



**MECHANISMS OF POPULATION ESTABLISHMENT IN INVASIVE SOUTH
AMERICAN TOMATO PINWORM *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA:
GELECHIIDAE)**

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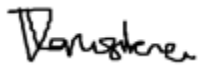
A Thesis Submitted to the Faculty of Science in Partial Fulfilment of the Requirements for the
Doctor of Philosophy in Biological Sciences of BIUST

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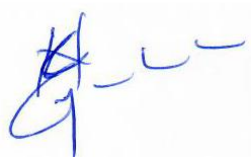
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CERTIFICATION

The undersigned certifies that they have read and hereby recommends for acceptance by the Faculty of Science a dissertation titled: **Mechanisms of population establishment in invasive South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)**, in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences at BIUST.



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DEDICATION

I would like to dedicate this thesis to my two biggest cheerleaders; my parents Prof. Moffat Tarusikirwa and Mrs. Gladys Tarusikirwa. They have been an endless source of inspiration, encouragement and their belief in my capabilities has been a great source of motivation during my hardest times.

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ABSTRACT

Global insect pest invasions have dramatically increased over the past decades, posing significant biosecurity threats to natural and managed ecosystems. As such, the mechanisms of spread and rapid establishment of invasive species have been a key issue for agricultural stakeholders. Similarly, while availability of suitable plant hosts is key to establishment, the eco-physiological mechanisms facilitating invasion amongst most invasive species are unclear. The South American tomato pinworm, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a devastating invasive global insect pest of tomato, *Solanum lycopersicum* L. (Solanaceae) accounting for ~80-100% yield losses. It has aggressively invaded the African continent since its first detection in North Africa in Morocco and Tunisia in 2008. Despite its economic importance and global pest status, little is known on eco-physiological mechanisms likely aiding its rapid establishment and spread. In addition, although diapause has not been reported in the pest's Mediterranean invaded areas, no studies have looked at its overwintering survival in sub-Saharan Africa. Against this background, this study assessed the role of eco-physiology in explaining part of the observed invasion success of this insect. Specifically, I investigated (i) the natural and low risk *T. absoluta* control strategies including scoping the native natural enemy guild of the pest insect in Botswana (Chapter 2) (ii) short- and long- term plastic responses to temperature (Chapter 3), (iii) basal cold hardiness and made inferences on overwintering strategy (Chapter 4), (iv) basal water balance and desiccation resistance (Chapter 5), and (v) integrated stress resistance (cross tolerance) to various abiotic co-occurring stressors as a potential survival mechanisms for this species (Chapter 6). Natural substances (NSs) vis pesticidal plant extracts, naturally occurring antagonists and related substances (e.g., biological control agents) for *T. absoluta* were reviewed and considered more environmentally friendly, bio-based and sustainable alternatives for management that can be used in combination with other low risk substances in a holistic way for successful pest control. Focus was also taken on the enabling and limiting factors that influence farmers in embracing the use of these NSs in an integrated approach. A scoping of the local natural enemy guild for *T. absoluta* showed a limited range of natural enemies that can be used for biocontrol of *T. absoluta* in integrated management systems. Chapter 2 also revealed that (i) different physiological mechanisms facilitating population establishment of *T. absoluta* in novel environments under rapidly changing environments and (ii) different natural- and low-risk substances and local natural enemies can be used to sustainably manage *T. absoluta* in agroecosystems. Results for the potential plastic responses to both short- and long-term acclimation showed that larvae are more thermally plastic than adults and can shift their thermal tolerance in short- and long- timescales. Larval plasticity advantage over adults reported here suggest asymmetrical ecological role of the larvae relative to adults in facilitating *T. absoluta* invasion. Empirical study on *T. absoluta*'s cold tolerance showed that larval lower lethal temperatures ranged from -1°C to -17°C for 0.5-4h durations and were more cold hardy than adults. Adults showed lower temperature activity limits than larvae albeit freeze strategy experiments showed neither life stages survived internal freezing indicating that both larvae and adults were chill-susceptible. In addition, fasting and dehydration pre-treatment depressed supercooling points (SCPs), although asymmetrically, conferring more negative SCPs for larvae while ramping rates and inoculative freezing also affected SCPs (Chapter 4). An investigation of water balance and desiccation tolerance showed body water content (BWC), body lipid content (BLC) and water loss rates (WLRs) significantly varied across life stages. Second instars recorded the lowest while 4th instars exhibited the highest BWC and BLC while they also had lowest WLRs. Desiccation resistance assays were consistent, showing that 2nd instars were the most vulnerable while 4th instar larvae and adults were the most desiccation

tolerant. This showed that *T. absoluta* 4th instar larvae were the most resilient developmental stage and potentially contribute the most to the invasion success of the pest in arid and semi-arid environments (Chapter 5). Integrated resistance assays showed context dependent (type of stress x developmental stage) integrated stress resistance that may heighten invasiveness in *T. absoluta*. Indeed, fasted individuals had a significantly higher desiccation resistance while desiccation acclimation improved starvation resistance. This cross tolerance indicates potential shared co-evolutionary mechanisms across divergent stressors and may help facilitate survival for invasive species under heterogeneous but stressful environments (Chapter 6). Unravelling such factors associated with successful invasion provide a basis for mitigating the introduction, establishment and spread of the pest species, especially when key life stages e.g., larvae and adults are targeted for experiments that inform early warning systems. This information is also important in modelling pest risk status, allows temporal life-stage specific targeting of management strategies and for developing early-warning system predictive models. Incorporation of these species-specific eco-physiological traits in predictive models can help refine invasive species potential spread and may help predict biogeographic patterns under changing climates.

CHAPTER 1

General Introduction

1.1. Climate change and insect pest invasion

A marked global increase in the numbers of invasive alien species has been observed and across varying geographical habitat types, with an estimated increase of up to 36% within the next three decades (Seebens et al., 2020, Cuthbert et al., 2021). These global biological invasions are partly a consequence of an increasingly more connected world, the rise in human population size and associated environmental disturbances thereof (Pyšek et al., 2020). Indeed, there is widespread agreement that increasing global connectivity, in particular the growing volumes and rates of movement of goods and people, is the most important factor influencing the increase in the number of macro- (insects and related organisms) and micro- invasive organisms (Fisher et al., 2012; Garnas et al., 2012; Liebhold et al., 2012; Boyd et al., 2013; Santini et al., 2013; Roy et al., 2014; Garnas et al., 2016). Biological invasions rank among the most significant threats to biodiversity and ecosystems and are considered the second most serious cause of species extinctions (Richardson et al., 2011; Castaño-Quintero et al., 2020). Invasive alien species can also have profound, negative effects on ecosystem functioning and the delivery of ecosystem services, native species richness and abundance, trophic networks, human health and welfare (Kumschick et al., 2015; Bellard et al., 2016; Jones, 2017; Ogden et al., 2019; Pyšek et al., 2020; Diagne et al., 2021). Moreover, they also generate a high but often undervalued economic burden to our societies as they are responsible for great economic losses due to monetary expenditures associated with their management, or lack thereof (Born et al., 2005; Jackson, 2015, Diagne et al., 2021).

Even though there is significant evidence of their devastating effects (of invasives across all taxonomic groups), the increase in numbers of alien species does not show any sign of saturation and most taxa even show increases in the rate of first records spatio-temporally (Seebens et al., 2017). Based on a recent spate of invasions of pests of agriculture, including forest plantations of pine and eucalyptus, it appears that many invasive insects are reaching global status much more rapidly than before (Hurley et al., 2016; Roques et al., 2016). For example, species such as *Sirex noctilio*, *Gonipterus* spp. and *Ctenarytaina eucalypti*, escaped their respective native ranges in the late 1800s/early 1900s and generally took between 50 and 100 years to reach true global distribution extents (Garnas et al., 2016). However, among more recently emerging invasive insects, several have achieved such distributions in little more than a decade or less e.g. *Leptocybe invasa*, a gall wasp on Eucalyptus, was first reported in Israel in 2000 (Mendel et al., 2004). At

that time, this wasp was completely unknown, but by 2008 it had spread throughout the North and South America, southern and southeast Asia, the Mediterranean and Africa in what appeared to be a more or less stepwise fashion (Nugnes et al., 2015; Garnas et al., 2016). Increases in the numbers, frequency and impacts of insect invasions have highlighted the importance of achieving an improved understanding of these invasions. This is especially important in the face of climate change and other anthropogenic influences which are set to modify the environments (e.g. habitats) and consequently species distributions and severity of insect pest invasions.

In addition to globalization, biological invasions are increasingly being exacerbated by climate change (Essl et al., 2020; Seebens et al., 2020; Diagne et al., 2021). In order to help control and limit the spread of invasive insect species, understanding the direct and indirect impacts of climate change on invasions is a critical exercise. For example, it has been demonstrated that over the second half of the 20th century increasing establishment rates of invasive insects in China were positively related to rising surface air temperature and the relationship remained significant after accounting for increase in international trade (Huang et al., 2011). Therefore, the increase in establishments of invasive alien insects could be explained only in part by introduction rate and propagule pressure. Indeed, from modelling results, it has been shown that even when propagule pressure is high, environmental suitability e.g. temperature remains an overriding factor for invasive species successful establishment (Kelley, 2014; Duncan, 2016; Renault et al., 2018). Warmer temperatures can favour establishment and spread of alien insects by providing new areas suitable for colonization, enabling insects to shift their geographic range poleward, and crossing barriers that previously limited their natural ranges (Cannon, 1998; Walther et al., 2009; Robinet and Roques, 2010). This brings about the need to consider global warming (e.g. temperature effects on biological processes) when designing strategies and policies to deal with insect invasions (Huang et al., 2011). It seems therefore plausible to focus, at least partly, on species traits related to temperature, to obtain insights into the mechanisms of insect invasions and improve predictions of future invaders.

The term global change embraces a range of natural and anthropogenic environmental changes (Sangle et al., 2015). According to the Intergovernmental Panel on Climate Change (IPCC), it is defined as “change in climate over time, either due to natural variability or as a result of human activity”. Global climate change predictions show an increase in temperature means, the

magnitude of seasonal and diel temperature variation and increase in acute thermal extremes (IPCC, 2014; Colinet et al., 2015). The global mean surface temperature is projected to increase by 1.4 to 5.8°C by 2100 if mitigation measures fail (IPCC, 2014; Sangle et al., 2015; Engelbrecht et al., 2015). This may cause some direct effects on the distribution and abundance of pest populations, and also indirect effects on the pests' host plants, competitors and natural enemies (Porter et al., 1991). Indeed, climate change inflicted bottom up and top-down effects may favor insect pest population abundance (Hance et al., 2007; Chidawanyika et al., 2019), resulting in failed production systems as a result of increased insect pest pressure.

Most researchers seem to agree that warmer temperatures in temperate climates will result in more types and higher populations of economic insects (Sangle et al., 2015). For example, as climate change increases global mean temperatures and variability, the threat of invasive insect species will increase as tropical and subtropical insects expand their range into temperate areas (Chown et al., 2007; Weldon et al., 2016). This marked increase in temperature coupled with inconsistent and limited rainfall, therefore, determines invasion success (Nyamukondiwa et al., 2010) and trends in insect population dynamics because it exerts potent environmental stressors such as heat, dehydration and starvation (Bale et al., 2002; Hoffmann et al., 2012; Bauerfeind and Fischer, 2013; Mutamiswa et al., 2018).

Among the key determinants of invasion success for insect species are climate suitability, propagule pressure and the availability of suitable hosts (Ward and Masters, 2007; Bacon et al., 2014). Climate change is expected to influence the distribution and abundance of invasive insects both directly (e.g. by altering where species and hosts can occur) and indirectly (e.g. via changes in population growth rates, propagule pressure, and spread), amongst other factors (Lantschner et al., 2014). However, the effects of climate change on the distribution and severity of invasive insects may be negative or positive (Ward and Masters, 2007; Bertelsmeier et al., 2015), resulting in so called 'winners' and 'losers' of climate change. For example, climate change alters the interactions between the insect pests and their host plants. As a result, some of the cultivars that are resistant to specific insect may become susceptible (Sangle et al., 2015). Adverse effects of climate change on the activity and effectiveness of natural enemies will be a major concern in future pest management programmes (Chidawanyika et al., 2019). The most affected will be higher trophic levels, including natural enemies (e.g. predators and parasitoids) and this may affect their

efficacy for biological control (Hance et al., 2007). The rate of insect multiplication might also increase with an increase in carbon dioxide (CO₂) and temperature, owing to the bottom-up effects associated with increase in e.g. plant host growth under optimal high CO₂ and temperature environments. Similarly, large scale changes in rainfall associated with changing climates will have a major effect on the abundance and diversity of arthropods (Sangle et al., 2015). Unraveling the effects of these biotic and abiotic interactions is thus significant in explaining the fate of invasive insect species under anthropogenically altered environments.

The tephritid flies (Diptera: Tephritidae), or “true fruit flies”, are some of the most successful invaders and are serious economic pests around the world (Aluja and Mangan, 2008; Papadopoulos et al., 2013; Karsten et al., 2015). Climate warming in temperate regions improve conditions for the flies to establish, through fewer frost days, a longer growing season and higher frequency of warm nights (Papadopoulos et al., 2013). Tephritids often have high dispersal ability and rapid growth and reproductive rates, as is characteristic of many invasive species and agricultural pests (Tscharrntke et al., 2005; Mutamiswa et al., 2020). Similarly, Nyamukondiwa et al. (2010) found that high basal thermal tolerance and the ability to remodel thermal phenotypes may also aid invasiveness in fruit flies. Thus, documenting the physiological effects of temperature and other abiotic stress factors may be essential for predictive distribution models of invasive insect species and may help inform pest risk analyses.

1.2. Insect adaptations to climate change

1.2.1. Insect adaptations

Environmental conditions can alter the form, function, and behaviour of organisms over short and long timescales, and even over generations (Srinivasan and Brisson, 2011). Adaptation of exotic species to novel environments and new species interactions is well documented, and much research effort has been placed in understanding exotic traits or conditions that facilitate or prevent invasion (reviewed in Mooney and Cleland, 2001; Strayer et al., 2006; Berthon, 2015; Wan and Yang 2016). Insects have been known to use diverse mechanisms to adapt to new environments, i.e. physiological (e.g. phenotypic plasticity, cross tolerance and/cross talk); behavioural (e.g. migration, micro- habitat selection, aestivation); molecular (e.g. transcriptional regulation and epigenetic modifications of DNA); morphological and evolutionary adaptations (Dillon et al., 2009; Nyamukondiwa et al., 2010; Sinclair et al., 2013; Chidawanyika et al., 2017; Sheikh et al.,

2017; Kellermann and van Heerwaarden, 2019; Qi et al., 2019; Zhang et al., 2019; Rodgers and Gomez Isaza, 2021).

1.2.2. Physiological responses to environmental stress

1.2.2.1. Thermal stress

Adaptation to high/low temperatures allows insects to spread further and outcompete species sharing the same niches (Wan and Yang, 2016). One of the most notable effects of global climate warming is an increase in the incidence of heat waves (IPCC, 2013; Stillman, 2019). As insects are ectotherms, they are particularly susceptible to thermal anomalies because their body temperature closely matches environmental temperature (Gray, 2013). Since body temperature directly affects performance, fitness and insect metabolic rate (Angilletta, 2009), organismal success is largely associated with the ability to alter phenotypes in response to environmental cues. As a result, insects employ various physiological adjustments to enhance fitness and survival rates in stressful environments (Mutamiswa et al., 2018).

Phenotypic plasticity is the ability of a genotype to express different phenotypes in different sets of environmental cues. It can vary among habitats depending on the levels of habitat cues and temporal and spatial heterogeneity (Liefting et al., 2009; Mathur and Schmidt, 2016). Phenotypic plasticity of some traits that protects organisms from stressed environments and increases its fitness under a particular circumstance could be defined as adaptive plasticity (Liefting et al., 2009) and it contributes as well to the maintenance of genetic variation within specific populations (Gomez-Mestre and Jovani, 2013; Qi et al., 2019). Although a single heat wave may not increase daily temperatures enough to impact the generation time of ectotherms (Cannon, 1998; Harrington et al., 2001), high temperatures for a few hours can nevertheless have negative effects on adult performance and offspring survival (Zhang et al., 2013; Liang et al., 2014). Short-term, acute exposure to sublethal temperatures is termed hardening (Bowler, 2005; Lagerspetz, 2006; Nyamukondiwa et al., 2010), whereas more chronic, longer term exposure to sublethal conditions is regarded as acclimation and can occur in both the field or in the laboratory (Huey and Berrigan, 1996; Hoffmann et al., 2003; Nyamukondiwa et al., 2010).

At short timescales, hardening allows individuals to adjust their phenotype temporarily to the environment they currently inhabit (Austin and Moehring, 2013) and this may be significant in

preserving key life-history traits (Shreve et al., 2004). Hardening can be induced by either stressful sub-lethal high or low temperatures. Rapid cold hardening (RCH) occurs when an insect experiences a rapid improvement in survival at a low lethal temperature after a brief pre-treatment with a sublethal temperature cold (Lee et al., 1987) and seldom heat shock (Nyamukondiwa et al., 2010). Rapid heat hardening (RHH) refers to the improvement in survival of a lethal acute high temperature following preconditioning at a stressful sub-lethal high temperature (Chown and Nicolson, 2004). These hardening responses have been shown to improve survival in several insects, including *Ceratitis capitata* (Nyamukondiwa et al., 2010) and *Zygogramma bicolorata* (Chidawanyika et al., 2017) and several *Drosophila* species (Nyamukondiwa et al., 2011).

Prolonged prior exposure to sublethal thermal environments commonly referred to as acclimation, can improve performance in otherwise lethal or stressful conditions (Nyamukondiwa et al., 2010; Chidawanyika and Terblanche, 2011). Acclimation improves survival through a rapid reversible change in phenotype in response to a relatively prolonged period (days or seasons) of pre-exposure to conditions similar to future potentially stressful thermal conditions (Terblanche et al., 2006). Under even more acute prior exposure (hardening), similar plastic responses that result in improved survival can also be induced (Yi et al., 2007). Acclimation temperature can have major effects on various stress resistance parameters, such as cold tolerance, heat tolerance and locomotor performance (Gibert et al., 2001; Ayriñac et al., 2004; Hoffmann et al., 2005; Fischer et al., 2010; Overgaard et al., 2011). Conditions eliciting acclimation have been reported to be highly complex, and differ across taxa, environments tested as well as across ontogeny (Bar-Ziv and Scharf, 2018; Kellermann et al., 2018; Rohr et al., 2018). Over longer timescales, acclimation of one developmental stage can have adaptive responses to a later developmental stage, a phenomenon called carry-over effect (see Moore and Martin, 2019). While phenotypic plasticity generally works within generation, acclimation effects on parents may have strong adaptive responses to later generations, a phenomenon called transgenerational plasticity (Sgró et al., 2016). This form of plasticity may be associated with changes in gene expression in different environments and sometimes called epigenetic inheritance. Thus, phenotypic plasticity is adaptive and may help organisms survive stressful environments and may cushion organisms against climate change (Kelley, 2014; Sgró et al., 2016; Wan and Yang, 2016; but see van Heerwaarden et al., 2016).

1.2.2.2. Low temperature stress

Cold tolerance is important in defining the distribution of insects (Overgaard and McMillan, 2017). Apart from plasticity, insects also resort to other mechanisms to adapt to low temperatures, i.e. cold tolerance/hardiness and overwintering strategy. Winter survival is particularly important for invasive insects as it ensures adequate propagules to sustain the insects in warmer climates, thereby aiding invasion success. An integration of behavioral and developmental adaptations, as well as physiological are required to achieve overwintering success. Individual species often exhibit considerable variation in the adaptations by which they achieve the ability to survive subzero temperatures (Lee, 2010; Storey and Storey, 2013). Two mechanisms are mainly employed, i.e., freeze tolerance and freeze avoidance (Sinclair et al., 2015; Andreadis and Athanassiou, 2017). Freeze tolerant insects tolerate the formation of ice in their internal body tissues while freeze avoidance insects cannot survive internal ice formation hence experience mortality upon freezing (Sinclair et al., 2015; Andreadis and Athanassiou, 2017). Studies of freeze tolerance and avoidance have revealed a range of fascinating physiological adaptations that involve accumulation of cryoprotectants, removal of ice nucleators, synthesis of antifreeze proteins, or use of severe dehydration, all of which allow insects to either tolerate or avoid freezing (Storey and Storey, 2013; Rozsypal, 2015; see review Overgaard and McMillan, 2017).

Some insects may display a programmed arrest of development called diapause during extremely cold seasons of the year. Diapause is a very common mechanism for overwintering and may be in response to food limitation, hence, is often associated with some degree of metabolic depression which is essential for conserving energy reserves. It is also accompanied by reduced activity, suppressed reproduction and development and a general upregulation of protective mechanisms, including antioxidants (Tauber et al., 1986; Sim and Denlinger, 2011; Sinclair et al., 2013). It is controlled by endogenous physiological factors (see Hand et al., 2016) e.g. reduced rate of water loss (e.g. Benoit and Denlinger, 2007), and heat shock proteins (HSPs) (e.g. Rinehart et al., 2007). Stress and immune responses are usually elevated during diapause, and mechanisms that protect against cold injury are commonly invoked (Hand et al., 2016).

1.2.2.3. Desiccation stress

Coping with climate change-related factors, especially temperature-humidity imbalance, is a global challenge for insects (Thorat and Nath, 2018). Most of the research has focused on adaptive

responses in thermal traits. However, changes in precipitation and water availability, in both cold and dry environments, will impose a significant stress to organisms, reduce fitness and influence distributions and survival (Kellermann et al., 2018) and especially in increasingly dry environments with climate change (Engelbrecht et al., 2015). As arthropods move from more mesic to xeric environments, they are faced with reduced rainfall, relative humidity (RH) and stressful desiccating environments (Weldon et al., 2016). Some species have the ability to counter dry periods through plastic responses (Kellermann et al., 2018). Rapid adult desiccation hardening has been described in *Drosophila* species (Hoffmann, 1991; Kalra et al., 2014), where a non-lethal exposure to desiccation stress increased resistance to subsequent exposures. Plasticity in desiccation resistance in response to environmental stress (i.e. rapid desiccation hardening) has been associated with changes to cuticular permeability (Hoffman, 1991; Bazinet et al., 2010; Stinziano et al., 2015; Krupp et al., 2020).

Most insects resort to desiccation tolerance and resistance which remain prime mechanisms employed to escape hostile consequences of fluctuating hydro periodicity patterns in their habitats (Edney, 1977; Chown and Nicolson, 2004; Thorat and Nath, 2018). These two main strategies can be defined according to how organisms conduct their water budgets to prevent the consequences of desiccating environments. Desiccation tolerance is when insects withstand the loss of a significant proportion of body water (Suemoto et al., 2004; Benoit et al., 2007; Pallarés et al., 2016), while desiccation resistance is avoiding desiccation through the reduction of water loss and increases in body water content (Gray and Bradley, 2005). Desiccation-resistant traits are diverse and have been linked to geographic locations and environmental conditions i.e. temperature and water availability (Thorat and Nath, 2018; Krupp et al., 2020). *Drosophila* species have been observed to potentially increase tolerance to desiccation stress through three main mechanisms i.e. reducing water-loss rates through the cuticle, increasing their tolerance to water-loss and increasing the amount of stored/metabolic water (Gibbs et al., 2003; Kellermann et al., 2018).

1.2.2.4. Multiple stressors

The ability of invasive species to rapidly expand into new regions is often largely attributed to their greater phenotypic plasticity, however, integrated stress resistance (e.g. cross-tolerance, - a mechanism by which tolerance to one particular stress can enhance tolerance to another contrasting stress), may contribute to the invasive success of some alien insects (Renault et al., 2015; Ricciardi

et al., 2021; Rodgers and Gomez Isaza, 2021). Multiple stressors, both abiotic and biotic (e.g. starvation, desiccation, heat/cold), are often experienced simultaneously by organisms in nature (Sinclair et al., 2013). For example, interactions between fluctuating precipitation trends and changes in temperature mean due to climate change and general heterogeneous environments encountered by insects as they invade new regions can cause insects to concurrently experience periods of high temperature and low RH. The persistence of their populations in these dynamic and multivariate environments greatly depends on their ability to deal with the interactive effects of different stressors occurring simultaneously (integrated stress resistance) or sequentially over short time scales (Gunderson et al., 2016).

1.3. Invader traits

Making predictions that capture how species will respond to climate change requires an understanding of how key traits and environmental drivers interact to shape fitness in a changing world (Kellermann and van Heerwaarden, 2019). Species attributes, such as high basal thermal tolerance, insecticide resistance, diverse reproductive structures, mutual facilitation and immune priming, host switching, high functional response, epigenetics and high propagule pressure are examples of common key traits found in invasive species (see Kelley, 2014; Wan and Yang, 2016; Cuthbert et al., 2019; Carneiro and Lyko, 2020). While these characteristics vary across taxonomic groups, notable trait overlaps are common across the most prolific insect pest invaders (Sakai et al., 2001; Weldon et al., 2018). Although, there are numerous studies that have found non-native species as better at adapting to effects of climate change, studies by Sorte et al. (2013) showed that in terrestrial (primarily plant) systems, native and non-native species responded similarly to environmental changes.

1.3.1. Functional responses

Functional Responses (FRs) have been identified as powerful predictive tools for forecasting the ecological impacts of existing, emerging and future invasive alien species (Cuthbert et al., 2019). The most harmful invasive insects exhibit some common characteristics, such as high reproduction, competitive dominance, high tolerance, benefit from mutualist facilitation interactions and have the ability to rapidly exploit and deplete resources (Strayer et al., 1999; Byers et al., 2002; Johnson et al., 2008; Morrison and Hay, 2011; Dick et al., 2012; Wan and Yang, 2016). Maintaining energy production across environmental heterogeneity is crucial, and

responses that have evolved for coping with stressful conditions also improve physiological resilience and insects' bioenergetics (Renault et al., 2018).

The relationship between resource density and consumption rate of invasive species is consistently of greater magnitude than those of taxonomically and/or trophically similar native species (Dick et al., 2012). Studies by Dick et al. (2012) showed that the FRs of the invasive predatory shrimp *Hemimysis anomala* an invader in Europe and North America, were generally higher in comparison to those of the local native analogues *Mysis salemaai* and *Mysis diluviana* in Ireland and Canada, respectively. Studies further indicated that *H. anomala* exhibited similar or higher attack rates, consistently lower prey handling times and higher maximum feeding rates compared to those of the two-native species, a result consistent with findings by Bollache et al. (2008) on *Dikerogammarus villosus*. Similarly, invasive species have high metabolic flexibility, and can regulate their metabolism under low resources and/starvation (Smit et al., 2021). Such mechanisms may help optimize fitness and survival under resource constraints associated with the invasion continuum.

1.3.2. Epigenetic inheritance

One of the ways an organism responds to its environment is by tweaking its gene expression to make the best use of the prevailing conditions which often contributes to phenotypic plasticity (West-Eberhard, 1989; Price et al., 2003; Ashe et al., 2021). In ectotherms, parental effects play an important role in offspring's phenotypic plasticity (Qi et al., 2019). Given that phenotypic plasticity is central to the adaptability of organisms to adapt to new environments, epigenetic mechanisms that generate plasticity and acclimation are important to consider in the evolutionary system (Ashe et al., 2021). Thus, the extent to which environmental effects can trigger epigenetic responses is particularly interesting for understanding the role of epigenetics in rapid adaptation (Carneiro and Lyko, 2020). Epigenetics explains how patterns of gene expression, instructed by extrinsic biotic or abiotic factors, can be passed to offspring through means other than the inheritance of DNA sequence (Srinivasan and Brisson, 2011). It is, therefore, one of the strategies utilized by invasive organisms to successfully adapt to new environments, outside a more prolonged natural selection mechanism (Jaenisch and Bird, 2003; Duncan et al., 2014; Carneiro and Lyko, 2020).

Invasive animals often show reduced genetic diversity, which is thought to limit the adaptive and evolutionary potential by constraining the availability of new gene variants (Chown et al., 2015). Natural selection acting on genetic variants explains how organisms can colonize ecological niches over evolutionary timeframes (Carneiro and Lyko, 2020). Nevertheless, invasive species are often highly successful in adapting to new and heterogeneous environments, with adaptive plasticity as an essential strategy for rapidly colonizing new habitats and out-competing native species (Carneiro and Lyko, 2020). The passing of the epigenetic states from parent to offspring can contribute to successful invasion as this ensures that the offspring develop towards a phenotype that is pre-adapted to current conditions (see review Ashe et al., 2021).

Various examples have been reported to show the contribution of epigenetics in invasion success. For example, *Drosophila mercatorum* parental temperature stress has been reported as having an influence on progeny phenotypes i.e., the progeny wing size and shape and relative mean centroid size increased with enhanced maternal temperature stress (Andersen et al., 2005; Rogilds et al., 2005; Kjærsgaard et al. 2007; Qi et al., 2019). The worldwide invasive pest *Drosophila suzukii* performs trans-generational prophylactic medication by adapting its oviposition behaviour in the presence of natural enemies (i.e. developing in hosts reared on atropine thereby strongly impacting the life-history traits of parasitoids) (Poyet et al., 2017). Transgenerational insecticide resistance was observed in the invasive Oriental fruit fly, *Bactrocera dorsalis* that was treated with an LC₂₀ of cyantraniliprole via the toxic feed feeding method when egg production increased, and male mating ability improved (Zhang et al., 2015; Li et al., 2021).

1.3.3. Propagule pressure

Event attributes are a significant factor in modulating invasive species establishment and spread. Insect invasions are typically influenced by the pathways that facilitate the movement of individuals, and the level of propagule pressure associated with each introduction (Hill et al., 2016). The pattern of propagule introduction, growth and development indeed is a significant driver to invasive population establishment (Ward and Masters, 2007; Bacon et al., 2014). In founder populations, the most extreme bottlenecks likely occur in invasive populations because of the relative scarcity of introduction events and reduced number of sampled propagules surviving through transportation (Renault et al., 2018). Propagule pressure is believed to be the key driver of invasion success because a single propagule could potentially lead to colonization and is often

important for the continued success of an invader after its introduction (Crawley et al., 1996; Colautti and MacIsaac, 2004; Lockwood et al., 2005; Eppstein and Molofsky, 2007).

Genetic diversity in invasive populations is not only linked to multiple introductions, but can also result from high propagule pressure, whether as a single event or as a result of sustained pressure over time (Garnas et al., 2016). For example, *Tuta absoluta* was introduced in Spain as a single initial Chilean population in the early 2000s (Guillemaud et al., 2015) but by the year 2006 it had successfully established and had started spreading to other European countries, thereby attaining global pest status (Desneux et al., 2010; Biondi et al., 2018). Studies on the population genetics data of *Vespa velutina* suggest that the distinct invasions of the wasp in France and Korea followed only a single initial introduction of some gravid females (Arca et al., 2015; Renault et al., 2018). Genetic background has been found to influence the reproductive rate, hence establishment success e.g. in the invasive whitefly (*Bemisia tabaci*) (Hufbauer et al., 2013; Renault et al., 2018). This trait has contributed to the successful spread of various other invasive insects globally. High supply and frequency of propagule introductions increase chance of successful invasion due to high genetic diversity, continual supplementation and increased probability of introduction to favourable environment (Lockwood et al., 2005; Colautti et al., 2006; Richardson and Pysek, 2006; Catford et al., 2009).

1.3.4. Host switching

Most plant eating insect species are host-specialists that consume only one or a few closely related plant species (Bernays and Graham, 1988; Forister et al., 2014; Christodoulides et al., 2017). However, studies have shown that most invasive species have the ability to feed on broad diets, i.e. polyphagous/generalists (Ross et al., 2012; Christodoulides et al., 2017). The availability of a variety of hosts can facilitate the expansion of such species in regions beyond the geographic scope of native hosts (Sylla et al., 2019) and ensure continual perpetuation of the populations even in the absence of the main host plant. This is particularly important during species introduction and ensures survival in new areas. Wan and Yang (2016) reported that ten of the most harmful invasive insect pests in China have a wide host range. This is a consistent observation on most invasive species, e.g. *Spodoptera frugiperda* has been reported on ~353 plants (76 families) (Montezano et al., 2018), *B. dorsalis* on more than 600 fruit species (Wan and Yang, 2016), *B. tabaci* on ~361 plant species (89 families) (Li et al., 2011) and *Liriomyza sativae* on at least 130 plant species (14

families) (Wan and Yang, 2016). Other factors/traits that are common across prolific invaders have been fully documented (see Kelley, 2014; Wan and Yang, 2016) and may improve invasiveness in pest insect species.

1.4. *Tuta absoluta* spread and global distribution

The South American tomato pinworm (or sometimes tomato leafminer), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered a key pest of greenhouse and open-field tomato (Desneux et al., 2010; Mansour et al., 2018). The status of the pest completely changed following its invasion of Europe in 2006 and the steady spread across the Afro-Eurasian supercontinent as it shifted from a pest restricted to South America toward a major threat to global tomato production (Desneux et al., 2010; Biondi et al., 2018). *Tuta absoluta* has increased its range radius by 800 km each year, in the last ten years, infesting almost 60% of the tomato cultivated area worldwide (Fig. 1.1) (Campos et al., 2017). Its migration is presumably aided by both long and short distance dispersal (Xian, 2017). Regardless of plant protection agency efforts, the pest quickly reached economic pest status on tomato in invaded areas (Desneux et al., 2011; Campos et al., 2017). In 2004, *T. absoluta* was added by the European and Mediterranean Plant Protection Organization (EPPO) to the A1 list of pests recommended for regulation (pests absent from the EPPO region), and in 2009 was transferred to the A2 list (pests locally present in the EPPO region), 3 years after its arrival in Spain (Urbaneja et al., 2007; Tropea Garzia et al., 2013).

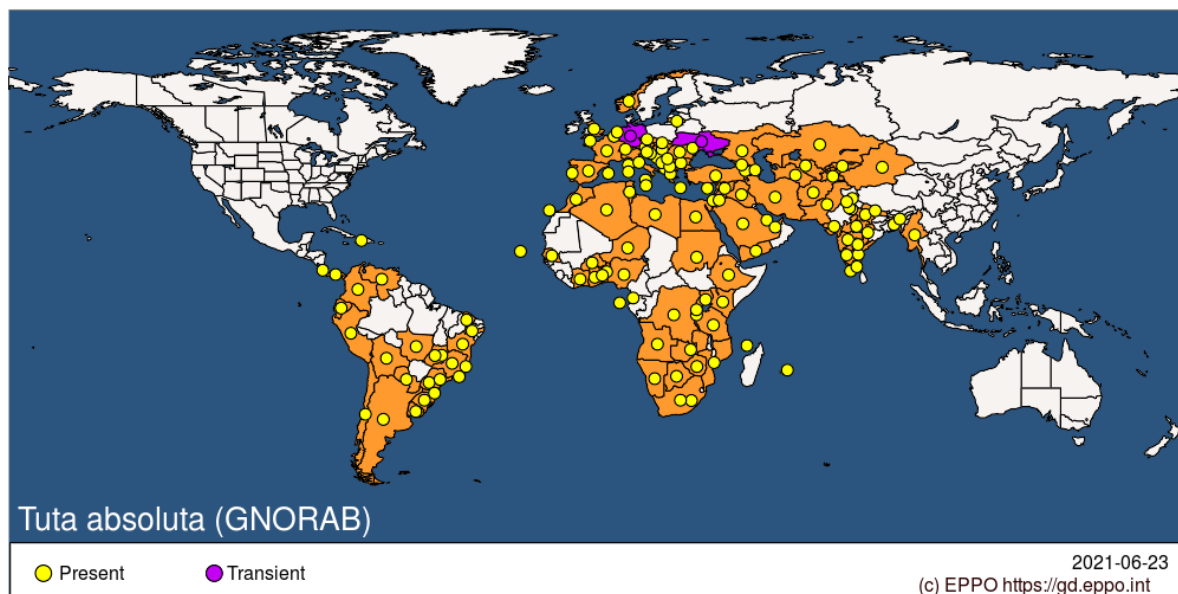


Figure 1.1. Current global distribution of the invasive pest *Tuta absoluta* (www.gd.eppo.int).

The pest affects tomatoes produced for both fresh and processed markets (Desneux et al., 2011; Arturo et al., 2012). The South American tomato pinworm damage results mainly from larval feeding, which causes aesthetic damage at low densities, reduces yield, and at high larval densities, can destroy plants (Desneux et al., 2010). The feeding habits of *T. absoluta* make its presence difficult to detect in the early infestation period, resulting in severe damage on young plants. Typical leaf mining damage is directly related to the reduction of plants' photosynthetic capacity and of production levels in both protected and open-field tomato crops. In addition, feeding activity on fruits directly affects the aesthetic appearance of harvested products and increases costs for fruit postharvest selection before marketing (De Castro et al., 2013). Yield losses can reach 80-100% if the pest is left uncontrolled (Campos et al., 2014; Alsaedi et al., 2017). Cost-benefit analysis of *T. absoluta* showed a significant increase in costs of management, attributed mainly to increased use of insecticides (Lietti et al., 2005; Nayana and Kalleshwaraswamy, 2015; Machekano et al., 2018).

1.5. Biology and ecology of *T. absoluta*

1.5.1. Biology

Tuta absoluta is a multivoltine species and the length of its life cycle depends on environmental conditions, particularly temperature (Tropea Garzia et al., 2012). Eggs are oval, creamy white coloured when laid and yellowish at maturity. They are laid individually by females in upper plant parts on young leaves, stems, or sepals (Cocco et al., 2015). Larvae normally hatch in the morning and wander about for 5–40 min before they start mining. Young larvae (1st–2nd instars) bore into the plant and, once mature (3rd–4th instars), they leave their mines and move to new locations for feeding. If food is available and climatic conditions are favourable, larvae feed almost continuously and generally do not enter diapause (Tropea Garzia et al., 2012). *Tuta absoluta* can overwinter as an egg, pupa or adult (the latter as long as food is available), with a prevalence of pupa in colder periods (Sannino and Espinosa, 2010). However, the pest has been reported to be susceptible to bottom-up effects, notably when tomato plants are subjected to nitrogen and water limitations; in such conditions, its juvenile survival rate decreases, and the developmental time increases (Han et al., 2016) owing to changes in larval food intake dynamics (Coqueret et al., 2017). Similarly, failure of biological control (e.g. by parasitoids) due to top down effects can also affect *T. absoluta* abundance (see discussions in Rundle et al., 2004; Hance et al., 2007;

Chidawanyika et al., 2019). Although Van Damme et al. (2015) reported no diapause in the pest, studies by de Campos et al. (2020) found that it has the ability to undergo facultative diapause. Mature larvae usually drop to the soil where they produce a thin, silky cocoon to transform into prepupae and pupae (Biondi et al., 2018).

Adults are crepuscular, and both sexes are active (sexually) by the first day of emergence; both sexes are polygamous and show almost no refractory period (Biondi et al., 2018). Mate communication relies on female sexual pheromones as well as physical cues (Lee et al., 2014). Females mate only once a day and are able to mate up to six times during their lifespan, with a single mating lasting between a few minutes to up to six hours (Lee et al., 2014). Adult lifespan ranges between 10 and 15 days for females and 6–7 days for males (Tropea Garzia et al., 2012). Females live longer than males, allowing them to be sexually mature when the males emerge (Fernandez and Montagne, 1990).

1.5.2. Host range, selection and oviposition

Tuta absoluta can be regarded as a polyphagous pest because while it is widely documented as a specific pest for tomatoes, it has garnered a broad range of plant hosts from many different genera and families (Husariu et al., 2017). The ovipositional choice of female *T. absoluta* is host plant species dependent. Ovipositional preference studies reported adult females showing a marked bias towards the tomato plant (Younes et al., 2018). Proffit et al. (2011) elucidated that females are attracted to and will only oviposit on a host plant because of volatiles emitted by that plant. Their study further pointed out that in the case of tomato, the leaf odours mainly include volatile terpenoid compounds which elicit in mated female's upwind orientation flight followed by landing as well as egg-laying. Adults have been reported to have crepuscular to nocturnal habits while remaining mostly hidden among vegetation during the day (Fernandez and Montagne, 1990), as well as auroral twilight dispersal, courtship and mating behaviour with more frequent oviposition during the daytime (Uchôa-Fernandes et al., 1995; Tropea Garzia et al., 2012). The majority of oviposition occurs 7 days after the first mating, and during this period a single female lays more than 70% of its eggs during the afternoon and twilight hours. The eggs are laid singly or in small groups, mainly on young leaves (73%) and secondly on stems (21%), sepals (5%) and green fruits (1%) (Tropea Garzia et al., 2012).

1.6. The importance of the tomato plant

The tomato plant (*Lycopersicon lycopersicum* Mill) is one of the most widely grown vegetable crops and leading non-grain commodity in the global production system (Ruben, 1980; Osata, 2003; Srinivasan, 2010; Tropea Garzia et al., 2012; Testa et al., 2014). It belongs to the family Solanaceae, which has over 3000 plant species of economic importance, including potato, eggplant, tobacco and pepper (Wakil et al., 2018). Tomatoes are grown for home consumption in the backyards of almost every homestead across Sub-Saharan Africa (SSA) and are an important source of vitamins and a cash crop for both small holder and medium scale commercial farmers (Ndor, 2018). The Food and Agriculture Organization of the United Nations (FAO) states that ~170 million tonnes of tomato are produced worldwide (FAOSTAT, 2017; Biondi et al., 2018). Tomato is grown for its fruits which are used in a variety of ways. Apart from being a source of income, both nutritional and medicinal uses of tomatoes have also been well documented (Kelley and Boyhan, 2014). They are a good source of minerals, vitamin C, potassium, an assortment of phytochemicals such as carotoids, natural phenols and compared to other fruits have the highest protein calorie ratio (Ndor, 2018). Tomatoes are known to be an important source of lycopene, which is a powerful antioxidant that acts as an anticarcinogen and reduces the risks of getting certain neurodegenerative diseases (Sies et al., 1992; Shi and Manguer, 2000; Srinivasan, 2010; Kelley and Boyhan, 2014).

1.7. Problem statement

Unprecedented rates of introduction and spread of non-native species pose burgeoning challenges to biodiversity, natural resource management, regional economies, and human health (Ricciardi et al., 2021). There is a high and often undervalued economic burden associated with invasive species, likely affecting development of policy for coordinated invasive species management. Nevertheless, it has been reported that by the year 2017 total global costs of invasions reached a minimum of US\$1.288 trillion (1970–2017), with an annual mean cost of US\$26.8 billion (Diagne et al., 2021). These values are often conservative and highly underestimated (Cuthbert et al., 2021). Also, there is often an increase in chemical pesticides as a first line of defense upon first introduction of invasive pests and this has detrimental effects on the environment and public health. *Tuta absoluta* has invaded Africa at a fast rate causing high losses to horticultural farmers across the continent and remain a global biosecurity threat. Tomato production serves as a source

of income and contributes to household and national food and nutritional security in Africa (Machekano et al., 2018). Therefore, unraveling mechanisms that facilitate its invasion may help in early warning predictive models and may help design efficacious pest management strategies.

1.8. Justification of the study

The current study seeks to address the increasingly urgent need to understand the factors that determine the success of invasive alien species. Although *T. absoluta* is of South American origin, it is currently globally recognized as a key pest in tomato production. It can cause crop losses of up to 100% and increases the cost of production cutting into the farmers profit because of increased pesticide usage. Due to its high biotic potential, such as multivoltine nature, short generation time, and increased resistance to insecticides, it attains the key pest status even in the new habitats. The pest has shown high invasion potential establishing in ~41 African countries in less than 10 years (see Fig. 1.1). Given the pest status of *T. absoluta*, and the effects of global climate change (e.g. increase in mean temperature, thermal variability, acute thermal extremes, low food resources and high desiccation stress), it is of paramount importance to carry out studies on the pest's environmental stress tolerance to temperature and desiccation, and potential evolutionary relatedness e.g. cross tolerance, that may suggest shared evolution of stress resistance mechanisms. Understanding the mechanisms affecting the successful establishment, dominance, and spread (i.e. invasion success) of this pest is central to developing effective management tools, e.g. mapping risk areas, predictive models and informing integrated pest management (IPM) programs.

1.9. Objectives

Unraveling physiological factors that likely aid invasion are key new fronts in invasion ecology (Kelley, 2014; Ricciardi et al., 2021), and may help advance invasion science amidst the rapidly changing climate environments. Thus, the overall aim of this study was to determine physiological responses to abiotic stress as fitness metrics that may facilitate establishment of a highly invasive global pest, *T. absoluta* in novel environments.

In the second chapter, a comprehensive literature review was conducted explaining current pest status of the pinworm, potential contributory factors to successful invasion in Africa, and various control mechanisms that can be used in combination with natural substances (NSs) in a holistic way for successful pest control. This was complemented with some survey data on native natural

enemies (Botswana), that may likely inform future integrated management systems locally, e.g. through conservation or augmentation. The specific objectives in this chapter were:

- To assess *T. absoluta* invasion pathways and mechanisms facilitating African invasion.
- To assesses potential use of diverse biological control agents, natural and low-risk substances in an integrated framework for more conservational control of the pest.
- To determine the enabling and limiting factors that influence farmers in embracing the use of NSs.
- To determine the ‘native’ natural enemy guild of *T. absoluta* in Botswana.

In the third chapter, the ability of *T. absoluta* developmental stages to remodel their thermal phenotypes in the short and more longer timescales was assessed through pest’s short- (RCH and RHH) and long -term (acclimation) plastic responses of fitness and survival. The specific objectives of this study were:

- To determine basal thermal tolerance for *T. absoluta*.
- To assess possible phenotypic plasticity of temperature tolerance following hardening and acclimation in *T. absoluta*, and by inference, how this may aid invasiveness.

The fourth chapter investigated the cold hardiness and overwintering adaptation strategy of *T. absoluta* larvae and adults. The specific objectives of this study were:

- To determine *T. absoluta* freeze strategy.
- To assess effects of various ecological factors, i.e. food limitation, desiccation, different diurnal cooling rates, on *T. absoluta* cold tolerance.

The main aim of chapter five was to explore the eco-physiological water balance and desiccation resistance traits across different *T. absoluta* developmental stages. The specific objectives of this chapter were:

- To assess the basal- body water and -lipid content, and the proportion of water to gross body mass in the pinworm.
- To examine the water loss rates of *T. absoluta*.
- To assess potential desiccation resistance in *T. absoluta*.

The sixth chapter assessed the possible multi-stress responses (integrated stress resistance) to various ecological stressors often experienced concurrently in nature to explain *T. absoluta* invasiveness. The specific objectives of this study were:

- To determine the effects of high temperature hardening on cold tolerance, desiccation resistance and starvation tolerance.
- To investigate the effects of low temperature hardening on high temperature tolerance, desiccation resistance and starvation tolerance.
- To determine the effects of desiccation acclimation on cold tolerance, high temperature tolerance and starvation tolerance.
- To investigate the effects of fasting pre-treatment on high temperature tolerance, cold tolerance and desiccation resistance.

1.10 Hypotheses

- Native natural enemies can be used for biological control against *T. absoluta* in Botswana and NS may be a significant component for *T. absoluta* integrated management.
- *Tuta absoluta* has high basal thermal tolerance and plasticity that may give it an advantage in invaded areas.
- Due to its tropical origin, *T. absoluta* is chill susceptible and that different ecological factors may affect its supercooling.
- *Tuta absoluta* is desiccation tolerant and may use one of the mechanisms e.g. high metabolic water to survive arid and semi-arid invaded environments.
- Integrated stress resistance to environmental stress e.g. cross tolerance may manifest in *T. absoluta* and helps the species survive heterogeneous invaded environments.

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CHAPTER 2

***Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on the ‘offensive’ in Africa: prospects for integrated management initiatives**

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2.1. Introduction

Invasive insect pests are widely recognized as major threats to agricultural production, biodiversity conservation and the maintenance of ecological integrity (Olson, 2006; Ragsdale et al., 2011; Campos et al., 2014). Increased trade, human travel and globalization have made crop production vulnerable to invasive alien insect pests (Williamson, 1996; Pimentel et al., 2001; Simberloff and Rejmanek, 2001). In Africa, the rate of alien species invasion has dramatically increased in the past decades (van Wilgen et al., 2020). For example, ‘recent’ pest insect invasive arrivals include *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Tams, 1932), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Dunstan and Magazini, 1981), *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) (Neuenschwander, 2001), *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Lux et al., 2003), *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Goergen et al., 2016) and indeed *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Mansour et al., 2018). Given that ~70-80% of African livelihoods depend on agriculture (FAO, 2002), these invasions represent a significant biosecurity as well as food and nutrition security threat.

Tuta absoluta commonly known as the South American tomato pinworm is one of the most invasive destructive insect pests of tomato *Solanum lycopersicum* (Solanaceae) globally (Desneux et al., 2010; Biondi et al., 2018; Mansour et al., 2018). It is thought to have originated from the Andes region in Peru before spreading to Latin American countries during the 1960s (Campos et al., 2017; Han et al., 2018). Although it is an endemic Neotropical insect pest whose initial distribution was restricted to its native range in South America, it has successfully extended its geographical range following unintentional introduction in the Mediterranean basin (Desneux et al., 2010; Ghoneim, 2014; Marchioro et al., 2016). It acquired global pest status after detection in eastern Spain in late 2006 (Urbaneja et al., 2007; Desneux et al., 2010) following introduction as a single initial Chilean population in the early 2000s (Guillemaud et al., 2015). Thereafter, it rapidly spread throughout Europe (Desneux et al., 2011; Guimapi et al., 2016). Since then, the pest has rapidly spread East- and -southward tracking favourable biophysical environments (Biondi et al., 2018). During the last 10 years, *T. absoluta* has also spread in the Middle East and Asia at lightning speed, resulting in extensive naturalised populations in India, Iran, Israel, Syria, Turkey, and Nepal (Abbes et al., 2012; Shashank et al., 2016; Bajracharya et al., 2016; Sankarganesh et

al., 2017; CABI, 2019). Following its invasion in most European countries, *T. absoluta* successfully invaded the Afrotropics reportedly via the Mediterranean Sea with first detections reported in Tunisia, Algeria and Morocco in 2008-09 (Desneux et al., 2010; Abbes et al., 2012; Mohamed et al., 2012; El-Arnaouty et al., 2014). Since then, further detections have been reported in Niger, Nigeria (2010), Senegal (2012) (Pfeiffer et al., 2013), Sudan, Ethiopia (2011) (Pfeiffer et al., 2013), Kenya (2013) (Tumuhaise et al., 2016), Tanzania (2014) (Chidege et al., 2016), Uganda (2015) (Tumuhaise et al., 2016), Zambia, Botswana, Mozambique, South Africa, Malawi (2016) (IPPC, 2016; Mutamiswa et al., 2017; Visser et al., 2017; Mansour et al., 2018) and Angola (2017) (Mansour et al., 2018) thereby elevating its continental pest status. This rapid north-south and downward incursion between 2008 and 2017 poses a biosecurity threat to natural and agroecosystems in pest-free countries. On a global scale, this pest is believed to have increased from primarily infesting only 3% of the worldwide tomato cultivated areas to 60% within 10 years (Biondi et al., 2018) with the most recent in China, a major tomato growing country (Zhang et al., 2020).

Underlying the invasion success of *T. absoluta* is its superior climate adaptation (Tarusikirwa et al., 2020a), polyphagous nature (Mohamed et al., 2015; Özgökçe et al., 2016; Machekano et al., 2018) and high biotic potential (Tonnang et al., 2015; Biondi and Desneux, 2019). In response to *T. absoluta* infestations, chemical control is often used as the first line of defence (Han et al., 2018; Machekano et al., 2020), providing a quick fix to pest pressure. It is relatively easy to apply, readily available and may be cost effective when applied at large scale. However, synthetic insecticides are expensive to many resource-constrained sub-Saharan African (SSA) farmers. The endophytic behavioural feeding and cryptic nature of *T. absoluta* larvae render the widely used contact insecticides ineffective (Silva et al., 2011; Cocco et al., 2013; Bawin et al., 2015). Moreover, synthetic insecticides also affect (i) non-target biological diversity e.g. natural enemies and pollinators thus disrupting desirable trophic interactions, (ii) development of pest resurgence, (iii) insecticide resistance development and (iv) public health owing to residual insecticide contamination (Margni et al., 2002; Abdollahi et al., 2004; Guedes and Picanço, 2012; Campos et al., 2014; Roditakis et al., 2018). Some of these vices have already been observed on *T. absoluta* chemical control, e.g. insecticide resistance (Lietti et al., 2005; Roditakis et al., 2015) and non-target effects on beneficial fauna (Biondi et al., 2012). The practical implications of the European Union Directive 2009/128/EC on the sustainable use of synthetic pesticides and the future

prospects that residue restrictions may become more stringent also necessitate the exploration of novel, sustainable non-chemical alternatives for the management of *T. absoluta* (van der Velden et al., 2012). As such, efficacious ecologically sound and safer biologically based or natural alternatives are required for sustainable management of *T. absoluta* for use in an integrated approach. Natural insecticides/substances (NSs) are thus compatible as they are cost-effective and environmentally friendly owing to their rapid environmental degradation (Mossa, 2016; Soares et al., 2019). However, studies on the NSs effective on *T. absoluta* are scattered in literature warranting an in-depth comprehensive review of current methods. This study, therefore, provides a comprehensive, gleaned compendium of potentially effective NSs on this invasive quarantine insect pest. The understanding of the role and potential of NSs in limiting *T. absoluta* economic damage is important in establishing a baseline for sustainable management options. NSs widely used in pest management include microbial (e.g. entomopathogens and entomopathogenic nematodes), botanical (e.g. insecticidal plants or their derivatives), and semiochemical agents (Witzgall et al., 2010; Tsoulnara and Port, 2016; Hikal et al., 2017; Kamali et al., 2017; Lengai et al., 2020). Despite availability of multiple potential options, current organic tomato production systems mostly rely on *Saccharopolyspora spinosa* (spinosad), *Azadirachta indica* (neem), and *Bacillus thuringiensis* toxins (Biondi et al., 2018; Guedes et al., 2019) signifying underutilisation of NSs. Moreover, identification and screening of effective locally available NSs remain scant in Africa (Deng et al., 2009; Stevenson et al., 2017; Lengai et al., 2020). Although NSs are environmentally friendly and have shown to be very effective in pest control, they have not been widely adopted by African farmers. Thus, this review also discusses *T. absoluta* invasion in Africa, possible mechanisms of invasion and the prospects of incorporating NSs as assets in an integrated management approach. We also discuss barriers to adoption of NSs use in African agricultural systems from a legislative, economic, ecological and social standpoint.

2.2. Economic impact of *Tuta absoluta* in Africa

Tomatoes are an important component of horticulture and a major pillar of sustainable development, with significant contribution to household and national food and nutritional security (FAO, 2012; Machekano et al., 2018). They are a cash crop grown for home consumption in the backyards of majority homesteads across SSA and are an important source of vitamins (Ndor, 2018). Tomato production also significantly contributes to economic development ascribed to its

high economic returns and ability to create employment (~ 60% of total labour force) and along the value chain (Weinberger and Lumpkin, 2007; Bala et al., 2019). About 170 million tonnes of tomatoes are produced worldwide (FAOSTAT, 2017; Biondi et al., 2018). Of this figure, Africa accounts for ~37.8 million tonnes annually. However, this figure is threatened by increased *T. absoluta* damage (CABI, 2019), with downstream consequences on African socio-economic value chains and household livelihoods (Aigbedion-Atalor et al., 2020).

Tuta absoluta larvae attack almost all aerial parts of tomato plants resulting in ~80-100% yield losses if left uncontrolled (Shahbaz et al., 2017). Larval feeding also reduces fruit quality through creating pin holes prone to secondary attack by pathogens, rendering them unmarketable (Kaoud, 2014). Increased cost of production has been reportedly experienced by small- and large-scale farmers in Africa due to increased costs for pesticides (Kamali et al., 2017). For example, recent evidence suggests decreased tomato yields and increased production costs (Aigbedion-Atalor et al., 2020). Highest losses are mostly experienced during early invasion owing to inadequate mitigation measures related to lack of preparedness (Han et al., 2018). Nigeria experienced up to 80% losses in tomato produce in 2016 due to unfamiliarity with the pest and management strategies (Bala et al., 2019). This reduced supply and catapulted an estimated 10-fold increase in tomato prices due to the laws of demand and supply (Sanda et al., 2018). Similarly, following *T. absoluta* invasion in South Africa (2016), pest-free countries banned importation of tomato and other Solanaceae crops from that country (Mansour et al., 2018) resulting in significant economic losses. Similar losses have also been reported in Southwestern Angola (Chidege et al., 2017). Thus, *T. absoluta* pest pressure has negatively affected agricultural enterprises in Africa through direct losses and increased costs in pest management (CABI, 2019). *Tuta absoluta* remains a pest of quarantine importance in countries under the Inter-African Phytosanitary Council (IAPSC) and has been reported on European and Mediterranean Plant Protection Organization (EPPO) A2 list as a regulated insect pest (EPPO, 2014).

2.3. *Tuta absoluta* Invasion Pathways and Distribution in Africa

Long-distance transmission and short-distance dispersal are the key drivers of *T. absoluta* invasion (Xian, 2017). International agricultural trade is a key long-distance transmission mode that may have contributed to a larger extent in the introduction of *T. absoluta* into Africa e.g. importation of fruits (e.g. tomatoes, egg plants) from pest-infested present areas (Karadjova et al., 2013; CABI,

2019). Other possible pathways for long-distance dissemination include production facilities and packaging materials (e.g. boxes, crates, pallets) from infested countries (Potting et al., 2013; Tonnang et al., 2015). Hence, production facilities repack and distribute infested fruits resulting in long-distance dissemination, reviewed in Illakwahhi and Srivastava (2017). In addition, propagule material (e.g. seedlings), farm equipment and transportation vehicles from pest-infested areas are also possible pathways for long-distance transmission (Karadjova et al., 2013; Retta and Berhe, 2015; Xian, 2017). From the foregoing, the rapid spread across Africa may have been exacerbated by porous port of entries, weak phytosanitary regulations and ineffective early surveillance in the region (Han et al., 2018). Furthermore, natural factors (e.g. wind, water), larval crawling and adult flight are possible key short-distance dispersal pathways (Xian, 2017). To date, *T. absoluta* has been reported in 41 of the 54 African countries (see CABI, 2019). The pest has not yet been officially reported in a few central and southwestern African countries (Mansour et al., 2018; Biondi et al., 2018). However, cognisance of the widespread presence in Africa, this absence may be a consequence of lack of surveillance and pest detection mechanisms.

2.4. Factors supporting *T. absoluta* invasion in Africa

2.4.1. African environments and *Tuta absoluta* niche

Interactions between the invaders and the recipient agro-ecological regions influence pest establishment and spread, with prevailing biophysical environment contributing to the invasion process (Davies et al., 2005; Richardson and Pysek, 2006; Guimapi et al., 2016). Tropical regions such as the majority of Africa are highly vulnerable to insect pest invasions (Crespo-Pérez et al., 2011; Osawa et al., 2013; Guimapi et al., 2016). Among the key determinants of invasion success are climatic suitability, propagule pressure, and the availability of suitable hosts (Ward and Masters, 2007; Bacon et al., 2014). Environmental matches between native and novel invasion areas play a pivotal role in invasion success (Walther et al., 2009). Modelling studies have shown that temperature, relative humidity (RH) and hosts are critical determinants of *T. absoluta* successful invasion (Tonnang et al., 2015; Guimapi et al., 2016; Santana et al., 2019). Research shows that Africa is a suitable biophysical niche for the pest (Tonnang et al., 2015; Guimapi et al., 2016; Machekano et al., 2018). Various models have been constructed to predict environmental suitability, potential and timing of *T. absoluta* spread in Africa (Tonnang et al., 2015; Guimapi et al., 2016; Santana et al., 2019). Indeed, these models suggest temperature and RH environments

for Africa are ideal for *T. absoluta* invasion and population establishment. However, it appears RH seems to have the strongest influence in *T. absoluta* invasion (Guimapi et al., 2016). A comparison of temperature and precipitation between its native range and invaded African region shows a similarity in temperature ranges. However, the native range experiences higher precipitation as compared to invaded Africa (Fig. 2.1). Nevertheless, reports suggest *T. absoluta* can thrive in high temperature and low precipitation environments as long as host plants are available (Tonnang et al., 2015). The optimal temperature for its development is 30°C, with egg, larval and pupal lower developmental thresholds at 6.9, 7.6 and 9.2°C, respectively (Barrientos et al., 1998; Martins et al., 2016). In addition, the upper developmental threshold from egg to adult cycle is 37.3 °C (Krechemer and Foerster, 2015). Low temperature and high altitudes (> 1000m) are limiting factors for its survival, and high RH is suitable for its development and life span (Notz, 1992; Cuthbertson et al., 2013; Tonnang et al., 2015). The negative impact of rainfall on population abundance of *T. absoluta* has been reported (Sylla et al., 2018). Based on these ecological requirements and evidence presented in Machekano et al. (2018), warm and humid conditions prevalent in Africa potentially elucidate its invasion success.

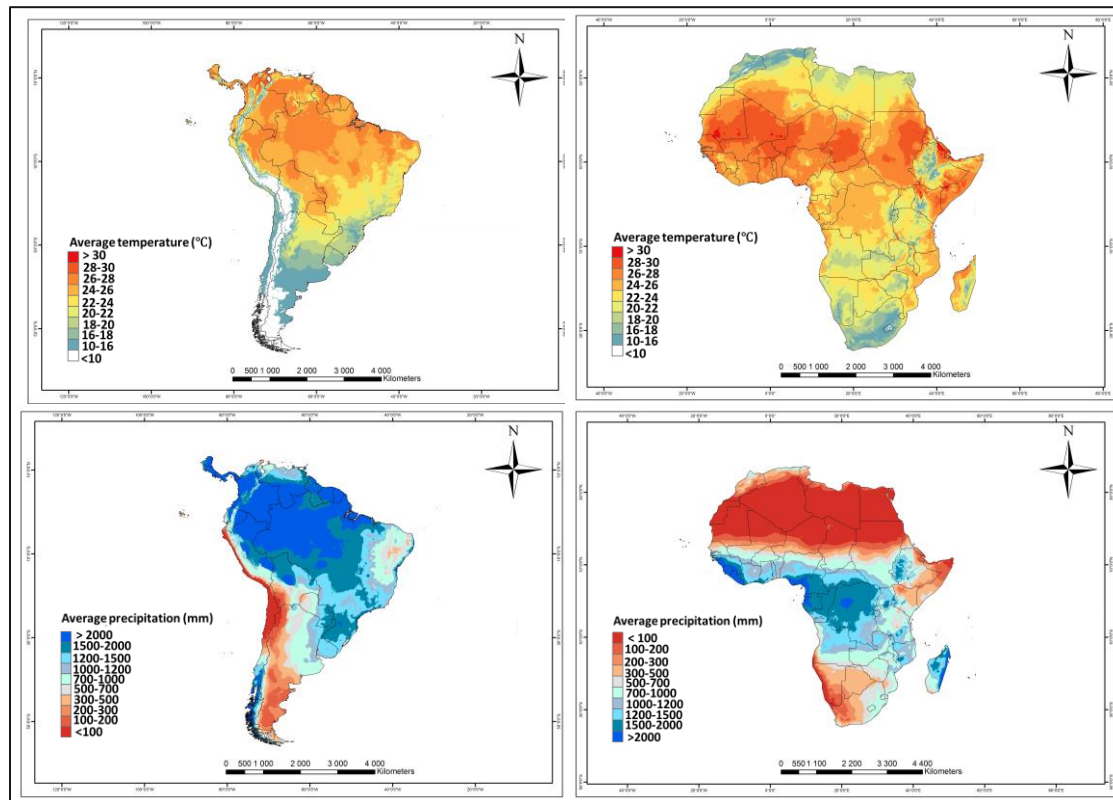


Figure 2.1. Temperature and precipitation comparison between *T. absoluta* native (South America) and invaded (Africa) regions. The maps generally show that *T. absoluta* niche is largely similar between the native and invaded region and thus successful establishment is possible. Maps were drawn in ArcGIS 10.3 from ESRI with data obtained from WorldClim Ver 2.0.

2.4.2. Physiological tolerance

Environmental adaptations enable invasive species to overcome variable stressful barriers along the invasion continuum and significantly contribute to alien species propensity (Lee, 2002; Nyamukondiwa et al., 2010; Weldon et al., 2016). Overcoming different stressful environmental barriers is the first of several potential factors determining whether a species may become established, naturalized and ultimately invasive (Davies et al., 2005). Temperature is the key abiotic ‘ecological filter’ (Crowl et al., 2008) for successful invasion in novel habitats (Olyarnik et al., 2009). As such, failure to mount any compensatory physiological resistance mechanisms against environmental barriers may offset invasion success (Chown and Terblanche, 2007; Machekano et al., 2018). Physiologically, some insect pests often increase invasion success

through employing either increased phenotypic plasticity or basal stress tolerance (Nyamukondiwa et al., 2010; Weldon et al., 2016; Mutamiswa et al., 2018; Tarusikirwa et al., 2020a). A previous study has shown that *T. absoluta* larvae are more thermally plastic than adults and can shift their thermal tolerance in short and long timescales (Tarusikirwa et al., 2020a). In addition, larvae showed a high basal heat tolerance than adults, whereas adults recorded superior basal cold tolerance relative to larvae (Machekano et al., 2018; Tarusikirwa et al., 2020a). This physiological tolerance may help elucidate the notion that ecophysiology may have aided the current invasion by *T. absoluta*.

2.4.3. Increased number of generations

Tuta absoluta is an r selected multivoltine species (Pereya and Sanchez, 2006) that remains active in Mediterranean and African winter climates (Tropea Garzia et al., 2012; Van Damme et al., 2015a; Tarusikirwa et al., 2020b). Its pest status is largely associated with high rate of reproduction, with each female producing up to 260 eggs during its lifetime (Uchôa-Fernandes et al., 1995). Under the projected warming in Africa (Engelbrecht et al., 2015), this promotes even shorter developmental time and higher voltinism. The life cycle of *T. absoluta* takes ~24-38 days at 27°C resulting in ~10 to 13 generations per year (Barrientos et al., 1998; Desneux et al., 2010; Tropea Garzia et al., 2012; Biondi et al., 2018). This rapid development may give *T. absoluta* a numerical advantage and propagule material that facilitates species proliferation.

2.4.4. New niche with limited natural enemies

The enemy release hypothesis postulates that invasive species likely have reduced biotic pressure (e.g. natural enemies) than their native counterparts (Middleton, 2019). It also follows that parasitoids and predators (see Lockwood et al., 2005; Sax et al., 2007) specific to the invasive species may be absent in the novel areas resulting in the pest not being suppressed (Keane and Crawley, 2002) owing to lack of co-evolved biological control agents following invasion (Selvaraj et al., 2013). This is likely the reason behind the limited natural biotic pressure on *T. absoluta* in Africa consequently contributing to its quick establishment (Aigbedion-Atalor et al., 2020). The enemy-free hypothesis also states that invading species often perform better and experience rapid population growth in new areas (Wolfe, 2002; Torchin et al., 2003; Colautti et al., 2004). This is because in the new habitats, invading species may at most, encounter opportunistic generalist natural enemies in their new range, but not more efficacious coevolved specialists (Keane and

Crawley, 2002; Torchin et al., 2003; Colautti et al., 2004; Meijer et al., 2016). Indeed, various indigenous generalist predators and parasitoids have been recorded as potential biological agents of *T. absoluta* in some parts of Africa, albeit with low efficacy (Mansour et al., 2018). However, these studies remain scant and constrained in space (Aigbedion-Atalor et al., 2020). Various authors have reviewed the natural enemy complex of *T. absoluta* (Desneux et al., 2010; Zappalá et al., 2013; Biondi et al., 2018; Salas Gervassio et al., 2019). Amongst these species, a few have been recorded in Africa. The few documented in the continent are mostly prevalent in North Africa (Mansour et al., 2018). The generalist mirid predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) has been the most reported, albeit also confined to North Africa (Mansour et al., 2018; Idriss et al., 2018). Other predators recorded include, *Macrolophus pygmaeus*, (Rambur) (Hemiptera: Miridae), *Macrolophus caliginosus* (Wagner) (Heteroptera: Miridae), *Dicyphus tamaninii* (Wagner) (Heteroptera: Miridae), *Rhynocoris segmentarius* (Germar) (Hemiptera: Reduviidae) and *Dicyphus errans* (Wolff) (Hemiptera: Miridae) (Mansour et al., 2018). In Africa, the parasitoids complex that has so far been recorded through field monitoring and surveys include larval braconid parasitoids of the genus *Apanteles* and *Bracon* in Nigeria, egg parasitoids of the genus *Trichogramma* in Nigeria, Tunisia, Algeria, Morocco (Mansour et al., 2018), several hymenopterous parasitoid species belonging to different families (Kenya) including *B. nigricans*, *B. hebetor*, *Dolichogenidea appellatory* (Telenga) (Hymenoptera: Braconidae), *Ecdamua cadenat* (Risbec) (Hymenoptera: Torymidae), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) in Sudan (Mansour et al., 2018). However, reports suggest these parasitoids cannot solely provide effective *T. absoluta* control (Mansour et al., 2018). Thus, use of other complementary NSs combined in an integrated approach may improve efficacy and sustainability in *T. absoluta* management (Fig. 2.3). To our knowledge, no egg parasitoids have been recorded in southern Africa. Thus, the inefficiency by current spacially isolated indigenous natural enemies coupled with total absence in some regions (Mansour et al., 2018) has provided an enemy-free platform for *T. absoluta* successful invasion and establishment.

2.4.5. Wide host range

Tuta absoluta is polyphagous, exploiting a wide range of alternate hosts (Zlof and Suffert, 2012; Husariu et al., 2017). This behaviour allows the continuous and spatial omnipresence of the pest. Although Özgökçe et al. (2016) reported 26 different host plant species for *T. absoluta*, it has a

strong preference for solanaceous species, with tomato, potato (*Solanum tuberosum*) and European black nightshade (*Solanum nigrum*) being the most preferred (Desneux et al., 2010; Abbes et al., 2016). In addition, it can also oviposit and develop on several plants belonging to the Amaranthaceae, Convolvulaceae, Fabaceae, and Malvaceae (Biondi et al., 2018). Since *T. absoluta* is rapidly and continually evolving, evidence suggest it is also expanding its host range (Husariu et al., 2017). For example, in Sudan, *T. absoluta* was recorded on watermelon (*Citrullus lanatus*) and alfalfa (*Medicago sativa*) (Mohamed et al., 2015) and on weeds such as thorn apple *Datura stramonium* (Husariu et al., 2017). In Algeria, Drouai et al. (2016) found *T. absoluta* on beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), as well as weed species *Chenopodium bonus-henricus* and *C. rubrum*. In South Sudan up to 50% *T. absoluta* damage was observed on potato foliage (Mohamed et al., 2012). In Botswana *T. absoluta* was spotted on some wild hosts *Solanum aculeatissimum* (Jacq.), *Solanum coccineum* (Jacq.), *Solanum supinum* (Machekano et al., 2018). Apart from the main solanaceous plant hosts, *T. absoluta* affects other crops of economic importance (Table 2.1). The availability of alternative host plants is an important factor that allows the sustainability of the pest in the absence of the primary tomato host (Machekano et al., 2018; Younes et al., 2018). Therefore, the presence of a wide range of both cultivated and wild host plants in African landscapes, creates a refuge opportunity for host switching and consequent pest success.

Table 2.1. Alternative host plants of *Tuta absoluta* reported in literature. The list may not be purely exhaustive but was compiled using literature available at the time of writing.

Family	Host plant	Reference
Solanaceae	<i>Solanum tuberosum</i> L.	Desneux et al., 2010
	<i>Solanum nigrum</i> L.	Abbes et al., 2016
	<i>Solanum melongena</i> L.	Gebremariam, 2015
	<i>Solanum aethiopicum</i> L.	Sylla et al., 2018
	<i>Solanum anguivi</i> Lam.	Retta and Berhe, 2015
	<i>Solanum macrocarpon</i> L.	Retta and Berhe, 2015
	<i>Solanum scabrum</i> Mill.	Retta and Berhe, 2015
	<i>Solanum villosum</i> Mill.	Retta and Berhe, 2015
	<i>Solanum aculeatissimum</i> (Jacq.)	Machekano et al., 2018
	<i>Solanum coccineum</i> (Jacq.)	Machekano et al., 2018
	<i>Solanum supinum</i> Dunal	Machekano et al., 2018
	<i>Solanum americanum</i> Mill.	Gebremariam, 2015
	<i>Solanum bonariense</i> L.	Galarza, 1984

	<i>Solanum elaeagnifolium</i> Cav.	Galarza, 1984
	<i>Solanum gracilius</i> Herter	Galarza, 1984
	<i>Solanum hirtum</i> Vahl	Fernandez and Montagne, 1990
	<i>Solanum pseudo-capsicum</i> L.	Galarza, 1984
	<i>Solanum sisymbriifolium</i> Lamb	Galarza, 1984
	<i>Solanum dulcamara</i> Linnaeus	Bawin et al., 2015
	<i>Solanum lyratum</i> Thunb.	Loni et al., 2011
	<i>Solanum puberulum</i> Nuttall ex Seemann	Loni et al., 2011
	<i>Nicotiana longiflora</i> Cav.	Husariu et al., 2017
	<i>Nicotiana tabacum</i> L.	Desneux et al., 2010
	<i>Nicotiana rustica</i> L.	Bawin et al., 2015
	<i>Nicotiana glauca</i> (Graham)	Retta and Berhe, 2015
	<i>Datura stramonium</i> L.	Husariu et al., 2017
	<i>Datura quercifolia</i> Kunth	Gebremariam, 2015
	<i>Datura ferox</i> L.	EPPO, 2005
	<i>Xanthium brasiliicum</i> Vell.	Husariu et al., 2017
	<i>Capsicum annum</i> L.	Özgökçe et al., 2016
	<i>Capsicum frutescens</i> L.	Sylla et al., 2018
	<i>Nicandra physalodes</i> (L.) Gaertner	Bawin et al., 2015
	<i>Lycium halimifolium</i> Mill.	Bawin et al., 2015
	<i>Lycium chilense</i> (Coralillo)	Loni et al., 2011
	<i>Lycium hirsutum</i> L.	Retta and Berhe, 2015
	<i>Physalis peruviana</i> L.	Garzia, 2009
	<i>Physalis angulata</i> L.	Fernandez and Montagne, 1990
Amaranthaceae	<i>Amaranthus spinosus</i> L.	Husariu et al., 2017
	<i>Amaranthus viridis</i> L.	Xian, 2017
	<i>Spinacia oleracea</i> L.	Drouai et al., 2016
	<i>Beta vulgaris vulgaris</i> L.	Portakaldali et al., 2013; Drouai et al., 2016;
	<i>Chenopodium bonus-henricus</i> (L.) Rchb.	Drouai et al., 2016
	<i>Chenopodium rubrum</i> (L.) S. Fuentes, Uotila and Borsch	Drouai et al., 2016
	<i>Chenopodium album</i> L.	Portakaldali et al., 2013
Fabaceae	<i>Phaseolus vulgaris</i> L.	Nitin et al., 2017
	<i>Medicago sativa</i> L.	Husariu et al., 2017
	<i>Vicia faba</i> L.	Husariu et al., 2017
Cucurbitaceae	<i>Citrullus lanatus</i> (Thunb.) Matsum. and Nakai	Husariu et al., 2017
Convolvulaceae	<i>Convolvulus arvensis</i> L.	Portakaldali et al., 2013
	<i>Calystegia sepium</i> (L.) Brown	Portakaldali et al., 2013
Malvaceae	<i>Malva sylvestris</i> L.	Bawin et al., 2015
Asteraceae	<i>Sonchus oleraceus</i> L.	Xian, 2017
	<i>Xanthium strumarium</i> L.	Borisade et al., 2017
Poaceae	<i>Sorghum halepense</i> (L.) Pers.	Xian, 2017
Brassicaceae	<i>Sinapis arvensis</i> L.	Borisade et al., 2017

2.4.6. Pesticide resistance

Synthetic insecticides are employed as the primary method of control against insect pest infestation (Williamson et al., 2008; Ragsdale et al., 2011). For example, Tunisia registered 18 new insecticides during 2009-11 following *T. absoluta* invasion although they all turned out ineffective (Abbes et al., 2012; Zekeya et al., 2017). Intensive use of synthetic insecticides for *T. absoluta* management coupled with insect biological traits, such as high reproductive potential and multivoltinism, endophytic larval feeding behaviour and mining habit as well as polyphagy has increased *T. absoluta* selection pressure for insecticide resistance (Siqueira et al., 2000; Lietti et al., 2005; Brévault et al., 2008). In South America and Europe, resistance has been reported against conventional insecticides such as organophosphates (OPs), pyrethroids, cartap, diamides, and avermectins (Siqueira et al., 2000; Silva et al., 2011; Haddi et al., 2012; Roidakis et al., 2013; Biondi et al., 2018). The main resistance mechanisms evolved through altered target-site sensitivity and/or enhanced detoxification, depending on the chemical class (Guedes et al., 2019). In northern Nigeria, resistance was reported in cyhalothrin (a Type II pyrethroid), propoxur and chlorpyrifos-methyl via enhanced detoxification, underlying the challenges in managing this invasive pest using pesticides (Bala et al., 2019). Given the prohibitive costs of synthetic pesticides for African farmers, evolution of pesticide resistance will further compound losses on already resource-constrained farmers.

2.5. Potential use of natural substances

2.5.1. Botanicals

Botanical insecticides are naturally occurring chemicals extracted from plants with insecticidal properties (El-Wakeil, 2013; Hikal et al., 2017). They can be classified based on their chemical constituents into categories namely; essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids (Bowers and Puttick, 1989; Gould and Lister, 2006; Regnault-Roger et al., 2012; Silva et al., 2015; Velu et al., 2015). Plant derivatives and bioactive compounds have been used to manage different crop pests with notable success (Lengai et al., 2020). Their physiological effects on insects vary depending on the target site and mode of action (Hikal et al., 2017) with most acting as repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants (Rajashekar et al., 2012; Hikal et al., 2017; Lengai et al., 2020). Plant parts used are dependent on the targeted bioactive compounds as well as their localised concentrations.

However, use of barks, leaves, roots, flowers, seeds, and stems is widely reported (Lengai et al., 2020). Common botanicals with reported insecticidal properties include neem (*A. indica*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and pyrethrum (*Tanacetum cinerariifolium*) (El-Wakeil, 2013; Lengai et al., 2020).

Given the wide availability of botanicals, ease of application and low operational costs, botanicals are a viable option for sustainable *T. absoluta* management. *Azadirachta indica* and *Jatropha curcas* extracts have been reported as efficacious on *T. absoluta* eggs and larvae (Kona et al., 2014). In an empirical study, four-day exposure to *J. caurcas* and *A. indica* seed extracts resulted in 18% and 25% egg mortalities respectively whilst 24-hour treatment elicited larval mortalities ranging 23.5 - 48.5% and 33 - 46.7% respectively. In another study, Abdel-baky and Al-Soqueer (2017) showed that simmondsin extracts, obtained from seeds of Jojoba, *Simmondsia chinensis* L. were effective in controlling 2nd instar larvae of *T. absoluta*. Field studies showed 71.82 and 74.26% larval mortalities following treatment using biopesticides, *A. sativum* and *A. indica* respectively (Shiberu and Getu, 2018). Furthermore, ethanolic leaf extracts of *Piper amalago* var. *medium*, *P. glabratum* and *P. mikanianum* significantly elicited *T. absoluta* larval mortalities (De Brito et al., 2015). *Piper* species have amides (e.g. piperamides) that are known to have neurotoxic and lipid metabolism effects (Dyer and Palmer, 2004) manifesting as knockdown, paralysis followed by death (Scott et al., 2007). Several other plant extracts have been found to be effective against *T. absoluta* (Table 2.2). Given the efficacy of some of the reported botanicals (e.g. Abdel-Baky and Al-Soqueer, 2017) (Table 2.2), using them in combination with soft pesticides and in an integrated approach could provide more optimized control. However, despite empirical support for botanicals' efficacy, their use in agriculture is currently limited in commercial use on vegetable and fruit crops with few prospects for commercial development of new products (El-Wakeil, 2013). Several factors affect the wide success of botanicals as conventional pesticides. Availability of plant material, solvent types, rapid environmental degradation, registration bureaucracy, market opportunities and availability of competing products are some of the barriers to successful use of botanical insecticides (El-Wakeil, 2013).

Table 2.2. Insecticidal plants used to make botanicals used against *Tuta absoluta* (Eggs- E; Larvae- L; Pupa- P; Adults- A). The records were obtained from literature at the time of writing and may not be purely exhaustive.

Natural Substance	Species	Host developmental stage	Reference
Botanicals	<i>Azadirachtin</i>	E, L, P	Kona et al., 2014
	<i>Petroleum ether extract</i>	E, L	Kona et al., 2014
	<i>Jatropha curcus</i>	E, L	Kona et al., 2014
	<i>Allium sativum</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Ocimum basilicum</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Thymus vulgaris</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Ricinus communis</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Eucalyptus sp.</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Melia azedarach</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Geranium sp.</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Allium cepa</i>	L	Abd El-Ghany et al., 2016; Birhan, 2018
	<i>Citrus aurantium</i>	L	Zarrad et al., 2013
	<i>Piper amalago var. medium</i>	L	De Brito et al., 2015
	<i>Piper glabratum</i>	L	De Brito et al., 2015
	<i>Piper mikanianum</i>	L	De Brito et al., 2015
	<i>Simmondsia chinensis</i>	L	Abdel-Baky and Al-Soqeer, 2017

2.5.2. Entomopathogens

Entomopathogens, such as *Metarhizium anisopliae* var. *anisopliae* (Metsch.) Soroki, *Beauveria bassiana* (Balsamo) Vuillemin, and *Bacillus thuringiensis* (Berliner), have shown efficacy against *T. absoluta* (Table 2.3) (Rodriguez et al., 2005; Gonzalez-Cabrera et al., 2011). The soil dwelling bacterium *B. thuringiensis* is one of the most important microorganisms with entomopathogenic properties (Youssef and Hassan, 2013). It is environmentally friendly and can be extensively used as part of an integrated approach to pest management (Illakwahhi and Srivastava, 2017). Apart from Lepidoptera, *B. thuringiensis* was reported to exhibit insecticidal effects on many other insect orders (Youssef and Hassan, 2013) making it ideal for sustainable integrated management. Indeed, both *B. bassiana* and *B. thuringiensis* have been used to control an array of pest insects including whiteflies, thrips and termites (Jacobson et al., 2001; Mburu et al., 2009). *Bacillus thuringiensis* has already been widely used in *T. absoluta* control (Gonzalez-Cabrera et al., 2011; Youssef and Hassan, 2013). Efficacy studies of *B. bassiana* and *B. thuringiensis* on *T. absoluta* showed that

third instar larvae were most susceptible (Tsoulmari and Port, 2016). Furthermore, their interaction effects were synergistic in *T. absoluta* control. By contrast, Gonzalez-Cabrera et al. (2011) found evidence that first instar larvae were the most susceptible to *B. thuringiensis* and that it could keep *T. absoluta* below economic thresholds (Alsaedi et al., 2017). Similarly, Biondi et al. (2018), reported that *Wolbachia* bacterial infection may potentially be efficacious for *T. absoluta* through its effects on reproduction. However, using *Wolbachia* has some potential disadvantages, e.g., horizontal transfer, mediating parthenogenesis in the pinworm, thus, compromising pheromone use against the species (Caparros-Megido et al., 2012). Spinosad, a fermentation product of *S. spinosa* has also been used in *T. absoluta* control (Racke, 2006; Baniamiri and Cheraghian, 2012; Biondi et al., 2013a). However, its continued use has been threatened by resistance (Caparros Megido et al., 2012). Thus, there is need for complementary control options to fight *T. absoluta*, if pest populations are to be maintained below economic threshold levels (Biondi et al., 2018). Regardless of sporadic resistance reports for these substances, they still remain reliable and efficacious sustainable options to pest control in integrated systems.

2.5.3. Entomopathogenic nematodes (EPNs)

Entomopathogenic nematodes (EPNs) (Table 2.3) are biological control agents that can kill insect pests using their coevolved mutualistic intestinal bacterium (Adams and Nguyen, 2002; Boemare, 2002; De Waal et al., 2011). Their use in pest management is already widespread and have shown efficacy in diverse taxa (Van Damme et al., 2015b). Entomopathogenic nematodes have been used against similar Lepidopterans, e.g. false codling moth (*Thaumatotibia leucotreta*), codling moth (*Cydia pomonella*) and the sugarcane borer (*Eldana saccharina*) (De Waal et al., 2011; Malan et al., 2011; Nthenga et al., 2014). Recent studies have shown EPNs *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* are effective against all larval instars of *T. absoluta* (Table 2.3) (Van Damme et al., 2015b). Similarly, Kamali et al. (2017) reported high efficacy for *S. carpocapsae* and *H. bacteriophora* against fourth instar larvae. These results suggest scope for EPNs in *T. absoluta* management and can be an intergap component of an integrated management approach.

Table 2.3. Microbials and other natural substances used as biopesticides against *Tuta absoluta*. (Eggs- E; Larvae- L; Pupa- P; Adults- A). The records were obtained from literature at the time of writing and may not be purely exhaustive.

Type of Microbial	Species	Host	Reference
Entomopathogens	<i>Bacillus thuringiensis</i>	L	Gonzalez-Cabrera et al., 2011; CABI, 2013
	<i>Bacillus thuringiensis kurstaki</i>	L	CABI, 2013
	<i>Beauveria bassiana</i>	L	CABI, 2013
	<i>Metarhizium beauveria</i>	L	Contreras et al., 2014
	<i>Metarhizium anisopliae</i>	L	Contreras et al., 2014
	<i>Baculoviruses</i> (NPVs)	L	Gomez et al., 2017
Entomopathogenic nematodes	<i>Saccharopolyspora spinosa</i>	L	Gozel and Kasap, 2015
	<i>Steinernema affine</i>	L	Gozel and Kasap, 2015
	<i>Steinernema carpocapsae</i>	L	Gozel and Kasap, 2015
	<i>Steinernema feltiae</i>	L	Kamali et al., 2017
	<i>Heterorhabditis bacteriophora</i>	L	Kamali et al., 2017
Other NSs	Pheromones	A	Mutamiswa et al., 2017; Witzgall et al., 2010
	Antimicrobial peptides (AMPs)	E, L, P	Broekaert et al., 1997

2.5.4. Semiochemicals

Semiochemicals are chemicals mediating interactions across organisms by eliciting behavioral responses in recipient organisms within and across species. Sex pheromones have been, by far, the most widely used semiochemicals in pest management (Witzgall et al., 2010). Semiochemical-based management of insects usually include pheromone lure and kill, mass trapping, and disruption of mating activities (Cocco et al., 2013; Caparros Megido et al., 2013). Pheromones can also be effectively utilised in population monitoring to determine action thresholds, early pest detection and other manipulations of insect pest behaviour (Mashaly et al., 2012). This form of control has been successfully implemented in South America, Europe, Asia and Africa in managing *T. absoluta* in greenhouses and open fields (Illakwahhi and Srivastava, 2017; Machekano et al., 2018). Using sex pheromones, Filho et al. (2000) recorded 233 males/trap/day under greenhouse conditions. These trap catches can be used as part of a mass trapping *T. absoluta* control strategy. Pheromone mediated control of *T. absoluta* is more recommended as a supplementary measure in combination with other management options. For example, a study in Egypt showed that a combined use of sex pheromone and other insecticides is effective against *T. absoluta* larvae (El-aassar et al., 2015). Similarly, Cherif et al. (2018) showed that mass trapping

in combination with *B. thuringiensis* and cyromazin significantly reduced *T. absoluta* numbers. Other behavior modifying strategies, e.g. the push pull strategies have worked successfully for the management of other Lepidopteran pest insects (Cook et al., 2007). However, to our knowledge, empirical studies showing the efficacy of push pull strategy in *T. absoluta* control in Africa is missing. Moreover, the use of pheromone-mediated management system in Africa is also low due to high cost and limited availability. Nevertheless, semiochemicals represent an efficacious and sustainable approach to pest management.

2.5.5. Sterile Insect Technique (SIT)

Sterile insect technique is an environmentally friendly control option aiming to suppress pest populations through F₁ generation sterility enabled by release of sterile male that mate with wild females (Dyck et al., 2005a) thereby producing non-viable offspring (Knipling, 1955; Dyck et al., 2005a; Carabajal Paladino et al., 2016). However, male sterilisation can reduce fitness thereby compromising field competitiveness (Suckling et al., 2011). As such, various mechanisms have been put in place to compensate for this reduced fitness and maintain optimal SIT efficacy in the field including the use of low dosage gamma radiation and thermal preconditioning/acclimation (Dyck et al., 2005a; Chidawanyika and Terblanche, 2011).

Sterile insect technique has been used successfully for control of Lepidopterans e.g. pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Cagnotti et al., 2016), false codling moth, *T. leucotreta* (Lepidoptera: Tortricidae) (Nepgen, 2014) and codling moth, *C. pomonella* (Lepidoptera: Tortricidae) (Bloem et al., 2005). The technique can be used in area-wide management approach, incorporating other options such as natural enemies, cultural control, and the application of bio-rational pesticides (Carpenter, 2000; Dyck et al., 2005b). Cagnotti et al. (2016) reported that the inherited sterility control of *T. absoluta* can be combined with the use of the predator *Tupiocoris cucurbitaceus* in pest management. Given the efficacy of SIT in pest management, its environmental soundness coupled with high compatibility with other control measures, SIT warrants exploration for use in *T. absoluta* management in Africa.

2.5.6. Host plant resistance

Development of tomato cultivars resistant to pests is an important strategy in pest control using NSs and is one of the fundamental pillars of an integrated management approach (Giustolin et al.,

2001). While few moderately resistant tomato cultivars have been reported, (Gharekhani and Salek-Ebrahimi, 2014; Han et al., 2019), the majority are highly susceptible to *T. absoluta* infestation. Three mechanisms may account for plant resistance to insect attack vis antixenosis, antibiosis and tolerance (Fernandes et al., 2011). Thus, tomato cultivars significantly differ in their susceptibility to *T. absoluta* (Oliveira et al., 2009; De Oliveira et al., 2012; Sohrabi et al., 2016; Ghaderi et al., 2017), and that resistance is positively associated with trichomes density and the diversity and concentrations of host secondary metabolites (Proffit et al., 2011; De Oliveira et al., 2012; Sohrabi et al., 2016). Indeed, tomato plants possess glandular trichomes which produce volatile and non-volatile secondary metabolites e.g. acyl sugars, terpenoids, phenylpropanoids, flavonoids and phenolic compounds (Kant et al., 2004; Oliveira et al., 2009). Therefore, breeding programmes have targeted lines with high acyl sugars and other secondary metabolites e.g. zingiberene for host resistance against the pest in tomato production (Borisade et al., 2017; Biondi et al., 2018). Use of resistant varieties may also offer a cost effective and sustainable approach to the fight against the *T. absoluta* scourge in Africa.

2.5.7. Use of predators and parasitoids

Several predators and parasitoid biological control agents have been demonstrated to suppress populations of the tomato pinworm below economic threshold levels (Table 2.4). Conservation and augmentative biological control programmes using predators and parasitoids have been developed for *T. absoluta* following its invasion in Europe (Biondi et al., 2018) and Africa (Urbaneja et al., 2012; Zappalá et al., 2013; El-Arnaouty et al., 2014; Luna et al., 2015). Hemipteran predators, notably anthocorids, geocorids, mirids, nabids, and pentatomids, have been identified as *T. absoluta* biocontrol agents in both native and invaded areas (Desneux et al., 2010; Biondi et al., 2018). The generalist predators *M. pygmaeus*, *N. tenuis* and *Dicyphus* spp. (Jaworski et al., 2015) are the most common antagonists in European greenhouses (Zappalá et al., 2013). Their use has been complemented with *B. thuringiensis* sprays against the early larval infestations (Mollá et al., 2011). Adults and nymphs of *M. pygmaeus* and *N. tenuis* prey on *T. absoluta* eggs and larvae (preferably first instar) (Arnó et al., 2009; Urbaneja et al., 2012). *Dicyphus tamaninii* has been reported as a predator of *T. absoluta* eggs and larvae in North Africa (Urbaneja et al., 2012). Other predators reported in Africa include *N. tenuis*; *M. pygmaeus*, *M. caliginosus*, *D. tamaninii*, *R. segmentarius* and *D. errans* (Mansour et al., 2018). In Spain, predatory lacewings

Chrysoperla carnea and mites *Amblyseius swirskii* Athias-Henriot and *A. cucumeris* (Oudemans) have been reported as egg and larval biocontrol agents. Furthermore, predatory ants, e.g. *Tapinoma nigerrimum* (Nylander) has been identified as biocontrol agent in North Africa (Table 2.4). Where available, conservation of these predators as NSs or mass rearing for use as augmentation or inoculative releases may offer a sustainable approach to the management of *T. absoluta*.

Hymenopteran egg parasitoids belonging to the genus *Trichogramma* have been reported as efficacious biological control agents in protected tomato crops (Parra and Zucchi, 2004; Chailleux et al., 2012). For example, *Trichogramma pretiosum* Riley and *Trichogramma achaeae* Nagaraja and Nagarkatti have been used in *T. absoluta* management in both native and invaded European regions (Pratissoli et al., 2005; Cabello et al., 2012). In Africa, egg parasitoids comprising especially *Trichogramma* spp. have shown potential for mass releases e.g. in Tunisia (Mansour et al., 2019). Indeed, reports suggest that the parasitoid complex for larval *T. absoluta* comprise approximately 20 hymenopteran species (Table 2.4) (Colomo et al., 2002; Luna et al., 2007; Luna et al., 2015). Similarly, several *T. absoluta* larval parasitoids including Eulophids, Braconids and Ichneumonids have also been reported in the Mediterranean basin (Urbaneja et al., 2012). For example, idiobiont *Necremnus artynes* parasitising third instars is widely documented (Mollá et al., 2008). Similarly, in Egypt, larval parasitoid *Stenomesus japonicus* has been reported a desirable NS for *T. absoluta* biocontrol (Urbaneja et al., 2012), while *N. formosa* (Westwood) has been reported in the Palearctic area, Asia, Africa and North America (Zappalá et al., 2013). As part of classical biocontrol larval parasitoid *Dolichogenidea gelechiidivoris* Marsh. (Syn.: *Apanteles gelechiidivoris* Marsh) was introduced from Peru into Africa (Aigbedion-Atalor et al., 2020). Progressive work has shown that *D. gelechiidivoris* prefers first and second instar larvae and is a highly efficacious parasitoids (Aigbedion-Atalor et al., 2020). Conversely, few *T. absoluta* pupal parasitoids have been reported (Biondi et al., 2018). Current research suggests Braconids, Chalcidids, Eulophids and Ichneumonids as potential pupal parasitoids (Marchiori et al., 2004; Desneux et al., 2010; Zappalá et al., 2013; Biondi et al., 2018) (see Table 2.4). Given the diversity of African landscapes, and the diversity of both indigenous and exotic natural enemies potent as NSs in pest control, using these in combination with other compatible NSs may be key to integrated management of *T. absoluta* in Africa.

Table 2.4. Natural enemies (parasitoids and predators) reported in literature for *Tuta absoluta*. The list may not be purely exhaustive but was compiled using available literature at the time of writing. (Eggs- E; Larvae- L; Pupa- P).

Natural Enemy	Species	Host	Reference
Parasitoids	<i>Agathis fuscipennis</i>	L	Loni et al., 2011
	<i>Apanteles dignus</i>	L, P	Desneux et al., 2010; Biondi et al., 2018
	<i>Apanteles gelechiidivoris</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Baryscapus bruchophagi</i>	L	Doğanlar and Yiğit, 2011
	<i>Brachymeria secundaria</i>	L	Doğanlar and Yiğit, 2011
	<i>Bracon lucileae</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Bracon lulensis</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Bracon sp.</i>	P	Desneux et al., 2010; Zappalá et al., 2013; Biondi et al., 2018
	<i>Bracon tutus</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Campoplex haywardi</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Capidosoma desantis</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Capidosoma koehleri</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Cheolras Semele</i>	-	Gabarra and Arnó, 2010
	<i>Closterocerus clarus</i>	L	Doğanlar and Yiğit, 2011
	<i>Closterocerus formosus</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Copidosoma desantisi</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Copidosoma koehleri</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Diadegma sp.</i> , <i>D. ledicola</i> and <i>D. pulchripes</i>	P	Desneux et al., 2010; Zappalá et al., 2013; Biondi et al., 2018
	<i>Diglyphus crassinervis</i>	L	Gabarra and Arnó, 2010
	<i>Diglyphus isaea</i>	L	Gabarra and Arnó, 2010
	<i>Dineulophus phthorimaeae</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Dolichogenidea litae</i>	-	Gabarra and Arnó, 2010
	<i>Elachertus inunctus</i>	L	Zappalá et al., 2012
	<i>Elasmus phthorimaeae</i>	L	Gabarra and Arnó, 2010
	<i>Encarsia porter</i>	E	Cáceres et al., 2011
	<i>Goniozus nigrifemur</i>	L	Desneux et al., 2010
	<i>Habrobracon didemie</i>	L	Doğanlar and Yiğit, 2011
	<i>Habrobracon hebetor</i>	L	Doğanlar and Yiğit, 2011
	<i>Habrobracon nigricans</i>	L	Biondi et al., 2013b; Biondi et al., 2018
	<i>Habrobracon osculator</i>	L	Zappalá et al., 2013
	<i>Halticoptera aenea</i>	L	Zappalá et al., 2012
	<i>Hemiptarsenus zilahisebessi</i>	L	Gabarra et al., 2013
	<i>Hockeria unicolor</i>	L	Gabarra and Arnó, 2010; Doğanlar and Yiğit, 2011
	<i>Horismenus sp</i>	P	Desneux et al., 2010; Zappalá et al., 2013; Biondi et al., 2018
	<i>Hyposoter didymator</i>	-	Boualem et al., 2012
	<i>Necremnus artynes</i>	L	Mollá et al., 2008; Gabarra et al., 2013
	<i>Necremnus metalarus</i>	L	Urbaneja et al., 2012
	<i>Necremnus tidius</i>	L	Riciputi, 2011
	<i>Neochrysocharis formosa</i>	L	Zappalá et al., 2013

	<i>Neochrysocharis formosa</i>	L	Zappalá et al., 2012; Biondi et al., 2013b
	<i>Neochrysocharis formosa</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Pnigalio cristatus</i>	L	Doğanlar and Yiğit, 2011
	<i>Pnigalio incompletes</i>	-	Doğanlar and Yiğit, 2011
	<i>Pnigalio soemius</i>	L	Gabarra and Arnó, 2010
	<i>Pnigalio sp. Soemius Complex</i>	L	Zappalá et al., 2012
	<i>Pseudapanteles dignus</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Pteromalus intermedius</i>	L	Doğanlar and Yiğit, 2011
	<i>Pteromalus semotus</i>	-	Gabarra and Arnó, 2010
	<i>Retisypiesis phthorimaea</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Retisypiesis phthorimaea</i>	L	Desneux et al., 2010; Biondi et al., 2018
	<i>Temelucha anatolica</i>	-	Gabarra and Arnó, 2010
Predators	<i>Trichogramma achaeae</i>	E	Cabello et al., 2012
	<i>Trichogramma achaeae</i>	E	Biondi et al., 2013b
	<i>Trichogramma bactrae</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma bourarachae</i>	E	Zouba et al., 2013
	<i>Trichogramma dendrolimi</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma exiguum</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma fasciatum</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma lopezandinensis</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma minutum</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma nerudai</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma pinto</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma pretiosum</i>	E	Pratissoli et al., 2005 Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma rojasi</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Trichogramma telengai</i>	E	Desneux et al., 2010; Biondi et al., 2018
	<i>Zoophthorus macrops</i>	-	Gabarra and Arnó, 2010
	<i>Amblyseius cucumeris</i>	E, L	Mollá et al., 2010
	<i>Amblyseius swirskii</i>	E, L	Mollá et al., 2010
	<i>Brachygastra lecheguana</i>	L	Desneux et al., 2010
	<i>Calosoma granulatum</i>	L, P	Desneux et al., 2010
	<i>Coleomegilla maculate</i>	E, L	Desneux et al., 2010
	<i>Cycloneda sanguinea</i>	E	Desneux et al., 2010
	<i>Dicyphus errans</i>	E, L	Ferracini et al., 2012
	<i>Dicyphus maroccanus</i>	E, L	Ferracini et al., 2012
	<i>Dicyphus. Tamaninii</i>	E, L	Mollá et al., 2010; Urbaneja et al., 2012
	<i>Doru lineare</i>	E	Desneux et al., 2010
	<i>Engytatus varians</i>	E	Mollá et al., 2010
	<i>Eriopsis conexa</i>	E	Desneux et al., 2010
	<i>Franklinothrips vespiformis</i>	L	Desneux et al., 2010
	<i>Labidura riparia</i>	P	Desneux et al., 2010
	<i>Lebia concina</i>	L, P	Desneux et al., 2010
	<i>Macrolophus pygmaeus</i>	E, L	Pérez-Aguilar et al., 2018
	<i>Nabis ibericus</i>	L	Cabello et al., 2009; Mollá et al., 2010
	<i>Nesidiocoris tenuis</i>	E	Pérez-Aguilar et al., 2018
	<i>Orius albidipennis</i>	-	Ferracini et al., 2012; Al-Jboory et al., 2012

<i>Orius insidiosus</i>	E, L	Desneux et al., 2010
<i>Podisus nigrispinus</i>	L	Desneux et al., 2010
<i>Polistes carnifex</i>	L	Desneux et al., 2010
<i>Polistes melanosoma</i>	L	Desneux et al., 2010
<i>Polistes versicolor</i>	L	Desneux et al., 2010
<i>Polybia ignobilis</i>	L	Desneux et al., 2010
<i>Polybia scutellaris</i>	L	Desneux et al., 2010
<i>Protonectarina sylveirae</i>	L	Desneux et al., 2010
<i>Protopolybia exigua</i>	L	Desneux et al., 2010
<i>Scolothrips sexmaculatus</i>	L	Desneux et al., 2010
<i>Solenopsis geminate</i>	L, P	Desneux et al., 2010
<i>Solenopsis saevissima</i>	L, P	Desneux et al., 2010
<i>Synoeca cyanea</i>	L	Desneux et al., 2010

2.6. Local natural enemy guild of Botswana and potential agents for biological control

Scoping of resident natural enemies is essential in informing inundative, inoculation and conservation biological control for sustainable pest management. Field surveys were conducted to determine the ‘native’ *T. absoluta* natural enemies in four districts of Botswana. Sampling was conducted during the 2018/2019 and 2019/2020 tomato production seasons. Natural enemies were sampled from infested tomato plants following methods by Mutamiswa et al. (2017); Moeng et al. (2018).

2.6.1. Field surveys and sampling

Field surveys for cultivated host plants were conducted in six tomato growing hotspots of Botswana, i.e., Central (Tonota), South East (Gaborone), North East (Francistown) and Ngamiland (Maun) districts (Fig. 2.2). Sampling involved the selection of tomatoes from six farms in each of the spatial production hotspots. A biased sampling procedure was employed to increase the chances of finding *T. absoluta*. Potential tomato host plants were inspected for leafminer damage symptoms such as presence of mines, larval entry holes in fruits, the presence of frass and mummified life stages (eggs, larva, pupa and adults). Leaves/fruits that exhibited damage symptoms were collected for subsequent incubation in the laboratory. Predatory insects, parasitoid cocoons or mummified larvae or pupa were collected and placed individually in labelled 20ml plastic vials. Pupae were placed in vials with a piece of wet cotton wad to prevent desiccation related mortalities. Following collection Geographic Positioning System (GPS) coordinates for each samples field were recorded. Whole parasitoid/predator samples were preserved in 70%

ethanol while mummified larvae and/or pupae were kept in vials containing fresh leaves (diet) and a wet cotton wool during incubation.

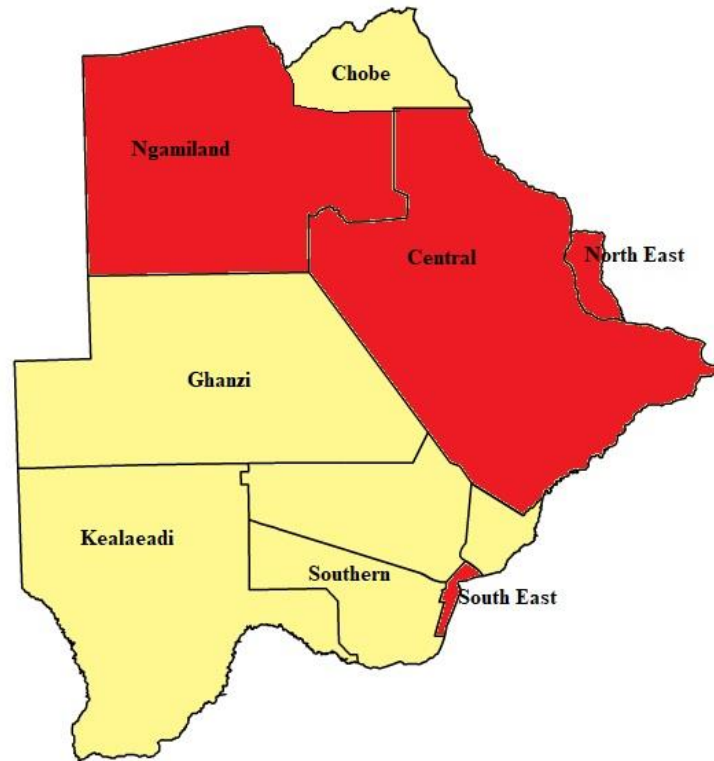


Figure 2.2. General map of Botswana showing the four tomato growing hotspots (red colour) surveyed for natural enemies.

2.6.2. Laboratory incubation

In the laboratory, all insects were incubated in climate-controlled chambers (HPP 260, Memmert GmbH +Co.KG, Germany) at 28 °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% ethanol for subsequent identification using morphological and molecular techniques.

2.6.3. Identification of specimens

Collected insect specimens were first identified using morphological features by Dr Simon Van Noort at Iziko South African Museum and then confirmed with molecular techniques following modified protocols from Buxton et al. (2019). Polymerase chain reaction (PCR) was used to amplify the cytochrome oxidase subunit I (COI) by use of universal primers LCO 1490 and HCO

2198, and in some specimens Pat-Jerry COI primer. Adults' appendages were used in the amplification process (Bass et al., 2007). The dissected appendages were added directly to a 50 µL PCR reaction mix consisting of 25 µL of 2× Phusion U Multiplex PCR Mix (Thermo Fisher Scientific, USA), 9 µL nuclease free water (VWR International LLC, USA) and 0.8 µM of each primer. The PCR conditions encompassed; initial denaturation at 98 °C for 30 seconds, 35 cycles of denaturation at 98 °C for 10 seconds, annealing at 48 °C for 30 seconds and extension at 72 °C for 30 seconds. Afterward, a final extension at 72 °C for 7 minutes was performed. Visualisation of amplicons were done under 1 % agarose gel stained with ethidium bromide. Purification of 40 µL PCR product was performed using a JET PCR purification kit (Thermo Fisher Scientific, USA) according to manufacturer's recommended protocols, followed by elution with 20 µL nuclease free water. Amplicons were sent to Inqaba Biotechnical Industries (Pretoria, South Africa) for sequencing. The sequences were trimmed and contigs were assembled using a CodonCode Aligner 8.0.2 software package and subjected to nucleotide blast for species homology identification. Sequences were further deposited in Genbank to obtain species accession numbers.

2.6.4. Local native natural enemies' results

Parasitoids recorded included *N. formosa*; *Brachymeria* species; *Anisopteromalus calandrae* (Howard); *Chelonus* species and *Goniozus omanensis* (Polaszek) (Table 2.5). Predators recorded were predominately ants *Pheidole tenuinodis* (Mayr) and *P. megacephala* (Fabricius); and the *Nabidae* species (Table 2.5).

Table 2.5. Native natural enemies (parasitoids and predators) recorded across four Botswana districts over the 2018/2019 and 2019/2020 tomato production seasons. C= Central, NE= North East, N = Northern, SE = South East; L = Larval, P = Pupal.

Parasitoids	Family	Life stage	District present	Coordinates
<i>N. formosa</i>	Eulophidae	L	C, NE, N	S21°8'54.239"; E27°38'48.42"
<i>Brachymeria</i> spp.	Chalcididae	L	C, NE, SE	S21°13'36.39864"; E26°26'49.30512"
<i>A. calandrae</i>	Pteromalidae	L/P	C, NE	S21°8'54.239"; E27°38'48.42"
<i>Chelonus</i> spp.	Braconidae	L	C, NE	S21°8'54.239"; E27°38'48.42"
<i>G. omanensis</i>	Bethylidae	L/P	C, NE	S21°8'54.239"; E27°38'48.42"
Predators				
<i>Nabidae</i> spp.	Nabidae	L	C, NE, N	S21°8'54.239"; E27°38'48.42"
<i>P. tenuinodis</i>	Formicidae	L	All sampled districts	
<i>P. megacephala</i>	Formicidae	L	All sampled districts	

A total of five parasitoids and three predators were recorded for the first time in four districts of Botswana indicating potential for natural biological control of this invasive insect pest. These results show that there are few natural enemies that are currently managing *T. absoluta* in Botswana. A review on the indigenous natural enemies of the pest in its native South America by Salas Gervassio (2019), reported more than 50 species or morphospecies of Hymenoptera associated with *T. absoluta*, albeit, only 23 were confirmed parasitising it. This is a relatively high number of recorded natural enemies compared to those recorded in this study. Results from the current survey are in agreement with Aigbedion-Atalor et al. (2020), who postulates that there is limited natural biotic pressure on *T. absoluta* in Africa. This may be one of the potential reasons for the fast establishment and spread of the pinworm in line with the enemy escape hypothesis (see Catford et al., 2009; Middleton, 2019). As a result, conservation biological control, which involves the use of farming practises that counter habitat loss and environmental disturbance associated with intensive crop production (Berg et al., 2017) is thus essential to increase the population of already existing natural enemies. This will in the long run lead to pest suppression and is an environmentally sustainable method that works well as part of IPM.

Some of the species recorded in this survey have also been observed in other regions, including South America. For example, Luna et al. (2011) reported the first record of *N. formosa* parasitizing larvae of *T. absoluta* in tomato crops in Argentina. The parasitoid reportedly parasitise the first, second and third instar larvae, with preference to the second instar (Guleria et al., 2020). It is, however, a polyphagous parasitoid that has been recorded as a primary natural enemy from over 100 host species in four different orders (i.e., Coleoptera, Diptera, Hymenoptera, Lepidoptera) (Luna et al., 2011). It was first recorded in Sudan (Mansour et al., 2018). *Brachmyeria* and *Chelonus* spp. have also been recorded attacking *T. absoluta* larvae in the current study in agreement with previous studies (Desneux et al., 2010; Doğanlar and Yiğit, 2011). Although Desneux et al. (2010) recorded *Goniozus nigrifemur* from Bethyridae family as a parasitoid of *T. absoluta* larvae, the current survey, reports *G. omanensis* for the first time on *T. absoluta*. The species is currently known only to attack the lesser date moth *Batrachedra amydraula* (Polaszek et al., 2019). This suggests that this parasitoid species may have a broader host range. However, there is need for further studies to ascertain its parasitism in *T. absoluta*. Although, *A. calandreae* has been previously reported as a parasitoid of stored products pests e.g. *Sitophilus zeamais* and *S. oryzae* (Smith, 1993; Burks et al., 1999), it was recorded on *T. absoluta* for the first time in the

current study indicating broader host range. Few predators were observed with only Formicidae and Nabidae species being recorded. Ants are well known as generalist predators and feed on a wide range of insect pests. A study by Cabello et al. (2009) showed that the zoophagous insect, the damsel bug *Nabis pseudoferus*, can be used for biological control of *T. absoluta*. The absence of the prominent egg parasitoid *Trichogramma* spp. was very unexpected as it has been reported in other parts of Africa (see review Mansour et al., 2018). This may be due to differential environmental conditions and sampling methodologies. Thus, more extensive and robust surveys are needed to document all *T. absoluta* natural enemies. Nevertheless, the detection of native natural enemies locally is the first step towards implementing biological control (e.g. through conservation and/augmentation), utilized in an IPM approach.

2.7. Use of synthetic pesticides and integrated pest management

Regardless of their limitations, chemical pesticides continue to be an important component of integrated pest management (IPM) and can be a crucial pillar in *T. absoluta* management. Common active ingredients registered for *T. absoluta* control include pyrethroids (Guedes and Picanço, 2012), organophosphates (Campos et al., 2014) and diamides (Roditakis et al., 2015). Laboratory bioassays using insecticides with some of these active ingredients showed very high efficacy for *T. absoluta* control (Braham and Hajji, 2012; Radwan and Taha, 2012; Deleva and Harizanova, 2014; CABI, 2019; Bala et al., 2019). Despite high laboratory efficacy, field *T. absoluta* optimal control remains a challenge owing to the cryptic leaf mining behavior that renders contact insecticides ineffective. In addition, continuous use of these pesticides has also led to resistance development. Insecticides may also have adverse effects on the environment, beneficial arthropods and public health (Lu et al., 2012). Thus, minimal insecticidal use is recommended, in an IPM approach complemented with more environmentally benign NSs.

IPM encourages the use of eco-friendly strategies, such as NSs in biological control, used in compatible combinations with other efficacious methods including use of selective insecticides for pest control (Smith, 2005). Thus, IPM comprises a cocktail of control practices, which may include cultural, chemical and biological control for the management of an economic pest species (Fig. 2.3) (Fusire, 2008). For *T. absoluta* management in Africa, and cognisant of all available control options discussed above, we propose the combination of compatible methods in an IPM approach and incorporating the use of sustainable NSs (see Fig. 2.3). This proposition is environmentally

friendly, conserves biological diversity including natural enemy populations and presents little potential public health negative implications. With the demerits associated with synthetic insecticides (e.g. Lietti et al., 2005; Alsaedi et al., 2017), IPM approaches may provide more lasting sustainable solutions to increased *T. absoluta* pest challenges (Desneux et al., 2010; Tsoulara and Port, 2016) and indeed other pest species.

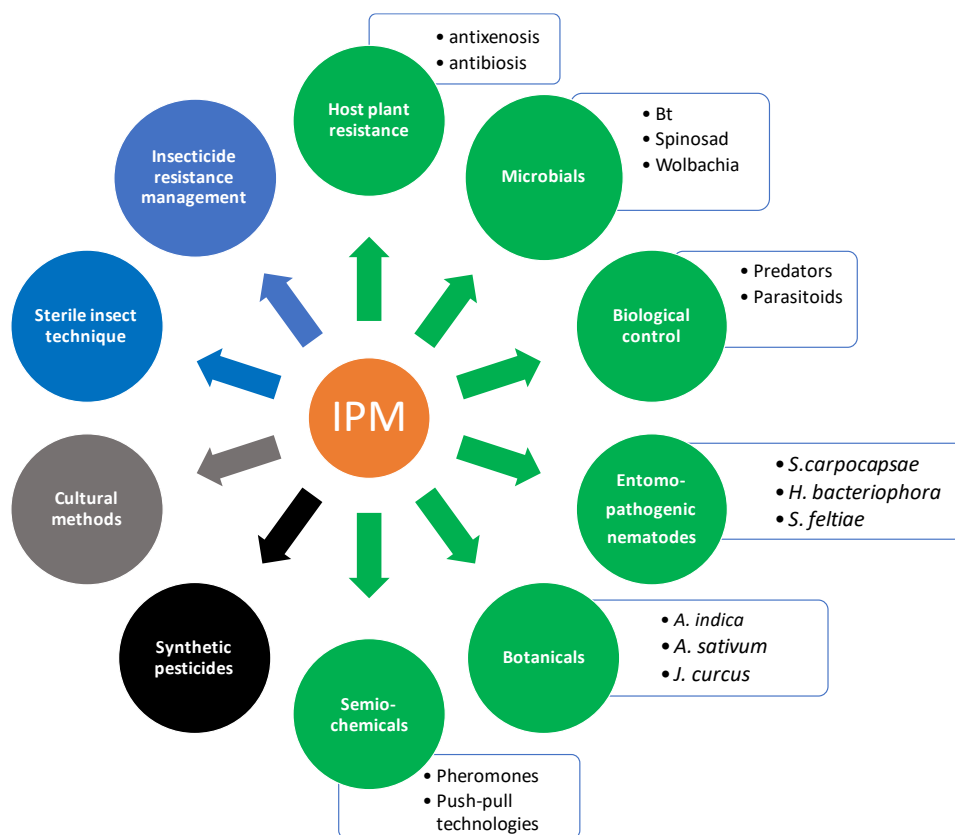


Figure 2.3. Schematic representation of proposed integrated pest management programme for *Tuta absoluta*.

2.8. Potential for natural substances in pest control; assets and liabilities

2.8.1. Legislative and regulatory frameworks

Despite being major pillars to an integrated approach to *T. absoluta* sustainable management, there are major liabilities and bottlenecks associated with NSs development and successful deployment in Africa. Some major liabilities for NSs include the increased number of complex guidelines, regulations and inadequate lobbying by biocontrol champions (Bale, 2011; Cherry and Gwynn, 2007; van Lenteren, 2012; Mason et al., 2017). Furthermore, negative, and often conflicting effects

on chemical industry profitability and general farmer overreliance on pesticides (Williamson et al., 2008; van Lenteren, 2012; Benjamin and Wesseler, 2016) are some of the barriers to NSs, biological and low-risk pest management options. Policy guidelines in the production, exportation or importation, shipment, environmental risk assessment and field application of NSs are bureaucratic (Bale, 2011; van Lenteren, 2012; Benjamin and Wesseler, 2016; Mason et al., 2017). Except for Kenya (Gwynn and Maniania, 2010), policy and legislation governing NSs in Africa are mostly based on templates for synthetic chemical pesticides with a single active ingredient, and thus does not permit registration of NSs with complex multiple active compounds (Gwynn and Maniania, 2010; Grzywacz et al., 2014). This impedes the ability of resource-poor small and medium enterprises (SMEs) active in the NSs crop protection industry in African countries to register their products (Cherry and Gwynn, 2007; Grzywacz et al., 2014). Governments need to create enabling legal and policy frameworks as pre-requisite for the promotion of NSs industry particularly promoting existing SMEs (Sola et al., 2014) relative to multi-national companies. Available evidence suggests that the multi-national companies in the agrochemical industry are ‘unwilling’ participants in creating an enabling legislative and registration environment for the NSs motion (Grzywacz et al., 2014). Benjamin and Wesseler (2016) suggest that slow adoption of NSs may be caused by prolonged and prohibitive regulations. Therefore, changes in regulatory policies governing NSs in SSA should aim to reduce this bottleneck and make the toxicological evaluation regulations light touch (Cherry and Gwynn, 2007; Gwynn and Maniania, 2010; Bale, 2011; Grzywacz et al., 2014). In light of this, African governments remain the main change agents (van Lenteren, 2012; Sola et al., 2014; Benjamin and Wesseler, 2016) through policy and regulatory adjustments that promote production, registration and marketing of NSs (Grzywacz et al., 2014; Sola et al., 2014).

In some cases, policies on NSs and IPM regulatory guidelines are elaborate on paper but lack implementation (Grzywacz et al., 2014; Sola et al., 2014; Naranjo et al., 2015). This in part, is supported by the lack of regulation on importation, trade and use as well as residue monitoring of unregistered/highly toxic pesticides (Grzywacz et al., 2014; Machekano et al., 2020). This may be largely due to lack of funding (van Lenteren, 2012; Sola et al., 2014). Therefore, monetary policies should enact direct funding of implementation of the proven NSs packages. Increased national funding will enhance research and development on NSs and their derivatives (Grzywacz et al.,

2014). However, research and developments funds are still limited in SSA due to restrictions attached to donor funds (Amoabeng et al., 2014; Grzywacz et al., 2014).

Bureaucracy, inefficiency and prolonged government legislative processes are additional impediments to swift changes in NSs policies and legislative regulations (Belmain and Stevenson, 2001; van Lenteren, 2012; Grzywacz et al., 2014). In addition, difficulty in harmonising regional policies due to fundamental mismatches or disagreements inhibits efficient movement of NSs across borders for purposes of research, propagation, pilot tests or field applications (Grzywacz et al., 2014). Sub Saharan Africa member states may need to adopt a common approach in interpretation of the Acts governing NSs, e.g. harmonised data requirements in toxicological studies or adoption of standard application forms and dossier formats for importation of NSs (Cherry and Gwynn, 2007; Bale, 2011; Mason et al., 2017). In addition, establishment of a central panel of pan-SSA experts may need to be developed to overlook NSs submissions (Mason et al., 2017). If data requirements are harmonised, NSs approved in one country may be accepted in another without the drudgery and costs of repeated efficacy evaluations (Cherry and Gwynn, 2007; Mason et al., 2017).

Encouragingly, related policy structural guidelines are in place for some countries (van Lenteren, 2012). For example, the organic production of fruits and vegetables, e.g. in Kenya (Gwynn and Maniania, 2010; Grzywacz et al., 2014) has very strict existing legislative measures that may be utilised as pedestals (asset) for building new guidelines for policy adjustments for NSs research and development (Cherry and Gwynn, 2007; van Lenteren, 2012; Amoabeng et al., 2014; Sola et al., 2014). Existing policies on conservation of biodiversity and protection of endangered species may be fundamental springboards for policy adjustments for the promotion of NSs. In addition, Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) guidelines are freely available for adoption and modification where necessary (FAO, 2017). The public outcry against pesticide contaminated food and environment (see review Carvalho, 2017; Thompson and Darwish, 2019) may also be used as bottom-up pressure to justify policy modifications promoting NSs use. For example, SSA governments can use the substitution principle, by replacing the most toxic pesticides (e.g. WHO hazard classes I and II) with the promising NSs (Cannell, 2007; van Lenteren, 2012). Alternatively, governments can increase tax on the most toxic pesticides and use that revenue to provide subsidies and financial incentives to early adopters or pesticide companies

involved in the development of NSs (De Groote et al., 2001; Cannell, 2007; van Lenteren, 2012). As the development and uptake of efficacious NSs increase, this may also positively affect farmer behavior towards shifting from conventional pesticides to adoption of NSs.

Limited adoption in use of authorised pesticides of NSs is partly attributable to lack of education and information (Jussaume and Glenna, 2009; Grogan, 2014; Wyckhuys et al., 2019; Machekano et al., 2020). However, evidence exists that some farmers in SSA are traditionally using NSs without anecdotal efficacy and safety validations (De Groote et al., 2001; Belmain and Stevenson, 2001; Cannell, 2007; Jussaume and Glenna, 2009; Grzywacz et al., 2009; Amoabeng et al., 2014; Grogan, 2014; Grzywacz et al., 2014; Sola et al., 2014; Naranjo et al., 2015; FAO, 2017; Carvalho, 2017; Thompson and Darwish, 2019; Wyckhuys et al., 2019). Based on this, SSA governments and other stakeholders alike need to develop and consolidate NSs oriented educational programs to increase ecological literacy and reduce adoption barriers (Jussaume and Glenna, 2009; van Lenteren, 2012; Wyckhuys et al., 2019). It is therefore in the interest of SSA countries to modify policies enabling the development of simplified testing and validation protocols to legitimise existing NSs for improved use and expansion for the greater good.

2.8.2. Economic dynamics

The economic feasibility of NSs is ingrained in a functional biopesticide industry which in turn is rooted in SMEs and small-scale farmers or farmer groups (Leng et al., 2011; Amoabeng et al., 2014; Sola et al., 2014;). This is because large companies are sceptical about the return on investment in NSs due to uncertainties regarding the market size (adoption issues), consistent supply of raw materials, uncertain patent issues and less than absolute efficacy (Grzywacz et al., 2009; van Lenteren, 2012; Sola et al., 2014). SMEs in SSA are not well developed due to financial constraints against the backdrop of prohibitive costs of investment on research and development for NSs (Bale, 2011; Amoabeng et al., 2014; Sola et al., 2014). Consequently, instead of local production, most SSA agrocompanies survive on distributing internationally sourced products (Grzywacz et al., 2009; Sola et al., 2014), missing huge local investments opportunities. For example, *B. thuringiensis* (FlorBac WG®) and Spinosad, *S spinosa* (Spintordust®) distributed by SSA subsidiary agrochemical companies are produced outside the continent (Grzywacz et al., 2014; Sola et al., 2014). This partly contributes to their premium prices, likely aiding their economic non-viability for the majority of resource constrained farmers in SSA.

Estimation of the full economic value of NSs as a pest management tool is complex and depends on several factors including the product being protected, the enterprise (e.g. farm), community structure to societal costs and benefits as reviewed in Naranjo et al. (2015) (Benjamin and Wesseler, 2016). However, empirical evidence exists in support of financial sustainability for NSs versus conventional insecticides. For example, Amoabeng et al. (2014) showed that pesticidal plants were more cost effective than synthetic insecticide in the control of the pests *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage. Similarly, McConnachie et al. (2003) showed that the net present value of using a biocontrol agent *Stenopelmus rufinus*, Gyllenhal (Coleoptera: Curculionidae) against the red waterfern *Azolla filiculoides* was US\$1093 per hectare and US\$206 million for the entire South Africa (with benefit–cost ratio ranging from 2.5:1 to 15:1). While this study documented noble cost savings, Naranjo et al. (2015), argues that the economic benefits of NSs are even higher. For example, by factoring avoided costs, e.g. public health protection, environmental damage costs, other maximum incremental social tolerable irreversible costs (MISTICs) and maintenance of essential ecological services (Benjamin and Wesseler, 2016; TEEB, 2020). Factoring all these variables thus reflects NSs may be way cheaper both in the short (Amoabeng et al., 2014) and long term (Pimentel, 2009; van Lenteren, 2012; Benjamin and Wesseler, 2016) than conventional pesticides. These arguments reinforce the overall economic viability of NSs as a critical pillar for IPM (McConnachie et al., 2003; van Lenteren, 2012; Benjamin and Wesseler, 2016).

The market for NSs in SSA is underdeveloped, small and highly fragmented due to lack of investment, low ecological consciousness, and farmers' skewed behavioral perceptions towards pesticides preference (e.g. De Groote et al., 2001; Amoabeng et al., 2014; Grzywacz et al., 2014), relative to NSs (Rahaman et al., 2000; Lacey and Georgis, 2012). For example, in Ghana, only 14-25% farmer population are using NSs (Amoabeng et al., 2014). While studies show that this value may increase with increased education on NSs (Grzywacz et al., 2009; van Lenteren, 2012; Amoabeng et al., 2014; Grzywacz et al., 2014; Benjamin and Wesseler, 2016), limited funding and stringent regulatory process still remain significant bottlenecks (Grzywacz et al., 2009; Grzywacz et al., 2014; Benjamin and Wesseler, 2016). We thus suggest the use of a combination of enabling policy adjustments, direct funding and incentives for biocontrol practitioners to improve NSs adoption. Through incentivising SMEs and mobilising farmer or community groups, SSA governments can encourage NSs enterprises (Amoabeng et al., 2014). This system will build

a strong localised production and trading system that can work as an encouraging baseline for private sector investment for scaling up (Grzywacz et al., 2014; Naranjo et al., 2015). Previous studies suggest scaling up is the main pitfall for research and development initiatives on NSs in SSA (Grzywacz et al., 2009). Thus, this can be circumvented by managing the production and trading of NSs using economic product development pathways and commercial imperatives wrapped around government supported SMEs.

2.8.3. Ecological perspectives

The main ecological assets of NSs in SSA include existing freely available rich biodiversity, known trophic systems and established harvesting and rearing/propagation protocols (Belmain and Stevenson, 2001; Grzywacz et al., 2014; Sola et al., 2014). Most NSs are highly specific, e.g. EPNs (Rahaman et al., 2000), microbial pesticides and parasitoids (Lacey and Georgis, 2012; Knox et al., 2015). Thus, they have low impact on beneficial and non-target species (Copping and Menn, 2000; Knox et al., 2015; Carvalho, 2017; Barratt et al., 2018) and hence contribute to maintenance of environmental integrity and conservation of biological diversity (van Lenteren, 2012; Grogan, 2014). In addition, production of NSs is ecologically benign and has low carbon footprint (Copping and Menn, 2000). Plant based NSs e.g. natural enemies have the ability to sustainably self propagate and disperse in space through inoculative releases (Belmain and Stevenson, 2001; Sola et al., 2014). This makes NSs a cost-effective management tool. Exhaustive benefits and assets of various NSs for the developing countries have fully been documented (Pimentel and Goodman, 1978; Cherry and Gwynn, 2007; Knox et al., 2015; Naranjo et al., 2015; Carvalho, 2017).

The liabilities of some NSs e.g. predators and parasitoids (Table 2.1-2.4) include dependency on the host pest insect for survival (Rahaman et al., 2000; Lacey and Georgis, 2012; Tena et al., 2016). This therefore means NSs need the presence of the pest, mediated by density dependent factors and thus allowing some level of tolerable pest damage. Thus, use of preventive measures for pest management are not possible when using NSs. The action of NSs may also be slow when compared to synthetic insecticides, allowing some degree of pest damage on products. This may be undesirable for pest outbreaks that require rapid efficacy to bring populations below economic thresholds. Furthermore, this may also offset the quality of cosmetic products e.g. fruit and vegetable industry whose Economic Injury Levels (EILs) are low (Grzywacz et al., 2014). In addition, some NSs like parasitoids, EPNs and baculoviruses are highly sensitive to the biophysical

environment (Rahaman et al., 2000; Lacey and Georgis, 2012; Knox et al., 2015; Chidawanyika et al., 2019), and thus their efficacy may vary in space. This makes the deployment of NSs challenging in largely rainfed, dry cropping systems in SSA (Grzywacz et al., 2014) or under rapidly shifting climate environments. Similar environmental limitations for NSs have been made for biocontrol agents *Romanomermis culicivorax* (Lacey and Georgis, 2012), *Steinernema carpocapsae* (Bélair et al., 2003) and other biocontrol approaches (Muniappan and Bamba, 2000; Myers, 2000; Georgis et al., 2006; Chidawanyika et al., 2019).

Other ecological limitations of NSs include lack of sound and sustainable mass rearing and/propagation technologies that facilitate reliable products supply and reduce costs of importation while safeguarding ecosystems (Grzywacz et al., 2014; Sola et al., 2014). Use of botanicals as natural pesticides has been criticised owing to unsustainable harvesting of bioactive plant species (Belmain and Stevenson, 2001; Amoabeng et al., 2014; Grzywacz et al., 2014). In some cases, effective pesticidal plants that are not native to production areas e.g. in a push-pull system (Cook et al., 2007) may become introduced weeds, thus, increasing cost of production. Lack of expertise and experience in the phytochemistry of plant based NSs for extraction of active ingredients has also been a barrier to success. This expertise is needed for robust evidence-based safety screening to improve research and development processes.

2.8.4. Farmer perceptions and social dynamics

Natural substances are readily available for most small-scale resource poor farmers thus argued to be the most appropriate technology compatible with SSA (Sola et al., 2014). The biggest asset for NSs is its relatively low public health risk. Small scale farmers are largely poorly advised on the appropriate use and disposal of toxic synthetic pesticides (Ngowi et al., 2007; Williamson et al., 2008; Machekano et al., 2020). Compounded by the aggressive promotion of synthetic pesticides that overshadows NSs (Grzywacz et al., 2009; Grzywacz et al., 2014; Benjamin and Wesseler, 2016; Goldberger and Lehrer, 2016) this makes use of pesticides a significant public health threat for livelihoods in SSA. There is also limited infrastructure bridging researchers and farmers to facilitate knowledge transfer. As such, most research outputs on NSs remain as pilot projects or scholarly papers that are inaccessible to the small-scale farmers (Grzywacz et al., 2009; Grogan, 2014; Benjamin and Wesseler, 2016). Nevertheless, behavioral studies show that while SSA farmers are solely used to pesticides, they are willing to adopt NSs if proven efficacious (Ngowi

et al., 2007; Williamson et al., 2008; Obopile et al., 2008; Machekano et al., 2020). Players in research and development thus need to lobby for uptake of newly developed NSs technologies through engagement with relevant stakeholders.

Fluctuations in supply in NSs e.g. botanicals often reduce reliability of this tool for pest management (Lacey and Georgis, 2012). Furthermore, vulnerability to the biophysical environment often compromises efficacy (Lacey and Georgis, 2012; Naranjo et al., 2015), leading to poor product quality and low market values (Amoabeng et al., 2014). The application of some NSs also requires significant adjustments in current farming methods, labour and skills (Goldberger and Lehrer, 2016). For example, the use of parasitoids requires shift from monoculture to habitat management that encourages biodiversity conservation (Lacey and Georgis, 2012; Wyckhuys et al., 2019). Without change in perception/behavior, ecological literacy and financial incentives, farmers may not be socially ready for changes facilitating use of NSs. Farmers' beliefs, attitude and behavior are major factors influencing adoption of NSs. Creative persuasion of the non-conformers to NSs is needed to change their choice of pest management approaches (van Lenteren, 2012; Benjamin and Wesseler, 2016; Goldberger and Lehrer, 2016). Indeed, Goldberger et al. (2016) showed that out of the three farmer categories, the 'environmental stewards' and the 'networking farmers' likely partake NSs than those farmers solely looking to maximise production. Similarly, mobilising farmers into networking groups may increase NSs adoption (Jussaume and Glenna, 2009; Goldberger and Lehrer, 2016), e.g. establishing social/networking groups that can implement specific NSs may be a fruitful endeavour for SSA (De Groote et al., 2001; Jussaume and Glenna, 2009). These groups also facilitate ease of training and awareness campaigns on ecological literacy, economic feasibility and other assets of NSs.

In conclusion, the continental invasion of *T. absoluta* represents a significant biosecurity threat that affects the majority of livelihoods dependant on agricultural sustenance. A number of biological and physiological factors discussed here may in part, contribute to its current spread in African natural- and agro- ecosystems. Insecticide use, against *T. absoluta* has been the common default response to pest pressure. However, pesticide misuse affects the environment, public health, ecosystem services and often leads to pest resistance development. Thus, use of NSs, low-risk and biocontrol methods in an integrated approach may be the sustainable solution to *T. absoluta* problem in SSA. Few natural enemies have been documented for the first time in Botswana including the widely reported *N. formosa*. In order to maximize their effect, there is

need to adopt good conservation biological control practises. The NSs also have their own liabilities as a pest management tool in African systems argued from a legislative, economic, ecological, and social standpoint. Elucidating these factors is critical in facilitating research and development of NSs in Africa and their consequent adoption as a sustainable tool for pest management.

2.9. References

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CHAPTER 3

Thermal plasticity in the invasive South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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3.1. Introduction

Global climate change characterised by increased magnitude and frequency of extreme climatic conditions such as heat waves, cold snaps, severe droughts and floods poses a great threat to biodiversity (Bozinovic et al., 2013; IPCC, 2014; Colinet et al., 2015). Such changes to the biophysical environments may result in changes in the abundance and geographic distribution of invasive species (Dukes and Mooney, 1999; Walther et al., 2009; Hulme, 2017). Owing to this is the notion that invasive alien species are more eurythermal, or able to maintain physiological functionality across variable temperatures. This is in contrast to stenothermy in non-invasive or indigenous species, which tolerate a narrower thermal range, and thus experience compromised fitness under changing thermal environments (Boher et al., 2016). Furthermore, climate change may also alter the spatio-temporal availability of biotic resources, creating bottlenecks in survival of organisms (Huey and Kingsolver, 2019). Overcoming these environmental challenges is the first of several potential barriers determining whether a species may become established, naturalised and, ultimately, invasive (Richardson and Pysek, 2006; Nyamukondiwa et al., 2010; Mbande et al., 2019). Given that most insects are ectotherms and their body temperature closely matches environmental temperature, dramatic changes in climate and more specifically weather, may affect their survival (Gray, 2013).

Indeed, temperature is one of the key abiotic factors known to directly influence reproduction, development, activity, fitness, survival and spatio-temporal distribution in insects (Bowler and Terblanche, 2008; Klepsatel et al., 2019). Thus, the potential to tolerate environmental temperature stress is important for fitness, survival and adaptation under global change (Karl et al., 2014; Boher et al., 2016). For example, insects have been reported to employ short and long term morphological, behavioural, physiological and molecular mechanisms to withstand extreme environmental conditions (Lachenicht et al., 2010). Physiologically, insects compensate through genetic adaptation which involves allele frequency changes (Karl et al., 2014) as well as phenotypic plasticity, the ability of a single genotype to change morphological, biochemical and physiological characteristics of an organism under heterogeneous environments (Whitman and Ananthakrishnan, 2009; Sgrò et al., 2016; Griffith et al., 2019).

Phenotypic plasticity regarding thermal tolerance encompasses a suite of mechanisms such as rapid cold- and heat-hardening (RCH and RHH respectively) (in the short term) (Chidawanyika

and Terblanche, 2011a), acclimation (under managed laboratory conditions) (Mutamiswa et al., 2018a) or acclimatisation (in the long term under field conditions) (Sgrò et al., 2016). The underlying basis of these mechanisms is the pre-exposure to sub-lethal temperatures (Chidawanyika and Terblanche, 2011a; Chidawanyika et al., 2017) which may lead to molecular responses such as the expression of heat shock proteins that act as chaperones against protein denaturation, in the case of high temperature exposure (Hoffmann et al., 2003; Nyamukondiwa et al., 2010). For cold tolerance, exposure to sub-lethal temperatures has been associated with increased polyols and lipid content, which protects the insects against extracellular freezing (see review Overgaard and MacMillan, 2017).

Phenotypic plasticity can be adaptive and has been reported to improve survival in Lepidoptera (Stotter and Terblanche, 2009; Chidawanyika and Terblanche, 2011a; Fischer et al., 2010; Mutamiswa et al., 2018a), Diptera (Overgaard and Sørensen, 2008; Kalosaka et al., 2009; Nyamukondiwa et al., 2010), Coleoptera (Chidawanyika et al., 2017; Nyamukondiwa et al., 2018) and related taxon e.g. Collembola (see Chown et al., 2007; Sengupta et al., 2017). Studies have also shown that phenotypic plasticity varies across ontogeny (Marais and Chown, 2008) and that immobile stages generally have inherent higher plasticity to compensate for their inability to behaviorally adapt through seeking benign microhabitats. During insect development, different life stages respond differently to abiotic stress due to the variable microenvironments they experience during their development. This creates differential adaptations across ontogeny, including differences in behavioral compensation abilities (Mutamiswa et al., 2019).

Different hypotheses have been postulated to explain how phenotypic plasticity facilitates adaptation under changing environments. The beneficial acclimation hypothesis has received considerable interest among insect physiologists and ecologists (Klepsatel et al., 2019) although it is debatable (Woods and Harrison, 2002b). This hypothesis posits that acclimation to a particular environment confers performance and fitness advantage to an organism in that environment over another organism that has not been exposed to that particular environment (Leori et al., 1994; Wilson and Franklin, 2002; Woods and Harrison, 2002a). The ‘hotter-is-better’ hypothesis states that organisms thermally adapted to warmer environments may have a better performance and fitness advantage than those adapted to colder or benign environments (Klepsatel et al., 2019). Similarly, the optimal developmental temperature hypothesis postulates that organisms raised at

optimal temperature have greater survival advantage across many thermal environments (Woods and Harrison 2002b; Klepsatel et al., 2019). In particular, beneficial acclimation and thermal plasticity may play an important role in facilitating the success of invasive pests, although the generality of these effects are not known.

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) native to South America is one of the key global destructive insect pests of tomatoes causing yield losses of 80–100% (Desneux et al., 2010; Brévault et al., 2014). Following its invasion into North Africa around 2008, the past decade has been marked by its rapid spread and establishment in East, West and Southern Africa threatening food security and livelihoods (Desneux et al., 2010; Biondi et al., 2018; Mansour et al. 2018). The successful spread and establishment of *T. absoluta* is a result of a number of reasons. These include a wide host range (cultivated and wild) (Machekano et al., 2018), high fecundity (Uchôa-Fernandes et al., 1995; Tropea Garzia et al., 2012; Biondi et al., 2018), trade globalisation and increased human travel (Williamson, 1996; Pimentel et al., 2001; Simberloff and Rejmanek, 2001) and tolerance to diverse environmental conditions including temperature (Tropea Garzia et al., 2012; Ponti et al., 2015; Santana et al., 2019).

Projected thermal variability under climate change may have contrasting effects on species adaptation, compromising plastic responses in some species thereby limiting their potential to spread and establish, while enhancing such responses in other species (Sgrò et al., 2016). One of the major points to emerge from recent work on thermal tolerance is that there may be a link between susceptibility to climate change and the magnitude of phenotypic plasticity (e.g. Kellett et al., 2005; Balanya et al., 2006; Calosi et al., 2008; Nyamukondiwa et al., 2011; though see Gunderson and Stillman, 2015; Gunderson et al., 2017). Given the dramatic increase in the risk and invasion rate globally in the past decades, an understanding of plastic responses of invasive species to thermal stress is of fundamental importance in predicting climate change effects on their population dynamics, spatio-temporal distribution (Pimentel et al., 2001; Hance et al., 2007; Ma et al., 2014) and potential establishment in new environments (Nyamukondiwa et al., 2010; Buckley and Huey, 2016).

While several reports have documented significant ecological effects of RCH (Stotter and Terblanche, 2009; Basson et al., 2011; Mutamiswa et al., 2018b), RHH (Chidawanyika and

Terblanche, 2011a; 2011b; Mutamiswa et al., 2018b) and acclimation (Lagerspetz, 2006; Fischer et al., 2010; Chidawanyika and Terblanche, 2011a; Sgrò et al., 2016), little is known on phenotypic plasticity of *T. absoluta* and its thermal tolerance (Han et al. 2018). Previous studies on *T. absoluta* have only focused on thermal requirements for development (Krechemer and Foerster, 2015; but see Van Damme et al., 2015). To the best of our knowledge, no study has been done to date with focus on its plastic responses to temperature, and likely contributory effects on invasion success. In addition, studies on acclimation responses to temperature treatment, a major mechanism used by insects to cope with temperature variation both in medium-term timescales (Overgaard and Sørensen, 2008; Nyamukondiwa and Terblanche, 2010; Nyamukondiwa et al., 2010) and over the longer term (e.g. seasonal timescales, Nyamukondiwa et al., 2013) also remain underexplored. In the present study, we investigated the thermal tolerance of *T. absoluta* larvae (4th instar) and adults (24–48 h old) through determining their short- (RCH and RHH) and long-term (acclimation) plastic responses of fitness and survival. I hypothesize that *T. absoluta* is thermally plastic at variable thermal regimes, i.e. under short and long-term acclimatory conditions. Understanding such factors associated with successful invasion provides a basis for a predictive framework important for mitigating the introduction, establishment or spread of potentially invasive pest species, and informing suitable control measures (e.g. when and where to focus efforts).

3.2. Materials and Methods

3.2.1. Insect culture

Wild populations of *T. absoluta* larvae were collected from infested tomatoes plants at Genesis Farm (S21°8'54.239"; E027°38'48.42"), Matshelegabedi village in North East District of Botswana. All specimens were collected in austral summer (December-February), so that all test organisms have the same environmental history. Larvae were reared in the laboratory on organically produced and pesticide-free excised fresh tomato (“Rodade”) leaves inside Bugdorm rearing cages (240cm³; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under optimum conditions, i.e. 28°C; 65±10% relative humidity (RH) (Guimapi et al., 2016) and 12L:12D photocycle in climate chambers (HPP 260; Memmert GmbH + Co.KG, Germany) until adult emergence (for adult experiments). For all the experiments, 4th instar wild larvae (field collected) and 24–48h old adults (eclosed from field collected larvae) were used.

3.2.2. Low temperature plasticity

Tuta absoluta hardening experiments were performed using established experimental protocols for Diptera and Lepidoptera (e.g. Nyamukondiwa et al., 2010; Mutamiswa et al., 2018b). To assess the effects of RCH and RHH on *T. absoluta* physiological traits, hardening temperatures for the two developmental treatment stages (larvae and adults) were established through preliminary assays. Cold hardening temperature for larvae was derived from critical thermal minima (CT_{min}) (Hoffmann et al., 2003; Lee and Denlinger, 2010) and was defined as 6°C below CT_{min} (Mutamiswa et al., 2018b) (Table 3.1). For adults, low hardening temperature was defined as 10°C above the lower discriminating temperature, consistent with other studies (see Nyamukondiwa et al., 2010) (Table 3.1). Lower discriminating temperature (-10°C), defined as the temperature that causes 80–100% mortality upon 2 h exposure to a stressful low temperature was derived from Machekano et al. (2018). These hardening temperatures suffice to elicit RCH responses in similar organisms under laboratory experimental conditions (Hoffmann et al., 2003; Lee and Denlinger, 2010). Larval and adult *T. absoluta* specimens were first placed in a climate chamber at 28°C and 65±10% RH for 30 min to allow for equilibration. Thereafter, the insects were placed in a zip lock bag and submerged in a programmed water bath (Systronix Scientific, Industria, South Africa) with 1:1 water: propylene glycol and hardened for 2 h at low hardening temperature [-2°C (larvae) and 1°C (adults)]. Following treatment, insects were allowed to recover under benign conditions (28°C; 65±10% RH) in a climate chamber for 30 min before measuring physiological traits. Control insects were kept at benign conditions during the same treatment before measuring thermal fitness traits.

As for RHH effects, hardening high temperature for larvae was derived from critical thermal maxima (CT_{max}) (Hoffmann et al., 2003; Lee and Denlinger, 2010) and defined as 7°C below CT_{max} , (see e.g. Mutamiswa et al., 2018b). For the adults, hardening high temperature was defined as 5°C below the discriminating high temperature (42°C), derived from Machekano et al. (2018). This hardening temperature is generally adequate to elicit an RHH response in similar insects (Hoffmann et al., 2003). Organisms were first temperature equilibrated in a climate chamber at 28°C and 65±10% RH for 30 min before being hardened for 2 h at high temperature (see Table 3.1). Following hardening, organisms were given 30 min recovery time to allow the *de novo* synthesis of heat shock proteins (HSPs) (Nyamukondiwa et al., 2010; Mutamiswa et al., 2018a)

before measuring physiological traits. Control organisms were maintained at optimum conditions (28°C; 65% RH) before measuring physiological traits. Traits measured include critical thermal minima (CT_{min}), chill coma recovery time (CCRT) and supercooling points (SCPs).

Table 3.1. Summary table showing hardening temperatures and duration for *T. absoluta* larvae and adults.

Developmental stage	Hardening high temperature (°C)	Hardening low temperature (°C)	Control temperature(°C)
Larvae	42 (2h)	-2 (2h)	28
Adults	37 (2h)	1 (2h)	28

Long term acclimation effects on low thermal tolerance were tested using standardised protocols (see Nyamukondiwa and Terblanche, 2010; Mutamiswa et al. 2018b). Acclimation temperatures were defined as 5°C above and below the optimal temperature (28°C), for low (23°C) and high (33°C) temperature pre-conditioning respectively. The control organisms were acclimated to an optimum temperature of 28°C and 65% RH. Following three-day acclimation, physiological traits were measured. Such temperature duration is also sufficient to elicit acclimation responses in similar insect taxa (Nyamukondiwa and Terblanche, 2010; Weldon et al., 2011; Mutamiswa et al. 2018a).

3.2.2.1. Critical thermal minima (CT_{min})

Critical thermal minima were measured on *T. absoluta* larvae and adults using standardized protocols as outlined by Nyamukondiwa and Terblanche (2009). Ten replicate organisms were individually placed in a series of numbered borosilicate glass tubes ('organ pipes') connected to an insulated double-jacketed chamber which was linked to a programmable water bath before decreasing the temperature at a rate of 0.25°C/min until their CT_{min} were recorded. This was repeated twice to yield sample sizes of $n = 20$ per treatment. CT_{min} was defined as the temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

3.2.2.2. Chill coma recovery time (CCRT)

Chill coma recovery time experiments were conducted following established protocols (e.g. Weldon et al., 2011; Nyamukondiwa et al., 2018). Ten organisms from each temperature treatment

i.e. larvae and adults were individually placed in 2ml Eppendorf tubes and then loaded into a zip-lock bag which was then submerged in a water bath (1:1 water: propylene glycol) set at 0°C (larvae) and -2 °C (adults) for 1 h. After 1 h under chill coma temperature, the vials were removed from the water bath and placed in a climate chamber set at 28°C, 65±10% RH for recovery. The chamber was connected to a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20; Hikvision Digital Technology Co. Ltd, Hangzhou, China) which was linked to a computer for recording observations. This was repeated twice to yield a sample size of $n = 20$. Chill coma recovery time was defined as the time (in min) required for the insects to regain consciousness, e.g. movement for larvae and standing upright on legs (for adults) following recovery from chill-coma.

3.2.2.3. Supercooling points (SCPs)

Supercooling points were assayed as outlined by Nyamukondiwa et al. (2013). Twenty organisms from each developmental stage (larvae and adults) and temperature treatment were individually placed into 2 ml Eppendorf tubes. These organisms were ‘fasted’ for 24 hours to clear gut contents and circumvent confounding effects the food gut particles may have on SCPs. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple, inserted through the gauzed lid of the vial and both the insect and thermocouple were secured in contact by a cotton wool. Thermocouples were connected to one of two 8-channel Picotech TC-08 thermocouple interfaces and temperatures were recorded at 1s intervals using PicoLog software for Windows. In all treatments the insects were first exposed to a temperature of 15°C for 10 min before decreasing the temperature at a rate of 0.5°C/ min until SCPs were recorded. SCP for each organism was determined as the lowest temperature recorded before a spike in temperature due to latent heat of crystallization (Nyamukondiwa et al., 2013).

3.2.3. High temperature plasticity

Hardening and acclimation experiments were conducted using standardised protocols, consistent with low temperature plasticity experiments (see section 3.2.2) (e.g. Nyamukondiwa et al., 2010; Nyamukondiwa and Terblanche, 2010; Mutamiswa et al., 2018b). Physiological traits were measured following hardening (RCH and RHH) and three-day acclimation at low (23°C), optimum (28°C) and high (33°C) temperature for both larvae and adults. Control organisms were also

maintained at optimum temperature (28°C; 65% RH) before measuring physiological traits. Fitness traits measured following acclimation treatments include CT_{max} and HKDT.

3.2.3.1. Critical thermal maxima (CT_{max})

Critical thermal maxima were measured using the same protocol as that for CT_{min} except that temperature was ramped up at 0.25°C/min until the endpoint. Critical thermal maximum was defined as the temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

3.2.3.2. Heat knockdown time (HKDT)

Heat knockdown experiments were assayed as outlined by Mutamiswa et al. (2018a). Ten replicate individuals in both larva and adult stage were individually placed in 0.65ml microcentrifuge tubes and placed in a climate chamber connected to a camera linked to a computer. The tubes carrying the insects were then exposed to an acute knockdown temperature (50°C) in the climate chamber. This knockdown temperature was selected basing on preliminary investigations of CT_{max} for larvae and adults ranging $49 \pm 0.13^\circ\text{C}$ and $48 \pm 0.2^\circ\text{C}$ respectively. This was repeated twice to yield sample sizes of $n = 20$. All observations were made from the climate chamber video recording system. Heat knockdown time was defined as the time (in minutes) at which insects lost activity following exposure to knockdown temperature in the climate chamber.

3.2.4. Statistical Analyses

Data analyses were done using STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0 (R Development Core Team, 2019). Critical thermal limits (CT_{max} , CT_{min}), SCP, CCRT and HKDT data were first checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests and met assumptions of ANOVA in all traits except for HKDT. Therefore CT_{max} , CT_{min} , SCP and CCRT results were analysed using factorial ANOVA in STATISTICA. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups. Heat knockdown time results were analysed using generalized linear models (GLZ) assuming a Gaussian distribution and an identity link function in R. Kruskal–Wallis *post hoc* tests were used to separate statistically homogeneous groups for HKDT data.

3.3. Results

3.3.1. Low temperature plasticity

3.3.1.1. Critical thermal minima

Hardening (RCH and RHH) significantly affected CT_{min} in both larvae and adults, but the direction of effect differed between the life stages (treatment x life stage interaction, $P < 0.001$, Table 3.2). Rapid cold hardening significantly improved CT_{min} in larvae while impairing it in adults (Fig. 3.1A). However, RHH significantly compromised CT_{min} in adults (Fig 3.1A), and marginally improved in larvae. Acclimation also significantly influenced cold tolerance (CT_{min}) ($P < 0.001$) such that low temperature acclimation (23°C) significantly improved CT_{min} in larvae while it had no effects on adults. Similarly, high temperature acclimation (33°C) had no significant effects for CT_{min} for both larval and adult *T. absoluta* (Table 3.2; Fig. 3.1B). As such, life stage × acclimation interaction effect was not significant ($P > 0.05$) (Table 3.2).

Table 3.2. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42°C; -2°C) and adults (37°C; 1°C) and three-day acclimation (23°C; 28°C and 33°C) on CT_{min} . SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	p
CT_{min}	<i>Hardening</i>					
	Intercept	191.77	1	191.77	345.80	< 0.001
	Life stage	117.81	1	117.81	212.43	< 0.001
	Treatment	177.68	2	88.84	160.19	< 0.001
	Life stage×Treatment	209.38	2	104.69	188.78	< 0.001
	Error	63.22	114	0.55		
	<i>Acclimation</i>					
	Intercept	34.03	1	34.03	68.47	< 0.001
	Life stage	503.07	1	503.07	1012.24	< 0.001
	Acclimation	8.66	2	4.33	8.72	< 0.001
	Life stage×Acclimation	1.03	2	0.52	1.04	0.36
	Error	56.66	114	0.5		

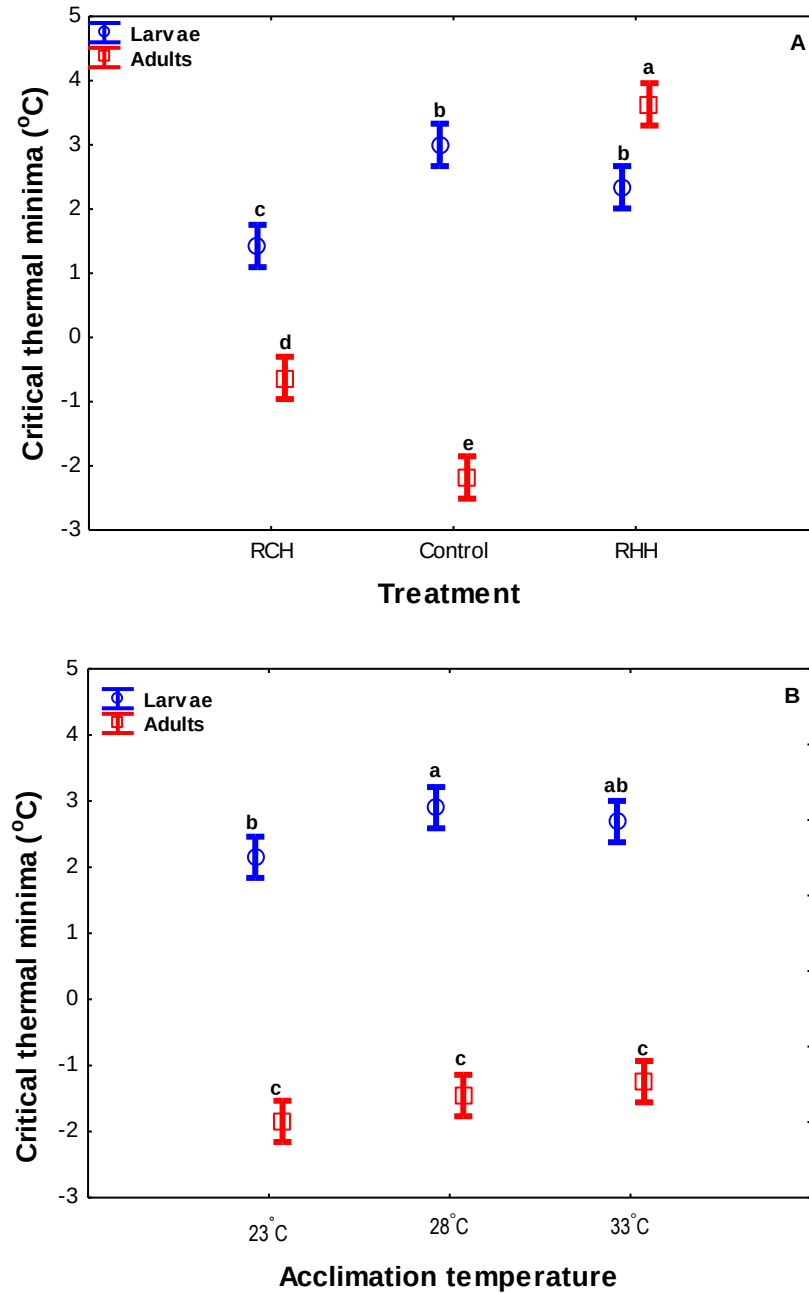


Figure 3.1. Effects of short-term acclimation- (A) 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42°C for RHH; -2°C for RCH) and adults (37°C for RHH; 1°C for RCH) and long-term acclimation (B)- three-day acclimation (23°C; 28°C and 33°C) on critical thermal minima (CT_{min}) Error bars represent 95% confidence limits (CLs) (n=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.

3.3.1.2. Chill coma recovery time

Hardening significantly influenced CCRT in larvae and adults ($P < 0.001$) (Table 3.3; Fig. 3.2A). Rapid heat hardening significantly compromised CCRT in adults, and marginally so in larvae, albeit the latter was non-significant (Fig. 3.2A). There were no significant differences in CCRT between larvae and adults following RCH, between the controls and RHH larval treatment ($P > 0.05$) (Fig. 3.2A). Nevertheless, larval CCRT did not significantly differ from the controls. There was a higher magnitude of difference in cold tolerance (CCRT) between larvae and adults following RHH (14.63 min) than RCH (0.23 min) (Fig. 3.2A). In addition, life stage $\times$ treatment interaction effect was significant ($P < 0.001$) (Table 3.3). Acclimation also significantly affected CCRT of *T. absoluta* larvae and adults ($P < 0.001$) (Table 3.3; Fig. 3.2B). Low temperature acclimation (23°C) marginally improved CCRT in larvae and adults (recovered faster from chill coma) whereas high temperature acclimation (33°C) compromised it (took more time to recover) in both developmental stages (Fig. 3.2B). Nevertheless, life stage $\times$ acclimation interaction effect was not significant ($P > 0.05$) (Table 3.3).

Table 3.3. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42°C; -2°C) and adults (37°C; 1°C) and three-day acclimation (23°C; 28°C and 33°C) on CCRT. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	Df	MS	F	p
CCRT	<i>Hardening</i>					
	Intercept	3714.64	1	3714.64	88.82	< 0.001
	Life stage	668.59	1	668.59	15.99	< 0.001
	Treatment	2037.07	2	1018.54	24.35	< 0.001
	Life stage $\times$ Treatment	1473.12	2	736.56	17.61	< 0.001
	Error	4767.79	114	41.82		
	<i>Acclimation</i>					
	Intercept	931.3969	1	931.4	932.89	< 0.001
	Life stage	0.8031	1	0.80	0.80	0.37
	Acclimation	44.4514	2	22.22	22.26	< 0.001
	Life stage $\times$ Acclimation	1.3589	2	0.68	0.68	0.51
	Error	113.82	114	0.99		

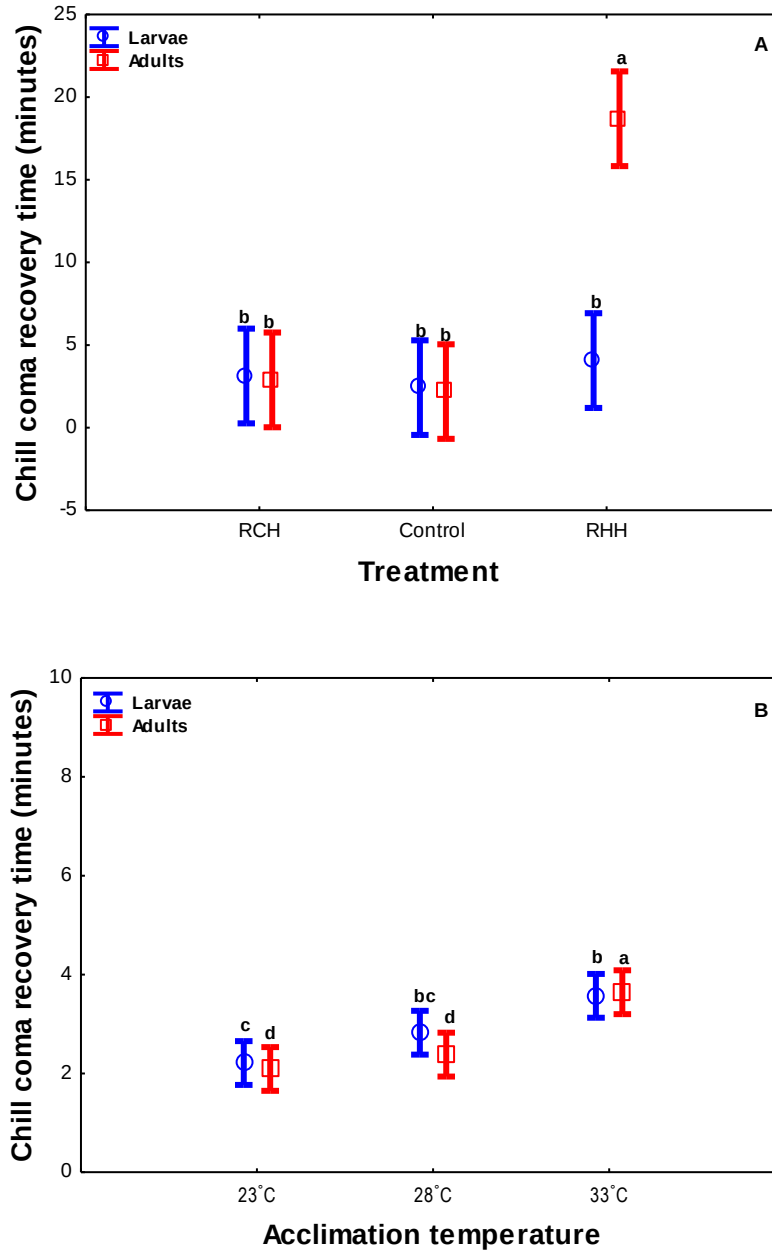


Figure 3.2. Effects of short-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42°C for RHH; -2°C for RCH) and adults (37°C for RHH; 1°C for RCH) and long-term acclimation (B)- three-day acclimation (23 °C; 28 °C and 33 °C) on chill coma recovery time (CCRT). Error bars represent 95% confidence limits (CLs) (n=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.

3.3.1.3. Supercooling points

Supercooling points for larvae and adults were significantly affected by hardening ($P < 0.001$; Fig. 3.3A). For larval *T. absoluta*, both RCH and RHH significantly impaired supercooling ability (more positive SCPs), while for adults, both hardening treatments had no significant effects (Table 3.4; Fig. 3.3A). There was a significant life stage $\times$ treatment interaction effect ($P < 0.001$) following hardening (Table 3.4). There were no significant acclimation effects on larval and adult SCPs (Table 3.4; Fig. 3.3B). For all developmental stages, there was no significant difference between the controls and that of the two treatments (23 and 33°C) (Fig. 3.3B). However, there was developmental stage related difference in SCPs following acclimation to high temperature (33°C), with larvae having more negative SCPs than the adults (Fig. 3.3B). In addition, life stage $\times$ acclimation interaction effect was significant ($P < 0.05$) (Table 3.4).

Table 3.4. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42°C; -2°C) and adults (37°C; 1°C) and three-day acclimation (23°C; 28°C and 33°C) on SCPs. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	<i>p</i>
SCP	<i>Hardening</i>					
	Intercept	35942.14	1	35942.14	50592.06	< 0.001
	Life stage	184.09	1	184.09	259.13	< 0.001
	Treatment	10.62	2	5.31	7.48	< 0.001
	Life stage $\times$ Treatment	10.45	2	5.22	7.35	< 0.001
	Error	80.99	114	0.71		
	<i>Acclimation</i>					
	Intercept	37655.36	1	37655.36	3564.70	< 0.001
	Life stage	139.04	1	139.04	13.16	< 0.001
	Acclimation	13.75	2	6.87	0.65	0.52
	Life stage $\times$ Acclimation	73.76	2	36.88	3.49	0.03
	Error	1204.23	114	10.56		

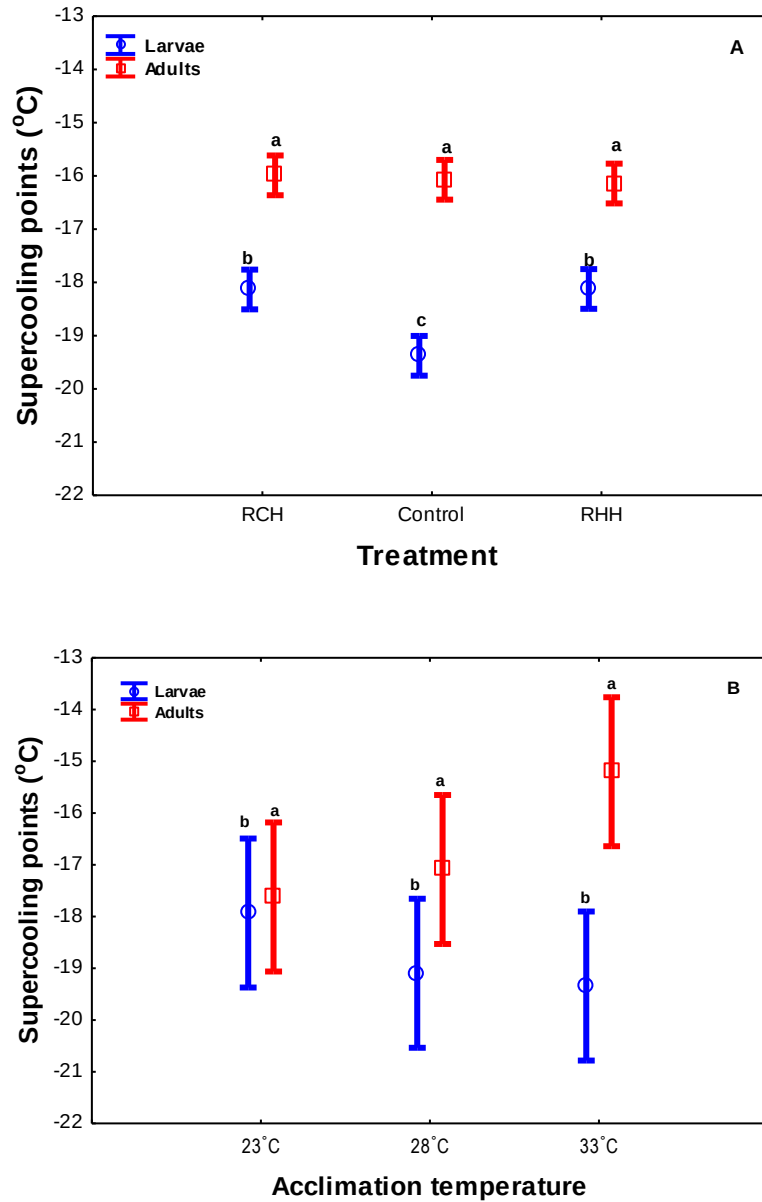


Figure 3.3. Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42°C for RHH; -2°C for RCH) and adults (37°C for RHH; 1°C for RCH) and long-term acclimation (B)- three-day acclimation (23°C; 28°C and 33°C) on supercooling points (SCPs). Error bars represent 95% confidence limits (CLs) (n=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.

3.3.2. High temperature plasticity

3.3.2.1. Critical thermal maxima

Heat tolerance (CT_{max}) in larvae and adults was significantly affected by hardening ($P < 0.001$) (Table 3.5; Fig. 3.4A). Hardening significantly reduced CT_{max} in adults, and more significantly so for the RHH treatment (Fig. 3.4A). However, there was no significant difference in CT_{max} for larvae following both RCH and RHH (Fig. 3.4A). Similarly, life stage $\times$ treatment interaction effect was significant ($P < 0.001$, Table 3.5). In addition, acclimation effects were not significant for larvae and adults CT_{max} (Table 3.5; Fig 3.4B). For both developmental stages, low (23°C) and high (33°C) temperature treatments did not significantly affect CT_{max} from the control level (Fig. 3.4B). Nevertheless, it appeared larvae had higher CT_{max} than adults across all the treatments (Fig. 3.4B). The interaction between life stage and acclimation was also not significant (Table 3.5).

Table 3.5. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42°C; -2°C) and adults (37°C; 1°C) and three-day acclimation (23°C; 28°C and 33°C) on CT_{max} . SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	Df	MS	F	p
CT_{max}	<i>Hardening</i>					
	Intercept	273435.6	1	273435.6	352409.3	< 0.001
	Life stage	168	1	168	216.6	< 0.001
	Treatment	33.9	2	17	21.9	< 0.001
	Life stage $\times$ Treatment	35.5	2	17.7	22.9	< 0.001
	Error	88.5	114	0.8		
	<i>Acclimation</i>					
	Intercept	274075.7	1	274075.7	204793.5	< 0.001
	Life stage	104.3	1	104.3	78	< 0.001
	Acclimation	3.2	2	1.6	1.2	0.31
	Life stage $\times$ Acclimation	1.6	2	0.8	0.6	0.56
	Error	152.6	114	1.3		

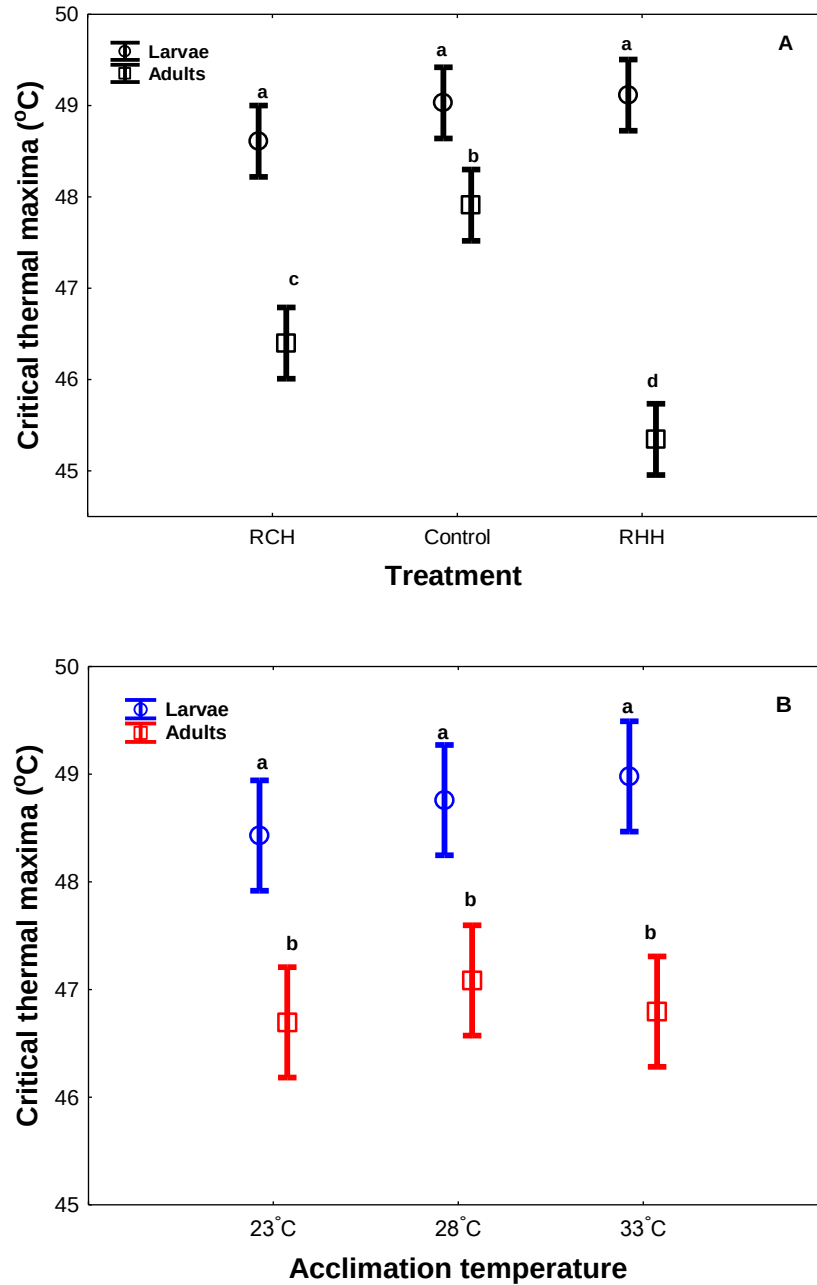


Figure 3.4. Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42°C for RHH; -2°C for RCH) and adults (37°C for RHH; 1°C for RCH) and long-term acclimation (B)- three-day acclimation (23°C; 28°C and 33°C) on critical thermal maxima (CT_{max}) Error bars represent 95% confidence limits (CLs) (n=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.

3.3.2.2. Heat knockdown time

As in CT_{max} , hardening significantly affected HKDT of larvae and adults ($P < 0.001$) (Table 3.6; Fig 3.5A). Rapid cold hardening significantly compromised HKDT for larvae, while it showed no significant effects for the adults (Fig. 3.5A). Similarly, no significant differences in HKDT were noted in adults following both hardening treatments, and between controls and RHH treatments for larvae (Fig. 3.5A). In addition, there was a significant life stage $\times$ treatment interaction effect ($P < 0.001$) (Table 3.6). Generally, larvae had higher HKDT (took longer to be knocked down following acute heat stress) than adults across all treatments (Fig. 3.5A). Acclimation also significantly influenced larvae and adults HKDT ($P < 0.001$) (Table 3.6; Fig 3.5B). Low temperature acclimation (23°C) significantly improved HKDT in both larvae and adults whereas high temperature acclimation (33°C) impaired it in both life stages (Fig. 3.5B). Similarly, there was a significant life stage $\times$ acclimation interaction effect (Table 3.6). Consistent with other traits, it also appeared larvae had significantly higher heat tolerance (higher HKDT) across all treatments (Fig. 3.5B).

Table 3.6. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42°C; -2°C) and adults (37°C; 1°C) and three-day acclimation (23°C; 28°C and 33°C) on HKDT. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	<i>p</i>
HKDT	<i>Hardening</i>					
	Treatment	380	2	190	11.44	< 0.001
	Life stage	7635	1	7635	459.94	< 0.001
	Treatment $\times$ Life stage	523	2	261	15.75	< 0.001
	Residuals	1892	114	17		
	<i>Acclimation</i>					
	Acclimation	3775	2	1888	28.1	< 0.001
	Life stage	5754	1	5754	85.67	< 0.001
	Acclimation $\times$ Life stage	2268	2	1134	16.88	< 0.001
	Residuals	7657	114	67		

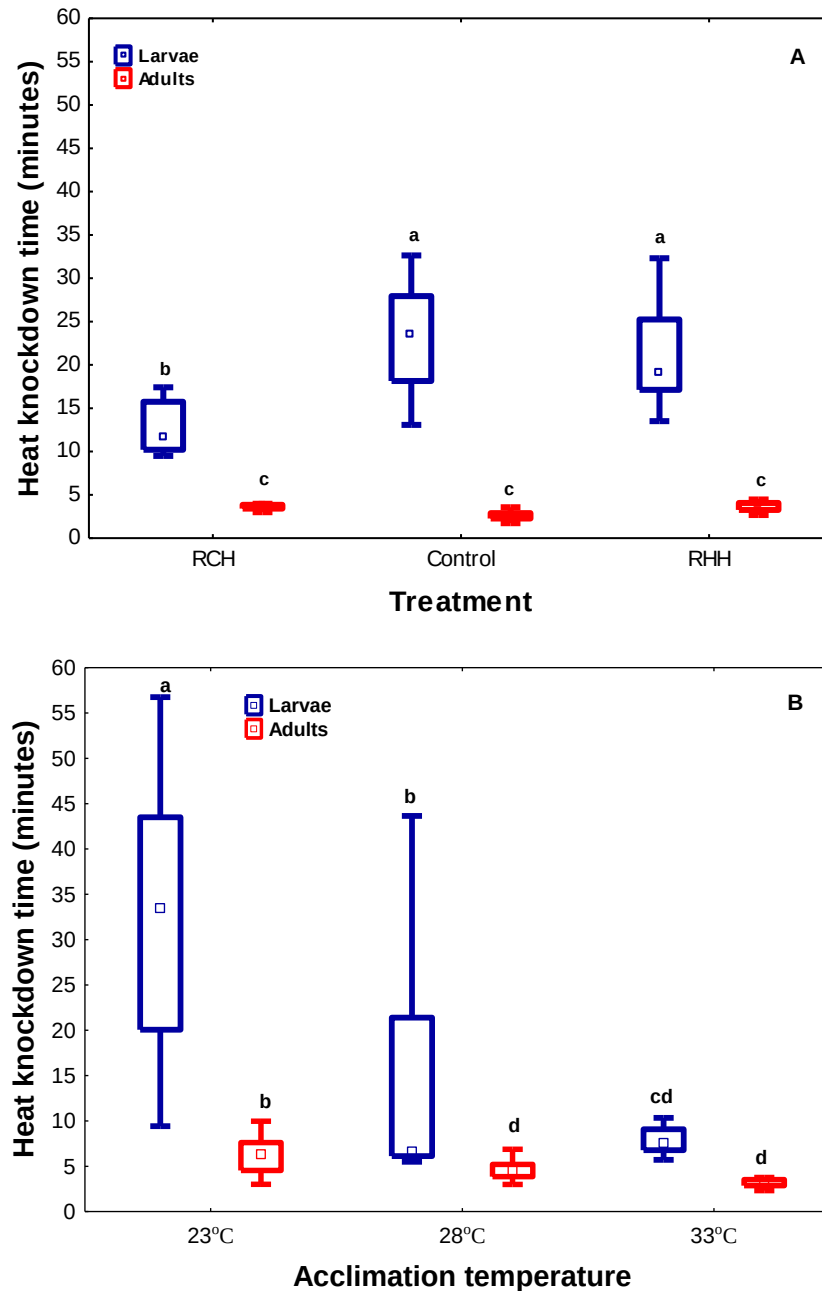


Figure 3.5. Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42°C for RHH; -2°C for RCH) and adults (37°C for RHH; 1°C for RCH) and long-term acclimation (B)- three-day acclimation (23°C; 28°C and 33°C) on heat knockdown time (HKDT). Error bars represent 95% confidence limits (CLs) (n=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively. The box covers the interquartile interval, where 50% of the data was found.

3.4. Discussion

The success of invasive species may stem from their ability to withstand stressful ambient novel environments. As such, basal and phenotypic plasticity traits play a pivotal role in invasive species' successful spread and establishment in new environments. Indeed, fitness and survival may be dependent on adjusting to novel environmental conditions through physiological acclimatization/acclimation and genetic adaptation (Webster et al., 2017; Castañeda et al., 2019; Griffith et al., 2019). It is increasingly being documented that invasive species may particularly have flexible life history traits (Agosta et al., 2018), and this potentially explains their success under heterogeneous environments.

The present study showed that rapid hardening and acclimation affects plasticity of thermal tolerance in *T. absoluta*, albeit asymmetrically for larvae and adults. Larvae appeared to be more thermally plastic than adults, suggesting that *T. absoluta* invasion propagules may be carried in the form of larvae under thermally extreme environments. Hence, this enhanced larval plasticity coupled with the limited one in adults may aid each other to enhance invasion success (Nyamukondiwa et al., 2010).

It appeared there was higher plasticity for low temperature compared to high temperature following both short- and long-term hardening. Changes in thermal limits as a result of acclimation are thought to be more pronounced at the lower- than upper -thermal thresholds (Klok and Chown, 2003; Weldon et al., 2011; Hoffmann et al., 2013). This is in agreement with our current results where both RCH and low temperature acclimation had a positive effect on CT_{min} of *T. absoluta* larvae. Coello Alvarado et al. (2015) reported improved CT_{min} in *Gryllus pennsylvanicus* adults following long- and -short term acclimation. Similar results have also been reported for *C. partellus* larvae, adult parasitoid, *Cotesia flavipes* (Mutamiswa et al. (2018a) and *Busseola fusca* larvae (Mutamiswa et al., 2018b).

In terms of low temperature tolerance (CCRT and CT_{min}), we found that adults had higher basal tolerance than larvae, corroborating a recent study by Machekano et al. (2018) who reported superior basal cold tolerance in adults. Interestingly, larvae showed strikingly higher plasticity of low temperature tolerance than adults. Insects often trade off basal low temperature tolerance for phenotypic plasticity (Nyamukondiwa et al., 2011), consistent with the current results which

showed compromised basal low temperature tolerance and improved plasticity of cold tolerance (CCRT and CT_{min}) for larvae. In addition, this also affirms the notion that insects with high basal thermal tolerance may be constrained in phenotypic plasticity of thermal traits (Stillman, 2003; Coello Alvarado et al., 2015). Studies have also shown that less or non-mobile life-stages are generally considered to have evolved inherent high phenotypic plasticity to abiotic stress, to compensate for limited ability of behavioural compensation (Vernon and Vannier, 1996; Klok and Chown, 2001; Jensen et al., 2007; Marais et al., 2009; Mutamiswa et al., 2019).

Although Coello Alvarado et al. (2015) reported improved CCRT in *G. pennsylvanicus* following cold acclimation and RCH, our results report no effects for both *T. absoluta* developmental stages for both RCH and low temperature acclimation. The reason for this absence is unknown but may, to a lesser extent be explained by the developmental stages we tested, and choice of acclimation treatments (see discussions in Mutamiswa et al., 2019). Nevertheless, this result indicates that plasticity of thermal tolerance in insects may be species or trait dependent (Nyamukondiwa et al., 2018). It also supports the notion that differences in responses to hardening and acclimation depend on duration of acclimation exposure hence this may consequently determine the direction of induced plasticity and any associated costs and/or benefits (Bowler and Terblanche, 2008). In addition, RHH and high temperature acclimation (33°C) generally impaired CT_{min} (more positive) in both developmental stages. This result is in keeping with Mutamiswa et al. (2018a; 2018b) who reported compromised cold tolerance (CT_{min}) in larvae and adults of *C. partellus* and *B. fusca* larvae following high temperature acclimation and RHH respectively. Nevertheless, impaired CCRT in adults following high temperature acclimation corroborates a previous study on *C. flavipes* adults (Mutamiswa et al., 2018a). A comparison of larvae and adults showed that the former recovered faster than latter from chill coma following RHH. This likely means that mechanisms associated with tolerance to low and high temperature for *T. absoluta* are decoupled (Hoffmann et al., 2003; Nyamukondiwa et al., 2011). Rapid cold hardening has also been shown to improve thermal fitness in other insect species e.g. *Drosophila melanogaster* (Overgaard and Sørensen, 2008); *Ceratitis rosa* (Nyamukondiwa et al., 2010) and *Cydia pomonella* (Chidawanyika and Terblanche, 2011a).

The thermal physiology of any organism is related to the range of environmental temperature where it evolved (Bryant et al., 2002; Hoffmann et al., 2003; Weldon et al., 2011; Ismail and

Brookes, 2016; Griffith et al., 2019). *Tuta absoluta* larvae dwell in tomato leaves and feed by mining the leaf mesophyll (Biondi et al., 2018), where it is somewhat buffered from environmental heterogeneity. Thus, the higher larval plasticity to low temperature reported here may be an evolutionary adaptation to cope with changing ambient environments. Faced with stressful environments, adults on the other hand, can adjust their behaviour through flight for instance (Bowler and Terblanche, 2008). This may help explain the limited plasticity reported here for the adults.

Both short- and long-term acclimation did not have any significant effects on SCPs in adults and larvae of *T. absoluta*. The results are in keeping with Mutamiswa et al. (2018a) who reported compromised SCPs for *C. partellus* pupae and adults following low and high temperature acclimation. Interestingly, a comparison of larvae and adults showed an enhanced SCPs (more negative temperature) for the larvae relative to adults across all treatments, suggesting higher cold hardiness for the larvae. Boardman et al., 2012 reported that 24 h fasting removes all gut contents in *Thaumatotibia leucotreta* larvae resulting in a decrease in SCPs. While clearance of gut contents was not tested after 24 h treatment in this study, we hypothesise this may likely not have affected our SCPs results here. Some studies have reported that *T. absoluta* may struggle to survive in low temperatures (e.g. Cuthbertson et al., 2013; Biondi et al., 2018), and that it may not be able to overwinter under sub-zero environmental conditions. Nevertheless, few empirical studies have tested its cold hardiness under laboratory or field conditions (but see van Damme et al., 2015; Han et al., 2018). This therefore calls for imminent studies looking at *T. absoluta* cold hardiness and overwintering physiology, including ontogenic freeze strategy to help explain how this may affect its current and future geographic distribution as well as population dynamics with a view to developing sustainable pest management programs (Tonnang et al., 2015; Santana et al., 2019).

Our study demonstrates effects of short- and long-term acclimation on *T. absoluta* high temperature tolerance. While RHH, RCH, low and high temperature acclimation did not have a significant effect on *T. absoluta* larvae, it appeared to come at a significant cost for adults (see Fig. 3.4A). Our results are in keeping with Mutamiswa et al. (2018b) who reported impaired heat tolerance (CT_{max}) in *B. fusca* and *Sesamia calamistis* larvae following RCH as well as low and high temperature acclimation. Some insects have been reported to trade off plasticity for other

physiological or fitness traits (Liefting and Ellers, 2008; Angilletta, 2009; Basson et al., 2011; Murren et al., 2015).

Thermal conditioning to a single environmental stress often has deleterious consequences upon exposure to a different or same stress (reduced fitness). This may partially be accounted for by the cumulative effects of the stress combinations (see discussions in Gotcha et al., 2017). Fitness maladaptations owing to cumulative abiotic stresses reported here somewhat vary and depend on methodology employed e.g. short versus long term acclimation and the magnitude of stress severity (see Mittler, 2006). Similarly, RCH also significantly reduced HKDT, suggesting similar heat tolerance costs of short-term low temperature acclimation (Fig. 3.5A). Conversely, acclimation to low temperature improved HKDT for both adult and larval *T. absoluta* larvae suggesting significant cross-tolerance effects. Phenotypic plasticity to one stress factor often confers fitness advantages to a different stressor, a phenomenon called ‘cross-tolerance’ (Sinclair et al., 2015). Such a phenomenon shows that shared co-evolutionary physiological stress resistance mechanisms exist across seemingly heterogeneous stress factors and may help species survive divergent environments.

In conclusion, we document larval and adult *T. absoluta* plasticity of thermal tolerance, and their likely contributions to invasion success. To our knowledge, this is the first study to describe short- and long-term plastic responses to temperature in this species, using field collected populations. Determining the invasion pathway and pest risk assessment is significant for management of invasive pest species. As such, determining risk factors and mechanisms facilitating invasive species upon introduction to novel environments is critical in focusing control efforts. Although our results show the effects of acclimation on *T. absoluta* are complex and may vary considerably depending on the traits and life stage examined, it appears larvae are generally more plastic under short- and long-term acclimation than adults. Therefore, we suggest that larval propagules may be contributing more to the ongoing invasion of *T. absoluta*, owing to their better ability to shift their thermal phenotypes under heterogeneous environments. More studies could explore the role of parental history including testing invasive versus non-invasive species or endemic versus invasive environments to tease apart the exact role of plasticity for invasion success (as in e.g. Jarošík et al., 2015). Furthermore, the limited plasticity in adults ought to be further explored, including the role of other temperature, time and RH combinations in eliciting acclimation (see

Mutamiswa et al., 2019). These results are important for pest risk analysis and for informing decision making in management this invasive species.

3.5. References

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CHAPTER 4

Cold hardiness of the South American tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae): both larvae and adults are chill-susceptible

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4.1. Introduction

Most insects are ectotherms, and any changes in environmental conditions may result in variable physiological responses (Overgaard and Sørensen, 2008; Gray, 2013). For example, stressful low winter temperatures induce different physiological responses depending on the nature of stress e.g. acute versus chronic, severity of exposure, life stage as well as environmental history e.g. plastic responses (Denlinger and Lee RE, 2010). As such, low temperatures are key drivers of fitness for many terrestrial insects. This makes overwintering strategy an important evolutionary adaptive mechanism for surviving harsh winters and species success through resumption of reproduction, growth and development when conditions become more favorable (Sinclair et al., 2015; Overgaard and MacMillan, 2017). Survival of these low temperatures is dependent on employing a suite of adaptive behavioural, morphological, molecular and physiological mechanisms, particularly in immobile diapausing (hibernating) and/or quiescent overwintering stages (Harrison et al., 2012; Teets and Denlinger, 2013; Williams et al., 2014; Knapp et al., 2018; Raza et al., 2020). Indeed, the Bogert effect entails less mobile developmental stages have inherent higher physiological fitness owing to their limited behavioral compensatory capacities and evolutionary inertia to fitness traits (Farallo et al., 2018). Exact mechanisms facilitating survival in hibernating organisms may vary but includes; body water loss (e.g. cryoprotective dehydration) (Sørensen and Holmstrup, 2011), regulation of protein expression (Tian and Xu, 2013), gut microbiota (Raza et al., 2020), feeding cessation, expulsion of gut contents, depressed supercooling points (SCPs), accumulation of carbohydrate cryoprotectants and selection of favorable overwintering microhabitats (reviewed in Denlinger and Lee RE, 2010; Andreadis and Athanassiou, 2017).

Cold hardiness refers to the ability of an organism to resist and survive injury associated with stressful low temperatures and allows organisms to survive temperatures that are even lower than the melting point of their bodily fluids (Lee, 1991; Cubillos et al., 2018). For insects, such resistance to cold is dependent on a variety of factors including taxa, developmental stage (Mutamiswa et al., 2019), diapause development, nutritional status (Chidawanyika et al., 2017), seasonality as well as severity and duration of cold exposure (Izadi et al., 2019). Cold hardiness facilitates organisms to overwinter, providing sufficient parent propagules for species emergence and proliferation the following summer and is important for setting a species' range limits (Bale, 2002; Sinclair et al., 2003). Overwintering insects are known to employ two key strategies for cold

survival, and these include freeze tolerance and freeze avoidance (Feng et al., 2018). Freeze tolerant insects can cope with intracellular ice formation through use of colligative (e.g. sorbitol, ribitol, glycerol) and non-colligative (trehalose and proline) cryoprotectants (Storey and Storey, 2012; Toxopeus et al., 2019). Other mechanisms involve removal of ice nucleators, synthesis of anti-freeze heat-shock proteins (e.g. Hsp70), and cryoprotective dehydration (Elnitsky et al., 2008; Lee, 2010; Storey and Storey, 2013; Sinclair et al., 2015; Andreadis and Athanassiou, 2017; Cubillos et al., 2018; Feng et al., 2018). Conversely, freeze intolerant/avoiding insects cannot withstand internal ice formation, thus they die upon freezing. They survive temperatures $\sim -50^{\circ}\text{C}$ without ice formation by supercooling, i.e. keeping their bodily fluids liquid at temperatures below freezing point (SCP) (Hance et al., 2007; Denlinger and Lee RE, 2010; Feng et al., 2018). Mechanisms enhancing supercooling include clearing gut contents, increase in antifreeze solutes, sugars and thermal hysteresis proteins (Denlinger and Lee RE, 2010). Most insects however die due to chilling injury before they freeze and are termed chill-susceptible (Lee, 2010; Marshall and Sinclair, 2012). Recent studies also showed that gut microbiota also play a pivotal role in improving insect cold hardiness through stimulating their metabolism and signaling pathways (Shin et al., 2011; Raza et al., 2020). For example, *Klebsiella michiganensis* BD177 has been reported to activate the arginine and proline metabolism pathway, of invasive *Bactrocera dorsalis* resulting in tolerance to extreme low temperature stress through influencing the functionality of the mitochondria (Raza et al., 2020). Given this background, it raises questions of how other invasive species overwinter and survive stressful low sub-zero winter temperatures.

Various measures of cold tolerance have been previously described in a wide range of insect orders, such as Diptera (Elnitsky et al., 2008), Coleoptera (Crosthwaite et al., 2011), Lepidoptera (Boardman et al., 2012), Hymenoptera (Hanson et al., 2013), and Orthoptera (Toxopeus et al., 2019). For example, chill susceptible larvae of the false codling moth, *Thaumatotobia leucotreta* (Lepidoptera: Tortricidae) freeze at temperatures between -13°C and -22°C but are killed by brief temperature exposures ranging from -8°C to -12°C (Boardman et al., 2012). The wood boring beetle, *Agrilus planipennis* (Coleoptera: Buprestidae) overwinters primarily as a freeze avoiding prepupa and its body fluids supercool to temperatures as low as -35°C , with most individuals being killed when freezing occurs at SCP (Crosthwaite et al., 2011). Similarly, the freeze-tolerant prepupa of the golden rod gall fly *Eurosta solidaginis* freeze at $\sim -8^{\circ}\text{C}$ but can survive with more than

60% of its body converted to ice, with mortality beginning below -25°C (Morrissey and Baust, 1976; Sinclair et al., 2015).

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most devastating invasive insect pests of tomato and can cause losses ranging ~80-100% (Desneux et al., 2010). It has a high invasion potential and has extensively spread from its native South America to cover Europe, Asia and Africa in less than a decade (Biondi et al., 2018). Its wide distribution coupled with continuous establishment in novel heterogeneous environments through remodelling thermal phenotypes shows that this invasive species can survive under challenging and heterogeneous environments (Tarusikirwa et al., 2020). While Van Damme et al. (2015) investigated the SCPs and lethal time for *T. absoluta* larvae, pupae and adults at 0°C and 5°C for a western European population, information on this insect's cold hardiness strategy and overwintering survival in arid and semi-arid African agro-ecosystems is currently limited. Here, we thus investigated the cold hardiness strategy of larval and adult *T. absoluta* wild populations to better explain the species' overwintering survival. Specifically, we measured lower lethal temperatures (LLTs), lower critical temperature activity limits and various ecologically relevant factors affecting SCPs *vis* hydration status, feeding status, desiccation and different diurnally synonymic temperature cooling rates. While previous studies reported that *T. absoluta* can survive slightly sub-zero temperatures for a short period (Potting et al., 2013; Van Damme et al., 2015) results may vary with space and across developmental stages tested. Moreover, to our knowledge, the exact freeze strategy for *T. absoluta* remains unknown. I hypothesize that *T. absoluta* is chill susceptible and that SCP is influenced by different ecological factors. This information is important in defining species' cold hardiness and overwintering strategies that can be critical in temporal ontogenetic targeted pest control, and as a predictive tool for forecasting outbreaks, determination of species range limits and in refining pest management.

4.2. Materials and Methods

4.2.1. Insect culture

Wild populations of *T. absoluta* larvae were collected during austral summer (September-November 2019) as predominantly first instar infested tomato leaves/plants at Borotsi Farm, Tonota village in North East District of Botswana (S $21^{\circ}24'0.621''$; E $27^{\circ}27'18.31752''$). Following hatching, *T. absoluta* first instar larvae tunnel the leaf mesophyll tissue, creating symptomatic

extensive mines or tunnels on the leaves. In the laboratory, larvae were fed on fresh tomato leaves (“Rodade”) inside Bugdorm rearing cages (240cm³ Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under optimum conditions, i.e. 28°C; 65% relative humidity (RH) and 12L:12D photocycle in climate chambers (HPP 260; Memmert GmbH + Co.KG, Germany). For all the experiments 4th instar wild larvae (field collected) and 24-48h old adults (eclosed from field collected larvae) were used.

4.2.2. Determination of lower lethal temperatures (LLTs)

Using direct plunge method in a programmable water bath (Systronix Scientific, Industria, South Africa), lower lethal temperatures (LLTs) were assayed according to established protocols (e.g. Chidawanyika and Terblanche, 2011; Boardman et al., 2012; Andersen et al., 2015; Mutamiswa et al., 2018). Ten larvae replicated thrice were placed into 60ml polypropylene vials which were then placed in a watertight zip lock bag (20 x 28cm) before being submerged into a programmed water bath filled with 99.9% circulating ethanol. Thereafter, the insects were subjected to a series of temperature/duration treatments, ranging from 0°C to -17°C for 0.5, 1, 2 and 4h until 0-100% mortality was recorded. For the duration of each treatment, digital thermometers (Fluke 53/54IIB, Fluke Cooperation, USA) were used to monitor the water bath temperature. Following treatment, tested larvae were placed in a climate chamber set at optimum conditions (28°C; 65% RH and 12L:12D photocycle) with *ad libitum* access to food (fresh tomato leaves) before scoring survival. Larval survival was defined as the ability to pupate following their respective treatments.

4.2.3. Critical thermal minima (CT_{min}) determination

Prior to CT_{min} determination, larvae and adults were exposed to two treatments, i.e. (1) fasted for 24h and (2) left with *ad libitum* access to food (tomato leaves for larvae and 10% sugar solution for adults) in a climate chamber under benign conditions (28°C; 65±10% RH; 12L:12D). Critical thermal minima were measured using standardized protocols as outlined by Nyamukondiwa and Terblanche (2009). This lower activity limits assay has widely been used in assessing cold hardiness in insects (Andersen et al., 2015). Ten replicate organisms were individually placed in numbered ‘organ pipes’ connected to a programmable water bath before decreasing the temperature at a rate of 0.25°C/min until their CT_{min} were recorded. Organisms were stimulated once every 0.5°C to monitor activity. This was repeated twice for each treatment to yield sample sizes of *N* = 20 per treatment. Critical thermal minimum was defined as the temperature at which

each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object (see Nyamukondiwa and Terblanche, 2009).

4.2.4. Determination of supercooling points (SCPs)

Supercooling points were assayed as outlined by Nyamukondiwa et al. (2013). The organisms were individually placed and secured in 2ml Eppendorf tubes. Thereafter, each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762-1121, Cambridge, UK), inserted through the gauzed lid of the tube and both the insect and thermocouple were secured in contact by a cotton wool. Thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) interfaces and temperatures were recorded at 1s intervals using PicoLog software for windows (Pico Technology, Cambridge, UK). The insects were first exposed to a temperature of 15°C for 10 mins before decreasing the temperature at a rate of 0.5°C/min until their SCPs were recorded. Supercooling point for each organism was determined as the lowest temperature recorded before a spike in temperature due to latent heat of crystallization (Nyamukondiwa et al., 2013).

4.2.5. Supercooling point mortality assay

In order to determine whether the SCP was indicative of the LLT, SCP mortality was assayed as outlined by Boardman et al. (2012). Fifteen organisms, in numbered Eppendorf tubes, were exposed to the standard SCP protocol (see section 4.2.4) with close monitoring of temperature. When half the larvae/adults had reached their SCPs, the water bath cooling program was terminated and then ramped back to 15°C at a rate of 1.4°C/min. Thereafter, the organisms were allowed to recover in a climate chamber set at optimum conditions (28°C; 65±10% RH and 12L:12D photoperiod) with food *ad libitum*. This experimental protocol was repeated twice to yield $N = 30$. Survival was scored at 24 and 48 h intervals after treatment, through gently stimulating the organisms using an insect brush. Larvae/adults were scored as dead if they did not have a controlled muscular response following gentle prodding.

4.2.6. Ecological factors affecting supercooling point

Feeding status - Prior to determination of SCP, *T. absoluta* larvae and adults were exposed to two different feeding regimes (fasting and feeding) as outlined by Boardman et al. (2012). Fasted organisms were removed from the food source and kept individually in 60ml polypropylene vials

at benign conditions (28°C; 65±10% RH; 12L:12D) in climate chambers for 24h, without access to food and water. Fed larvae and adults were provided with food *ad libitum* (i.e. tomato leaves and 10% sugar solution respectively). Assays started at a set point of 15°C for 10min before ramping the temperature down at a rate of 0.5°C/min until SCPs were recorded (see protocol description in section 4.2.4).

Diurnal cooling rates - To assess whether SCPs change e.g. through plasticity, different diurnal cooling rates (synonymous to changing seasons) on SCPs were assessed. Both larvae and adults were subjected to three independent cooling rates (0.12, 0.25, and 0.5°C/min) following standardized protocols (see section 4.2.4). The intermediate cooling rate (0.25°C/min) represented the relative control. In all cases, assays started at a set point of 15°C for 10min (to allow for insects' temperature equilibration) before ramping the temperature down at the treatment cooling rates. This was repeated twice to yield sample sizes of $N = 20$ per ramping rate treatment for each life-stage.

A modified plunge protocol which exposed the insects directly to -30°C was also used to investigate the time it would take for both larvae and adults to freeze. Twenty individual organisms were placed into a water bath set at -30°C and the SCP as well as the time taken to freeze, was recorded.

Inoculative freezing - To assess the possible effects of freezing induced by contact with external ice on *T. absoluta*, a piece of wet cotton wool (soaked in distilled water) was placed in each Eppendorf tube with the organism (larva/adult) and thermocouple during a SCP experiment ($N = 20$). For the control organisms, dry cotton wool was placed in each of the SCP experimental tubes.

Hydration status - In order to investigate the effect of water content and fasting on SCP, larvae were removed from tomato leaves and kept under a modified RH using silica gel (150g) for 24h ($N = 20$) in a glass sealed desiccator. Adults were also exposed to the same dehydrating conditions and duration. Preliminary assays showed this duration elicits ~30% body water loss from both larvae and adults. Thermocron iButtons (Dallas Semiconductors, Model DS1920) were used to monitor temperature and RH inside the desiccator at 30-min intervals during the pre-treatment. Following dehydration pre-treatment, SCPs were determined using the same protocols (see section

4.2.4). Control organisms were allowed to feed *ad libitum* under benign conditions (28°C; 65 ± 10% RH) prior to SCP determination.

4.2.7. Data 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, 2019). First, data was checked for normality and equality of variance using the Shapiro-Wilk and Hartley-Bartlett tests respectively. Supercooling point and CT_{min} data met assumptions of ANOVA, thus were analysed using factorial ANOVA in STATISTICA. Lower lethal temperature data did not meet the assumptions of ANOVA, therefore, were analyzed using a generalized linear model (GLZ) with a binominal distribution and a logit link function in R (R Development Core Team, 2019). Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups.

4.3. Results

4.3.1. Lower lethal temperature assays

The effects of both temperature and duration of low temperature exposure on *T. absoluta* larvae survival were significant ($P < 0.0001$) (Table 4.1). An increase in low temperature severity and duration of exposure resulted in increased larval mortalities (Fig. 4.1). Nevertheless, duration of exposure × temperature interaction effects were not significant ($P > 0.05$) (Table 4.1). Lower lethal temperatures ranged from -17 to 0°C for 0.5, 1, 2 and 4 h durations (Fig. 4.1). While temperatures -8 to 0°C appeared to cause general larval mortality for the 4 h duration treatment, rapid mortality started at temperature beyond -9°C across all durations. No larva survived -17°C across all experimental treatment durations.

Table 4.1. Summary of results of generalized linear model (GLZ) (using a binominal distribution and a logit link function), for the effects of low temperature, duration and their interaction effects on survival of *T. absoluta* larvae.

Effect	χ^2	df	<i>p</i>
Duration	177.77	3	<0.0001
Temperature	1251.59	9	<0.0001
Duration x Temperature	7.16	27	0.1

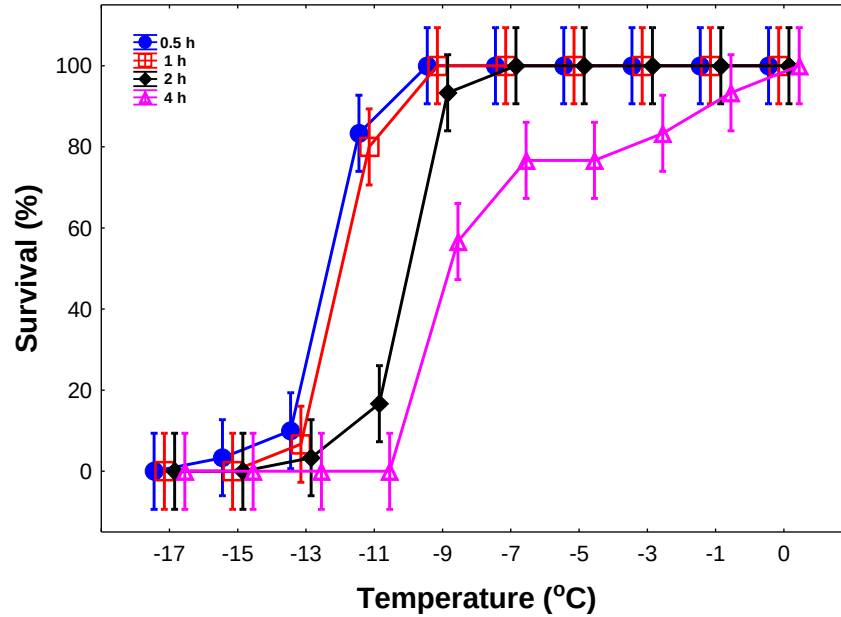


Figure 4.1. Survival (%) of *T. absoluta* 4th instar larvae under exposure to different low temperatures and time durations. Each data point represents mean $\pm$ 95% CLs of three replicates (N = 30). Lines indicate mean survival for each duration (0.5, 1, 2 and 4hrs).

4.3.2. Supercooling point (SCP) mortality

The SCP mortality assay showed frozen larvae never recovered following supercooling and discoloured from green to black within 24 h post treatment. Unfrozen larvae seemed somewhat responsive to mild prodding 24 h post treatment but died within 48h after treatment. All 30 adults did not recover following freezing after 24h at benign conditions. These results suggest both larvae and adults cannot withstand internal ice formation and are chill susceptible.

4.3.3. Ecological factors affecting supercooling point

4.3.3.1. Feeding status

There was no significant difference between CT_{min} of controls (fed) and treatment (fasted) for both larvae ($3.0 \pm 0.2^{\circ}C$ vs. $3.3 \pm 0.2^{\circ}C$) and adults ($-1.9 \pm 0.2^{\circ}C$ vs. $-1.5 \pm 0.1^{\circ}C$) ($P > 0.05$) (Table 4.2; Fig. 4.2A). A comparison of life stages showed significant differences in CT_{min} ($P < 0.001$) between fed larvae and adults as well as fasted life stages (Table 4.2; Fig. 4.2A). Larvae were significantly more cold hardy (lower CT_{min}) than adults. In addition, life stage $\times$ feeding status effect was not significant ($P > 0.05$) (Table 4.2).

Fasting significantly depressed SCP in larvae (Fed: $-19.1 \pm 0.2^{\circ}\text{C}$ vs. Fasted: $-21.4 \pm 0.5^{\circ}\text{C}$) ($P < 0.001$) (Table 4.2; 4.3; Fig. 4.2B). In contrast, there was no significant difference between the SCPs of fed and fasted adults ($-16.9 \pm 0.2^{\circ}\text{C}$ vs. $-16.5 \pm 0.2^{\circ}\text{C}$) (Table 4.3; Fig. 4.2B). Significant differences in SCPs ($P < 0.001$) were noted between fed as well as fasted larvae and adults (Table 4.2; Fig. 4.2B). In addition, there was a significant life stage $\times$ feeding status interaction effect ($P < 0.001$) (Table 4.2). In both treatments, larvae and adults were unable to recover 24h post supercooling.

Table 4.2. Summary results on the effects of different treatments and their interaction effects with developmental stages (larvae and adults) on low temperature tolerance in larval and adult *Tuta absoluta*. Treatments tested comprise effects of (1) feeding status (i.e. fasted and fed) on critical thermal minima ($CT_{\min}$), (2) feeding status (i.e. fasted and fed) on supercooling points (SCPs), (3) different cooling rates (i.e. 0.12°C , 0.25°C and 0.5°C) on SCPs, (4) effects of inoculative feeding on SCPs and (5) effects of dehydration pre-treatment on SCPs (see methods section for more comprehensive account of treatments). SS = sum of squares, df = degrees of freedom, MS = means of squares.

Effect	SS	df	MS	F	p
<i>Effects of feeding on $CT_{\min}$</i>					
Intercept	39.9031	1	39.9031	62.4366	<0.0001
Life stage	471.9061	1	471.9061	738.3932	<0.0001
Feeding status	2.2111	1	2.2111	3.4598	0.067
Life stage x Feeding status	0.0781	1	0.0781	0.1222	0.728
Error	48.5715	76	0.6391		
<i>Effects of feeding on SCPs</i>					
Intercept	27260.99	1	27260.99	16101.60	<0.001
Life stage	249.57	1	249.57	147.41	<0.001
Feeding status	18.45	1	18.45	10.90	<0.001
Life stage x Feeding status	33.88	1	33.88	20.01	<0.001
Error	128.67	76	1.69		
<i>Effects of cooling rates on SCPs</i>					
Intercept	39553.21	1	39553.21	39545.07	<0.0001
Life stage	142.05	1	142.05	142.02	<0.0001
Cooling rate	3.22	2	1.61	1.61	0.204

Life stage x Cooling rate	27.26	2	13.63	13.63	<0.0001
Error	114.02	114	1.00		
<i>Effects of inoculative freezing on SCPs</i>					
Intercept	14059.05	1	14059.05	8547.877	<0.0001
Life stage	0.05	1	0.05	0.032	0.859
Inoculative freezing	2104.43	1	2104.43	1279.489	<0.0001
Life stage x Inoculative freezing	37.41	1	37.41	22.748	<0.0001
Error	125.00	76	1.64		
<i>Effects of dehydration pre-treatment on SCPs</i>					
Intercept	27272.81	1	27272.81	12719.46	<0.0001
Life stage	49.77	1	49.77	23.21	<0.0001
Dehydration pre-treatment	12.69	1	12.69	5.92	0.017
Life stage x Dehydration pre-treatment	12.75	1	12.75	5.95	0.017
Error	162.96	76	2.14		

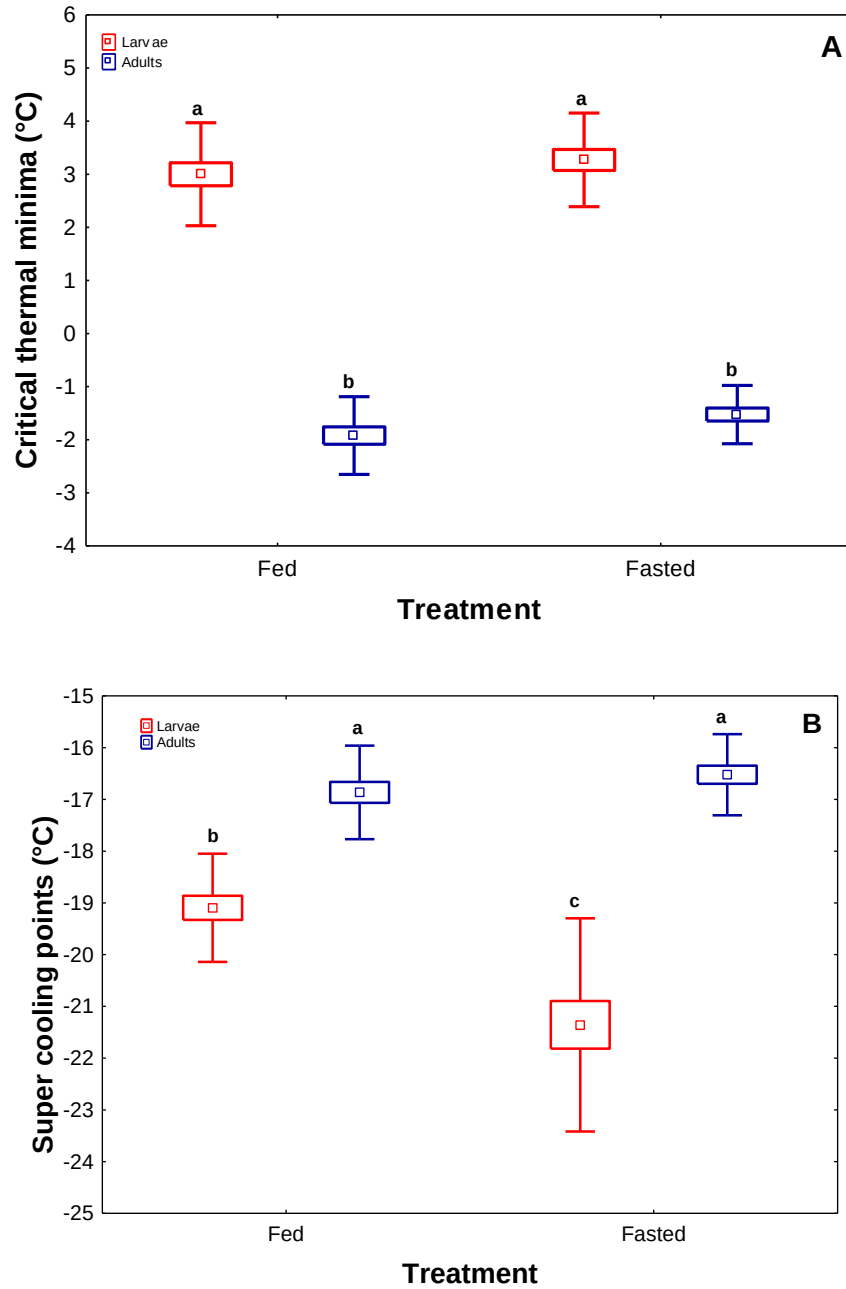


Figure 4.2. Effects of different feeding status on *T. absoluta* 4th instar larvae and 24h- 48h old adults critical thermal minima (CT_{min}) (A) and supercooling points (SCPs) (B). N=20 per group. Means with the same letter are not significantly different from each other.

Table 4.3. Summary of supercooling point (SCP) results after different treatments (i.e. fasting, exposure to different cooling rates, direct plunge to -30°C, inoculative freezing and dehydration pre-treatment). Means $\pm$ SEM with same letters are not significantly different.

Treatment	Mean $\pm$ SEM	
	Larvae	Adults
Control	-19.1 $\pm$ 0.2 ^a	-17.7 $\pm$ 0.2 ^b
Fasted	-21.4 $\pm$ 0.5 ^a	-16.5 $\pm$ 0.2 ^b
Fed	-18.5 $\pm$ 0.3 ^a	-16.9 $\pm$ 0.2 ^b
Slow ramp rate (0.12°C/min)	-19.8 $\pm$ 0.3 ^a	-16.3 $\pm$ 0.1 ^b
Medium ramp rate (0.25°C/min)	-18.9 $\pm$ 0.2 ^a	-17.3 $\pm$ 0.2 ^b
Fast ramp rate (0.5°C/min)	-19.1 $\pm$ 0.2 ^a	-17.7 $\pm$ 0.2 ^b
Direct plunge (-30°C)	-22.0 $\pm$ 0.5 ^a	-18.8 $\pm$ 0.5 ^b
Inoculative freezing	-7.5 $\pm$ 0.3 ^a	-8.8 $\pm$ 0.3 ^b
Desiccation	-20.3 $\pm$ 0.3 ^a	-17.7 $\pm$ 0.2 ^b

4.3.3.2. Diurnal cooling rates

Cooling rate effect on SCPs was not consistent between larvae and adults for the different ramping rates, but developmental stage differences were evident at each cooling rate ($P > 0.05$) (Table 4.2). Supercooling points significantly improved (more negative) relative to the control with slower cooling rate in larvae whereas in adults, slower cooling rates compromised SCPs (more positive) (Fig. 4.3). Faster cooling rate (0.5°C/min) also marginally improved SCP in larvae but not in adults (Fig. 4.3). There was a significant difference observed between the slowest cooling rate and the intermediate rate (0.25°C/min) on both larval and adult SCP but no significant effect was noted between the rates; 0.25°C/min and 0.5°C/min for larvae (Table 4.2; Fig. 4.3). Life stage $\times$ cooling rate effect was significant ($P < 0.0001$) (Table 4.2). At all cooling rates, larvae also had significantly more negative SCPs relative to adults (Fig. 4.3). It generally took larvae longer time to freeze (≤ 10 min; median: 6.2min) than adults (≤ 6 min; median: 3.4min) following direct -30°C plunge treatment.

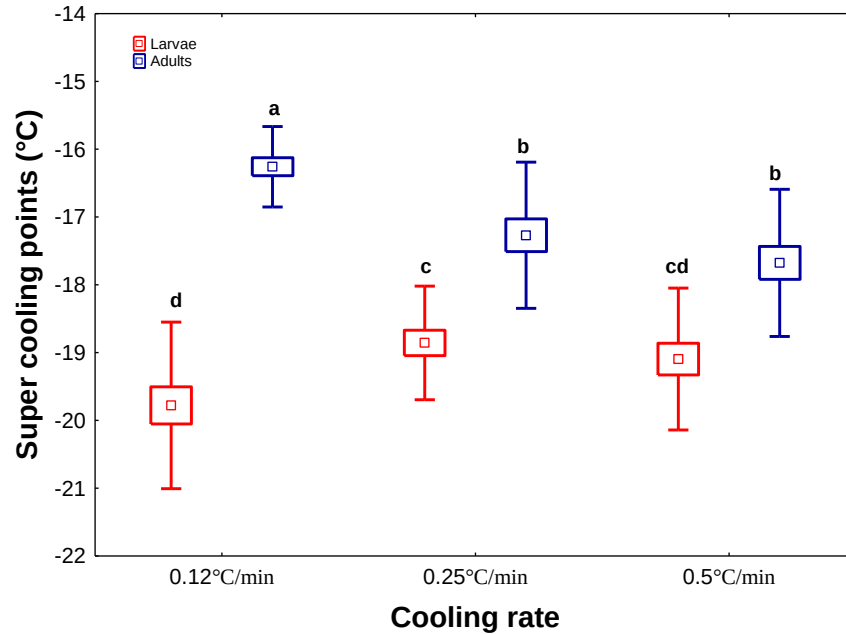


Figure 4.3. Effects of different cooling rates (0.12°C; 0.25°C and 0.5°C) on *T. absoluta* 4th instar larvae and 24h- 48h old adults supercooling points (SCP). N=20 per group. The intermediate ramping rate (0.25°C/min) served as relative control. Means with the same letter are not significantly different from each other.

4.3.3.3. Inoculative freezing

Inoculative freezing significantly influenced both larval and adult SCPs ($P < 0.0001$) such that treated insects recorded a higher (more positive) SCP (larvae: $-7.5 \pm 0.3^{\circ}\text{C}$; adults: $-8.8 \pm 0.3^{\circ}\text{C}$) relative to controls (larvae: $-19.1 \pm 0.2^{\circ}\text{C}$; adults: $-17.7 \pm 0.2^{\circ}\text{C}$) (Table 4.2; 4.3; Fig. 4.4A). The magnitude of temperature difference between treated larvae and controls was $\sim 12^{\circ}\text{C}$ whereas a $\sim 9^{\circ}\text{C}$ temperature difference was recorded between treated adults and their controls (Fig. 4.4A).

4.3.3.4. Hydration status

Dehydration pre-treatment significantly influenced SCP in larvae ($P < 0.001$). Larvae that were dehydration pre-treated recorded improved SCP ($-20.3 \pm 0.3^{\circ}\text{C}$) than controls ($-19.1 \pm 0.2^{\circ}\text{C}$) (Table 4.2; Fig. 4.4B). There was no significant difference in SCP ($P > 0.05$) between dehydrated pre-treated adults ($-17.7 \pm 0.2^{\circ}\text{C}$) and controls ($-17.7 \pm 0.2^{\circ}\text{C}$) (Fig. 4.4B). Consistent with other results, significant differences in SCPs ($P < 0.001$) were recorded between treatment larvae and adults as well as controls (Fig. 4.4B).

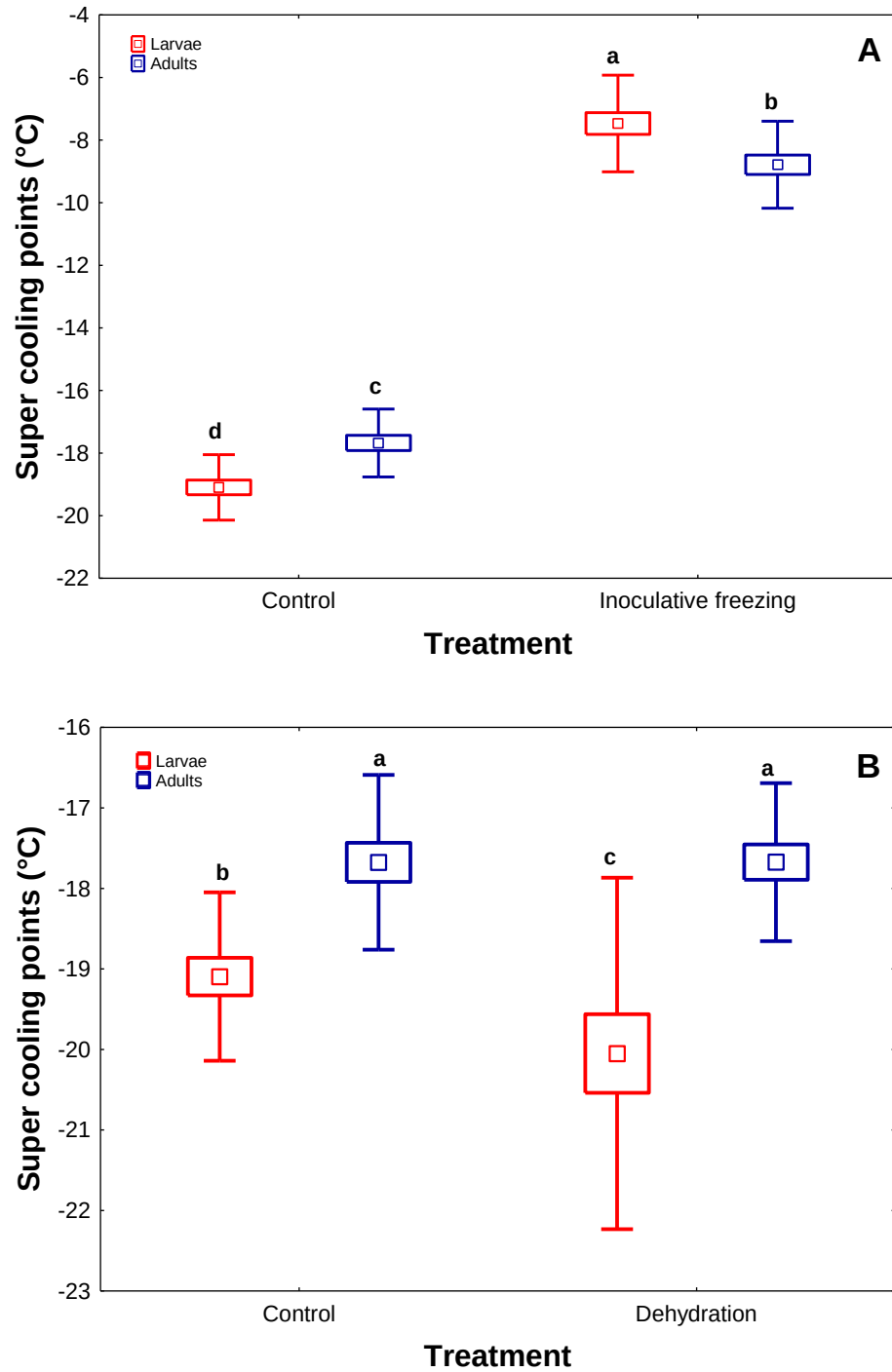


Figure 4.4. Effect of inoculative freezing (A) and 24h dehydration pre-treatment (B) on *T. absoluta* 4th instar larvae and 24h- 48h old adults supercooling points (SCPs). N=20 per group. Means with the same letter are not significantly different from each other.

4.4. Discussion

Our results show that chilling injury in *T. absoluta* larvae and adults appear to be irreversible at temperatures below -17°C , and always results in mortality. Supercooling results show that larvae and adults cannot tolerate internal ice formation and coupled with LLT mortality, these two developmental stages are thus chill susceptible. Larvae nevertheless appeared in all cases to be more cold hardy than adults, congruent with the Bogert effect (Farallo et al., 2018) and supporting the notion larval thermal tolerance edge may enhance *T. absoluta* fitness under stressful environments (Tarusikirwa et al., 2020). Supercooling points were significantly affected by different ecological factors tested here vis feeding status, cooling rates, inoculative freezing and hydration status. This points to the role of ecological factors in modulating supercooling, a mechanism critical for cold hardiness (Denlinger and Lee RE, 2010). While the current results support *T. absoluta* may survive African winter climates as both larvae and adults, we suggest that population re-establishment may be driven to a larger extent by larvae relative to adults, owing to their superior cold hardiness. This study extends valuable knowledge of physiological cold tolerance and overwintering potential of *T. absoluta* larvae and adults under changing environments.

Our LLTs ranged from 0 to -17°C (Fig. 4.1) with mortality increasing as temperature x duration increased (see e.g. Tewksbury et al., 2008). This result is also consistent with Machezano et al. (2018) who showed that adult *T. absoluta* chilling injury occurred at temperatures below -12°C with the LLTs ranging -1°C to -12°C . Minimum winter temperatures experienced in Botswana (see Machezano et al., 2018) rarely go beyond sub-zero and are thus unlikely to kill both *T. absoluta* larvae and adults through direct or indirect low temperature mortality. Similarly, general African climates, and their rapidly warming surfaces (see Engelbrecht et al., 2015) may also mean that both larvae and adults may survive throughout winter, albeit development may be slowed. This implies that, if host plants are available, *T. absoluta* larvae and adults may continue activity during winter without undergoing diapause (Van Damme et al., 2015). The inability to diapause is a fitness advantage for this invasive species, since it continues to develop, multiply and potentially spread during winter. Indeed, all-year round activity has been reported in other African agro-ecosystems. For example, in Northern Africa, pheromone-based monitoring revealed activity of the pest all year round and at high population density (Ouardi et al., 2012; Mansour et al., 2019).

Studies have also shown that Africa hosts diverse wild and cultivated *T. absoluta* host plants that are available all year round (Cocco et al., 2015; Tonnang et al., 2015; Mohamed et al., 2015; Drouai et al., 2016; Mutamiswa et al., 2017; Machekano et al., 2018; Mansour et al., 2018; Cherif et al., 2019). These can facilitate *T. absoluta* host switching, impairing diapause and thus aiding survival through winter. Host switching coupled with improved cold hardiness reported here increases parent propagules post winter, thus improving *T. absoluta* early summer pest proliferation.

Although Van Damme et al. (2015) reported no significant differences in SCPs between larvae and adults, our results showed larvae had significantly lower SCPs than adults. Numerous studies have shown a marked difference in supercooling in insects depending on diets and feeding status (Sømme, 1982; Leather et al., 1993; Suh et al., 2002; Boardman et al., 2012). Most of these studies indicate a reduction in supercooling ability in fed insects probably due to initiation of freezing by nucleating agents in food residues (Leather et al., 1993; Suh et al., 2002). In nature, most overwintering insects of the temperate climates evacuate the digestive tract as part of a multifactorial process that maximizes supercooling in winter (Leather et al., 1993). Depressed SCPs are often attributed to absence of ice nucleating compounds as well as accumulation of cryoprotective elements in the gut (Milonas and Savopoulou-Soultani, 1999; Boardman et al., 2012; Raza et al., 2020;). Fasting eliminates gut contents resulting in reduction of the number of gut ice nucleating agents and depressed SCPs (Chown and Nicolson, 2004; Boardman et al., 2012). This is consistent with current larval results showing depressed SCPs with fasting. Contrary to larval response, no fasting effects were recorded for adult SCPs. The reasons for this observation are largely unclear. However, as digestion proceeds in a fasting insect, food may become secluded in certain parts of the digestive tract, where its minimally potent as a nucleator source (Leather et al., 1993). Our results are thus congruent to Suh et al. (2002) who found no differences in SCPs between fed and fasted weevils. In addition, non-food contents in the gut have also been shown to affect insect supercooling. Castrillo et al. (2000) reported that the presence of ice-nucleating bacterial strains in the gut of diapausing Colorado potato beetles, *Leptinotarsa decemlineata*, significantly elevated their SCPs. A comparison of larval and adult cold tolerance indicated more depressed SCPs for larvae relative to adults (Fig. 4.2B). The reason behind this variation is unknown but may be related to differential cryoprotectants in the gut and a developmental stage related difference in supercooling. Cryoprotectants are the key drivers to insect tolerance to low

temperature stress (Raza et al., 2020) and these may differ with developmental stage and likely with differences in diet. For example, the presence of *K. michiganensis* gut symbionts reportedly stimulated cryoprotectants' production and cold hardiness in *B. dorsalis* (Raza et al., 2020). Studies have shown that dietary components and gut food placement may differentially affect insects' SCPs (Leather et al., 1993). For example, proline and arginine augmented diets improved cold hardiness in *Drosophila melanogaster* larvae and adults (Kostal et al., 2012; Kostal et al., 2016). We conclude, with caveats that variation in feeding mechanisms between *T. absoluta* larvae and adults and likely the Borget effect may account for the SCP differences reported here. Future studies should look into these potential mechanisms, e.g. differences in cryoprotectants' expression across different developmental stages and diets.

Variation in cooling rates has been shown to have significant effects on insect cold hardiness (Salt, 1966; Kelty and Lee, 1999; Carrillo et al., 2004; Boardman et al., 2012; Mutamiswa et al., 2017). Our results showed slower cooling rate improved SCPs in larvae but compromised it in adults (Fig. 4.3). Contrary to Boardman et al. (2012), improved SCP following slower cooling rate suggests that *T. absoluta* larvae may have capacity to adjust their lower temperature tolerance through phenotypic plasticity, in keeping with previous reports (Kelty and Lee, 2001; Tarusikirwa et al., 2020). However, lack of significant SCP depression effects for adults is consistent with previous studies that have shown asymmetrical thermal plasticity in *T. absoluta* developmental stages, appearing to be higher in larvae than adults (Tarusikirwa et al., 2020). Larvae and adults subjected to direct plunge treatment (-30°C) froze within a period of 10 min (median: 6.2min) and 6 min (median: 3.4min) respectively, further suggesting larvae are more cold hardy than adults. While previous studies report *T. absoluta* overwinters as eggs, pupae and adults (EPPO, 2005; Van Damme et al., 2015), current data suggest larvae may also have a role in overwintering. However, larval *T. absoluta* could not survive freezing and are thus chill susceptible, a result in agreement with other related Lepidopteran species, e.g. *T. leucotreta*, (Boardman et al., 2012), *Spodoptera exigua* (Atapour and Moharramipour, 2014), and *Cydia pomonella* (Neven, 1999; Khani et al., 2007).

Most insects are known to overwinter in natural microhabitats such as upper soil layer and leaf litter with some degree of environmental buffering. However, these microhabitats are often humid posing a risk of inoculative freezing (Rozsypal and Košťál, 2018). Studies have shown that contact

with moisture may rapidly result in inoculated ice formation through the cuticle (Sømme, 1982). This may lead to an increased freezing risk, and negative fitness consequences for non-freeze tolerant species (Boardman et al., 2012). Consistent with Boardman et al. (2012), we found inoculative freezing significantly increased SCPs (more positive temperatures) for both larvae and adults and has fitness consequences for *T. absoluta* living in humid environments. Our results also show *T. absoluta* larvae can survive cooling to -5°C but has limited survival at temperatures around -10°C following exposure to external ice (Fig. 4.4A). This is consistent with Rozypal (2015) findings on *C. pomonella* larvae where SCPs ranged from -15°C to -26°C but averaged -3°C when larvae were placed on ice. Since *T. absoluta* adults do not require tomato plants as food source, they have been reported to overwinter in empty greenhouses (Van Damme et al., 2015). Given that tomato production in Africa is done in both greenhouses and open fields (see Mansour et al., 2018) where winter temperatures rarely reach SCPs recorded here, even those following inoculative freezing, larval and adult winter survival is highly likely. Given that *T. absoluta* is highly fecund (Tropea Garzia et al., 2012; Guimapi et al., 2016; Biondi et al., 2018) even 10% overwintering population survival can result in an upsurge the following growing season (Van Damme et al., 2015). The ability of both adults and larvae to overwinter without diapause reported here suggest potential synergistic and increased pest risks for Solanaceous plants production in African agro-ecosystems.

Dehydration pre-treatment significantly improved the SCP of the larvae from -19.1°C in the control to -20.3°C in dehydrated insects. This result resonates well with the effects of inoculative freezing and supports the ecological significance of water in supercooling. There is a close positive relationship between dehydration tolerance and cold tolerance with some insect species utilising dehydration as a cold hardiness strategy (Ring and Danks, 1994; Holmstrup et al., 2002). Dehydration improves cold tolerance through partial removal of water from cells (cryoprotective dehydration) (e.g. Sørensen and Holmstrup, 2011) that improves supercooling (Rozsypal, 2015). For example, dehydration pre-treatment has been reported to improve cold tolerance in freeze avoidant Collembola (Bayley et al., 2001; Sjørnsen et al., 2001). In addition, most insects have been reported to upregulate the synthesis of heat shock proteins following dehydration (e.g. Feder and Hofmann, 1999), a mechanism likely accounting for results reported here. Furthermore, studies have shown that organisms undergoing dehydration accumulate polyhydroxy compounds, which facilitate improved cold hardiness (see e.g. Rozsypal, 2015). This may partly account for the SCPs

differences reported here. However, exact mechanisms for larval *T. absoluta* improvement in SCPs reported here are unknown and thus warrant further investigation. Nevertheless, the largely arid and semi-arid Africa may confer an enhanced cold hardiness for *T. absoluta*. Dehydration pre-treatment did not affect SCPs in adults, consistent with other observations on ecological effects on SCPs reported here. Thus, this result partially supports the notion that extreme cold winters and lack of soil moisture may be key factors that lower the risk of permanent establishment in northern Europe, Russia, and Canada (Tonnang et al., 2015).

Improvement in cold hardiness following dehydration pre-treatment may also indicate cross-tolerance (Sinclair et al., 2013), an indication of shared co-evolved physiological resistance mechanisms between dehydration and cold stress. This result is in keeping with Mutamiswa et al. (2018) who reported improved cold tolerance for *Chilo partellus*, *Busseola fusca* and *Sesamia calamistis* larvae following dehydration pre-treatment. This potential cross tolerance can be a significant survival mechanism under overlapping and heterogeneous stressful climates that may explain *T. absoluta* survival in different novel and climatically heterogeneous environments. Because these stresses are regularly encountered together, it is not surprising those insects that are cold hardy also tend to be tolerant of dehydration (Ring and Danks, 1994; Danks, 2000; Kellermann et al., 2012).

In conclusion, our results show that (1) *T. absoluta* larvae and adults are chill susceptible and successfully overwinter in warm African environments, (2) larvae appear more cold hardy than adults likely due to evolutionary inertial physiological fitness advantage and (3) ecological factors e.g. inoculative freezing, diurnal cooling rates, feeding- and hydration are key drivers of *T. absoluta* cold hardiness. While our results show that *T. absoluta* may overwinter as both larvae and adults, larvae seem to play a greater part of overwintering propagules, through their improved enhanced cold hardiness. However, more studies should investigate exhaustive ontogenetic temporal cold hardiness, to better explain the effects of developmental stages and timing in shaping overwintering survival. These results are important in determining species range limits, population phenology and modelling pest risk status and allows temporal and life-stage specific targeting of management strategies.

4.5. References

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CHAPTER 5

Water balance and desiccation tolerance of the invasive South American tomato pinworm

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5.1. Introduction

Despite an increasing awareness of the ecological, economic, and sociological effects of human mediated translocations of insects (Ward and Masters, 2007; Meyerson and Mooney, 2007; Bradshaw et al., 2016; Cuthbert et al., 2021; Renault et al., 2021), the rate of alien invasion continues to rise significantly (Seebens et al., 2017; 2018; Bailey et al., 2020). Various studies have shown that increase in temperature coupled with inconsistent and limited rainfall due to climate change has a substantial influence on insect invasion dynamics (Nyamukondiwa et al., 2010; Lantschner et al., 2014; Weldon et al., 2016). As a result, tolerating climate change-related factors, e.g. humidity-temperature stress, has become a challenge in both natural and agroecosystems and from native to exotic systems (Bellard et al., 2012; Boggs, 2016; Thorat and Nath, 2018). Although most studies focused on changing temperatures, water availability trends have also been changing (Chown et al., 2011). Indeed, Engelbrecht et al. (2015) showed that droughts may increase in frequency, arid environments will become drier, precipitation seasonality may change, and extreme events will likely increase, should climate change mitigation fail. Given the large surface area to volume ratio of insects, water balance is a key stressor in increasingly arid terrestrial environments (Weldon et al., 2013a; Everatt et al., 2014). Dehydration is known to affect organismal function through its direct effects on metabolism. For example, it may lead to changes in enzyme functionality and membrane potential, decrease in circulation of nutrients and hormones, and movement retardation in hydrostatic skeleton insects (Harrison et al., 2012; Weldon et al., 2019). As a result, management of body water content remains a key survival mechanism under stressful and changing environments.

As arthropods move from more mesic to xeric environments, they are faced with stressful desiccating environments (Weldon et al., 2016). The ability to employ stress response mechanisms is thus important for the maintenance of key fitness traits, survival and reproduction, and may inform predictions of invasion success (Kellermann et al., 2012; Everatt et al., 2014; Kellermann et al., 2020). The ability of an insect to maintain constant levels of body water is termed water balance (Yoder et al., 2003). It plays a significant role in determining energy budgets, activity patterns, and population dynamics and hence geographic distribution (Kleynhans and Terblanche, 2011; Tee and Lee, 2015). Maintaining water balance, which is dependent on a state of equilibrium

between environmental water gain and loss, is thus fundamental for insect survival and invasion success (Addo-Bediako et al., 2001; Pallarés et al., 2016; Weldon et al., 2016).

The ability of insects to gain water is highly dependent on the relative humidity (RH) in the environment (Guedes et al., 2015). For example, dry environmental conditions pose a threat to insect water balance due to the interplay between temperature and water availability. Insects gain water via several mechanisms such as drinking, extraction from food, absorption from the atmosphere (through cuticle), and metabolically (e.g. through lipid hydrolysis) (Hadley, 1994; Chown and Nicolson, 2004; Guedes et al., 2015). In contrast, water is lost by cuticular and respiratory transpiration, oral or anal secretions, and excretion (Addo-Bediako et al., 2001; Chown et al., 2011; Guedes et al., 2015). The capacity to prevent water loss from the body varies greatly among invertebrate species and across developmental stages. Nonetheless, insects have an adaptive and highly restrictive water economy and diverse mechanisms to reduce water loss (Zachariassen, 1996). For example, insects have evolved a variety of behavioral, morphological, molecular and physiological adaptations that increase water storage, restrict water loss, enable tolerance to water loss (e.g. anhydrobiosis [see Watanabe et al., 2002]) and cope with desiccation stress (Chown and Nicolson, 2004; Weldon and Taylor, 2010; Hershkovitz and Gasith, 2013; Strachan et al., 2015). Physiologically, insects suppress water loss through deposition of extra cuticular lipids, cuticular structure modification, metabolism reduction, alteration of surface area to volume ratios and increasing levels of cryoprotectants (Benoit et al., 2007; Lopez-Martinez et al., 2009). Similarly, reduction of cuticular permeability is one other mechanism allowing reduction of insect water loss rates and is reported to play a critical role in explaining species distribution (Winston, 1967; Appel et al., 1991). Studies have shown that some physiological mechanisms linked to desiccation stress resistance such as the control of water loss rates have an important plastic and adaptive component (Chown et al., 1999; Hoffmann and Harshman, 1999; Pallarés et al., 2016). Therefore, desiccation resistance is perceived as the main strategy often employed by insects under stressful xeric environments (Weldon et al., 2013a; Pallarés et al., 2016; Weldon et al., 2016; Bujan et al., 2016).

Desiccation resistance occurs when an organism avoids desiccation through one or more of the following mechanisms, e.g. reduced respiration rate, increased tolerance to water loss, reduction of water loss (e.g. decreased cuticular permeability), increase in body size (increased body water

content) or via the management of water loss by lowering the critical water content (Chown and Nicolson, 2004; Gray and Bradley, 2005; Benoit and Denlinger, 2010; Weldon et al., 2016; de la Vega and Schilman, 2017). Desiccation tolerance is characterized by an organism's ability to withstand the loss of a significant proportion of its body water (Suemoto et al., 2004; Benoit et al., 2007; Guedes et al., 2015; Pallarés et al., 2016). Desiccation tolerance has been reported to significantly vary across clines (Gibbs and Matzkin, 2001; Guedes et al., 2015), sex (Benoit et al., 2005; Tejeda et al., 2014), developmental stage and species (Weldon et al., 2013b; 2016). Indeed, differences have been observed in desiccation tolerance across congeneric species inhabiting different precipitation environments (Strachan et al., 2015). Similar studies showed that *Ceratitis capitata* (Wiedemann) was more desiccation resistant than congeneric *Ceratitis rosa* (Karsch) owing to higher lipid reserves, serving as metabolic water (Weldon et al., 2016). In addition, *Drosophila* species from more xeric environments exhibited lower metabolic rates and cyclic carbon dioxide emission, likely reducing respiratory water loss (see Gibbs et al., 2003; Weldon et al., 2016). Increased body size also improved desiccation resistance in *Anastrepha ludens* (Loew) (Tejeda et al., 2014) and terrestrial ants (Hood and Tschinkel, 1990; Bujan et al., 2016). Furthermore, desiccation resistance decreased with age in *Bactrocera tyroni* (Froggatt) (Weldon and Taylor, 2010) and varied across entire ontogeny (Benoit et al., 2007). However, to our knowledge, no work has looked at the variation in water balance and desiccation resistance across *T. absoluta* developmental stages, despite being significant in explaining species distribution. Explaining the role of water stress in insect ecology may help explain species biogeography and is significant for pest risk modelling.

Tuta absoluta is a highly polyphagous invasive pest of tomato that has extensively spread its global distribution occupying new niches (Pfeiffer et al., 2013; Mutamiswa et al., 2017; Biondi et al., 2018). A comparison of precipitation patterns between its native and invaded African regions showed that the native environments experience higher precipitation than Africa (see Tarusikirwa et al., 2020a). Modeling studies have shown that although temperature and hosts are critical determinants of *T. absoluta* successful invasion, RH may have the strongest influence (Tonnang et al., 2015; Guimapi et al., 2016; Santana et al., 2019). Various studies have shown that desiccation resistance indeed plays a pivotal role in successful invasion and niche partitioning (Weldon et al., 2016; Pallarés et al., 2016; de la Vega and Schilman, 2017; Kellermann et al., 2020). Although *T. absoluta* has shown a high invasion potential under changing and diverse

environments, to our knowledge, no work has investigated its water balance and desiccation resistance. Research has only focused on basal temperature tolerance and plastic responses (see e.g. Machekano et al., 2018; de Campos et al., 2020; Tarusikirwa et al., 2020b). Nevertheless, high temperature and desiccation stress are co-occurring stressors and thus both warrant investigation, given increasing incidence of both stresses with climate change (see Engelbrecht et al., 2015). Thus, the goal of this study was to explore eco-physiological water balance and desiccation resistance traits across different *T. absoluta* developmental stages and provide in-depth knowledge on how these life stages survive under xeric environments, as well as in the context of novel invasion environments. We tested water balance, desiccation resistance and metabolic water across *T. absoluta* developmental stages to make inference on its invasion success and realized geographic niche. Due to its prolific spread, we hypothesize that *T. absoluta* is highly desiccation resistant and that this may have a contributory role (among other factors) in its current invasiveness in arid/semi-arid African regions. A favorable result will give insight on *T. absoluta* niche partitioning and explain biogeographic patterns. This information is highly useful in models predicting species distribution and can help in pest risk assessments.

5.2. Materials and Methods

5.2.1. Insect culture

Wild populations of *T. absoluta* larvae were collected from infested tomato plants at Genesis Farm (S21°8'54.239"; E027°38'48.42") in Matshelegabedi village, North East District of Botswana. *Tuta absoluta* first instar larvae tunnel into and feed through leaf mesophyll tissue. Symptomatic plants, infested tomato leaves and branches were therefore handpicked and taken to the laboratory, where larvae were later collected using soft forceps. To maintain uniform environmental history, all test insects were collected in austral summer (January - February 2020). Larvae were reared in the laboratory according to Tarusikirwa et al. (2020b), i.e. on organically produced excised fresh tomato leaves inside Bugdorm rearing cages (240cm³; Bugdorm - BD43030F, Megaview Science Co., Ltd, Taiwan). They were reared under optimum conditions, 28°C, 65 ± 10% RH and 12L:12D photocycle in climate chambers (HPP 260; Memmert GmbH + Co.KG, Germany) until adult emergence (for adult treatment groups). For all the experiments 2nd, 3rd and 4th instar larvae and 24h - 48h old adults (eclosed from field collected larvae) were used. These two developmental stages were chosen because (1) the larval stage is the key destructive developmental stage of

tomato plants while (2) adults are responsible for propagation. Larval instar stage was determined from measuring body length and head capsule following methods by Rasheed et al. (2018) (see also Table 5.1).

Table 5.1. *Tuta absoluta* instar body length and head capsule measurements (Rasheed et al., 2018).

Instar stage	Body length (mm)	Head capsule (mm)
2 nd	2.66 ± 0.04	0.30 ± 0.03
3 rd	4.22 ± 0.06	0.38 ± 0.03
4 th	7.59 ± 0.09	0.58 ± 0.04

5.2.2. Basal body water content

Body water content was determined gravimetrically for larval instars as well as adults. Ten individual insects from different larval instars and adults' treatments were individually placed in pre-weighed 2 mL Eppendorf tubes and weighed (to 0.1mg) on an analytical balance (AS220.R2, Radwag, Poland) to determine the initial body mass (wet mass). Initial mass of each organism was calculated by subtracting the empty tube from total tube mass (i.e. tube + insect). Thereafter, the insects were dried in a fan-forced laboratory oven (UF160, Memmert, Germany) at 60 °C for 48h and weighed to determine dry mass. This was repeated thrice per treatment group (i.e. one replicate run comprised 10 individuals). Basal body water content was calculated as the difference between initial body mass and dry mass.

5.2.3. Body lipid content

Ten individual insects from each developmental stage (larval instars and adults) were placed in pre-weighed 2 mL Eppendorf tubes, weighed (to 0.1mg) and dried in an oven set at 60 °C for 48 h. Body water content was then calculated as specified in the above section. Thereafter, 1.5 ml of diethyl ether was added to each tube, and the tubes were gently agitated at 250 rpm for 48 h at 37 °C using an orbital shaking incubator (MaxQ 4450, Thermo Fisher Scientific, USA). Subsequently, the diethyl ether was decanted out and the insects were dried at 60 °C for 24 h, and reweighed. This process was replicated thrice per treatment to yield 3 replications with ten insects each). The lipid content of each insect was calculated by subtracting the lipid free dry mass from the initial dry mass, following methods by Ballard et al. (2008).

5.2.4. Basal water loss rates

To assess water loss rates, ten individuals for each life stage were individually placed into perforated 2mL Eppendorf tubes and their initial mass determined (see section on body water content measurement for details). The insects were then placed in a climate chamber set at optimum temperature (28°C) in a modified RH ($5 \pm 2\%$) using silica gel (150g) for different time durations, (6, 12, 24 and 48 h) in a glass-sealed desiccator. Thermocron iButtons (Dallas Semiconductors, Model DS1920) were used to monitor the RH inside the desiccator at 30-min intervals. After each respective time duration (6, 12, 24 and 48 h), each of the replicate treatment insects were weighed (to 0.1 mg) to calculate weight loss. Insects were used only once per treatment and discarded before commencing the next treatment with fresh ones (i.e. the same individual was not measured at different time points). Insects were then dried and weighed to determine body water and dry mass after each respective treatment. This was repeated thrice for each time duration/treatment to yield sample sizes of $n = 30$ per duration/treatment. The same procedure was conducted for the control insects; however, they were maintained at optimum conditions, i.e. 28°C, $65 \pm 10\%$ RH. Water loss rate (mg hour^{-1}) was calculated by subtracting final mass from initial body mass divided by the time (experimental duration in hours).

5.2.5. Desiccation resistance

Desiccation resistance assays were assessed following an established protocol by Weldon et al. (2016). Initial mass of each replicate insect was determined following methods outlined in the body water content section (see above). Insects from each treatment were then exposed to a low RH treatment i.e. $5 \pm 2\%$ ($n = 30$ per treatment group). Relative humidity was manipulated by placing 150g of silica gel inside an airtight glass desiccator. Thereafter, the insects were maintained at optimum temperature (28°C) for the entire duration of the experiment in a climate chamber. Control insects were maintained in a climate chamber under benign conditions, i.e. 28°C, $65 \pm 10\%$ RH. Insects were checked for mortality after 24, 48 or 72 h intervals. Desiccation resistance was thus examined with respect to mortality rates following different exposure durations.

5.2.6. Data analyses

Body water and lipid contents were analysed using one-way analysis of variance (ANOVA) as functions of life stage. Proportional BWC and BLC, i.e. (1) basal body water (g) divided by wet mass (g), and (2) body lipid (g) divided by insect dry mass (g) were similarly analyzed using one-

way ANOVA. Three-way ANOVA was used to examine water loss rates as a function of life stages, treatments and durations, including all possible interactions. Proportional survival of different life stages among treatments and durations was analysed using generalised linear models (GLMs) assuming a binomial error distribution and logit link, with all possible interactions. Bias reductions were used in GLMs owing to complete separation among certain treatments (Kosmodis, 2020). Shapiro-Wilk and Levene's tests were used to ensure residuals met assumptions of normality and variances were homogenous in all ANOVA models (Fox and Weisberg, 2019). Non-significant terms were removed backward stepwise to obtain the most parsimonious model in all cases. Following Crawley (2007), non-significant terms were thus removed sequentially starting with the highest-order interaction, in order of significance, such that the final model included only significant terms. Tukey adjustments were used for all models to compute pairwise post hoc tests where terms were found to be statistically significant at the 95% confidence level (Length, 2020). All analyses were undertaken in R v4.0 (R Core Team, 2020).

5.3. Results

5.3.1. Body water and lipid contents

Body water content was significantly different ($F = 14.94$; d.f. = 3, 8; $P = 0.001$) among life stages. It ranged from 0.08mg to 1.07mg across tested developmental stages. Second instar larvae had the lowest body water content (0.16 ± 0.06 mg) (Fig. 5.1a) and levels were significantly lower than 4th instars ($P < 0.001$) and adults (both $P = 0.01$), but not 3rd instars ($P = 0.19$). Fourth instar larvae exhibited highest body water content (0.84 ± 0.12 mg), and these were significantly higher than 2nd ($P < 0.001$) and 3rd instars ($P = 0.01$), but not adults ($P = 0.19$) (Fig. 5.1a). In contrast, proportional BWC was highest in the adult life stage, with results significantly differing among life stages ($F = 14.90$; d.f. = 3, 8; $P = 0.001$). Adult exhibited significantly higher proportional BWC than 2nd ($P = 0.002$), 3rd ($P = 0.002$) and 4th ($P = 0.01$) instars. There were no significant differences recorded among the all the three larval instars tested (Fig 5.1c).

Body lipid contents differed significantly ($F = 22.82$; d.f. = 3, 8; $P < 0.001$) across life stages ranging from 0.15 mg to 0.74 mg, with 2nd instars exhibiting lowest levels that differed significantly from 4th instar ($P < 0.001$) and adult stages ($P = 0.02$), but not 3rd instars ($P = 0.06$) (Fig. 5.1b). Fourth instars displayed significantly highest body lipid content (0.69 ± 0.05 mg), that was significantly greater than 2nd instars ($P < 0.001$), 3rd instars ($P = 0.004$) and adults ($P = 0.01$)

(Fig. 5.1b). Adults and 3rd instars were not statistically different ($P > 0.05$). Proportional BLC also differed significantly among stages ($F = 12.63$; d.f. = 3, 8; $P = 0.002$). The smallest 2nd instar larvae recorded the highest proportional BLC and was not significantly different from adults ($P = 0.08$) but differed from 3rd ($P = 0.002$) and 4th ($P = 0.005$) instars. Third instar larvae recorded the lowest proportional BLC, which was, however, not significantly different from 4th instar larvae ($P = 0.93$) or adults ($P = 0.11$).

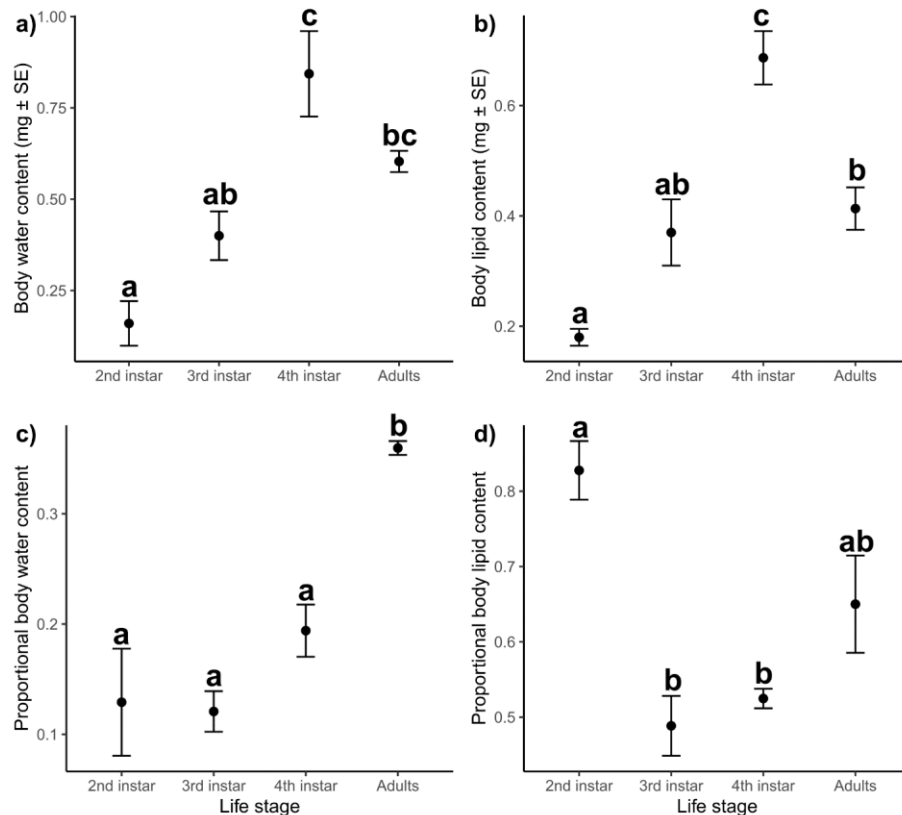


Figure 5.1. *Tuta absoluta* basal body water content (a) and body lipid content (b), for larvae (2nd, 3rd, 4th instars) and adults, (c) proportional body water content, (d) proportional body lipid content. Bars represent mean $\pm$ SE (n=3 replications per treatment group).

5.3.2. Water loss rates and desiccation resistance

Water loss rates were predicted by two significant interaction effects between life stage and treatment ($F = 10.56$; d.f. = 9, 76; $P < 0.001$) and life stage and duration ($F = 5.94$; d.f. = 9, 76; $P < 0.001$). Desiccation pre-treatment significantly increased water loss rates in 2nd instars ($P = 0.03$), 3rd instars ($P < 0.001$) and adults ($P = 0.01$), but 4th instars had significantly higher water loss in

controls ($P = 0.01$). In turn, the effects of duration on water loss rates differed among life stages, where effects were strongest at shorter durations (Fig. 5.2a). Following 6 h, control 4th instars had significantly highest water loss rates (1.03 ± 0.09 mg/h) compared to all other stages (all $P < 0.001$); 3rd instars had significantly greater water loss rates than 2nd instars and adults (both $P < 0.001$); but 2nd instars were statistically similar to adults ($P = 0.53$). Similarly, after 12 h, increasing instar stage always caused a significant increase in water loss (all $P < 0.05$), but adults had significantly lowest water loss (-0.028 ± 0.07 mg/h) compared to all immatures (all $P < 0.01$). After 24 h, 4th and 3rd instars had significantly higher water loss rates than 2nd instars and adults (all $P < 0.01$); 2nd instars were similar to adults ($P = 0.89$), while 4th instars were similar to 3rd instars ($P = 0.98$) (Fig. 5.2a). Lastly, after 48 h, life stages were more similar, with water loss rates by controls and desiccated 4th instars only significantly greater than adults ($P = 0.01$) (Fig. 5.2a).

Mortality was significantly higher at longer experimental durations overall ($X^2 = 69.54$; d.f. = 2; $P < 0.001$), increasing incrementally with greater durations (all $P < 0.05$) (Fig. 5.2b). The effects of treatment significantly interacted with life stage ($X^2 = 26.49$, d.f. = 3; $P < 0.001$), with desiccation only causing significant mortality compared to controls at the 2nd instar juvenile stage ($P < 0.001$), whilst other instar stages were not significantly affected (3rd instar: $P = 0.20$; 4th instar: $P = 0.49$) (Fig. 5.2b). For adults, there was zero mortality in control groups, and treatment significantly increased mortality ($P = 0.01$) (Fig. 5.2b). Overall, consistent with the body water content and body lipid content, 4th instar larvae were significantly more desiccation resistant than other instar stages. Conversely, 2nd instar larvae were the most vulnerable to desiccating conditions (Fig. 5.2b).

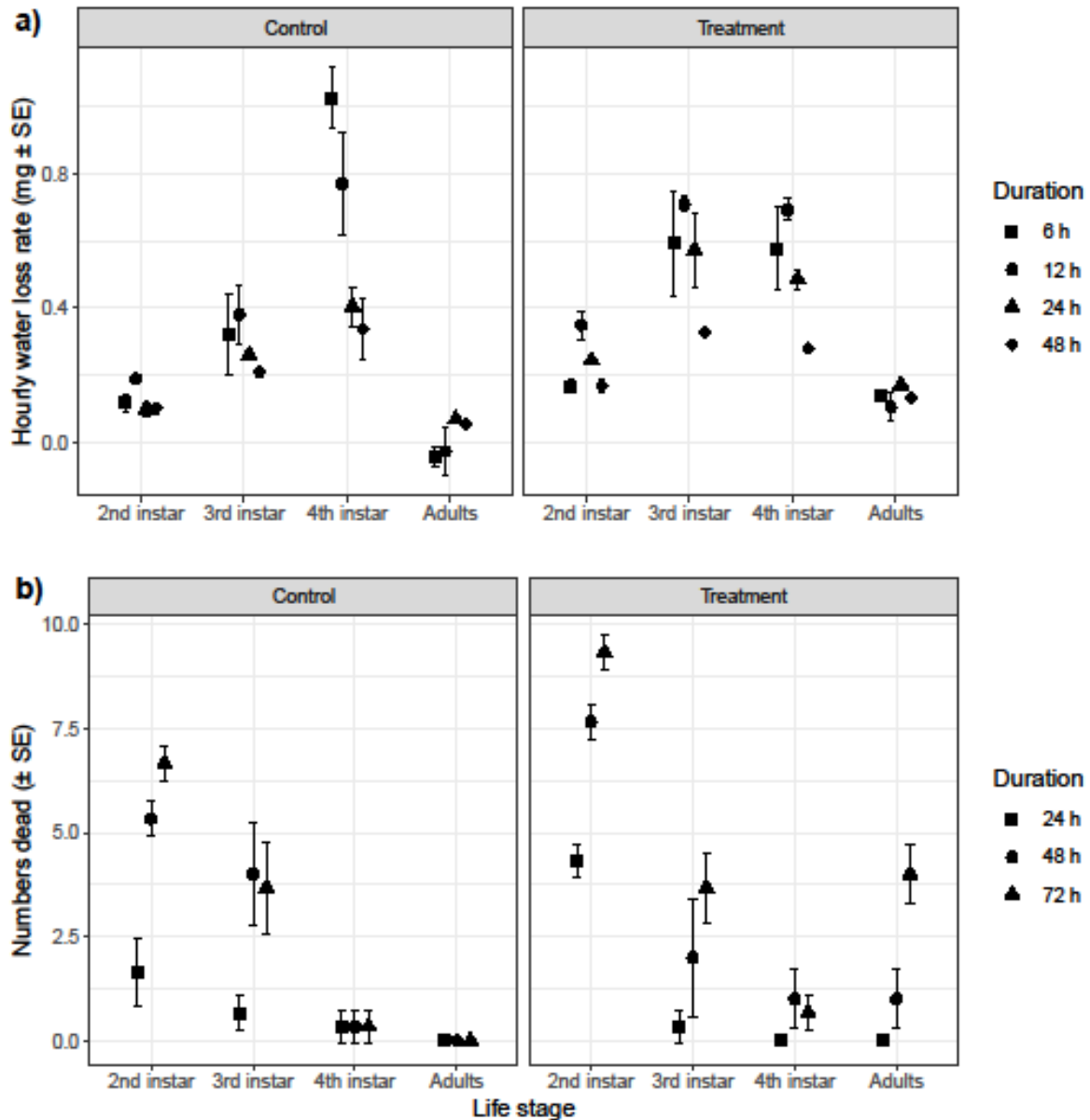


Figure 5.2. Water loss rates (a) and desiccation resistance (b), for *T. absoluta* larvae (2nd, 3rd, 4th instars) and adults between control and treatment groups. Bars represent mean $\pm$ SE (n=3 replications per treatment group).

5.4. Discussion

Increasing environmental stress, such as overlapping high temperature and low precipitation, threaten the survival of many organisms (Parmesan, 2006). For insects, this is true since temperature and RH are the most important abiotic factors affecting organismal function (Chown and Nicolson, 2004). Insect species differ in their level of resistance to desiccation in many

complex ways, with consequent effects on fitness (Chown et al., 2011; Kleynhans et al., 2014; Guedes et al., 2015; Kellermann et al., 2018). Here, we show that various developmental stages of *T. absoluta* exhibited different levels of desiccation resistance and water balance traits, indicating that low precipitation environments may differentially affect *T. absoluta* life stages, and that not all stages contribute equally to invasion success in arid environments. Specifically, our results show that (1) body water content and body lipid content increased with age amongst larval instars, with 2nd and 4th instar larvae recording the lowest and highest body water content/body lipid content, respectively. Second, adults recorded the lowest water loss rates across the developmental stage treatments, albeit at 48h, differences were less statistically clear. Third, we show that 4th instar larvae consistently exhibited higher desiccation tolerance than other larval instars, but a similar tolerance compared to adults. These results suggest that 4th instar larvae may be the most desiccation tolerant instar stage and may play a substantial role in population persistence under low precipitation environments, while adults also had low water loss rates across the treatments.

The increase in body water content with body size in larval instars recorded here is not surprising. Small body size limits body water reserves and increases evaporative water loss due to a higher surface area to body mass ratio (Krupp et al., 2020). Differences in body water content with increasing instar size recorded here may thus be attributed to body size. The water content of terrestrial insects may vary both within and between species (Edney, 1977). Adults recorded the highest proportional BWC, while no significant differences were noted among the larval instars. This finding is in contrast with Benoit et al. (2007) who reported higher body water content in 3rd instar larvae of *Beligica antarctica* relative to 4th instars. Previous reports showed that more desiccation-resistant ants were significantly larger (Bujan et al., 2016), a result that supports the current finding. Low body water content of the early larval stages makes them more vulnerable to desiccation stress. Furthermore, results from adult tephritid flies also showed higher desiccation resistance with increased body size (Weldon and Taylor, 2010; Weldon et al., 2013a; 2016). However, insects also compensate through behavioural adaptations and microclimates may buffer them to stressful environments (Princebourd and Woods, 2020). This may be true for 1st and 2nd instar *T. absoluta* larvae, which mine through the leaf mesophyll and can be buffered from more desiccating outside environments. A comparison of 4th instar larvae and adults showed that 4th instars had higher body water content than adults. This result is consistent with Yoder et al. (2003) who reported higher body water content in *Stethorus nigripes* (Kapur) larvae than adults. Higher

body water content of 4th instar larvae of *T. absoluta* may also be attributable to its tomato fruit diet. This developmental stage tends to move from tomato leaves to fruits, where moisture is readily available. This is the most damaging stage of the pest, as the larvae invests in nutrition in preparation for pupation. Thus, it is highly likely that 4th instar larvae obtain a significant amount of their body water content from the fruits (Noble-Nesbitt, 1990). Adults, on the other hand, may have the ability to seek microclimates through flight. Proportional BWC was highest in adults than the larval instars (which were not significantly different from each other). The environment inhabited by adults may be drier than that of larvae, which thrives in fleshy plant stems, leaves and fruits. Thus, it is not surprising that adults would invest much of their body size on water while larvae would trade off proportion of BWC for other potentially significant fitness-defining resources. This means increased BWC may be a trait strongly selected for evolutionary adult fitness under arid terrestrial environments.

The importance of epicuticular lipids has been clearly demonstrated across many insect species (Hadley, 1989; Gibbs, 2011; Krupp et al., 2020). Insect epicuticular lipids comprise primarily of hydrocarbons whose composition varies at all levels of insect organization, from species to individual level (Krupp et al., 2020). These cuticular hydrocarbons play a pivotal role in reducing water loss (Benoit et al., 2007) by minimizing evaporative water loss. Furthermore, hydrolysis of these lipids also produces metabolic water, a significant mechanism used by insects in surviving desiccation environments (Fraenkel and Blewett, 1944; Downer and Matthews, 1976). Body lipid content results were consistent with those of body water content, increasing with age amongst larval instars and differing significantly across life stages tested. Second and 4th instar larvae showed significantly lowest and highest body lipid content respectively, while adults were intermediate. In contrast, 2nd instar larvae and adults recorded the highest proportional BLC. This result suggests that *T. absoluta* body lipid content is developmental stage-specific. Smaller developmental stages may invest more on lipids as metabolic water to compensate for their inability to accommodate large BWC. Such high lipid content relative to body size may lead to possible trade-offs between BLC with e.g. BWC. This water balance adjustment may facilitate their survival in dry environments amidst their low BWC and higher surface area to volume ratios. Dehydration-resistant arid and semi-arid insects usually have higher lipid content or a heavily waterproofed cuticle that serves to reduce water loss, and also serves as metabolic water (Benoit et al., 2007). In the current study, 4th instars displayed highest body lipid content and desiccation

resistance indicating the significance of lipids in reducing cuticular water loss. This result confers a survival advantage for 4th instar larvae relative to other instar stages under desiccating environments. Tarusikirwa et al. (2020c) further reported that *T. absoluta* 4th instar larvae were more cold hardy than adults. Given the relationship between low temperature and desiccation stress tolerance (Sinclair et al., 2013), the current results may further sustain *T. absoluta* 4th instar larvae under low temperature stress. Given higher body water content and body lipid content of 4th instar larvae, our results thus suggest that this developmental stage achieves greater water balance through maintaining higher body and metabolic water relative to other developmental stages.

Fourth instar larvae showed reduced water loss rates following treatment relative to the other larval instars and adults. This result is in keeping with Yoder et al. (2003) who reported adult *S. nigripes* exhibiting lower water loss rates than larvae. The 4th instar larvae also lost more water in the control treatment as compared to desiccation pre-treatment. This reduction in water loss following desiccation pre-treatment could be attributed to desiccation plasticity, where a non-lethal exposure to desiccation stress can increase resistance to a subsequent exposure (Kellermann et al., 2018). Rapid desiccation hardening is associated with changes to cuticular permeability. Incidentally, the 4th instar recorded the highest level of body lipid content. Given that lipids are one of the main components that make up the cuticle, the different water loss rates could be attributed to differences in cuticular thickness and permeability (Appel et al., 1991; Mazer and Appel, 2001). This is in agreement with Gibbs et al. (1997) who found that different desiccation resistant lines of *Drosophila melanogaster* (Meigen) have reduced water permeability of the cuticle as a consequence of altered lipid composition, among other factors. The cuticle is the first portal of water loss in insects and the differential desiccation tolerance patterns in other related insects such as *Polypedilum vanderplanki* (Hinton) vs. *Paraborniola tonnoiri* (Freeman) and *Chicoreus ramosus* (Linnaeus) vs. *D. melanogaster* have been attributed to differences in cuticular thickness and thus permeability (Nakahara et al., 2008; Thorat et al., 2017). The trends in water loss rates were, however, seldom not very apparent. For example, 2nd instar larvae water loss rates were highest at 12 h of exposure to desiccating conditions. The rate of water loss began to reduce at 24 h and reduced further at 48h. The same trends were observed in both the 3rd and 4th larval instars. The reduced water loss rates following more prolonged duration to desiccating environments may offer desiccation preconditioning and thus show a potential time course to response for desiccation

acclimation (Hoffmann, 1990). A study by Hoffmann (1990) showed that *D. melanogaster* females developed a response to desiccation acclimation within a 2 h period of initial exposure to low RH. This acclimation response persisted for more than 28 h post-stress exposure. Insects can also experience a decrease in the respiratory water-loss via a reduced metabolic rate (Kellerman et al., 2018). A reduction in metabolic rate can therefore account for the reduced water loss rates after a longer exposure to dry environments. Desiccation acclimation of *T. absoluta* developmental stages, including the time course of eliciting positive acclimation responses and reversal, thus warrants future investigation. Nevertheless, the present study shows that *T. absoluta* may have acclimatory ability to desiccation conditions as water loss rates decreased with treatment duration; however, water loss rates tended to peak for desiccated larvae at 12 h. Osmoregulatory mechanisms in similar lepidopteran species have also demonstrated the homeostatic control to readjust hemolymph osmolality triggered by body water loss (Willmer, 1980; Thorat and Nath, 2018). Thus, future studies may assess the osmoregulatory system of *T. absoluta* that underpins the mechanisms contributing to reduced water loss rates reported here. Overall adults exhibited lower water loss rates across all developmental stages tested. Desiccation resistance for this developmental stage is particularly important as the insect often spreads in the adult form through flight assisted by wind currents. This adult reduced water loss rates may thus facilitate long term dispersal and successful establishment in novel arid and semi-arid environments (Mutamiswa et al., 2017).

Desiccation resistance amongst larval instars increased with age such that 3rd and 4th instars exhibited lower mortality than 2nd instars, albeit 4th instars and adults were significantly the most desiccation resistant. This result among juveniles may be associated with higher body water content and body lipid content linked with the 4th instars, that helps in desiccation resistance (Weldon et al., 2013a). Desiccation resistance in adults also decreased with greater duration of exposure, with highest level of mortality recorded after 72 h. Indeed, mortality at stressful environments is a function of the interaction between time and severity of the stressful conditions (Chown and Nicolson, 2004). This thus implies that prolonged exposure to desiccating conditions, typical under current changing climates (see e.g. Parmesan, 2006; Chown et al., 2011; Engelbrecht et al., 2015), will negatively influence *T. absoluta* survival and population dynamics. A comparison of 4th instar larvae and adults showed larvae were slightly more desiccation resistant than adults at the longest duration. This shows that larvae may have a physiological edge to adults

under increasingly dry arid and semi-arid environments. Similar trends have been reported for the two developmental stages, albeit for traits of low and high temperature (see Tarusikirwa et al., 2020b). Nevertheless, contrary to our findings, Weldon et al. (2016) found that adult physiological traits may play a bigger role in desiccation resistance in *Ceratitis* species. This therefore point to (1) species and (2) developmental stage-related differences in responses to desiccation stress. Although this study did not investigate immobile developmental stages (eggs and pupae), we strongly feel the results for mobile life stages (larvae and adults) reported here represent the stages that are highly economically impactful as they contribute directly to plant damage (larvae) and reproduction (adults). However, future studies should look into ontogenetic water balance and desiccation resistance in this invasive insect pest, and also measure other life history traits, e.g. BLC following desiccation acclimation. In addition, while sex was not considered in the current study, future studies should investigate sex related effects on water balance.

In conclusion, our results document developmental stage differences in desiccation resistance and water balance in *T. absoluta* and they have significant implications for its invasion potential. First, we show that body water content and body lipid content consistently increased with age, and that 4th instar larvae had the highest body water content and body lipid content. Second, we show that water loss rates varied across developmental stages, albeit the trends became less apparent through time, with adults tending to show the lowest water loss rates across the developmental stages. Third, our results document that 4th instar larvae and adults had higher desiccation resistance than other larval stages and may thus play pivotal role in invasion success in arid environments. The differential desiccation resistance and water loss rates amongst *T. absoluta* developmental stages indicates that water balance in this invasive species is life-stage dependent. However, 4th larval instar, the most economically damaging pest stage, is more desiccation tolerant and may thrive more under dry conditions. Further, the resistance of adults may aid aerial dispersal and spread to novel environments. Coupled with lower water loss rates of adults, 4th instar larvae and adults may thus substantially contribute to population persistence in arid environments. More studies should investigate the osmoregulatory mechanisms employed by *T. absoluta* in desiccation resistance, plastic responses to desiccation and the time-course of these plastic responses. Incorporation of these species and developmental stage-specific eco-physiological traits in predictive models can help refine invasive species potential biogeography under changing climates and is vital for elucidating pest insect invasions under changing climates.

5.5. References

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CHAPTER 6

Acclimation stress and life stage-dependent integrated stress resistance may heighten invasiveness in the global pest invader, the South American tomato pinworm

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6.1. Introduction

Anthropogenic climate change has generically been associated with increased mean temperatures and variability (Stillman, 2019; Xu et al., 2020). However, increased global temperature extremes also likely elicit additional abiotic environmental stressors such as desiccation and food limitation (Nguyen et al., 2017) as well as pathogens (Hector et al., 2021) in natural and managed ecosystems. Given that most terrestrial arthropods often experience these stressors across space and time in nature, the ability to mount compensatory mechanisms is fundamental for fitness and survival under changing environments (Kelley, 2014; Renault et al., 2015; Kalra et al., 2017; Mutamiswa et al., 2018). Indeed, the global invasiveness and distribution of insects may be driven by mounting a diverse set of responses against environmental stress in novel environments (Renault et al., 2015). Chief among them include inherent basal and plastic thermal tolerance (Kelley, 2014), mutualistic interactions with the biotic environment, metabolic flexibility and others (reviewed in Wan and Yang 2016; Smit et al., 2021) as well as integrated stress resistance (see e.g. Gotcha et al., 2018; Hector et al., 2021; Ricciardi et al., 2021). As a result, invasion success is dependent on an invader's ability to pass through the 'ecological filters' (i.e. biotic and abiotic) of its recipient environment (Crowl et al., 2008; Kelley, 2014) and indeed positive interactive effects across diverging stressors.

As invasive species move from their native regions expanding into novel territories, they often experience various stressors concurrently (Richardson et al., 2000). For example, temperature extremes and the temporary deprivation of food or water can have downstream impacts on organismal performance and fitness. Hence, tolerating these stressors is essential for optimising fitness for insects inhabiting environmentally heterogeneous landscapes (Mitchell et al., 2017; Nguyen et al., 2017). The persistence of these populations after introduction in dynamic and multivariate environments greatly depends on their ability to deal with the interactive effects of different stressors occurring simultaneously or sequentially over short to longer timescales (Gunderson et al., 2016). These interactive stressors may influence insects in an additive (linear effects), synergistic or antagonistic (non-linear) way (Todgham and Stillman, 2013; Cheng et al., 2015). One of the mechanisms that may elicit non-linear effects is cross-tolerance/integrated stress resistance (ISR), a physiological adaptation where tolerance to one environmental stressor results in increased tolerance to another stress (Sinclair et al., 2013; Cheng et al., 2015). This likely shows

co-evolution to stress resistance mechanisms (Kalra & Parkash, 2016; Pallares et al., 2017; Gotcha et al., 2018), and reportedly contributes towards invasion success of some alien insect species (Renault et al., 2015). Conversely, non-linear effects may be derived from cross-susceptibility, where an exposure to one stressor may have no or negative response effect on another stress (Sinclair et al., 2013; Nguyen et al., 2017). Cross tolerance responses indicate common mechanisms underlying tolerance to different types of stress and (e.g. Heat shock protein (Hsps) (Lee et al., 2004; Gracey et al., 2008; Kelley, 2014) or shared regulatory or signalling pathways that activate separate mechanisms of protection against different stresses i.e. ‘cross talk’ (Sinclair et al., 2013; Renault et al., 2015). While Hsps were generally considered to be expressed in response to thermal stress, increasing evidence suggest their expression indeed vary and in response to other non-thermal stress factors e.g. desiccation and starvation stress (Eckwert and Köhler, 1997; Pyza et al., 1997; Köhler et al., 1999; Knigge and Köhler, 2000; Köhler et al., 2000; Karouna-Renier and Zehr, 2003; Arts et al., 2004; Haap and Köhler 2009; Jiang et al., 2012; Haap et al., 2016; Augustyniak et al., 2017).

Cross tolerance is considered adaptive and optimizes fitness in dynamic multivariate environments (Pallares et al., 2017). In seasonally and temporally varying climate environments, insects often survive sub-optimal climatic conditions through within and across generational acclimation to different environmental stressors (Chown and Nicolson, 2004; Chown et al., 2011; Harrison et al., 2012; Sgró et al., 2016; Kalra et al., 2017). Cross tolerance is thought to be widespread in insects, particularly with regards to environmental stressors that concomitantly occur. For example, low temperatures and desiccation, longevity and starvation and desiccation and high temperature stresses (Ring and Danks, 1994; Harshman and Hoffmann, 2000; Bayley et al., 2001; Wu et al., 2002; Vermeulen and Loeschcke, 2007; MacMillain et al., 2009). However, cross tolerance responses may differ with species and thus the interactive effects of one stress on different phenotypes for a single species cannot be generalized across different taxa. Thus, more studies are needed to determine the ISR in insects and how it may help predictive models for species distributions.

Acclimatization (coordinated phenotypic response to a specific stressor under field conditions) of ectothermic organisms under natural environments often involves the joint impact of multiple stressors (Schou et al., 2015; Gotcha et al., 2018). Studies have also shown positive correlations

between resistance to cold and desiccation as well as between resistance to heat and desiccation (Bayley et al., 2001, Wu et al., 2002, Phelan et al., 2003, Bubliy and Loeschcke, 2005; Hayward et al., 2007; Singh and Prasad, 2020). Cold and desiccation have many similar effects at a cellular level and mechanisms that protect against cold stress also protect against desiccation. Therefore, responses to cold and dehydration stress likely evolved as cross-tolerance interactions among regulatory pathways (i.e. cross-talk) (Sinclair et al., 2013). Both stressors result in similar injuries and physiological challenges, including reduction of the fluidity, and thus stability and function of plasma membranes (Bayley et al., 2001). Many physiological mechanisms of seasonally acquired tolerance to cold and desiccation overlap, e.g. upregulation or production of molecular chaperone proteins (Rinehart et al., 2007) and cryoprotectants (i.e. cryoprotective dehydration) (Kostal et al., 2007; Rinehart et al., 2007), as well as modification of the structure of cellular membranes (Holmstrup et al., 2002; Sinclair et al., 2013). Studies have also found positive associations between desiccation stress and heat tolerance (Benoit et al., 2009a; Bubliy et al., 2012), largely attributed to the upregulation of Hsps (Feder and Hofmann, 1999).

Intermittent food shortages are commonly encountered in the environment (Zhang et al., 2019), and seldom simultaneously with heat stress. Nutritional stress may influence thermal tolerance primarily through reductions in body lipid- (Mitchell et al., 2017) or sugar levels (Nyamukondiwa and Terblanche, 2009). Reductions in lipid levels are generally associated with starvation across all organisms (McCue, 2010), thus lipid levels and the composition thereof are thought to underpin the link between starvation and thermal tolerance (Mitchell et al., 2017). The reduction in lipid levels may result in increased water uptake (e.g. Raubenheimer and Gäde, 1993) which can be beneficial for improving heat tolerance by increasing the water stores available for evaporative cooling or generally withstanding desiccation stress (Chown and Nicholson, 2004; Mitchell et al., 2017). For example, Gotcha et al. (2018) found an improvement in heat tolerance following starvation of *Ceratitis rosa*. Thus, metabolic flexibility is one factor that may likely contribute to insect invasiveness (see Smit et al., 2021) and its positive interaction with other stress factors may improve fitness and heighten invasive competitiveness in globally significant pest insect species.

Integrated resistance to environmental stress traits, or cross tolerance, has been observed in numerous invasive species. For example, the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), an invasive stored product beetle pest showed cross tolerance between traits of

desiccation and cold stress (Renault et al., 2015). This integrated resistance is believed to have facilitated its successful establishment in temperate areas e.g. Europe (Klejdysz and Nawrot, 2010). Another stored product pest the Khapra beetle, *Trogoderma granarium* is among the most cold hardy insect pests although it originates from the typically hot and dry regions of the Indian subcontinent (Fields, 1992; Wilches et al., 2017; Shivananjappa et al., 2020). Studies conducted on this pest suggest that the physiological mechanisms that protect diapausing larvae from desiccation may also increase cold tolerance, even though these insects may rarely be exposed to extreme low temperatures in their native environment (Shivananjappa et al., 2020). Similarly, desiccation and fasting acclimation improved cold tolerance in invasive *Chilo partellus* (Lepidoptera: Crambidae) (Mutamiswa et al., 2018). We thus speculate, with caveats that integrated stress resistance may, to a large extent increase insect pests' fitness and optimise key life history traits in environmentally heterogeneous natural and managed habitats of colonised areas (see also Renault et al., 2015) thereby facilitating invasion success (Renault et al., 2015).

The highly invasive *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) has rapidly spread throughout the globe and successfully established in both temperate and tropical regions (Desneux et al., 2010; Biondi et al., 2018; Tarusikirwa et al., 2020a; Zhang et al., 2020). Mechanisms behind its successful invasion are largely speculated, however, with the exception of a few empirical support (see e.g. Lietti et al., 2005; Roditakis et al., 2015; 2018; Wan and Yang, 2016; Machekano et al., 2018; Tarusikirwa et al., 2020b; 2020b). Studies have shown that the pest possesses most of the characteristics that have been observed in good invaders (see Kelley 2014; Wan and Yang 2016), e.g. high propensity to develop pesticide resistance (Lietti et al., 2005; Roditakis et al., 2015; 2018; Wan and Yang, 2016), phenotypic plasticity (Tarusikirwa et al., 2020b); high basal thermal tolerance (Machekano et al., 2018; Tarusikirwa et al., 2020b); facultative diapause (de Campos et al., 2020), host switch and metabolic flexibility (Bawin et al., 2015; reviewed in Tarusikirwa et al., 2020a) and high fecundity and propagule pressure (Guillemaud et al., 2015). However, even though the *T. absoluta* has successfully established in strikingly heterogeneous environments, no studies have looked into potential for integrated stress resistance to these common stress factors and traits. Cognisant of its economic importance, it remains unknown how multiple environmental stressors may influence its physiological fitness in the face of climate change. Here, I assessed the integrated resistance of *T. absoluta* traits of cold- critical thermal minima (CT_{min}), heat- critical thermal maxima (CT_{max}), desiccation and starvation -stress

following acclimation to each respective stressor. I hypothesise that in addition to beneficial acclimation (see Wilson and Franklin 2002), acclimation to one stress may offer protection to a totally different stressor (cross tolerance; see Sinclair et al., 2013). This phenomenon may likely explain the global invasiveness of *T. absoluta*. Incorporation of the capacity for integrated resistance in predictive models may help refine invasive species distribution and is vital for informing more consolidative pest management strategies.

6.2. Materials and methods

6.2.1. Insect culture

Natural populations of *T. absoluta* larvae were collected between April - June 2021 from infested tomato leaves/plants at Gurus Farm (S23°7'58.6614"; E27°30'51.6042"), Machaneng village in Central district of Botswana. In the laboratory, larvae were maintained according to Tarusikirwa et al. (2020b), i.e. fed on pesticide-free fresh tomato leaves (“Rodade”) inside Bugdorm rearing cages (240cm³ Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under optimum conditions, i.e. 28°C; 65±10% relative humidity (RH) and 12L:12D photocycle in climate chambers (HPP 260; Memmert GmbH + Co.KG, Germany). Acclimation and hardening trials were conducted differentially across the different life stages (see Table 6.1) and were randomized across treatments. This allowed for stress resistance tests to be conducted on insects of the same age, i.e. 3rd instar larvae and six-day old adults (eclosed from field collected larvae) were tested across all stress tests. Larval instar stage was determined from measuring body length and head capsule following methods by Rasheed et al. (2018).

Table 6.1. Pre-treatment age for *T. absoluta* larvae and adults for the pre-treatments (i.e. Rapid heat hardening = RHH; Rapid cold hardening = RCH; Desiccation; Fasting; Control).

Pre-treatment	Age	
	Larvae	Adults
RHH	3rd instar	6 days
RCH	3rd instar	6 days
Desiccation	3rd instar	4 days
Fasting	2nd instar	1day
Control	3rd instar	6 days

6.2.2. High temperature hardening

Third instar larvae and six-day old moths were placed in 30ml plastic vials (n=10). Hardening experiments were performed using established experimental protocols (e.g. Mutamiswa et al., 2018; Tarusikirwa et al., 2020b). Rapid heat hardening temperature was developmental stage dependant and was derived from CT_{max} (Hoffmann et al., 2003; Lee and Denlinger, 2010) and was defined as 7°C below CT_{max} (see e.g. Nyamukondiwa et al., 2011; Mutamiswa et al., 2018) (i.e. 41.7 °C for larvae; 38.5 °C for adults). This hardening temperature is generally adequate to elicit an RHH response in similar insects (Hoffmann et al., 2003). The insects were first temperature equilibrated in a climate chamber at 28°C and 65±10% RH for 30 min before being hardened for 1 h at high temperature inside a climate chamber set at 41.7 °C (larvae) and 38.5 °C (adults). Following hardening, organisms were given 1 h recovery time before measuring stress tolerance (see Fig. 6.1). Control organisms were maintained at optimum conditions (28°C; 65% RH) before measuring stress resistance traits (Fig. 6.1). A total of 100 organisms were hardened for both larvae and adults, respectively.

6.2.3. Low temperature hardening

Third instar larvae and six-day old moths were placed in 30ml plastic vials (n=10). Hardening experiments were performed using established experimental protocols (following e.g. Mutamiswa et al., 2018; Tarusikirwa et al., 2020b). Cold hardening temperature was derived from CT_{min} (Hoffmann et al., 2003; Lee and Denlinger, 2010) and was defined as 6°C below CT_{min} (Nyamukondiwa et al., 2011; Mutamiswa et al., 2018) (i.e. -4 °C – larvae; -6 °C – adults). These hardening temperatures are suffice to elicit RCH responses in similar organisms under laboratory experimental conditions. Larval and adult *T. absoluta* specimens were first placed in a climate chamber at 28°C and 65±10% RH for 30 min to allow for equilibration. Thereafter, the insects were placed in a zip lock bag and submerged in a programmed water bath (Systronix Scientific, Industria, South Africa) with 1:1 water: propylene glycol and hardened for 1 h. Following treatment, insects were allowed to recover under benign conditions (28°C; 65±10% RH) in a climate chamber for 1 h to allow the *de novo* Hsps synthesis (Hoffman et al., 2003) before measuring stress tolerance (Fig. 6.1). Control insects were kept at benign conditions during the same treatment before measuring stress tolerance. A total of 100 organisms were hardened for both larvae and adults, respectively.

6.2.4. Desiccation acclimation

Third instar larvae and four-day old moths were individually placed in 30ml perforated plastic vials (n=10). The vials were then placed in a modified humidity inside a desiccator. Humidity was modified to $1\pm1\%$ RH by placing 150 g of silica gel inside the desiccator. The desiccator was then placed in a climate chamber set at 28 °C for a period of 24 h. This duration was suffice to elicit 40% body water loss in test insects (e.g. see Tarusikirwa et al., 2020c). Thermocron iButtons (Dallas Semiconductors, Model DS1920) were used to monitor the temperature and RH inside the desiccator at 30-min intervals. After 24 h of desiccation stress, the organisms were removed from the desiccator and given a recovery period of 24 h with food and water at 28 °C; 65% RH before stress resistance tests. The control organisms were maintained under benign conditions before conducting stress tests (Fig. 6.1). A total of 100 organisms were subjected to desiccation acclimation for both larvae and adults, respectively, following methods by Gotcha et al. (2018).

6.2.5. Starvation acclimation

Second instar and one-day old moths were placed in 30ml perforated plastic vials (n=10). A damp cotton wad was placed inside the vial to prevent mortality associated with desiccation (Parkash et al., 2014). The organisms were then placed in a climate chamber under optimum conditions (i.e. 28°C; 65% RH) for 48 h (larvae) and 72 h (adults), with no access to food, i.e. “fasting acclimation”. After the respective acclimation durations of food deprivation, the organisms were given a 24 h recovery period (provided with water and food, at optimum conditions). Control organisms were sorted in the same way as treatment organisms, but were however, placed in 30ml vials with *ad libitum* access to food (tomato leaves for larvae and 10% sugar solution for adults) under optimum conditions for subsequent stress tolerance tests (see Fig. 6.1). A total of 100 organisms were subjected to fasting pre-treatment for both larvae and adults, respectively.

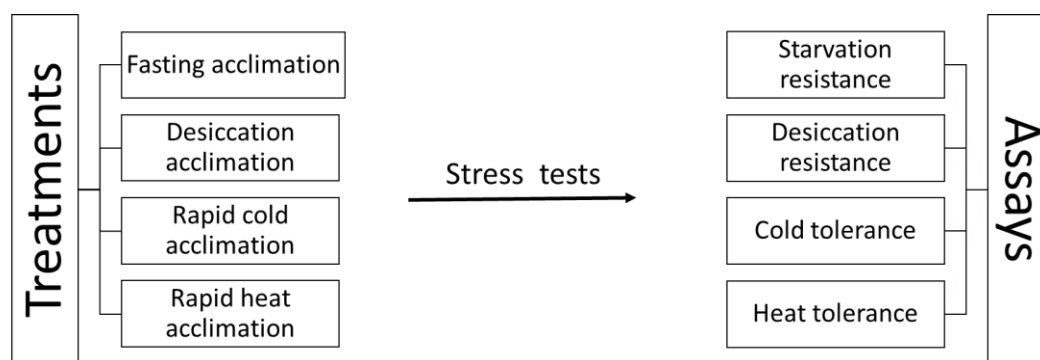


Figure 6.1. Schematic diagram showing the acclimation treatments and subsequent resistance and tolerance tests carried out to test potential cross tolerance in *Tuta absoluta*. Cold tolerance was measured using critical thermal minima (CT_{min}), while heat tolerance was measured using critical thermal maxima (CT_{max}).

6.2.6. Stress tolerance and resistance tests

After subjecting the organisms to their respective pre-treatment conditions, they were exposed to various stress tolerance/resistance tests to measure tolerance and resistance to high and low temperatures, desiccation and fasting, respectively, i.e. CT_{max} , CT_{min} , starvation resistance and desiccation resistance (Fig. 6.1).

Critical thermal maxima – CT_{max} were measured on *T. absoluta* larvae and adults using standardised protocols as outlined by Nyamukondiwa and Terblanche (2009). Ten replicate organisms were individually placed in a series of numbered ‘organ pipes’ connected to an insulated double-jacketed chamber which was linked to a programmable water bath before ramping up the temperature from a setpoint temperature of 28°C (10 minutes) at a rate of 0.25°C/min until their CT_{max} were recorded. This was repeated twice to yield sample sizes of $n = 20$ per pre-treatment. Critical thermal maximum was defined as the upper temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

Critical thermal minima – CT_{min} were measured using the same protocol as that of CT_{max} (Nyamukondiwa and Terblanche 2009) except that temperature was ramped down from a setpoint temperature of 28°C (10 minutes) at a rate of 0.25°C/min until the CT_{min} endpoint. Critical thermal

minimum was defined as the lowest temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

Desiccation resistance – Following the recovery period from the various acclimation pre-treatments, organisms were placed in desiccators with modified humidity, $1\pm1\%$ RH (150g silica gel). Thirty replicate organisms were used for each pre-treatment ($n=30$). Thereafter, the desiccators with test organisms were transferred and maintained at optimum temperature (28°C) for the entire duration of the experiment in a climate chamber. Control organisms were maintained in a climate chamber under benign conditions, i.e. 28°C , $65 \pm 10\%$ RH. Organisms were checked for mortality after 24, 48, 72 and 96 h intervals. Desiccation resistance was thus examined with respect to mortality rates following these different desiccation exposure durations.

Starvation resistance – Thirty replicate insects for each developmental stage exposed to pre-treatments were used for starvation resistance tests. Both treated and control organisms were placed into 30ml perforated plastic vials. The vials were then placed inside a climate chamber under optimum conditions and monitored for four days ($n=30$ pre-treatment). To avoid mortality associated with desiccation while ‘starving’ moist cotton wad was placed inside each vial (Bubliy et al., 2012; Parkash et al., 2014). Mortality was assessed after every 24 h for four-days - a period in which most larvae were either dead or had pupated (survived) (Gotcha et al., 2018).

6.2.7. Data analyses

Differences in CT_{max} and CT_{min} for the five pre-treatments were examined using separate Kruskal-Wallis tests, each for larval and adult stages, as data violated assumptions of parametric tests. Dunn tests were used for post-hoc pairwise comparisons in cases where treatment was found to be significant (Ogle et al., 2020). Factorial binomial generalised linear models with logit links were used to examine the effects of life stage, treatment and duration, separately according to the desiccation and starvation survivability experiments. Bias reductions were used to account for complete separation in the data, where full survival and/or mortality was exhibited in certain conditions (Kosmidis, 2020). In each model, stepwise reductions were used to remove non-significant terms until the final model included only significant predictors, with Type III analysis of deviance used to compute coefficients in the final model (Fox and Weisberg, 2019). Residuals were checked for overdispersion by comparing fitted values with simulations (Hartig, 2020).

Estimated marginal means were used to compute post-hoc tests for significant terms in generalised linear models (Lenth, 2020). All analyses were computed in R v4.0.2 (R Core Team, 2020).

6.3. Results

6.3.1. Thermal tolerance

There was no significant improvement in heat tolerance in both life stages following the various acclimation pre-treatments, i.e. RHH, RCH, desiccation, and starvation (Fig. 6.1a). In adults, heat tolerance (CT_{max}) differed significantly according to treatment ($\chi^2 = 10.286$, $df = 4$, $P = 0.036$; Fig. 6.1a), such that RCH significantly reduced CT_{max} while RHH marginally improved it ($P = 0.043$). No other treatments were significantly different pairwise (all $P > 0.05$) (Fig. 6.1a). Conversely, treatment did not have a significant effect on CT_{max} at larval stages ($\chi^2 = 6.607$, $df = 4$, $P = 0.158$), and thus all treatment pairs were statistically similar (all $P > 0.05$), but these were significantly higher than adults (mean range adults: 44.590 – 45.705 °C; mean range larvae: 48.490 – 48.805 °C; $\chi^2 = 147.610$, $df = 1$, $P < 0.001$; Fig. 6.1a).

In adults, all treatments did not improve cold tolerance (CT_{min}) as no treatment groups significantly differed from the control (all $P > 0.05$) (Fig. 6.1b). Critical thermal minima for adults again differed significantly according to treatment ($\chi^2 = 12.527$, $df = 4$, $P = 0.014$; Fig. 6.1b). The RCH treated adults exhibited significantly reduced cold tolerance (i.e. higher CT_{min}) compared to desiccated ($P = 0.030$) and fasted ($P = 0.028$) ones. Larvae also exhibited significant differences in CT_{min} ($\chi^2 = 29.629$, $df = 4$, $P < 0.001$). The RCH treated larvae exhibited significantly greatest cold tolerance (i.e. lowest CT_{min}) ($1.29 \pm 0.13^\circ\text{C}$) compared to all other treatments (all $P < 0.05$) (Fig. 6.1b). All other treatment pairs were statistically similar (all $P > 0.05$). Adults were significantly more cold tolerant than larvae overall (mean range adults: -0.450 – -1.255 °C; mean range larvae: 2.465 – 1.290 °C; $\chi^2 = 148.28$, $df = 1$, $P < 0.001$; Fig. 6.1b).

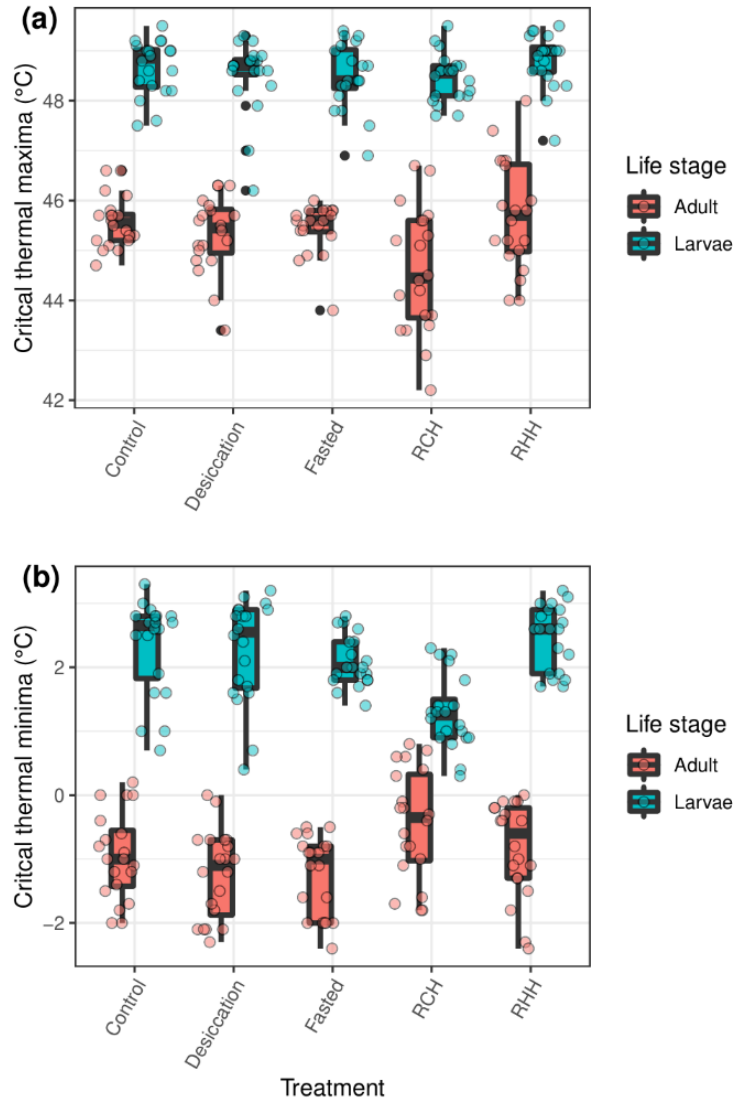


Figure 6.2. Critical thermal maxima (a) and minima (b) according to treatment (desiccation and fasting acclimation, rapid cold hardening [RCH] and rapid heat hardening [RHH]) and life history stage. In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range.

6.3.2. Desiccation and starvation resistance

For desiccation resistance, the effects of pre-treatment significantly interacted with life stage in driving mortality (Table 6.2; Fig. 6.2). In adults, fasted ($P < 0.001$) and RHH ($P = 0.012$) groups exhibited significantly greater and lower desiccation resistance than controls, respectively (Fig. 6.2). Desiccated and RCH treated adults were more similar to controls (both $P > 0.05$). Fasted

adults showed significant cross tolerance, exhibiting greater survival than desiccated, RCH and RHH treated adults (all $P < 0.001$). In turn, desiccated individual adults exhibited significantly greater survival than RCH ($P = 0.028$) or the RHH ($P < 0.001$) treated ones. The RCH ($P = 0.035$) group also exhibited greater survival than RHH treatments. In larvae, fasted groups, conversely to adults, exhibited significantly lowest survival (all $P < 0.001$). All other groups were statistically similar to the controls ($P > 0.05$). Desiccation exposure did not increase desiccation survival significantly for adults ($P = 0.076$) or larvae ($P = 0.913$) compared to controls. While prior stressors (i.e. starvation) provided an advantage to adults in terms of desiccation resistance, the opposite thus occurred for larvae. Survival was also significantly affected by exposure duration (Table 6.2; Fig. 6.2), falling significantly with all increasing exposure increments overall (all $P < 0.005$). There were no other significant terms or interactions driving desiccation tolerance (Table 6.2).

Table 6.2. Binomial generalised linear model coefficients considering (a) desiccation and (b) starvation effects on survivability. Terms are computed stepwise following backward deletion of non-significant effects. Significant terms are emboldened.

Trait	Effect	χ^2	Df	<i>p</i> -value
(a) Desiccation	Stage	287.370	1	< 0.001
	Treatment	34.970	4	< 0.001
	Duration	390.860	3	< 0.001
	Stage × Treatment	205.75	4	< 0.001
	Stage × Duration	3.074	3	0.380
	Treatment × Duration	13.715	12	0.319
	Stage × Treatment × Duration	5.339	12	0.946
(b) Starvation	Stage	0.05	1	1.000
	Treatment	25.09	4	< 0.001
	Duration	421.58	3	< 0.001
	Stage × Treatment	270.718	4	< 0.001
	Stage × Duration	4.612	3	0.203
	Treatment × Duration	29.116	12	< 0.001
	Stage × Treatment × Duration	8.376	12	0.755

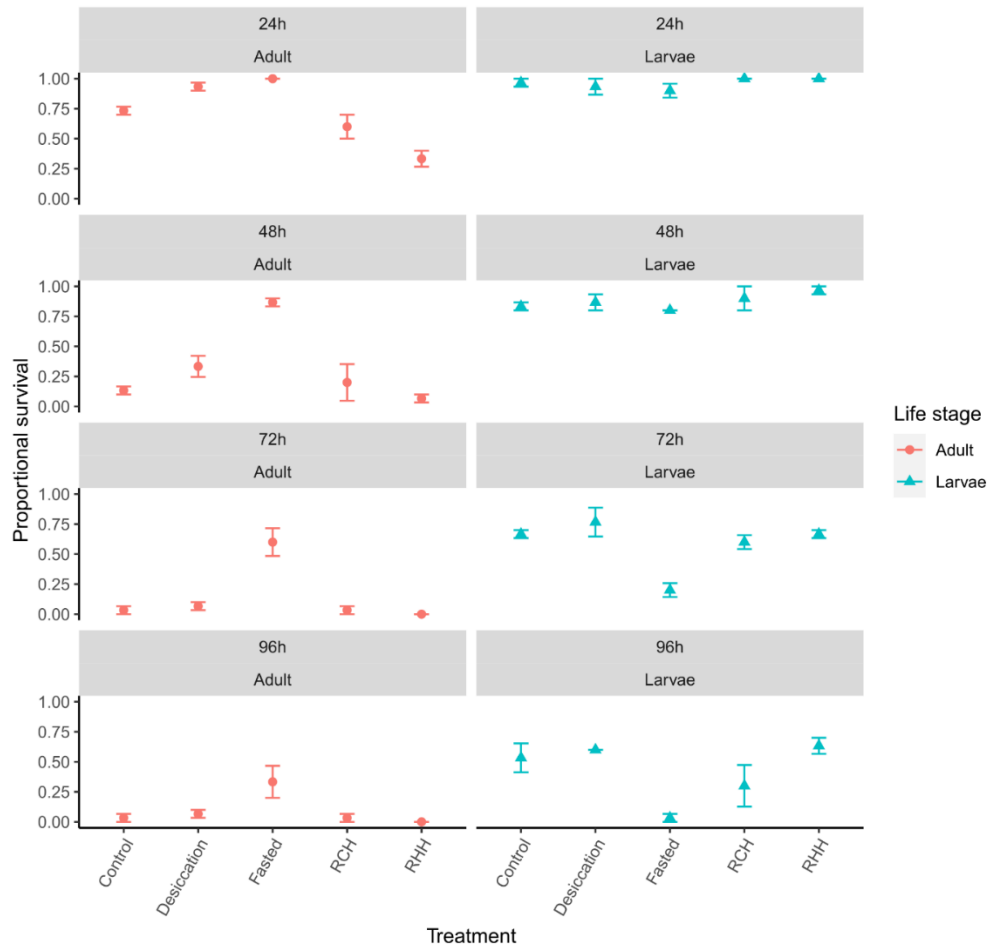


Figure 6.3. Mean ($\pm$ standard error) survival to desiccation between adult and larval life stages, following exposure to various prior stressor treatments (desiccation and fasting acclimation, rapid cold hardening [RCH] and rapid heat hardening [RHH]) and following different durations.

Similar to desiccation, starvation-induced resistance was significantly affected by treatment in interaction with life stage (Table 6.2; Fig. 6.3). For adults, fasted groups exhibited significantly greater survival than controls ($P < 0.001$), while all other groups were statistically similar to controls (all $P > 0.05$) (Fig. 6.3). Fasted adults also exhibited significantly higher survival than desiccated, RCH and RHH treated ones (all $P < 0.001$), while desiccated individuals also survived significantly better than RCH ($P < 0.001$) and RHH ($P < 0.001$) treated adults. For larvae, conversely to adults, fasted individuals exhibited significantly lower survival than controls ($P < 0.001$), with all other treatment groups statistically similar to controls (all $P > 0.05$). Fasted groups also exhibited significantly lower survival following starvation compared to all other groups (all

$P < 0.001$), with all other groups statistically similar (all $P > 0.05$). Accordingly, starvation conferred an adaptive advantage to adults after starvation exposure, but a disadvantage to larvae. Duration significantly affected survivability in interaction with treatment (Table 6.2; Fig. 6.3), again significantly increasing with exposure increments overall (all $P < 0.001$). There were no other significant terms or interactions driving starvation tolerance (Table 6.2).

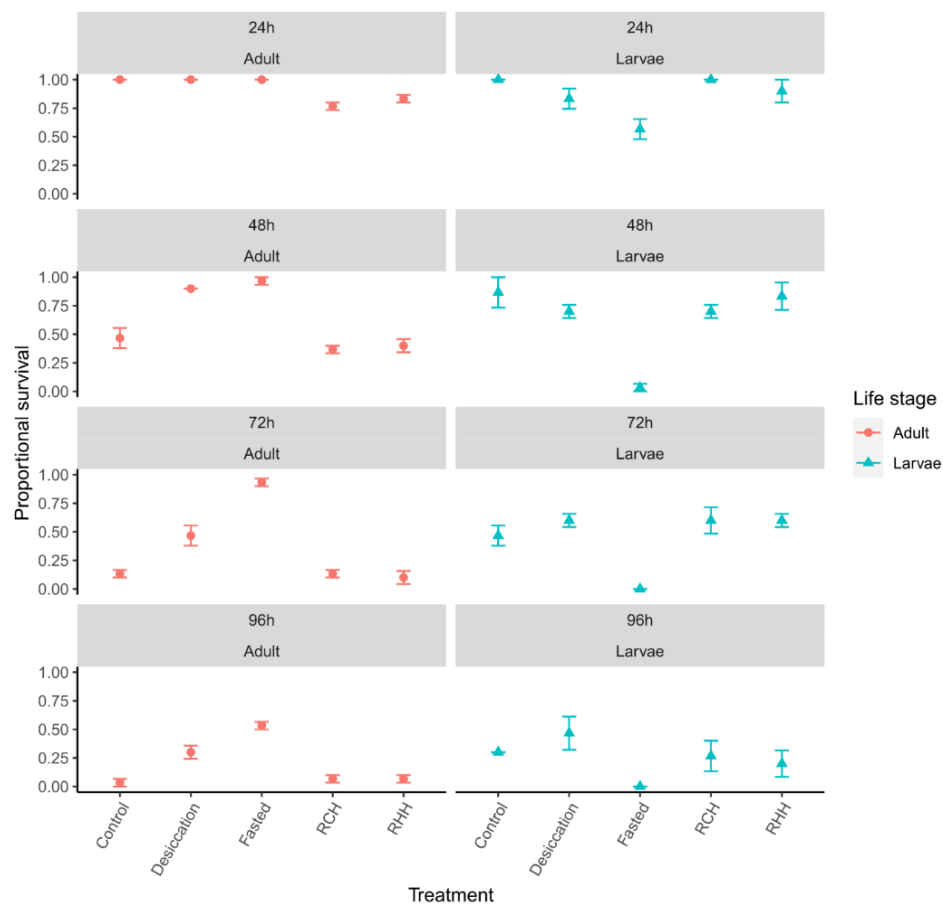


Figure 6.4. Mean ($\pm$ standard error) survival to starvation between adult and larval life stages, following exposure to various prior stressor treatments (desiccation and fasting acclimation, rapid cold hardening [RCH] and rapid heat hardening [RHH]) and following different durations.

6.4. Discussion

Cross tolerance is adaptive and likely shows shared co-evolved response mechanisms to totally contrasting environmental stressors. This integrated resistance likely saves energetic costs likely associated with the mounting of divergent stress responses for co-occurring environmental stresses and likely heighten invasiveness in pest insects (Renault et al., 2015). Our results show novel

context-dependence between life stages, with certain prior stressors imparting an advantage to adults but a disadvantage to larvae. For example, we show that fasting improves desiccation tolerance in adults (cross tolerance), while showing a negative effect (cross susceptibility) in larvae. This shows that interaction between divergent stressors may be dependent on developmental stage context. Second, we show beneficial acclimation for CT_{max} and CT_{min} in adults and larvae respectively, but not for desiccation and starvation resistance. This likely resonates well with the rejection of the beneficial acclimation hypothesis for short term acclimation responses, consistent with Ramniwas et al. (2020). Third, we show that acclimation to one particular stress may come at a cost of another divergent environmental stress (cross susceptibility). For example, RCH came at a cost of heat tolerance (CT_{max}) in adults and that RCH was maladaptive for CT_{min} .

This study provides insights on developmental stage related responses to multiple environmental stressors and their interaction thereof in *T. absoluta*. The results generally show lack of support for the beneficial acclimation hypothesis (BAH) and differential developmental stage resistance and thermal tolerance following exposure to environmental stresses. This result is in consonance with recent literature debating the BAH and the rejection thereof for short term plastic responses (Raminwas et al., 2020). Indeed, RHH had no significant effects on CT_{max} , a result that is in keeping with *Zygogramma bicolorata* (Chidawanyika et al., 2017) and *Drosophila nepalensis* (Raminwas et al., 2020). When different stressors activate distinct molecular pathways, exposure to one may have no effect on response to the other contrasting stress or may come at a cost due to the energetic demands of responding to multiple stressors simultaneously (Sinclair et al., 2013; Todgham and Stillman, 2013; Nguyen et al., 2017). While RHH, desiccation and fasting acclimation did not have significant effects on heat tolerance in both larvae and adults, RCH had a cost to adult CT_{max} indicating cross-susceptibility effect. This result is in agreement with reports on related lepidopteran species, *C. partellus*, *Busseola fusca* and *Sesamia calamistis* (Mutamiswa et al., 2018). Similarly, dehydration acclimation and RHH had no effects on *Cimex lectularius* heat tolerance (Benoit et al., 2009b; Everatt et al., 2014). However, our results are in contrast with the notion that episodes of heat stress often coincide with water restriction, and thus responses to either heat or desiccation may promote resistance to both stresses (Renault et al., 2015). A comparison of both life stages showed larvae exhibited higher CT_{max} than adults following all treatments, indicating that larvae were more heat tolerant than adults. Exact mechanisms for this difference

are unknown and warrant further investigation. However, larvae have higher body water content and lipid content than adults (see Tarusikirwa et al., 2021). This may likely point to the role of body water to heat tolerance (Huey and Kearney, 2020).

None of the pre-treatments, i.e. RHH, starvation and desiccation elicited a cross tolerance response to low temperature tolerance (CT_{min}). Rapid cold hardening had significant beneficial effects on larval CT_{min} while it was significantly maladaptive for adult cold tolerance. This result suggests that acclimation to low temperature is developmental dependent, in keeping with Mutamiswa et al. (2019). The cross susceptibility between RCH and cold tolerance (CT_{min}) in adults suggest that RCH may come at a cost of low temperature tolerance in adult *T. absoluta* likely owing to the additive thermal injury associated with both acclimation and stress exposure (see discussions in Jørgensen et al., 2021). This result is nevertheless in agreement with Tarusikirwa et al. (2020b) and Keosentse et al. (2021) who reported differences in RCH between larval and adult *T. absoluta* and *Spodoptera frugiperda* respectively. There is a close association between desiccation and cold such that dehydration stress often confers improved cold tolerance (Yi et al., 2017). This correlation is linked to similar cellular damage inflicted by both stresses and overlapping mechanisms e.g. Hsps and cryoprotectants (Holmstrup et al., 2002; Kostal et al., 2007; Rinehart et al., 2007; Sinclair et al., 2013). Contrary to the current results, various studies have shown that desiccation acclimation has a positive effect on cold tolerance, e.g. exposure to mild desiccation was reported to increase cold tolerance in the springtail, *F. candida* (Bayley et al., 2001).

The effect of fasting pre-treatment is consistent with previous study by Bubliy and Loeschcke (2005) who detected an increase in starvation tolerance in cold-selected flies but no improvement in cold tolerance in starvation-selected flies. This shows that the response mechanism from cold acclimated organisms might have a positive effect on starvation tolerance, but the reverse is true for starvation acclimation. However, this asymmetrical cross tolerance is debatable and variable across species. For example, MacMillan et al. (2009) found contrary results that did not detect any effect of selection for cold tolerance on starvation tolerance. Furthermore, fasting reduced cold tolerance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Scharf et al., 2015). Hoffmann et al. (2005) showed that flies selected for starvation resistance have decreased resistance to cold, whereas those selected for increased cold resistance show decreased starvation resistance (Singh and Prasad, 2020). Contrastingly, a comparison of *T. absoluta* larvae and adults showed adults had

higher cold tolerance (more negative CT_{min}) than larvae across all treatments. This suggests that physiological responses to environmental stressors in nature are life stage and trait dependent (Mutamiswa et al., 2019), and shows that different developmental stages may select for synergistic resistance to different stressors under natural conditions. Results also showed no significant effects of RHH for CT_{min} . Mechanisms required to adapt to a specific type of stress might conflict with mechanisms required to adapt with other kinds of stress, leading to trade-offs across stress resistance traits (Kellett et al., 2005; Overgaard et al., 2006; MacMillain et al., 2009; Scharf et al., 2015). This may also mean that mechanisms for heat and cold resistance are highly decoupled, and that stressful and injurious effects between RHH and cold tolerance may be additive (Jørgensen et al., 2021).

One striking result of the current study is that fasted adults had significantly higher desiccation tolerance while the same acclimation effect was maladaptive in larvae. This may mean that during the invasion process, invader 'propagules' arriving in a novel environment may have already been exposed to stressors which promote their survival in new environments. In particular, organisms deprived of food, e.g. during anthropogenically-associated propagule translocation may have improved desiccation tolerance, a positive cross tolerance effect that may improve survival in arid environments, and likely facilitating invasion (Renault et al., 2015). However, this cross tolerance is context dependent, as larval fasting came at a cost of desiccation tolerance. This result is in consonance with Bubliy et al. (2012) who reported 21% increase in *D. melanogaster* desiccation resistance following starvation acclimation. Studies by Raubenheimer and Gäde (1993) showed that there is a possible link between the reduction in lipid levels and an increase in water uptake. Generally, there is a decrease in lipid levels following starvation. This can lead to an increase in water stores available for the insect to utilize in withstanding desiccation stress (Mitchell et al., 2017). However, other acclimation pre-treatments had no effects on desiccation tolerance between both developmental stages. This is in agreement with Bubliy et al. (2012) who reported cross-susceptibility between traits of cold acclimation on desiccation resistance of *D. melanogaster*. However, the result is also contrary to Gotcha et al. (2018) who reported improved desiccation resistance following cold acclimation *C. rosa* suggesting context complexity in teasing cross tolerance effects.

The present study showed cross-susceptibility between RHH and desiccation resistance. This result is also in keeping with Bubliy et al. (2012) who reported cross susceptibility in desiccation resistance in *D. melanogaster* following heat hardening. However, Kalra et al. (2017) reported cross tolerance between heat hardening and desiccation tolerance in *Zaprionus indianus*, indicating that this integrated resistance may be species dependent. A comparison between larvae and adults showed larvae had significantly higher desiccation resistance across all durations and following acclimations. This implies that larvae are more desiccation resistant than adults, a result resonating well with Tarusikirwa et al. (2021). Desiccation acclimation did not improve desiccation tolerance, and as such, we reject the BAH for desiccation tolerance, with caveats, at least for the current study context. Nevertheless, this study adds to the current debate on the BAH and the rejection thereof for short term stress responses (Ramniwas et al., 2020).

Low and high temperature hardening pre-treatments did not improve starvation resistance in both larval and adult *T. absoluta* indicating cross susceptibility effect. This is in keeping with Gotcha et al. (2018) who reported lack of improvement in starvation resistance following cold acclimation and heat hardening in *C. rosa*. Similar trends were also reported by Bubliy et al. (2012) on *D. melanogaster*. Cross susceptibility may in caveats arise from an insect's failure to produce Hsps that can work in multiple regulatory pathways, thereby, inducing resistance to various stressors simultaneously. It has also been suggested that the accumulation of metabolic energy reserves underlies multiple-stress resistance (Bubliy et al. 2012), therefore, insects that experience cross susceptibility might in some cases have high metabolism. Desiccation and fasting acclimation significantly improved starvation resistance in adults following 48, 72 and 96 h exposure but not so in larvae. This significant integrated stress resistance between desiccation and starvation tolerance in adults suggest that continuous bouts of low food resources and RH in nature may give a survival advantage in adults, that may optimise fitness and invasiveness under anthropogenically changing environments. Given that adults are responsible for propagation, this survival advantage may lead to population perpetuation when they encounter increasingly low food resources and desiccation stress under arid tropical environments. Geographic dispersal of *T. absoluta* has been reported to be primarily driven by short and long-distance transmission (Xian, 2017; Tarusikirwa et al., 2020a). While international agricultural trade (e.g. imports and exports of infested fruits, propagule material and packaging materials) and natural factors (wind and water) are the key long and short distance dispersal mechanisms (Tarusikirwa et al., 2020a), there is a high likelihood of

mobile life stages (larvae and adults) experiencing multiple environmental stressors in transit. Thus, integrated stress resistance reported here may be adaptive and likely improves *T. absoluta* competitiveness and invasiveness in invaded environments.

In conclusion, the results show that acclimation to one particular stressor may confer improved (cross tolerance) or impairment/no effect (cross susceptibility) to other contrasting stressors. In particular, I show novel *T. absoluta* cross tolerance context-dependence. For example, fasting improved desiccation tolerance in adults (cross tolerance), while showing a negative effect (cross susceptibility) in larvae. Similarly, desiccation acclimation temporarily improved starvation tolerance in adult *T. absoluta*. This integrated stress resistance may strengthen pest insect invasiveness amidst increasingly arid and environmentally heterogeneous invaded environments and represents another facet of the arsenal supporting the invasion success (Renault et al., 2015). Second, I also show context beneficial acclimation for $CT_{\max}$ and $CT_{\min}$ in adults and larvae respectively, but not for desiccation and starvation resistance, resonating well with the rejection of the BAH. Third, I show that acclimation to one particular stress may come at a cost of another divergent environmental stress (cross susceptibility) suggesting some stress responses may be multiplicative. Considering that different stressors may affect *T. absoluta* interactively in various possible ways, the results are important in modelling its risk status and developing sustainable management options under changing environments. Incorporating these integrated stress responses into predictive distributional models may help refine invasive pest species potential biogeographical niches.

6.5. References

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CHAPTER 7

General Discussions

The systematic investigation of the causes and consequences of biological invasions is a rapidly evolving interdisciplinary field. (Ricciardi et al., 2021). One of the key questions in ecological invasion is what makes an invading species successful? (Kelly, 2014). A wide range of factors drive the processes responsible for range dynamics following a successful invasion (Wan et al., 2019). Studies have shown that a variety of life-history traits and other biological characteristics promote invasion success (Kelly, 2014; Wan and Yang, 2016). For example, several common intrinsic biological and physiological traits have been reported across prolific invaders and may help explain their increased invasiveness. Chief among them include; superior bio-physical competitive ability relative to native species (Callaway and Ridenour, 2004), wide host range (Bawin et al., 2015), high functional responses (Cuthbert et al., 2019), phenotypic plasticity (Nyamukondiwa et al., 2010), metabolic flexibility (Smit et al., 2021) and high propagule pressure (Simberloff, 2009) and others (Wan and Yang, 2016). Environmental attributes are a key factor that dictates whether a species will establish following invasion of new environments. Invasion and consequent establishment are thus dependant on insect adaptations, to both the biotic and abiotic environment upon introduction to a novel environment (Mutamiswa et al., 2018). Such flexibility allows an organism to thrive short to long term episodes of stress in the face of a novel heterogeneous environment. The characterization of physiological phenotypes that may play a part in the establishment of non-native species can, therefore, broaden our understanding of the ecology of species invasion (Kelley, 2014). This study focused on understanding the mechanisms behind establishment and spread of *T. absoluta* following its recent invasion in Africa. Specifically, I investigated its physiological adaptations to climate change through measuring various eco-physiological traits, key attributes for understanding invasion biology. These standardised traits have increasingly been employed in insect thermal biology and are useful proxies dictating species' distributions (Chown and Nicolson, 2004; Andersen et al., 2015; Sinclair et al., 2015).

Tuta absoluta has aggressively invaded the African continent (Mansour et al., 2018). Since its first detection in North Africa in Morocco and Tunisia in 2008, it has successfully invaded the entire southern, eastern and western Africa, where it has been on the offensive, causing significant damage to solanaceous food crops. Coupled with a survey on *T. absoluta* natural enemies in Botswana, an in-depth literature review unravelled the pest's invasion pathways and the potential factors that favoured its successful invasion in Africa (Chapter 2; Tarusikirwa et al., 2020a). The study showed that the pest had traits that most good invaders possess e.g. high propensity for

pesticide resistance (Lietti et al., 2005), multivoltine with many generations per year (Tropea Garzia et al., 2012), wide host range (Barwin et al., 2015; Machekano et al., 2018) and high propagule pressure (Guillemaud et al., 2015). Coupled with absence of natural biotic pressure that comes with natural enemies in native environments, this may help explain why this pest exploded within a short time. Thus, *T. absoluta* exhibits most of the traits typical of a good invader. Similarly, complementary traits that may improve *T. absoluta* invasiveness were uncovered in Chapter 3 (i.e. phenotypic plasticity and high basal thermal tolerance) and Chapter 4, 5 and 6 (i.e. integrated stress resistance [cross tolerance]). A survey of native natural enemies showed few natural enemies for this pest species, supporting the natural enemy escape hypothesis in pest establishment. Nevertheless, reported natural enemies represents a positive step in natural biological control e.g. through conservative and augmentative releases and applied in integrated approaches. As the chapter also sought to come up with good IPM strategies for *Tuta* control, this information shows natural enemies can indeed be included in the program. The literature review also covered the assets and liabilities that influence farmer adoption of natural substances as part of integrative pest control practices. The information showed that more awareness still needs to be put in place to encourage farmer adoption as farmer perceptions and social dynamics were some of the reasons for the failure in African farmer setups. Governments also need to invest in this endeavour as well because most of the legislation that affects farmer adoption is implemented at government level.

Temperature is often regarded as the most important abiotic factor in determining species distribution owing to its impact on biochemical and cellular processes (Somero, 2005; Johnston and Bennett, 2008), which, in turn, affect organismal performance (Pörtner, 2002; Kelley, 2014). To cope with rapidly changing environments, insects often employ a suite of mechanisms at both acute and chronic time-scales, thereby improving fitness at sub-optimal thermal environments (Nyamukondiwa et al., 2010). Environmental effects and physiological responses may differ across insect life stages and this can have implications on population dynamics. Against this background, I investigated short- and long-term plastic responses to temperature of *T. absoluta* larvae (4th instar) and adults (24–48 h old) field populations (Chapter 3: Tarusikirwa et al., 2020b). Results showed that plastic responses were life-stage dependent with larvae being more thermally plastic than adults and showing a potential to shift their thermal tolerance in short- and long-

timescales. Larval plasticity advantage over adults reported here suggest asymmetrical ecological role of the larvae relative to adults in facilitating *T. absoluta* invasion.

For many insects, including invasive species, overwintering survival is achieved behaviourally (e.g. through migration) or physiologically by entering diapause, a state of arrested physiological development that may be accompanied with depressed supercooling points (SCPs) (Sinclair et al., 2015). Diapause allows *in situ* adaptation to adverse environmental conditions, providing sufficient parent propagules for insect pest proliferation when optimal conditions resurface. Previous studies by Van Damme et al. (2015) reported the absence of diapause in the tomato pinworm. Therefore, I investigated cold hardiness of *T. absoluta* larvae and adults and its overwintering survival in tropical arid and semi-arid African agro-ecosystems, i.e. Botswana (Chapter 4; Tarusikirwa et al., 2020c). Results showed that *T. absoluta* larvae and adults are chill-susceptible, hence, this does not have any negative implications on overwintering in the tropics as temperatures generally do not digress to less than sub-zero. This indicate that both stages may successfully overwinter in agreement with Chapter 3 results (i.e. superior performance/ fitness in larvae) showing that larvae were more cold hardy than adults. Ecological factors may also affect cold hardiness, with desiccation pre-treatment improving cold tolerance strategy (SPCs). This showed a cross tolerant response in *T. absoluta* consistent with various studies that found a positive correlation between desiccation and cold tolerance (see Sinclair et al., 2013). This likely shows that traits of desiccation cold tolerance likely co-evolved in this species and may share response pathways/mechanisms. Temperatures experienced in the sub-Saharan Africa rarely drop below 0°C, however, the study showed that SPCs for the pest can be as low as -17°C. This shows that the pest can thrive well in its invaded African areas even during the winter season.

Temperature and dehydration stress are two major co-occurring environmental stressors threatening the physiology, biochemistry and ecology of insects (Weldon et al., 2016). As such, understanding adaptive responses to desiccation stress is critical for predicting climate change impacts, particularly its influence on insect invasions. I further assessed water balance and desiccation resistance of the tomato pinworm and results were used to infer how eco-physiology shapes its niche (Chapter 5; Tarusikirwa et al., 2021). Results showed that 4th instar larvae and adults are the most resilient developmental stages and potentially contribute most to the invasion success of the pest in arid environments. Incorporation of these species-specific eco-physiological

traits in predictive models can help refine invasive species potential spread under changing climates.

In nature, insects are concurrently faced with multiple divergent but often overlapping environmental stressors, a scenario likely increasing with climate change (Renault et al., 2018). Upon exposure to these environments, insects often employ behavioural or physiological adjustments to optimise fitness. Exposing insects to a particular stressor may enhance or impair tolerance to a subsequent divergent stress (cross tolerance and cross susceptibility respectively) due to energetic demands associated with responses to various stressors simultaneously. This integrated stress resistance often improve fitness and drive invasive species biogeographical success. As a result, I investigated potential cross tolerance to abiotic stress in *T. absoluta* 3rd instar larvae and adults (Chapter 6). There was a positive correlation between desiccation and starvation resistance. The results showed context dependent integrated resistance to stress traits which may potentially enhance its invasion success. Unravelling this information is vital for determining the underpinnings of multi-stressor responses which are fundamental in forecasting species responses to changing environments.

In conclusion, *T. absoluta* invasion may be attributable to its several physiological traits' superiority, trait attributes often missing in native species. Its superior basal thermal tolerance, phenotypic plasticity, water balance and integrated stress resistance allows it to thrive and gain pest status in new heterogeneous environments. Incorporating this information in predictive models of potential invasions may help refine invasive species distribution under global change. The major problem in population ecology and IPM has been to define the reasons why insect population rates change over time and the consequences of that change on the dynamics of pests, host plants, and natural enemies (Gutierrez and Wilson, 1989). Since the origins of the IPM concept in the late 1950s, a rich set of mathematical models have emerged in the literature focusing on different features of IPM based applications (Chávez et al. 2017). Modelling of pest distribution is an important technique available for biologists and ecologists to understand and predict the past, current or future presence of species based on ecological observations and/or laboratory data (Tonnang et al. 2015). However, for these models to be developed, there is need for studies that look into factors that may influence insect population dynamics. Temperature and RH are the two major abiotic factors that affect insect survival and thus population dynamics, hence it is important

to get temperature and RH thresholds in which different insect pests can survive. To help and better prioritize management strategies, species distribution models (SDMs) are often used to predict the potential invasive range of introduced species. Some models have already been developed for *T. absoluta* e.g. Tonnang et al. (2015) looked into the geo-reference of incidence records of *T. absoluta* and this was combined with data on the biology and ecology of the pest to estimate its climatic suitability and potential spread at regional and global scale. A CLIMEX model was developed and used for the global prediction of current and future climate-induced changes in the distributional shifts of *T. absoluta*. This will allow for pre-emptive controls measures to be put in place. Much work still needs to be carried out with regards to more effective control e.g. conservative, augmentative and classical biological control. Furthermore, molecular mechanisms underpinning this successful biological edge may need to be investigated e.g. the role of small RNAs in invasive species thermal and stress adaptation. Explaining these may help design molecular tools for invasive pest management. Some fruitful angles for future work may include 1) more work on conservation biological control to maximize effects of native natural enemies; 2) construction of predictive models to assist in pest outbreak predictions to minimize farmers losses; 3) population dynamics of the pest during summer and winter seasons.

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Appendix 1

Fluctuating environments impact thermal tolerance in an invasive insect species *Bactrocera dorsalis* (Diptera: Tephritidae)

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1.1. Introduction

There is increasing evidence of changes in the global climate system due to anthropogenic activities (IPCC, 2014). An emerging concern is that the incidence and severity of climate ‘pressures’ and ‘pulses’ such as extreme variation in temperatures and rainfall, heatwaves, cold snaps, drought and floods are increasing globally (Meehl and Tebaldi, 2004; Thuiller, 2007; IPCC, 2014; Furlong and Zalucki, 2017; Harris et al., 2018). Given that most arthropods are ectotherms, exposure to these extreme thermal perturbations may compromise fitness, resulting in mortality associated with direct and indirect effects of environmental stress (Parmesan, 2006; Dillon et al., 2009; Gray, 2013; Zhang et al., 2015). Although literature often depicts climate change factors in the form of mean monthly and annual temperature and/or rainfall, extremes brought by diurnal and seasonal fluctuations are as potent in determining ectotherm fitness and survival (Paaajmans et al., 2013; Chidawanyika et al., 2017). As such, abiotic factors e.g. temperature and relative humidity (RH) are therefore key in determining the reproduction, development, abundance, longevity, distribution and survival of insects (Hoffman et al., 2012; Nguyen et al., 2014). Dry seasons are often associated with high temperature and high vapour pressure deficit (VPD), defined as the drying power of the air, resulting in insects facing thermal and hydric stress (Bujan et al., 2016). Hence, insects experiencing variable suboptimal temperatures and RH often employ physiological, morphological and behavioral mechanisms to enhance their fitness (Lachenicht et al., 2010). In order to mitigate the risk of desiccation under drier environments, insects can increase body water content through rapid formation of metabolic water by conversion of stored body fat (Bujan et al., 2016). Furthermore, insects can increase body water conservation through rhythmic opening and closing of spiracles during discontinuous gas exchange (Williams et al., 2016) and increased rectal water reabsorption (Bujan et al., 2016). Similarly, insects employ a range of acute and chronic mechanisms to survive thermal stress mediated by both genetic adaptations (Lachenicht et al., 2010) and/or phenotypic plasticity, the ability of a single genotype to express variable phenotypes under different environments (Xue and Leibler, 2018). Such plasticity can be realized through activation of biochemical responses that aid resistance to stressful thermal regimes following an acute (hardening) or relatively chronic (acclimation) exposure to suboptimal temperatures (e.g. Chidawanyika and Terblanche, 2011; Sgrò et al., 2016; Mutamiswa et al., 2018a). Moreover, over longer timescale, insects facing such multiple environmental stresses simultaneously e.g. high temperature and low RH often evolve shared physiological response mechanisms (cross tolerance)

(see Bubliy et al., 2012; Sinclair et al., 2013; Gotcha et al., 2018) or signal pathways (cross talk) (see Sinclair et al., 2013). Such mechanisms help buffer organisms against rapidly changing and overlapping stressful environments (Bubliy et al., 2012).

The magnitude and frequency of temperature and RH fluctuations is key in determining insect physiological responses (Cavieres et al., 2018; Mutamiswa et al., 2019). High fluctuating temperature regimes ranging from 27-37°C significantly influenced survival and reproduction of *Nezara viridula* nymphs and adults, respectively (Chanthy et al., 2015). Similarly, Chidawanyika et al. (2017) reported improved thermal tolerance in *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) that were acclimated under fluctuating temperatures compared to constant temperatures. In addition, a decrease in RH resulted in increased mortality in *Tribolium castaneum*, *T. confusum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) following exposure to four different moisture regimes (Jay et al., 1971; Norhisham et al., 2013). Similarly, high RH enhanced fecundity and longevity in *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) females (Ouedraogo et al., 1996). In *Spodoptera frugiperda* (Lepidoptera: Noctuidae), fluctuating temperature or relative humidity showed little effect on survival and development relative to constant thermal and moisture regimes (Simmons, 1993). Furthermore, at 20 and 80% RH, higher egg mortalities in *Dinoderus minutus* (Coleoptera: Bostrichidae) were recorded compared to 40-75% moisture regimes (Norhisham et al., 2013). These results point to ambivalent fitness effects of RH fluctuations across different taxa. Moreover, there is limited information on how temperature and RH likely interact, e.g. through associated VPD changes, and effects on insect thermal fitness under rapidly changing environments. Nevertheless, most studies have often dwelt on the extent to which mean and fluctuating temperatures affect insect fitness under changing environments (see Chidawanyika et al., 2017; Cavieres et al., 2018), without accounting for changing RH gradient. Using adult *Chilo partellus* (Lepidoptera: Crambidae), Mutamiswa et al. (2019) recently reported effects of larval, pupal and adult acclimation under variable temperature and RH regimes on thermal tolerance. In this study, interactive costs and benefits of developmental stage, temperature and RH in driving thermal plasticity in *C. partellus* were evident (see Mutamiswa et al., 2019). Cognisant of increasing extreme events, climate variability, and overlapping stressful environments with global climate change, this raises an interesting question of how simultaneous fluctuations in both temperature and RH may influence fitness and population dynamics among arthropods. This is particularly significant for invasive

pests where geographic range expansion is often associated with superior environmental stress resistance (Mutamiswa et al., 2017).

Experiments mainly focusing on average temperatures without including their variabilities may not be exhaustive in explaining the key effects of fluctuating temperature regimes on insect thermal fitness (Bannerman et al., 2011; Vasseur et al., 2014; Williams et al., 2016; Kingsolver and Buckley, 2017). Therefore, an understanding of how fluctuations in temperature and RH influence thermal fitness in insects is important in providing insights on their future distribution as well as ecological and evolutionary responses under changing environments (Stephens et al., 2007; Vasseur et al., 2014; Chidawanyika et al., 2019; Mutamiswa et al., 2019). Recent global change has shown that extreme events (pulses) are gradually getting superimposed on ongoing changes in climate trajectories (presses) and their magnitude and frequency is projected to increase. Although most insects have developed adaptation mechanisms through evolution to cope with short and long-term variabilities in climate, the magnitude of encountering multiple interacting climate ‘presses’ and ‘pulses’ has increased. This has resulted in higher likelihoods of experiencing overlapping survival as well as extinction extremes in nature (Harris et al., 2018). While Mutamiswa et al. (2019) reported on the thermal fitness of *C. partellus* following acclimation to static temperature and RH regimes, this approach could have resulted in the masking of potential climate presses and pulses rendered by combined thermal and RH fluctuations. In line with this background, this study thus investigated the effects of climate variability on the thermal fitness of adult *B. dorsalis*, a key developmental stage responsible for reproduction and dispersal. *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae), native to Asia is a key destructive invasive insect pest of vegetables and fruits in sub-Saharan Africa (Lux et al., 2003; Manrakhan et al., 2015). Because of its high invasion potential, it was first reported in Africa around 2003 in Kenya before rapidly spreading and establishing in East, Central, West and Southern Africa (Lux et al., 2003; Magagula et al., 2015; Manrakhan et al., 2015). Its successful spread and establishment in novel environments, in part, stems from wide host range of cultivated and wild fruits and perhaps superior environmental stress resistance (Han et al., 2011; Magagula et al., 2015; Pieterse et al., 2017). Specifically, we thus investigated how combined fluctuating and interaction of temperature and RH may influence its low and high temperature tolerance through measuring their ecologically relevant proxies such as critical thermal minima (CT_{min}), chill coma recovery time (CCRT), critical thermal maxima (CT_{max}) and heat knockdown time (HKDT) (Andersen et al., 2015). We combine

low and high temperature responses with calculated VPD to understand the significant effects of these variabilities on *B. dorsalis* fitness in the face of climate change. The results in this study are important in informing predictive models for species responses to climate change and may help inform pest management under shifting abiotic environments.

1.2. Materials and Methods

1.2.1. Study insects and rearing conditions

Bactrocera dorsalis initial colony was obtained from the International Centre for Insect Physiology and Ecology (ICIPE), Kenya as pupae. This outbred colony had been in the laboratory for ~20 generations with regular augmentation with wild caught flies to minimize inbreeding depression and genetic drift. The colony was reared in Bugdorm rearing cages (240cm³; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) housed in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) set at $28 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH and 12L:12D photoperiod. Upon emergence, adult flies had access to water *ad libitum* from a cotton wick, a mixture of sugar and yeast for adult nutrition, along with perforated apples for oviposition (Barnes et al., 2007). After every two days, perforated apples from the adult cages were removed and placed in plastic food bowls (10cm x 15cm x 25cm) with artificial larval rearing diet modified from Barnes et al. (2007), with an additional 100 g of carrot powder per kg of mix. The food bowls were placed in a plastic box with vermiculite at the bottom and incubated at the above optimal conditions (28°C , 65% RH) (Pieterse et al., 2017). For pupation, third instar larvae jumped from the bowls with rearing diet into the vermiculite on the bottom of the plastic box. To maintain uniformity among test insects, vermiculite was sifted daily to remove the pupae, seven days after placing the apple on the rearing artificial diet. Pupae were placed in petri dishes which were later placed in new rearing cages housed in climate chambers at benign conditions. In this study, sex was not considered a factor since it has been reported not to affect traits of thermal tolerance in closely related species (e.g. Nyamukondiwa and Terblanche, 2009; Bertoli et al., 2010).

1.2.2. Temperature and relative humidity treatments

Adult *B. dorsalis* flies (24-48h old) were acclimated under 12L: 12D for four days in programmed Memmert climate chambers at four different fluctuating thermal ($\delta 3$, $\delta 6$, $\delta 9$ and $\delta 12^\circ\text{C}$) and moisture ($\delta 7$, $\delta 14$, $\delta 21$ and $\delta 28\%$) regimes around optimum conditions (28°C ; 65% RH) (see Fig.

1.1). Control flies were placed in a programmed chamber set at constant 28°C (±0°C), 65% RH and 12L: 12D for the same duration as treatments (Fig. 1.1). Acclimation for this duration is generally sufficient to elicit some plastic responses in other similar insect taxa e.g. *Ceratitis capitata* (Weldon et al., 2011). Rate of temperature increase or decrease varied during ramping up and down depending on temperature-RH amplitude treatment (Fig. 1.1). In all treatments, the thermal fluctuations exposed insects to warmer temperatures relatively longer than cold conforming to the notion that future climate change scenarios will expose organisms to more high than low temperature stress in future (see Stillman, 2019; Xu et al., 2020). Thermocron iButtons (Dallas Semiconductors, Model DS1920) were used to verify and monitor actual temperature and RH in each cage. Vapour pressure deficit, a measure of the drying power of the air and dependent on both temperature and RH (Bujan et al., 2016) was determined as follows:

$$VPD = (1 - (RH/100)) * SVP$$

where VPD is Vapour pressure deficit (kPa), RH is relative humidity and SVP is Saturated vapour pressure (kPa). Following acclimation, critical thermal minima (CT_{min}), chill coma recovery time (CCRT), critical thermal maxima (CT_{max}) and heat knockdown time (HKDT) were measured.

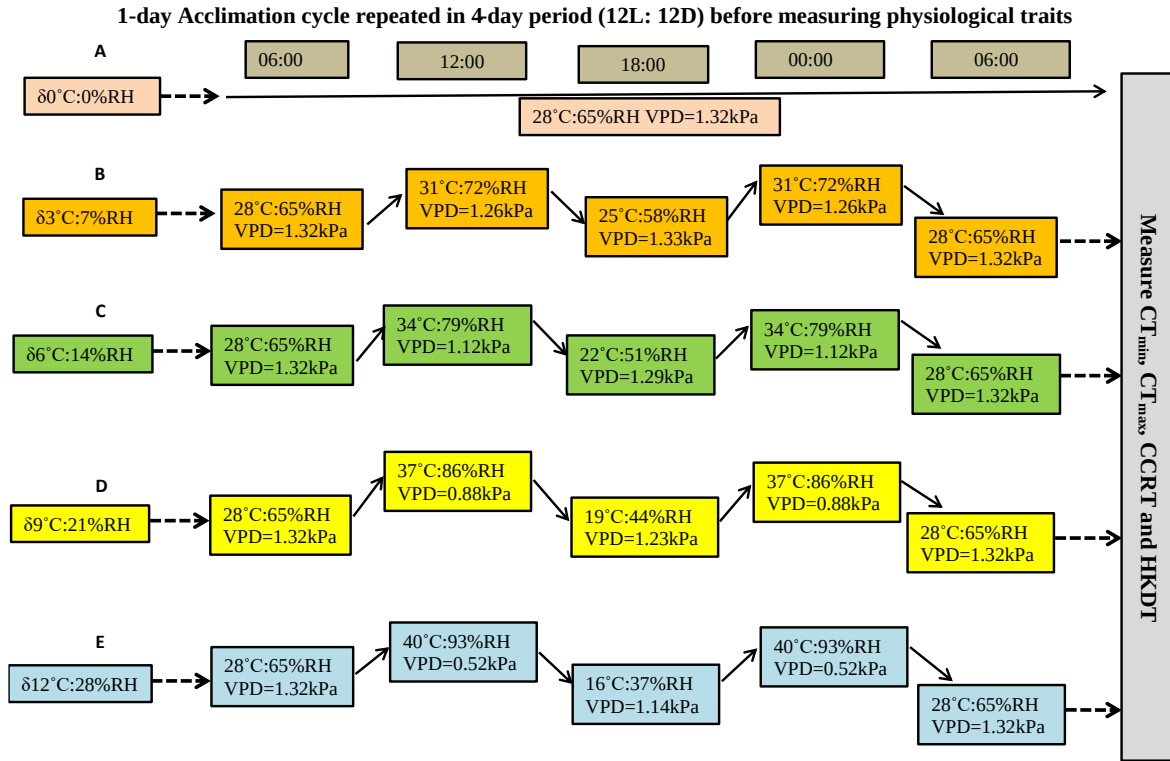


Figure 1.1. Schematic diagram of 4-day acclimation treatments of adult *Bactrocera dorsalis* to variable temperatures and relative humidities oscillating at different amplitudes around 28°C; 65% RH. Treatments were categorised based on magnitude of fluctuations of both temperature and RH as follows: (A) 0°C and 0% RH (control), (B) 3°C and 7% RH, (C) 6°C and 14% RH, (D) 9°C and 21% RH and (E) 12°C and 28% RH. Physiological stress decreased with amplitude such that $\delta 3^{\circ}\text{C}; 7\% \text{ RH}$ (0.07kPa VPD window) elicited the most stress on test organisms followed by $\delta 6^{\circ}\text{C}; 14\% \text{ RH}$ (0.2kPa VPD window), $\delta 9^{\circ}\text{C}; 21\% \text{ RH}$ (0.44kPa VPD window) and $\delta 12^{\circ}\text{C}; 28\% \text{ RH}$ (0.8kPa VPD window). VPD = vapour pressure deficit, CT_{min} = critical thermal minima, CT_{max} = critical thermal maxima, CCRT = chill coma recovery time, HKDT = heat knockdown time, RH = Relative humidity.

1.2.3. Temperature and relative humidity fluctuation effects on low temperature tolerance

1.2.3.1. Critical thermal minima

Critical thermal minima were measured using dynamic protocol as outlined by Nyamukondiwa and Terblanche (2009). Firstly, ten acclimated individual flies were placed randomly in a series of test tubes ('organ pipes') connected to an insulated double-jacketed chamber that was linked to a

programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol before being subjected to a constant cooling rate. Thereafter, the flies were held for 10 minutes to equilibrate at 28°C (optimal temperature) before ramping down temperature at a rate of 0.25°C min⁻¹ until their critical thermal minima were recorded. The chamber temperature was verified and monitored by a thermocouple (type K 36 SWG) inserted into centre tube and connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA). The flies were stimulated every minute (0.25°C) upon showing signs of incapacitation. The same experiment was repeated twice to yield sample sizes of $n = 20$. In this study, critical thermal minimum was defined as the lowest temperature at which each individual fly lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (like mild prodding with a soft thermally inert camel-hair brush) (e.g. Nyamukondiwa and Terblanche, 2010).

1.2.3.2. Chill coma recovery time (CCRT)

Chill coma recovery time experiments were assayed following four-day acclimation using established protocols (Weldon et al., 2011; Mutamiswa et al., 2018b). A total of 10 replicate adult flies were individually placed in numbered 0.65ml microcentrifuge tubes and then placed in a large watertight zip-lock bag. Thereafter, the zip-lock bag 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 × time treatment has been reported to elicit chill coma in other like insect taxa (e.g. Weldon et al., 2011; Sinclair et al., 2015). After 1 h at chill coma temperature, the tubes were immediately transferred from the water bath to the climate chamber set at optimum conditions (28°C, 65% RH) for recovery. This was repeated twice to yield sample sizes of $n = 20$ per each treatment. All observations and data were recorded from a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China), connected to the climate chamber and also linked to a computer. Chill coma recovery time was defined as the time (in minutes) required to regain coordinated movement ability (Milton and Partridge, 2008).

1.2.4. Temperature and relative humidity fluctuation effects on high temperature tolerance

1.2.4.1. Critical thermal maxima

Following four-day acclimation, critical thermal maxima were performed using standardized protocol as outlined by Nyamukondiwa and Terblanche (2009). As in CT_{min} experiments, 10 replicate adult flies were individually placed in numbered ‘organ pipes’ connected to a programmable water bath before ramping the temperature up at a rate of $0.25^{\circ}C/min$ until their critical thermal maxima were recorded. This was also repeated twice to yield sample sizes of $n = 20$ per treatment. The flies were also stimulated once every minute ($0.25^{\circ}C$) to monitor activity. Critical thermal maximum was defined as the highest temperature at which each individual fly lost coordinated muscle function, consequently losing the ability to respond to mild stimuli (like mild prodding with a soft thermally inert camel-hair brush) (e.g. Nyamukondiwa and Terblanche, 2010).

1.2.4.2 Heat knockdown time (HKDT)

As in CCRT, HKDT experiments were assayed as outlined by Mutamiswa et al. (2018b). Ten adult flies were individually placed in numbered 0.65ml microcentrifuge tubes and placed in a climate chamber connected to a computer linked camera. In the chamber, the tubes were then exposed to a knockdown temperature of $49 \pm 0.3^{\circ}C$; 65% RH. This knockdown temperature ($49^{\circ}C$) was selected based on preliminary upper thermal activity limits data from temperature and RH fluctuation treatments. This was repeated two times to yield sample sizes of $n = 20$ for each treatment. As in CCRT, observations from the climate chamber were also recorded from a video recording system. In this study, HKDT was defined as the time (in minutes) at which flies lost activity following exposure to acute heat stress ($49^{\circ}C$) in the climate chamber.

1.2.5 Statistical Analyses

Data analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma). Before analysis, data were first checked for normality and equality of variances using the Shapiro-Wilk test and Hartley-Bartlett tests respectively. In all cases, linear model assumptions of constant variance and normal errors were met (Shapiro-Wilk and Hartley-Bartlett tests, $P > 0.05$). Therefore, results were analysed using one-way analysis of variance (ANOVA) with CT_{min} , CT_{max} , CCRT and HKDT being the dependent variables while temperature and RH acclimation were the categorical factors. Tukey-Kramer’s *post-hoc* tests were used to separate statistically heterogeneous groups. Linear regression was performed to test the relationship between amplitude

($\delta 3^{\circ}\text{C}; 7\% \text{ RH}$ $\delta 6^{\circ}\text{C}; 14\% \text{ RH}$, $\delta 9^{\circ}\text{C}; 21\% \text{ RH}$ and $\delta 12^{\circ}\text{C}; 28\% \text{ RH}$) and traits of thermal tolerance ($\text{CT}_{\min}$, CCRT, $\text{CT}_{\max}$ and HKDT).

1.3. Results

1.3.1 Temperature and relative humidity fluctuation effects on low temperature tolerance

1.3.1.1. Critical thermal minima

Temperature and RH fluctuations significantly affected $\text{CT}_{\min}$ in *B. dorsalis* adults ($P < 0.001$) (Table 1.1; Fig. 1.2). There was a marginal but non-significant improvement in cold tolerance ($\text{CT}_{\min}$) following $\delta 6^{\circ}\text{C}; 14\% \text{ RH}$ and $\delta 9^{\circ}\text{C}; 21\% \text{ RH}$ amplitudes and significantly compromised $\text{CT}_{\min}$ following $\delta 3^{\circ}\text{C}; 7\% \text{ RH}$ and $\delta 12^{\circ}\text{C}; 28\% \text{ RH}$ amplitudes relative to controls ($\delta 0^{\circ}\text{C}; 0\% \text{ RH}$) (Fig. 1.2). Significant differences in $\text{CT}_{\min}$ were recorded between controls and $\delta 3^{\circ}\text{C}; 7\% \text{ RH}$ as well as $\delta 12^{\circ}\text{C}; 28\% \text{ RH}$ amplitudes ($P < 0.05$) (Fig. 1.2). However, there were no significant differences in $\text{CT}_{\min}$ between controls and $\delta 6^{\circ}\text{C}; 14\% \text{ RH}$ as well as $\delta 9^{\circ}\text{C}; 21\% \text{ RH}$ amplitudes ($P > 0.05$) (Fig. 1.2). In addition, no significant correlation was recorded between amplitude and $\text{CT}_{\min}$ $r(1) = 0.12$, $P = 0.27$ (Fig. 1.4).

Table 1.1. Summary statistical results from one-way ANOVA showing effects of temperature and relative humidity fluctuations on critical thermal minima, chill coma recovery time, critical thermal maxima and heat knockdown time of *Bactrocera dorsalis*.

Trait	Effect	SS	df	MS	F	p
$\text{CT}_{\min}$	Intercept	6,385.61	1	6,385.61	15,349.4	<.001
	Treatment	19.26	4	4.815	11.57	<.001
	Error	39.521	95	0.416		
CCRT	Intercept	1,407.25	1	1,407.25	2,400.54	<.001
	Treatment	29.03	4	7.26	12.38	<.001
	Error	55.69	95	0.59		
$\text{CT}_{\max}$		213,702.8		213,702.8		
	Intercept	0	1	0	449,621	<.001
	Treatment	25	4	6.2	13.1	<.001
HKDT	Error	45.2	95	0.5		
	Intercept	1,896.31	1	1,896.31	5,917.84	<.001
	Treatment	10.86	4	2.72	8.48	<.001
	Error	30.44	95	0.32		

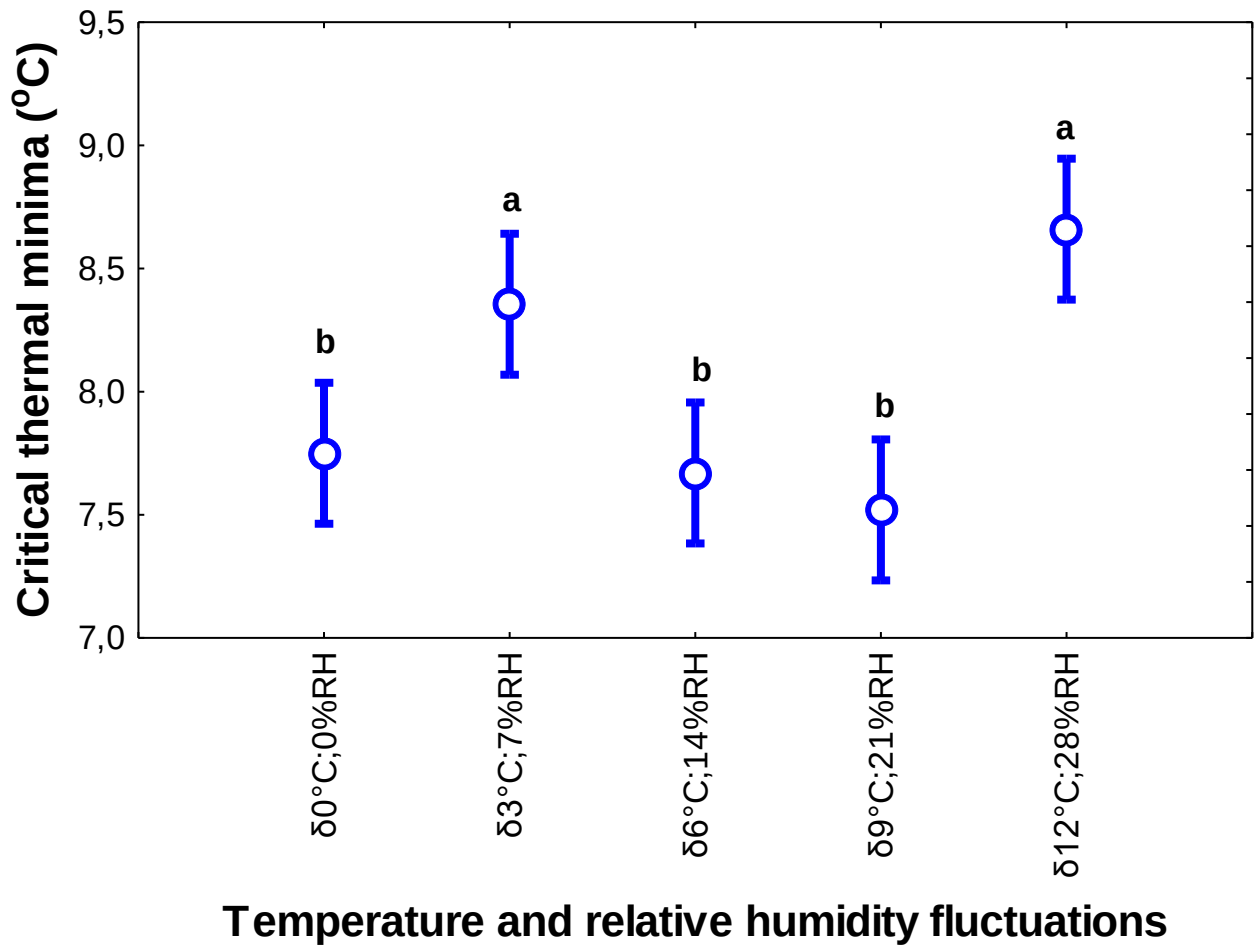


Figure 1.2. Effects of temperature and relative humidity fluctuations on critical thermal minima of *Bactrocera dorsalis* adults. Error bars denote $\pm 95\%$ confidence limits. Means with the same letter are not statistically different.

1.3.1.2. Chill coma recovery time

Chill coma recovery time of *B. dorsalis* adults significantly varied across treatments (Table 1.1; Fig. 1.3). There were no significant differences in CCRT between controls and $\delta 6^{\circ}\text{C}; 14\%$ RH, $\delta 9^{\circ}\text{C}; 21\%$ RH and $\delta 12^{\circ}\text{C}; 28\%$ RH amplitudes (Table 1.1; Fig. 1.3). However, significant difference in CCRT was recorded between controls and $\delta 3^{\circ}\text{C}; 7\%$ RH amplitude (Fig. 1.3). Adult flies acclimated at $\delta 3^{\circ}\text{C}; 7\%$ RH took more time to recover from chill coma (compromised cold tolerance) relative to controls, $\delta 6^{\circ}\text{C}; 14\%$ RH, $\delta 9^{\circ}\text{C}; 21\%$ RH and $\delta 12^{\circ}\text{C}; 28\%$ RH amplitudes (Fig. 1.3). When pooled together, there was a negative correlation between amplitude and CCRT $r(1) =$

0.5, $P < 0.05$ such that an increase in amplitude resulted in improved cold tolerance (decreased CCRT) (Fig 1.4).

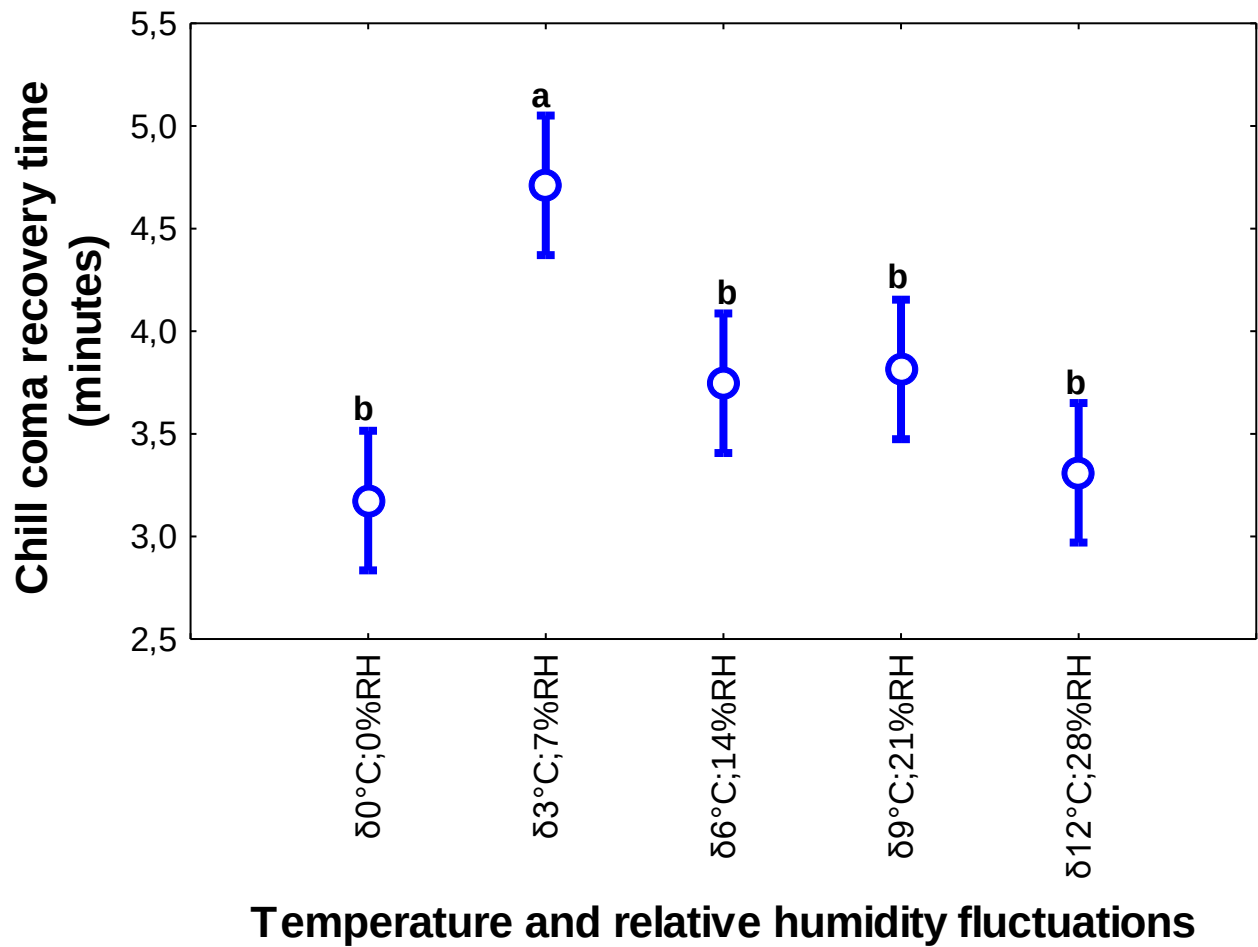


Figure 1.3. Effects of temperature and relative humidity fluctuations on chill coma recovery time of *Bactrocera dorsalis* adults. Error bars denote $\pm 95\%$ confidence limits. Means with the same letter are not statistically different.

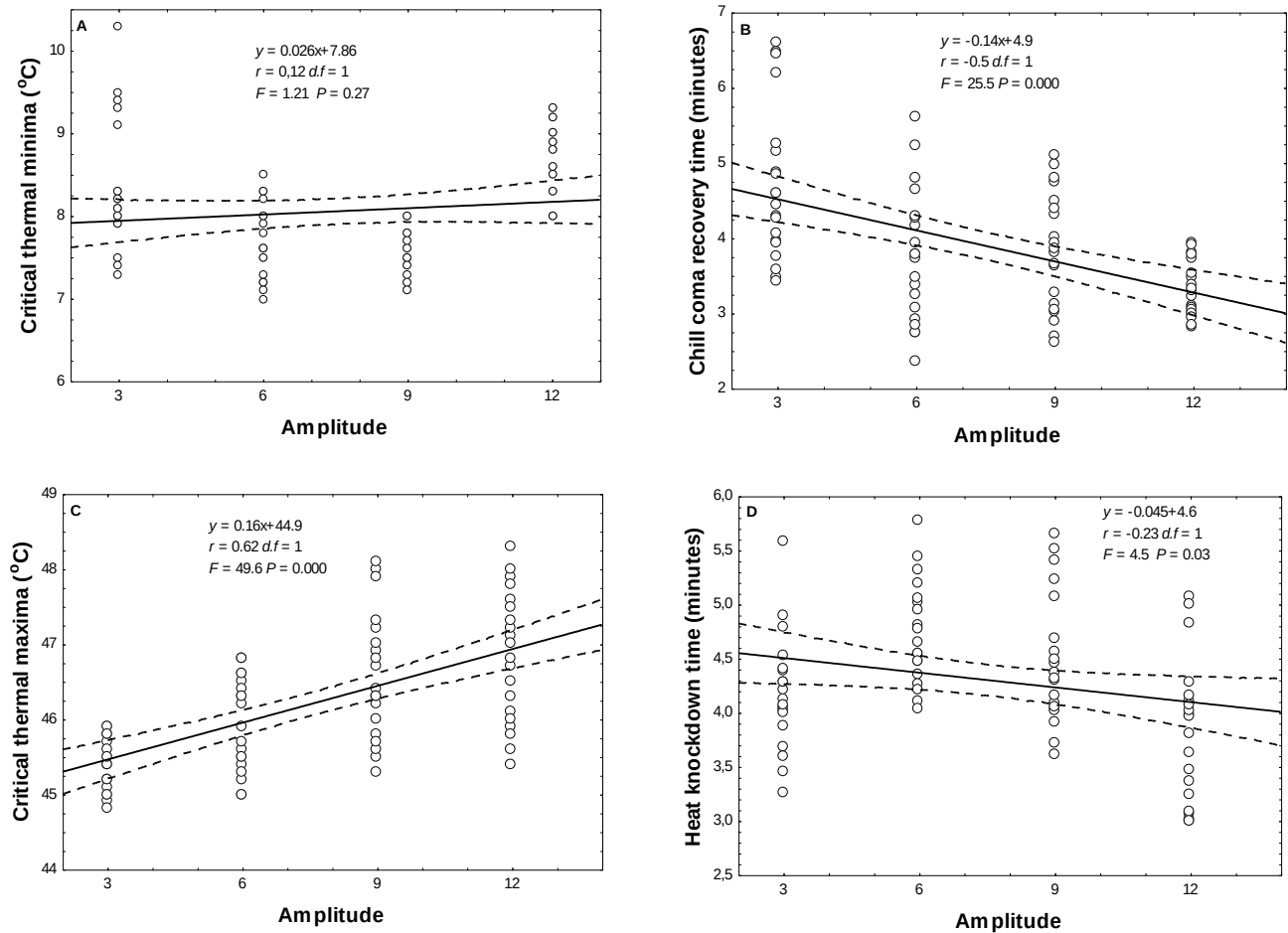


Figure 1.4. Relationships between amplitude and (A) critical thermal minima, (B) chill coma recovery time, (C) critical thermal maxima and (D) heat knockdown time. Amplitude 3 = $\delta 3^{\circ}\text{C}; 7\%$ RH, Amplitude 6 = $\delta 6^{\circ}\text{C}; 14\%$ RH, Amplitude 9 = $\delta 9^{\circ}\text{C}; 21\%$ RH and Amplitude 12 = $\delta 12^{\circ}\text{C}; 28\%$ RH.

1.3.2. Temperature and relative humidity fluctuation effects on high temperature tolerance

1.3.2.1. Critical thermal maxima

Bactrocera dorsalis adults' $CT_{\max}$ were significantly influenced by temperature and RH fluctuations (Table 1.1; Fig. 1.5). Heat tolerance improved with increase in thermal and RH breadths such that $\delta 12^{\circ}\text{C}; 28\%$ RH amplitude recorded the highest $CT_{\max}$ (Fig. 1.5). There was a significant difference in $CT_{\max}$ between controls and $\delta 3^{\circ}\text{C}; 7\%$ RH amplitude (Table 1.1; Fig. 1.5), with the latter having significantly lower heat tolerance. However, no significant differences were recorded between controls and $\delta 6^{\circ}\text{C}; 14\%$ RH, $\delta 9^{\circ}\text{C}; 21\%$ RH and $\delta 12^{\circ}\text{C}; 28\%$ RH amplitudes

(Table 1.1; Fig. 1.5). In addition, there was a positive correlation between amplitude and CT_{max} ($r(1) = 0.62$, $P < 0.05$) such that an increase in amplitude resulted in improved heat tolerance (increased CT_{max}) (Fig 1.4).

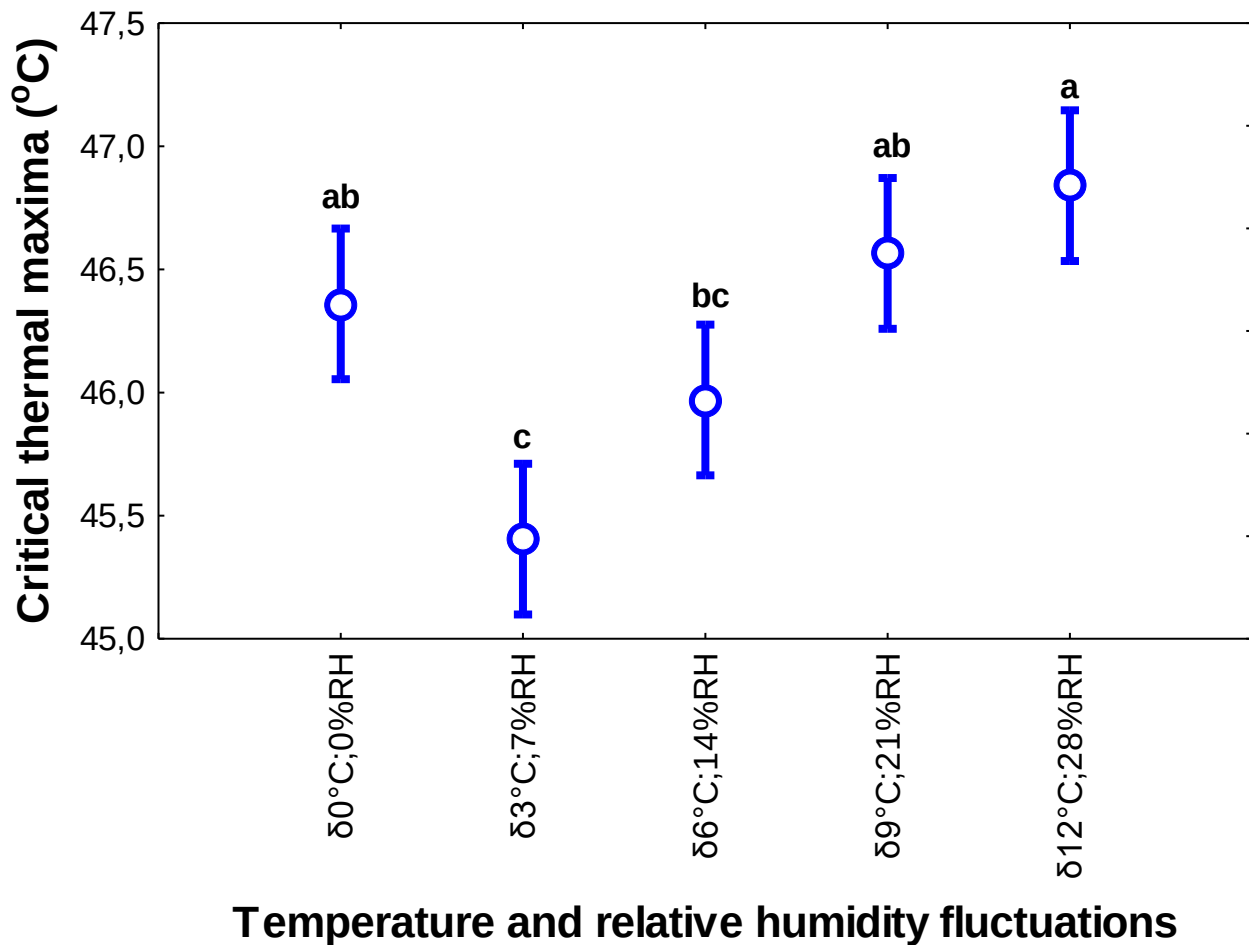


Figure 1.5. Effects of temperature and relative humidity fluctuations on critical thermal maxima of *Bactrocera dorsalis* adults. Error bars denote $\pm 95\%$ confidence limits. Means with the same letter are not statistically different.

1.3.2.2. Heat knockdown time

As in CT_{max} , temperature and RH fluctuations significantly affected *B. dorsalis* adults HKDT ($P < 0.001$) (Table 1.1; Fig. 1.6). Adult flies acclimated at $\delta 12^{\circ}\text{C}$; 28% RH were knocked down significantly faster (compromised heat tolerance) than controls, $\delta 6^{\circ}\text{C}$; 14% RH and $\delta 9^{\circ}\text{C}$; 21% RH amplitudes following exposure to acute heat stress (49°C) (Fig. 1.6). There was a significant

difference in HKDT between controls and $\delta 12^{\circ}\text{C}; 28\%$ RH treated adults (Table 1.1; Fig. 1.6). However, no significant differences in HKDT were recorded between controls, $\delta 3^{\circ}\text{C}; 7\%$ RH, $\delta 6^{\circ}\text{C}; 14\%$ RH and $\delta 9^{\circ}\text{C}; 21\%$ RH amplitudes (Fig. 1.6). When pooled together, a negative correlation was recorded between amplitude and HKDT $r(1) = 0.23$, $P < 0.05$ (Fig 1.4).

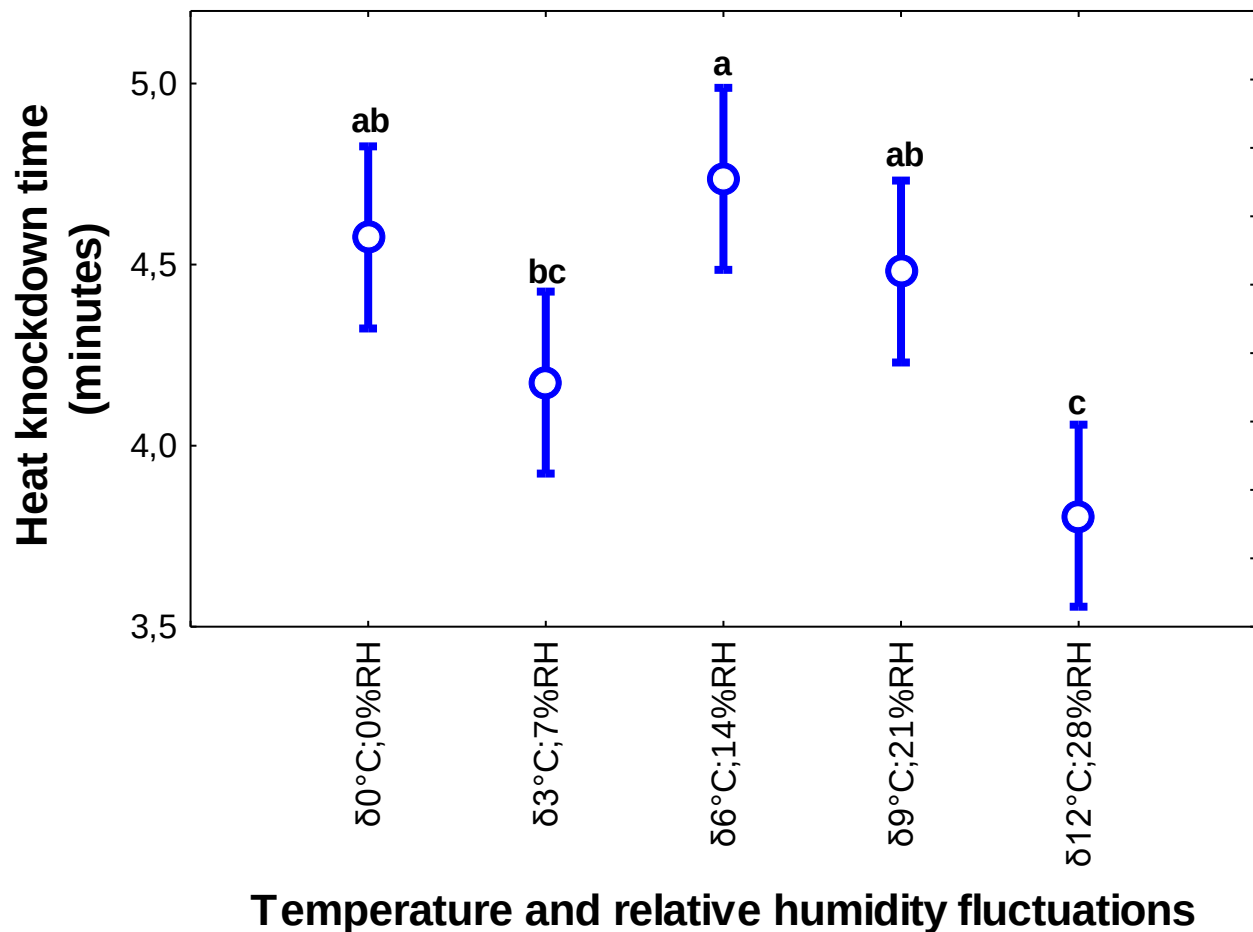


Figure 1.6. Effects of temperature and relative humidity fluctuations on heat knockdown time of *Bactrocera dorsalis* adults. Error bars denote $\pm 95\%$ confidence limits. Means with the same letter are not statistically different.

1.4. Discussion

Our results showed that temperature and RH fluctuations significantly influenced low and high temperature fitness of adult *B. dorsalis*. Temperature and RH fluctuations ($\delta 3^{\circ}\text{C}; 7\%$ RH and $\delta 12^{\circ}\text{C}; 28\%$ RH amplitudes) significantly reduced cold tolerance measured as ($\text{CT}_{\min}$) and CCRT

($\delta 3^{\circ}\text{C}$; 7% RH amplitude). Similarly, $\delta 3^{\circ}\text{C}$; 7% RH amplitude significantly reduced heat tolerance (CT_{max}) while flies acclimated at $\delta 12^{\circ}\text{C}$; 28% RH and $\delta 3^{\circ}\text{C}$; 7% RH had significantly lower heat tolerance (significantly lower HKDT) than the controls. This result is congruent to the notion that climate variability impairs insect fitness across spatio-temporal scales. Unless organisms shift their stress tolerance either through phenotype remodeling or genetic adaptation, survival is significantly affected (Cavieres et al., 2018). Temperature and RH fluctuations are highly dependent (Bujan et al., 2016), seldom through the former's influence on VPD. Both abiotic factors affect insect survival through direct and indirect effects on development, activity and survival (Chown and Nicolson, 2004; Masó et al., 2019).

Vapour pressure deficit is generally proportional to evaporative loss in organisms even at various air temperatures (Ghazy et al., 2012). A combination of high temperature and low humidity results in high drying power (high VPD), which leads to organismal high-water loss (Huang et al., 2019). An increase in temperature by 3°C can result in 45% increase in VPD since a temperature rise generally leads to reduction in RH (Huang et al., 2019). This level of VPD increase was not observed in the present study as it was masked by fluctuating RHs around the optimum (28°C ; 65% RH) which were coupled with simultaneous decrease and increase in temperatures (see Fig. 1). The present study shows that VPD decreased with increased amplitude such that fluctuations ranged from 1.26-1.33kPa, 1.12-1.32kPa, 0.88-1.32kPa, and 0.52-1.32kPa for $\delta 3^{\circ}\text{C}$; 7% RH, $\delta 6^{\circ}\text{C}$; 14% RH, $\delta 9^{\circ}\text{C}$; 21% RH and $\delta 12^{\circ}\text{C}$; 28% RH amplitudes respectively. This indicates VPD windows of 0.07kPa, 0.2kPa, 0.44kPa and 0.8kPa for $\delta 3^{\circ}\text{C}$; 7% RH, $\delta 6^{\circ}\text{C}$; 14% RH, $\delta 9^{\circ}\text{C}$; 21% RH and $\delta 12^{\circ}\text{C}$; 28% RH amplitudes respectively. Based on these results, it shows that the level of physiological stress in test insects decreased with increased amplitude. As such, organisms acclimated at $\delta 3^{\circ}\text{C}$; 7% RH experienced a narrow VPD window indicating that they were the most physiologically stressed followed by $\delta 6^{\circ}\text{C}$; 14% RH, $\delta 9^{\circ}\text{C}$; 21% RH and $\delta 12^{\circ}\text{C}$; 28% RH acclimations. This is confirmed by a reduced low (CT_{min} and CCRT) and high (CT_{max} and HKDT) temperature tolerance for *B. dorsalis* adults acclimated at $\delta 3^{\circ}\text{C}$; 7% RH relative to other acclimations (see Figs. 2, 3, 5 and 6). This indicates *B. dorsalis* thermal fitness losses under fluctuating temperatures and RHs. Significant effects of these combinational factors for fitness suggest the significance of incorporating these in future empirical studies for better explaining the responses of organisms in the face of climate change.

In a previous study, we reported significant interactive effects of static temperature and RH on *C. partellus* thermal tolerance following developmental acclimation (Mutamiswa et al., 2019). This is in corroboration with the present study where dynamic interaction of thermal and moisture regimes influenced *B. dorsalis* thermal tolerance. Critical thermal limits are dependent on methodological context (Terblanche et al., 2007) and are highly considered as ecologically relevant measures of thermal tolerance in insects. In the present study, an increase in amplitude did not significantly improve cold tolerance (CT_{min}) in adult *B. dorsalis*. The results are in keeping with previous study by Terblanche et al. (2010) which reported a loss in basal and plastic responses in a related invasive species, *C. capitata* following increased amplitude of temperature fluctuations.

The onset of heat waves is often associated with more sunshine and higher VPD (Bannerman et al., 2011). Consequently, insects experiencing high temperature stress environments are often simultaneously faced with desiccation stress. In our study, acclimation to $\delta 3^{\circ}\text{C}$; 7% RH (0.07kPa VPD window) was associated with fluctuating high VPD (Fig. 1.1) suggesting adult *B. dorsalis* may have experienced more desiccation stress under that treatment than other acclimations. Although Bayley et al. (2001) reported an increased cold tolerance in springtails, *Folsomia candida* following desiccation stress, our results show otherwise. Our experiments showed that adult *B. dorsalis* exposed to $\delta 3^{\circ}\text{C}$; 7% RH coupled with desiccation had compromised cold tolerance (CT_{min} and CCRT). These findings therefore suggest that adult *B. dorsalis* was unable to develop any plastic mechanisms to withstand stress and improve fitness under these conditions ($\delta 3^{\circ}\text{C}$; 7% RH). This is in keeping with Pieterse et al. (2017) who reported a lack of plasticity in larval *B. dorsalis*. While phenotypic plasticity is nearly universal in insects (Whitman and Ananthakrishnan, 2009), and that it may facilitate invasiveness (Mutamiswa et al., 2018c) and adaptation to changing environments (Chown and Nicolson, 2004), it may appear that it has little or no effect on the invasion edge of *B. dorsalis* (also see discussions in Pieterse et al., 2017).

Our results also showed that an increase in environmental amplitude (temperature and RH) may result in impaired thermal fitness (CT_{min} and CCRT). Amplitudes $\delta 6^{\circ}\text{C}$; 14% RH (0.2kPa VPD window), $\delta 9^{\circ}\text{C}$; 21% RH (0.44kPa VPD window) and $\delta 12^{\circ}\text{C}$; 28% RH (0.8kPa VPD window) compromised both CT_{min} and CCRT in adult *B. dorsalis*. These results are in consonance with previous study by Nyamukondiwa et al. (2018) which reported compromised low temperature

(CT_{min}) response of *Scarabaeus zambeianus* at higher thermal amplitudes (δ6 and δ9°C; 65% RH). Interestingly, *B. dorsalis* low and high temperature responses were inversely related such that the most positive CT_{min} (compromised cold tolerance) and highest CT_{max} (enhanced heat tolerance) were recorded at the highest environmental amplitude (Figs. 1.2; 1.5). This suggests a trade-off between traits of heat and cold tolerance for adult *B. dorsalis*. In consequence, heat tolerance improvement associated with increasing environmental stress amplitude may likely come at a fitness cost to cold tolerance (see discussions in Nyamukondiwa et al., 2011). The relationship between amplitude and low temperature tolerance varied among traits tested such that CCRT was negatively correlated with amplitude whereas CT_{min} was not significantly correlated. An increase in amplitude improved cold tolerance (reduced CCRT) while it had no effects for CT_{min}. Given this variation and that CCRT and CT_{min} are proxies for low temperature tolerance, it indicates that amplitude and low thermal tolerance relationship in this invasive insect species is trait dependent.

The current results also show differential sensitivity to thermal and moisture variability in some closely linked traits of high thermal tolerance. Under fluctuating temperature regimes ranging 19.9 – 28.3°C, *S. frugiperda* recorded faster developmental rate relative to constant regimes (Simmons, 1993). Although Nyamukondiwa et al. (2018) reported compromised high temperature tolerance following increased temperature variability, our results indicate otherwise since increased thermal and moisture fluctuations resulted in improved CT_{max}. This indicates a higher level of plasticity and synergistic effect between low to high temperature and low to high RH regimes in enhancing heat tolerance. However, for HKDT, results showed variations in high temperature tolerance with the highest amplitude recording the lowest knockdown time. This therefore suggests that heat tolerance in *B. dorsalis* may be trait dependent, consistent with Mutamiswa et al. (2019). Exposure to increased amplitude and variability in extreme temperatures has also been reported to influence performance in *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Bozinovic et al., 2013) as well as development and population growth in aphids (Bannerman et al., 2011). These findings are in keeping with our results since exposure to δ3°C; 7% RH negatively influenced both low and high temperature tolerance in *B. dorsalis* adults. If insect mean body temperature is close to or higher than thermal optimum, cooling and warming may reduce its performance (Bozinovic et al., 2011). In our study, *B. dorsalis* adults exposed to δ3°C; 7% RH amplitude experienced temperatures which were near and slightly above their optimum conditions. This resulted in compromised low and

high temperature tolerance in agreement with Bozinovic et al. (2011). Desiccation stress following exposure to a wide array of sub-lethal low and high temperatures has been reported to impair upper thermal limits in *Drosophila melanogaster* (Diptera: Drosophilidae) (Da Lage et al., 1989). Given that acclimation at $\delta 3^{\circ}\text{C}$; 7% RH was associated with fluctuating high VPD with high likelihood of dehydration, it is highly likely that reduced CT_{max} recorded in the present study may have been triggered by desiccation stress, in keeping with Da Lage et al. (1989). In *Aphaenogaster picea* (Hymenoptera: Formicidae), desiccation stress resulted in reduced HKDT following exposure to acute knockdown temperature of 40.5°C (Nguyen et al., 2017). Our HKDT under increasing temperature and moisture amplitude ($\delta 3^{\circ}\text{C}$; 7% RH) resulted in compromised high temperature tolerance. With the high VPD recorded under increased amplitude ($\delta 3^{\circ}\text{C}$; 7% RH) coupled with desiccation stress, our results agree with Nguyen et al. (2017). As in low temperature tolerance, the relationship between high temperature tolerance and amplitude also varied among tested traits such that HKDT was negatively correlated with amplitude whilst CT_{max} was positive. This also indicates that amplitude and high temperature tolerance relationship in *B. dorsalis* is trait dependent.

In conclusion our results show that variation in abiotic environment (temperature and RH) influences thermal fitness (for both low and high temperature traits) of adult *B. dorsalis*. Thermal tolerance levels significantly varied across environmental amplitudes and physiological traits measured, indicating that the effects may also be trait dependent. These differential thermal fitness levels indicate the significant roles of combined thermal and moisture fluctuations on insect fitness and survival under changing environments. This implies that (1) daily and seasonal variations in thermal and hydric environmental conditions projected under climate ‘pulses’ and ‘presses’, may confer fitness losses in adult *B. dorsalis* and that (2) future experiments predicting insects’ responses to climate change should incorporate both fluctuating temperature and RH environments (than constant abiotic stress conditions) when forecasting species population dynamics. This may be especially significant for predictive models targeting invasive pest species population establishments under changing climates and may help early warning systems and pest management.

1.5. References

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