta* sample and other samples deposited in the Genebank, and between 12.67-12.84 between *T. absoluta* samples and the outgroup *Ephysteris promptella*. The molecular data confirmed the present specimens as *T. absoluta*.

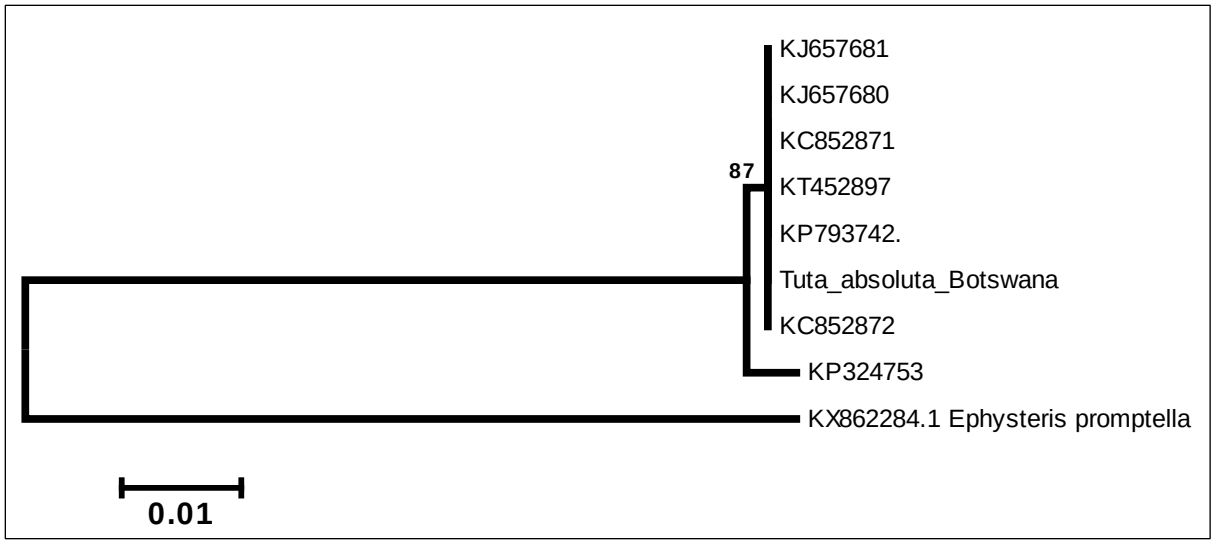


Figure 2.9: A comparison of the sequences of Botswana specimens with existing sequences on GenBank; KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872, KP324753 and an outgroup KX862248. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.13050185 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 510 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013).

2.4 Discussion

The recent invasion and rapid geographic spread of *T. absoluta* poses a major threat to both natural and agroecosystems (Desneux *et al*., 2010) in the African region. While *T. absoluta* has been reported in east, north and west Africa (Desneux *et al*., 2010; Abbes *et al*., 2012; Ouardi *et al*., 2012), to my knowledge, this is the first scientific report, detailing the presence of this pest in Botswana and generally Southern Africa.

Tuta absoluta was caught at all the sites where the traps were set in the two districts of Botswana suggesting that there are high chances of widespread distribution of the insect pest in these and other districts. This is also supported by the detection of moths at distant places from the core detection site (Genesis farm) and in natural habitats. Indeed, this finding agrees with previous study by (Desneux *et al*., 2010) who detected adult *T. absoluta* at ~10 kilometers from the tomato fields. Presence of *T. absoluta* in the wild may explain its host polyphagy and also means it may be capable of migrating distances beyond 10km. Indeed, the adult moths have been reported to fly distances of up to 100km (CFIA, 2016), and given their small size, they are easily blown by wind currents. This characteristic further improves spread of propagules upon introduction in new habitats and thus invasion success.

The mitochondrial COI (cytochrome c oxidase subunit I) sequencing through DNA Barcoding linked the Botswana trap catches to *T. absoluta* samples from Tunisia (Accession number JQ749676) and India (Accession number KP793741), the characteristic host damage symptoms and sex specific pheromone trapping consequently supporting morphological identification that was done in Botswana and South Africa. This confirmed moth identity as *T. absoluta*, suggesting that this alien pest is highly invasive and is expanding its geographic distribution. The insect pest has been reported being on a downward incursion from north Africa towards the south, and this may have been promoted by its high reproductive capacity (Tonnang *et al*., 2015), wide host range (Retta & Berhe, 2015), wide developmental thermal windows (Krechemer *et al*., 2015; Martins *et al*., 2016), continuous vegetable production across political borders, absence of effective surveillance and monitoring systems, lack of effective sanitary and phytosanitary measures and increase in intra-continental trade (Mohamed, 2014). Moreover, resistance to conventional and new chemicals (Gontijo *et al*., 2013; Campos *et al*., 2014) has been reported as a contributing factor towards its invasion success (Lietti *et al*., 2005; Roditakis *et al*., 2015). In addition, African ecological and climatic conditions are similar to those of South American countries (Desneux *et al*., 2010), suggesting that *T. absoluta* may establish and invade other countries in the southern African region.

Most of the farmers in Botswana who are into tomato production rely on importing seedlings from neighbouring countries. In addition, retailers are known to import tomato fruits from Zambia, Zimbabwe and South Africa for supplying domestic market (personal

observation). Zambia reported its first detection of *T. absoluta* in May 2016 (IPPC, 2016a) while South Africa reported hers in September 2016 (IPPC, 2016b). Therefore, there are high chances that *T. absoluta* may have found its way into Botswana through tomato fruits, seedlings from its trade partners through e.g. packing materials, boxes, crates, pallets and to some extent outdoor markets selling the fruits from infested areas. Furthermore, the insect may have migrated across borders as flying adult moths. Given its high invasion potential, the moth has been reported to drift with wind currents (Desneux *et al*., 2010), fly up to 100 kilometres and move between non screened greenhouses and outdoor crops (CFIA, 2016), suggesting that they can move long distances in a country or across borders colonizing novel environments. The recent invasions by *T. absoluta* in Africa were reported in Tanzania (Chidege *et al*., 2016), Uganda (Tumuhaise *et al*., 2016), Zambia and South Africa (IPPC, 2016a, 2016b; Visser *et al*., 2017). Botswana therefore, records the latest detection of the invasive species in the southern African region. This implies that all the countries bordering Botswana e.g. Namibia and Zimbabwe, and others e.g. Mozambique, Malawi, Angola may be at significant risk. It may also imply that, the pest may have already invaded these countries but not yet reported hence the need for strict surveillance and quarantine regulations in these countries. Although tomato is the preferred host by *T. absoluta*, this invasion poses a threat to other solanaceaous crops in the country and region such as *Solanum tuberosum*, *Capsicum annuum*, *Nicotiana tabacum* and leguminous *Phaseolus vulgaris*. Given the socioeconomic values of these commodities to the African communities, the current *T. absoluta* invasion may have negative consequences on the agricultural export market, household and national food security and thus livelihoods.

The detection of *T. absoluta* in Botswana specifically on tomatoes poses a threat to tomato production in the country and region thereby affecting food and nutritional security. This is because it prefers tomatoes to other solanaceous crops (Karadjova *et al*., 2013; Tumuhaise *et al*., 2016). Given the rate at which this pest is spreading across the continent (Pimentel *et al*., 2001; Pfeiffer *et al*., 2013; Tumuhaise *et al*., 2016) and its potential damage on tomato plants, there is need to come up with effective management options to avoid further invasions. In this regard, I recommend effective monitoring of its spread, conducting pest risk assessments for the country and region, developing effective sanitary and phytosanitary measures, awareness campaigns, farmer trainings, countrywide surveys to determine pest free zones and introduction of biological control agents. In addition, coordinated efforts amongst stakeholders, research

specialists and extension officers in Botswana and across the Southern African region should be employed to implement effective monitoring systems and area-wide pest management.

2.5 Conclusion

I conclude that morphology of the male genitalia, and confirmation through molecular data, positive sex specific pheromone lure trapping and host plant damage symptoms all confirmed association with *T. absoluta*. Therefore based on these findings I confirm the first record of *Tuta absoluta* in Botswana.

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