



Faculty of Sciences

Department of Biological Sciences and Biotechnology

**PLASTIC RESPONSES AS DRIVERS OF FITNESS IN *SITOTROGA*
CEREALELLA (OLIVIER)**

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BSc Biological Sciences and Biotechnology (BIUST)

A Thesis Submitted to the Faculty of Science in Partial Fulfilment of the Requirements
for the Award of the Degree of Master of Science in Biological Sciences and
Biotechnology of the BIUST

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APRIL, 2023

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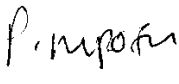
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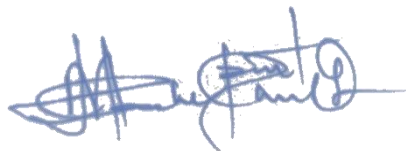
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Plastic responses as drivers of fitness in *Sitotroga cerealella* (olivier), in fulfilment of the requirements for the degree of Master of Science *Biological Sciences and Biotechnology* of the BIUST.



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Dedications

I would like to dedicate this thesis to my mother Tambudzai Patricia Mpofu.

Acknowledgement

Words are inadequate to express my thanks to Prof. Casper Nyamukondiwa and Dr Honest Machekano who served as my supervisors, for their invaluable patience and feedback. Without their support and generosity in sharing their knowledge and experience, I would not have been able to go on and complete this journey. In addition, this project would not have been possible without the kind funding provided by BIUST.

I am also grateful to Dr Nonfo Gotcha for her mentoring and moral support. Many thanks should also go to my research lab colleagues who impacted positively and inspired me.

Lastly, I would be remiss in not mentioning my mother. Her belief in me has kept my spirits and motivation high during this process.

Abstract

Models predict increase in both global temperatures, means and the magnitude of seasonal and daily temperature variations. These changes are likely to impact insect species physiology and phenology. However, the ability of insect species to adapt to these shifts in temperature remain a mystery. Nevertheless, it has been documented that insects use a wide variety of mechanisms such as phenotypic plasticity to cope with extreme environmental stressors. This mechanism has been reported to allow the organisms to cope with environmental unpredictability and or heterogeneity. Despite this knowledge, it is unclear how much plasticity in thermal tolerance will impact insect response to global warming, particularly over generations. Insects in the visited local storage facilities are frequently exposed to high temperature stress due to the enclosed nature and relatively small size of grain storage facilities. However, the possibility for this climate induced continuous pre-conditioning to create heat resilient storage pest phenotypes through beneficial acclimation remain unknown and is also likely to worsen under current and projected climate change. This work was therefore aimed at investigating carry over effects of heat and starvation acclimations (chapter 3) and cold and desiccation acclimations (chapter 4) on the thermal tolerance of adult *Sitotroga cerealella* across generations. I found out that, heat acclimation (acute and dynamic) showed reduced heat tolerance measured as both CT_{max} and Heat knockdown time on *S. cerealella*. These results are however not surprising as although insects are able to quickly respond to changing environmental conditions through phenotypic plasticity, phenotypic plastic responses have been reported to be reversible as well as not fixed. I also found that fasting (food deprivation) acclimation did not significantly affect CT_{max} , indicating a lack of evidence for cross tolerance and/or susceptibility. Similarly to starvation acclimation, both cold and desiccation acclimation showed reduced thermal tolerance, suggesting lack of inter-generational crosstalk in heat stress following both cold and desiccation acclimation in *S. cerealella*. This work provides knowledge on how phenotypic plasticity is likely to contribute to the survival of *S. cerealella* under the ongoing global warming. The information is critical in that it can be used in the improvement of grain treatment methods, thereby contributing towards global food security.

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Chapter 1:

General Introduction

1.1. Background

Models predict increase in both global temperature means and the magnitude of seasonal and diel temperature variations (Colinet *et al.*, 2015). These changes may also concomitantly bring other environmental stressors such as dehydration and starvation, that likely impact differentially on species, and across ontogeny and consequently affect insect population phenology and dynamics (Mutamiswa *et al.*, 2018). Abiotic factors such as temperature and humidity have been reported to directly influence insect population dynamics (Selvaraj *et al.*, 2013). For example, global temperature increase accelerates development rate, survival, reproduction, and population size of insects (Selvaraj *et al.*, 2013). As such, heat tolerance may thus determine the fate of tropical organisms in the face of climate change (Machekano *et al.*, 2021). Indeed, the ability of an organism to remain active under extreme heat conditions has been reported to be a significant component of fitness (Loeschcke & Hoffmann, 2007).

Insects have been documented to use a wide variety of mechanisms to cope with extreme environmental stressors (Sinclair *et al.*, 2003; Kliot & Ghanim, 2012; González Tokman *et al.*, 2020). One such mechanism is phenotypic plasticity, which is the expression of a wide range of phenotypic traits by a single genotype in response to variable environmental conditions (Schlichting & Smith, 2002; Whitman & Agrawal, 2009; Benito Garzón *et al.*, 2019; Pfennig, 2021). This mechanism has been reported to allow the organisms to cope with environmental unpredictability and or heterogeneity (Gotcha *et al.*, 2018), and has been suggested to be an important driver of evolutionary change (Manfredini *et al.*, 2019), although its role in adaptive evolution remains debatable (de Jong, 2005; Deere *et al.*, 2006; Moczek, 2010; Sommer *et al.*, 2017). Nevertheless, the ability of insect species to adapt to shift in temperature is the main challenge in a warming world (Manfredini *et al.*, 2019). This is because although thermal tolerance can be plastic (Schou *et al.*, 2017), it is unclear how much plasticity in thermal tolerance will impact insect response to global warming, particularly over generations. Studies have shown that thermal plasticity can vary within and across insects' species, and that the degree or extent to which plasticity can buffer insects from extreme temperatures varies among species (Whitman & Agrawal, 2009; Gunderson & Stillman, 2015; van Heerwaarden *et al.*, 2016; Machekano *et al.*, 2021). Furthermore, the effect of phenotype plasticity can happen at distinct time scales thus, during development/ developmental acclimation or during adulthood/ adult acclimation (Parkash *et al.*, 2014). In addition, plasticity in one developmental stage can manifest in another stage or can be passed on across generations- transgenerational effects/plasticity (TGP) (Sgrò *et al.*, 2016; Salis *et al.*, 2018).

Safe storage of staple foods improves household resilience and stability, aids long-term household planning, reduces ‘worry for food’ and thus, enables investment (of time and resources) into other interventions that improve quality of life both at household and national levels. The prolonged storage of food has become more important under the Covid-19 lockdown restrictions that limited access to fields, reduced access to grain suppliers and hindered input deliveries thus, negatively impacting on production. In Botswana, the demand for staple sorghum and maize is continually increasing as the population increases. Almost 96% of people in Botswana depend on sorghum as their major staple food and animal feed (Boling and Eisener, 1981; APHLIS, 2021). This has increased the need for farmers to store their own grain products for future consumption, seed, and livestock feed until the next good harvesting season to ensure food and nutritional security (Wambugu *et al.*, 2009). However, the loss of stored products due to pest insects attack is a major economic constraint worldwide. The damage and loss on sorghum caused by one of the most economic storage insect pests, the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera, Gelechiidae), (Togola *et al.*, 2010) has made it difficult for farmers and grain traders to store their cereal grain until the next harvest.

Although TGP has been studied in many other species (Eriksen *et al.*, 2006; Marshall, 2008; Murray *et al.*, 2014; Shama & Wegner, 2014) very few studies have focussed on stored product pest species. Research shows that insects in storage facilities are constantly exposed to sub-lethal high temperature stress due to the enclosed nature and relatively small sizes of grain storage environments in which they dwell (Angelovič *et al.*, 2018). Depending on the storage environment and location, this is likely worsening under current and projected climate change. The potential for this climate induced continuous pre-conditioning to create heat resilient storage pest phenotypes through beneficial acclimation over generations (TGP) is unknown (Sgrò *et al.*, 2016; Tougeron *et al.*, 2020). In addition, stored product pests inevitably increase their immediate grain environmental temperature due to their own respiration and constrained space, creating high but stochastic storage temperatures through ‘hot spots’ (Jian & Jayas, 2012; Wang *et al.*, 2021). This exposes the insect pests not only to sustained high constant sub-lethal heat stress, but also fluctuating (variable) temperatures, conferring potential transgenerational plasticity (TGP) responses, assuming beneficial acclimation effects holds (see Angilletta, 2009; Sgrò *et al.*, 2016). Other non-thermal stressors may also be passed on through TGP, for example, extended periods of no stored grain expose insects to food deprivation (fasting acclimation). Similarly low humidity in stored grain environments expose insects to desiccation conditions likely eliciting acclimation responses. The consequent effects on cross-temperature tolerance remain unknown. Against this background, I thus assessed TGP of a model and economically important storage pest species, *S.*

cerealella, post-exposure to acute and dynamic sub-lethal heat stress as well as fasting acclimation.

1.2. Research gap

Sitotroga cerealella is one of the storage insect pests of economic importance. It has been reported to be established in diverse habitats across the globe. Nevertheless, despite its pest status, there is lack of information on its thermal tolerance, plasticity as well as TGP. Physiological mechanisms aiding species adaptation to changing environments are increasingly becoming elucidated in various insects (see Nyamukondiwa *et al.*, 2010; Mutamiswa *et al.*, 2018; Gotcha *et al.*, 2021; Machekano *et al.*, 2021). These mechanisms are important since they can influence pest establishment and persistence into new environments. Furthermore, due to climate change and potential warming of enclosed storage facilities, *S. cerealella* may likely become ‘acclimated’, to heat, hence the need to investigate effects of environmental acclimation treatment on the thermal tolerance in this species. In addition, given overwhelming evidence of co-occurring multiple environmental stressors (see e.g., Gunderson *et al.*, 2016), it is therefore necessary to investigate possible mechanisms that could aid this insect pest adaptation to changing environments, as well as cross tolerance to multiple stressors, both within and across generations.

1.3. Justification of study

Sitotroga cerealella larvae inhabit grain environments, as such, providing protection from insecticides used to manage the pest. However non-chemical alternatives like heat treatment of grain have been widely used in the management of various storage insect species and were shown to be effective (Akdoğan *et al.*, 2005; Tilley *et al.*, 2007). Knowledge on thermal biology, especially effects of acclimation on the heat tolerance of *S. cerealella* will be very beneficial in that it can be used in population forecasting and pest management relative to prevailing temperature environments. This is because information obtained from this study can be used to explain how plastic responses of *S. cerealella* may influence population phenology and population resilience of this storage insect species. Furthermore, the results from this study can be used in explaining fitness costs and benefits in future *S. cerealella* generations and may help explain the evolution of plastic responses in storage pests for more informed, non-chemical and safer pest management alternatives. Overall, the results of this study may be used to design and/or refine heat grain treatments/phytosanitary disinfestation, as such ensuring safe and long storage of grains by farmers hence contributing towards food security.

1.4. Aim

The aim of this study was to assess TGP of temperature (high and low), starvation and desiccation acclimation on F₂ generation of *S. cerealella*.

1.5. Objectives

The specific objectives of this study were to;

- i). Determine the effects of high temperature at a constant relative humidity (65 ± 10 %) and starvation acclimation of F₁ on the heat tolerance F₂ generation in adult *S. cerealella*.
- ii). Determine the effects of low temperature at a constant relative humidity (65 ± 10 %) and desiccation acclimation of F₁ on the heat tolerance of F₂ generation in adult *S. cerealella*.

1.6. Hypotheses

- i). High temperature and starvation acclimation in F₁ will improve heat tolerance of the F₂ generation
- ii). Low temperature and desiccation acclimation in F₁ will improve heat tolerance of the F₂ generation.

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Chapter 2: Literature Review

2.1. The biology, ecology, and distribution of *S. cerealella*

The Angoumois grain moth was first described by Guillaume-Antoine Olivier in France in the year 1789 (Mishu, 2015). Because of its synanthropic properties which makes it simple to transport across the world in grain shipments, this pest has a near cosmopolitan distribution. It can thrive on a diversity of hosts, facilitating spread globally (Mishu, 2015). In subtropical and warm temperate parts of the world, the Angoumois grain moth is among the main pests of storage grain (Germanov, 1982). Moths are reproductively mature ~24 hours post eclosion and begin mating (Akter et al., 2013). The female lays between 150 to 400 eggs under optimal conditions (Singh & Bindu, 2014). These eggs hatch between 4-7 days under optimal temperature conditions (Akter et al., 2013; Singh & Bindu, 2014). The small larva burrow into the grain by moving about and typically entering through a fracture or abrasion in the pericarp. It lives its entire life there, feeding on the kernel. The larvae are around 5.0 mm long when fully mature. Larval stage typically lasts between 2-3 weeks depending on prevailing ambient environment (Singh & Bindu, 2014). However, a thin layer of the coat is left intact as it only consumes a small portion of the seed's exterior awaiting pupation period. A reddish-brown pupa is created inside a silky cocoon. The moth then encloses through a thin seedcoat of the grain that the larvae had left behind after pupating. The larva and pupa are wholly contained inside the grain in this manner. The pest typically has 3–4 broods per year, although occasionally 8 broods have been recorded under warmer tropical environments (Samiksha, 2016). Reports show *S. cerealella* has a global distribution that cuts across tropical, subtropical, and temperate regions (CABI, 2021), raising questions about physiological traits and adaptation mechanisms that enable its survival across multiple and climatically divergent environments. In Botswana, *S. cerealella* is described as 'present and widespread' (CABI, 2021; Champ and Dyte, 1977) indicating that its pest status and economic importance in Botswana needs not to be underestimated. Given that Botswana experiences moderate to high temperatures all year round, this pest can thus thrive all year round, as has been reported elsewhere (see Trematerra, 2015).

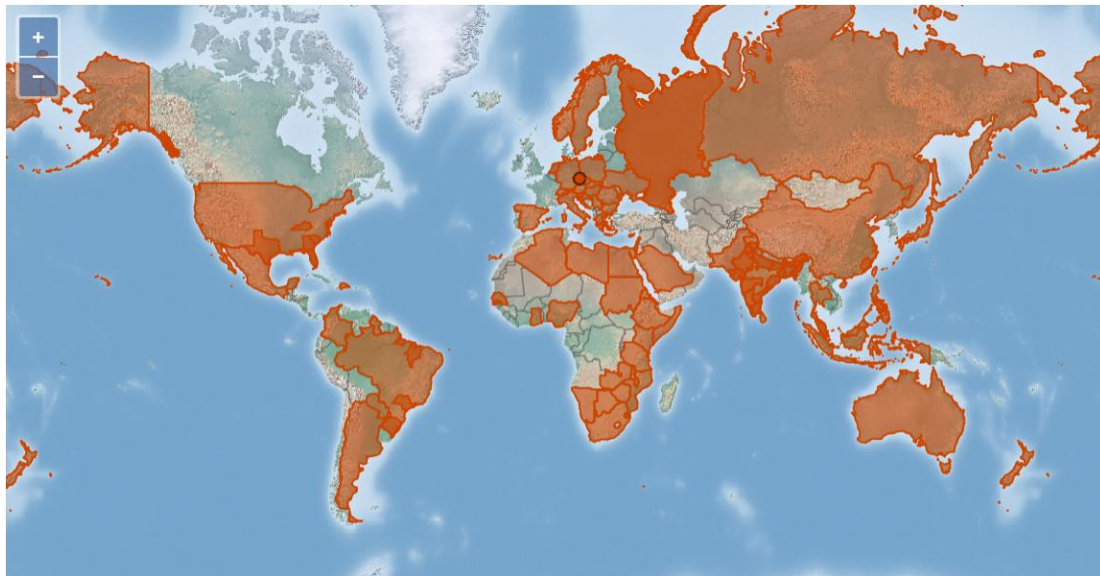


Figure 1.The Global distribution of *Sitotroga cerealella* (Olivier)(source: CABI Compendium) (<https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.50238#REF-DDB-10852>).

2.2. Economic importance of *S. cerealella*

Postharvest losses in stored sorghum grain associated with storage pests, particularly *S. cerealella*, are a major challenge to cereal grain farmers. Loss of grain that has already been harvested nullifies the economic justification for the inputs (seeds, fertilizers, labor, and chemicals), time (single season in a year for Botswana and southern Africa) and other human and material resources used in production. Therefore, safer storage of harvested grain is the only way to best justify resource utilization and derive optimum benefits from the resources used in production. *Sitotroga cerealella* is one of the most harmful storage pests of grain (Yadu et al., 2000; Pathak & Jha, 2003). It is regarded as a pre- and post-harvest grain insect pest (Togola *et al.*, 2010) that infests grain at considerably high moisture content in the field. Thus, *S. cerealella* lays eggs on semi-dry mature sorghum heads or maize cobs with ~50-55% moisture content and they complete their development as the maize dries to <31% moisture (Subramanyam and Hagstrum, 2018). During the harvest time, the crops show no signs of infestation, however, after some weeks the first adults start to emerge in storage facilities (Bushra and Aslam, 2014). The pest thrives at temperatures 25-30 °C and 60-75 % RH with a 15-35 °C developmental threshold temperature. Thus, apart from tropical and subtropical climates, this pest can also infest stored grain in cooler climates (Fig. 1). . Once grain has been infested, the burrowing by the larvae in stored grain damages about 25-60% of the seeds translating to 25-60% losses in food value and/or profits. As a result, most of these seeds do not germinate. About 25–60% of the seeds are damaged by the larvae's burrowing in grain that has been stored. The larvae eat the interior contents of grains as they bore through them, leaving the grains both unable to germinate and unsuitable for human consumption.

These damaged cereals are susceptible to secondary insect attack leading to nutrient value decline, microbial contamination and tanning making the cereal product unsuitable for marketing human or livestock consumption (Demissie et al., 2015).

2.3. Management of *S. cerealella*

Biological control

Biological control involves the use natural substances or derivatives thereof to control insect pest populations including parasitic or predatory insects and microbes to control pests (Bale et al., 2008; Van Lenteren et al., 2018). Many stored grain pests, including weevils, rusty grain beetles, maize weevils, lesser grain borer, sawtooth grain beetles, and grain moths, are now being controlled by using antagonistic insects such as hymenopterous parasitoids. These beneficial parasites are used to control large numbers of storage pests however, this does not fully safeguard the grains if they have suffered severe harm (Ahmad et al., 2021). Parasitoids such as *Seenopinus fenestral* feed on many stored grain pests and help to decrease their infestation. Another example is *Theocolax elegans*. This parasitoid wasp mainly controls grains by attacking the grain pests whose immature stages grow inside seed kernels, such as the lesser grain borer, weevils, and the Angoumois grain moth (Flinn et al., 2006). *Trichogramma spp.* has been successfully utilized to control storage pests in peanuts, wheat, bakeries, warehouses, and retail establishments in Europe (Grieshop et al., 2014). Biological control has limited application in stored grain management due to the high aesthetic value of stored products, very low economic thresholds (ET) and economic injury levels (EIT). In addition, it is difficult to implement at the small-scale farmer household level. Thus, physical control could be an alternative control option.

Chemical control

Since the discovery of Dichlorodiphenyltrichloroethane (DDT), synthetic pesticides have been recognised as one of the most dependable and effective pest controlling method in the world (Isman, 2006). Their preference may amane from their relatively faster mode of action. However, farmers use them indiscriminately without taking any preventative procedures, making occupational and oral exposure rampant. This indiscriminate use of synthetic pesticides has also resulted in several negative consequences, including resistance development and damage to non targets including the environment and its constituents at large (Fields & White, 2002). Fumigation is one of the chemical ways of controlling storage pests. However, fumigation should be done with caution as some gases such as phosphine are flammable and will explode if fire is brought close to them (Ahmad et al., 2021). Despite the option of using chemicals to control pests, not all

chemicals are allowed in the management of storage pests due to the high public health risks. For example, chemicals such as organochlorides and organophosphates such as primiphos-methyl and dichlorvos are not safe for management of storage pests and of the deployment of some organochloride pesticides like DDT has since been banned (McGinn, 2002; Seagren, 2005; Rahman, 2013). This pesticide has been linked to endocrine disorders, impacts embryonic development, and changes lipid metabolism (Nicolopoulou-Stamati et al., 2016). Furthermore, although glyphosate is the most widely used pesticide made from organophosphates, this category also includes dimethoate, malathion, and parathion. Such organophosphorus pesticides have the potential to be endocrine disruptors. They hinder the ability of cholinesterase enzymes to function, as well as interfere with normal cellular metabolism of proteins, carbohydrates, and lipids, and decrease insulin production (Jaga & Dharmani 2003; Karami-Mohajeri & Abdollahi 2011; Mnif et al. 2011).

Cultural methods

Cultural control is a well-established way of altering the environment to make it unsuitable for pest survival and reproduction (Calatayud et al., 2014). Indeed, it is the most applicable and cost-effective approach pest management accessible for low-income farmers (Van den Berg et al., 1998). This pest control approach is one of the oldest traditional procedures and is considered the first line of defence against pests. Cultural control involves practices such as crop rotation, intercropping, sanitation, and related methods. Human beings have consistently evolved different traditional strategies to safeguard stored grains from insect damage throughout time. Some farmers use bamboo, wooden planks, straw, dirt, bricks, cow dung, leaves of numerous plants, and related materials to safeguard the quality and quantity of stored foods (Suleiman & Rugumamu, 2017). One of the most prevalent ways employed by farmers was the use of plant parts or plant extracts as natural pesticides and repellents. Plant species such as *Nicotiana tabacum*, *Derris elliptica*, *Lonchocarpus spp.*, *Juglans regia*, *Azadirachta indica*, and *Chrysanthemum cineraria folium* are utilized to produce plant extracts such as nicotine, derris dust, rotenone, Juglans, Azadirachtin, and pyrethrum to help control storage pests (Dales, 1996; Sola et al., 2014).

High and low temperature-based pest control

Physical control involves reduction of pest population by using devices which affect them physically or alter their physical environment, e.g., manipulation of temperature, humidity and/or light. Temperature control is commonly employed in postharvest to slow down product deterioration caused by storage insects (Vincent et al., 2003). Both high and low temperatures can

both be effective for pest control (Fleurat-Lessard & Torc'h, 2001; Fields, 2001). Heat treatment (HT) has been reported as an eco-friendly way to effectively control insect pests in agro-industrial grain storage buildings or phytosanitary disinfestation for grain trade (Akdoğan et al., 2005; Tilley et al., 2007). This method is often performed by raising the mills' interior air temperature to levels that can eradicate all the insect life stages (Porto et al., 2017). During heat treatment, gas, electric, or steam heaters are used to raise the temperature within a mill to between 50-60°C (Roesli et al., 2003), with heat treatment durations usually ranging between 24-36 hrs (Imholte & Imholte-Tauscher, 1999). High temperatures required to kill stored insect pests thus vary, as has been reported for the red flour beetle *Tribolium castaneum*, bruchids, the grain moth *S. cerealella*, the cigarette beetle *Lasioderma serricorne* and the flat grain beetle *Cryptolestes pusillus* (Oosthuizen, 1935; Murdock & Shade, 1991; Dowdy & Fields, 2002; Roesli et al., 2003; Machekano et al., 2018). Nevertheless, there is limited knowledge on the effects of acclimation on the thermal tolerance of most stored insects both within and across generations. However, the application of kinetic models to characterise intrinsic thermal mortality is a viable strategy for implementing high-temperature, short-duration thermal treatments in a quarantine setting (Tang et al., 2000) for storage pest management. Similarly low temperature treatments have also been employed for the treatment of grain to control pests (Beckett et al., 2007). These thermal methods are very beneficial particularly for developing countries where the cost of pesticides is high. Nevertheless, thermal treatments are however difficult to execute in the field because heat transport is difficult to control and the changes in thermo-sensitivity between grain and pests might be subtle (Laguë & Péloquin, 2001).

2.4. Insects and heat tolerance

Heat tolerance refers to the ability to tolerate high temperatures (González-Tokman et al., 2020). It determines an individual's ability to survive in warming environments (Colinet et al., 2015). The most used dynamic techniques to determine high temperature tolerance is the determination of the critical thermal maxima CT_{max} (Duffy et al., 2015; González Tokman et al., 2020), heat knockdown time (HKDT) (Sgrò et al., 2010; Bauerfeind et al., 2018; Nyamukondiwa et al., 2018; Jørgensen et al., 2019; Mutamiswa et al., 2020; Gotcha et al., 2021) and lethal temperature limits (LTLs) (Hoffmann et al., 2013; Duffy et al., 2015). However, critical thermal limits are considered more ecologically relevant (Somero 2005; Terblanche et al., 2007) and have been assayed in various insect species (Lutterschmidt & Hutchison, 1997; Bowler & Terblanche, 2008; Hoffmann et al., 2013). Critical thermal maximum, which is highest temperature an individual can withstand before losing muscular control is easy to measure and is often assessed using dynamic protocols e.g., by heating an organism until it loses its righting response (Terblanche et

al., 2007) or using static plunge protocols (see Chown & Nicolson, 2004). When measuring CT_{max} , insects are subjected to ramping protocols, where the temperature is heated at a specific rate, until CT_{max} is reached (Terblanche *et al.*, 2007; Gotcha *et al.*, 2018; Nyamukondiwa *et al.*, 2018). Heat knockdown time on the other hand is a measure of heat tolerance whereby an organism is heated in a temperature-controlled column, and the time taken for an insect to be knocked down by acute heat stress is recorded (Sørensen & Loeschcke, 2001; Weldon *et al.*, 2011; Gotcha *et al.*, 2018).

Heat tolerance has been investigated in a variety of insect species including storage pests (Boina & Subramanyam, 2004; Machekano *et al.*, 2018). Several storage insect pests have been reported to be heat tolerant (Beckett & Morton, 2003; Machekano *et al.*, 2018). For example, the CT_{max} of *S. cerealella* adults were determined to be 46.09 °C (Machekano *et al.*, 2018). However, heat tolerance also varies across ontogeny, clines (Sinclair *et al.*, 2012) and environmental history (Nyamukondiwa & Terblanche 2010). Some of the factors affecting heat tolerance include age, sex, body size, body colour, individual condition, food availability, oxygen availability, environmental condition, and life stage (Krebs & Loeschcke, 1994; Bowler & Terblanche, 2008; Kingsolver *et al.*, 2011; Adamo *et al.*, 2012; Baudier *et al.*, 2015). For example, it has been shown that different life stages differ in their ability to withstand high temperatures. In *Tribolium confusum*, Jacquelin du Val (Coleoptera: Tenebrionidae) the adults were found to be more susceptible to higher temperatures as compared to other developmental stages (Boina & Subramanyam, 2004). In another study, younger larvae of *T. castaneum* were found to be more heat tolerant than other life stages when exposed to high temperatures of 50°C (Mahroof *et al.*, 2003). This difference in thermal tolerance among life stage of an insect species poses a challenge on heat treatment methods as one temperature may not be effective enough to kill all the life stages rendering the treatment method ineffective. Nevertheless, thermal tolerance across the different life stages across *S. cerealella* has not been fully elucidated (see Machekano *et al.*, 2018). Therefore, determining the limits to activity is an important first step in understanding the ways in which environmental variation affects fitness and the dynamics of a given population.

When assessing thermal tolerance, it has been documented that various factors such as photoperiod, ontogeny, exposure temperature and duration, temperature acclimation, rate of temperature change and play a role in the thermal limits of an organism (David *et al.*, 2003; Mahroof *et al.*, 2003; Boina & Subramanyam, 2004; Terblanche *et al.*, 2005; Worland, 2005; Rako & Hoffmann, 2006; Gotcha *et al.*, 2021). In unrelated insect species, it has been documented that nocturnal insect exhibit lower heat tolerance compared to diurnal species (Jayatilaka *et al.*, 2011; Gotcha *et al.*, 2021). In addition, within a given insect species, a similar relationship between

environmental temperature and thermal tolerance has been found across many species whereby the thermal threshold depends on the temperature and the duration of exposure (Piyaphongkul et al., 2014). For example, temperature and duration of exposure significantly affected adult *Cydia pomonella* survival with more extreme temperatures or longer durations proving to be more lethal (Chidawanyika & Terblanche, 2011). In another study when adult whiteflies were exposed to high temperatures (37, 39, 41, 43 and 45 °C) for 1 hour, it was found that survival was significantly reduced at temperatures higher than 43°C, indicating reduced thermal tolerance at higher temperatures, despite the same duration (Cui et al., 2008). Furthermore, longer exposures to sublethal temperatures have been documented to result in significant reduction in survival compared to shorter durations (Li et al., 2011), suggesting mortality is an interaction of time and magnitude of temperature severity.

According to the beneficial acclimation hypothesis, pre-exposure to environmental stress enhance survival for that particular stress at a later stage in life. This pre-exposure to environmental stress, can occur in nature (acclimatization), or during laboratory simulation (acclimation). During acclimation, organisms are exposed to either static or dynamic sub-lethal temperatures (Stockton et al., 2018; Sørensen et al., 2019). Static temperature acclimations involve exposing organisms to constant low/high temperature (Wallingford & Loeb, 2016; Stockton et al., 2018; Mutamiswa et al., 2020; Mpofu et al., 2022). Studies have shown that pre-exposure to mild heat results in improved CT_{max}/HKDT in various insect species. However, it has been argued that in nature, temperature is not static, hence the introduction of dynamic temperature acclimations (Chidawanyika & Terblanche, 2011). During dynamic temperature acclimations, individuals are subjected to variable temperature programs (Chidawanyika & Terblanche, 2011; Sørensen et al., 2016; Stockton et al., 2018). It has been found that when compared to dynamic acclimation, the continuous heat exposure that occurs in dynamic acclimation protocols may limit Heat shock proteins (Hsps) expression (Wang et al., 2008). Therefore, this may lead to a compromise in heat tolerance following dynamic acclimation protocols compared to acute protocols (Mpofu et al., 2022).

2.5. Phenotypic plasticity as a mechanism against heat stress

Insects survive extreme temperature variation at short to long-temescales using a host of morphological, behavioral, physiological, and biochemical mechanisms (Hoback & Stanley, 2001; Bale & Hayward, 2010; Kingsolver & Buckley, 2017). Since morphological forms of adaptation are resource and time consuming, insects often adapt to changing climates by changing their behavior, e.g., moving to new environments (Hoffmann & Sgrò, 2011; Munday et al., 2013).

Physiological adaptation can either be through phenotypic plasticity or by natural selection (Hoffmann & Sgrò, 2011). Natural selection involves change in the genotype of an organism and, as such, acts over a long time (Oms *et al.*, 2017; Svensson *et al.*, 2020). Phenotypic plasticity is the environment-dependent expression of alternative phenotypes by the same genotype (Chevin *et al.*, 2013; Svensson *et al.*, 2020). It does not involve any change in genetic makeup but allows organisms to respond quickly to changing environmental conditions (Oms *et al.*, 2017; Macagno *et al.*, 2018). Thus, phenotypic plasticity originated as a means of enhancing fitness in an environment to which the organism has adapted.

A form of phenotypic plasticity which involves changes in the physiological phenotype of an organism, enabling organisms to increase their resistance after pre-exposure to some form of environmental stress is referred to as acclimation/hardening (Bubliy *et al.*, 2012). Acclimation refers to more long-term (days to weeks) of environmental pre-conditioning while hardening refers to short-term (minutes to hours) exposure to sub-lethal conditions, that confers pre-conditioning (Chown & Nicolson 2004) and improves survival to an otherwise lethal stress environment. This phenomenon is known as "beneficial acclimation hypothesis" (BAH) (Kristensen *et al.*, 2008; Angilletta, 2009). Acclimation to sublethal high temperatures has been reported to increase the heat tolerance in stored product insect pests such as *Tribolium castaneum* (Lü & Liu, 2017; Mahroof *et al.*, 2003) and *Prostephanus truncatus* (Mutamiswa *et al.*, 2021). However, despite *S. cerealella* cosmopolitan nature, and being one of the most destructive pests of cereal grains, the effects of acclimation on its heat tolerance remain unknown. The BAH has been questioned in literature since organisms frequently adapt to their environment improperly and incur fitness consequences as a result (Wilson & Franklin, 2002; Angilletta, 2009). Thus, phenotypic plasticity may not always improve an organism's fitness in its natural environment (Gibert *et al.*, 2001). For instance, *Drosophila melanogaster* reared at high temperatures did not exhibit superior fitness characteristics to those reared at intermediate and low temperatures (Gibert *et al.*, 2001). In fact, the optimal developmental temperature hypothesis argues that the opposite is true, and that optimally developed organisms have a fitness advantage at both thermal extremes (Bahrndorff *et al.*, 2016).

While plasticity is a near ubiquitous phenomenon in insects, and likely adaptive, other species nevertheless lack the ability to adjust phenotypes (Chown & Nicolson, 2004; Nyamukondiwa *et al.* 2010). Thus, even for closely related insect species, the degree of plasticity always varies (Chown & Nicolson, 2004; Mutamiswa *et al.*, 2018). However, the causes of, or the extent of this lack of plasticity are frequently poorly understood. Empirical evidence shows that organisms often

trade off some of their traits for others, or basal temperature resistance for plasticity (Nyamukondiwa et al., 2011; Basson et al., 2012) in what is known as the trade-off theory (Gunderson & Stillman, 2015). It has been reported that, since metabolic demands vary under different thermal conditions, this can lead to trade off in resource allocation that can affect different history traits such as mating and courtship, cell and tissue maintenance, body size, egg laying as well as heat tolerance (Basson et al., 2012; Overgaard & Sorensen, 2008; Marshall & Sinclair 2010). In a related study, *Drosophila melanogaster* traded off plasticity for high basal heat tolerance (Van Heerwaarden et al., 2016). Similarly, dung beetle *Allogymnopleurus thalassinus* exhibits high basal heat tolerance for limited thermal plasticity (Machekano et al., 2021).

Acclimation to one stress can also lead to improved resistance to other stressors in what is known as the cross resistance (Bubliy et al., 2012). Cross tolerance or multiple-stress resistance refers to a phenomenon in which one type of stress can confer a plastic response leading to increased resistance to a different kind of stress (MacMillan et al., 2009; Bubliy et al 2012; Sinclair et al., 2013). Thus, an improved survival at low temperatures can also occur after mild high temperature treatments (Chidawanyika & Terblanche 2011) indicating cross tolerance/resistance. Cross tolerance has been investigated in several insect species (Bayley et al., 2001; Holmstrup et al., 2002; Chidawanyika & Terblanche 2011; Bubliy et al., 2012; Boardman et al., 2015; Kalra et al., 2017) including storage pests such as *P. trancutus* (Mutamiswa et al., 2021), *Alphitobiud diaperinus* (Renault et al., 2015), *T. castaneum* (Scharf et al., 2015) and *Trogoderma granarium* (Shivananjappa et al., 2020). Desiccation acclimation was found to also improve heat tolerance in *P. trancutus*, showing evidence of cross tolerance (Mutamiswa et al., 2021). Nevertheless, cross tolerance in storage insect pests remain limited. Storage insect pests can undergo desiccation acclimation in dry grain environments especially during storage, however how combined effects of both desiccation and changes in temperature (heating or cooling) can affect this species thermal plasticity remain uninvestigated. In addition to other environmental stressors, insect pests can also undergo prolonged periods of food deprivation or starvation. Although starvation is commonly known as lack of food, starvation can also refer to access to poorly nutritious food which can occur once all nutritious food has been consumed. Similarly, effects of pre-exposure to this food deprivation on the thermal tolerance in this species also remain unknown. Research has shown that plasticity can be induced either during development (developmental acclimation), during adulthood (adult acclimation) (Parkash et al., 2014), in one developmental stage and manifest in another stage in what is known as carry-over effects (Stoks & Cordoba-Aguilar, 2012; Salis et al.,

2018) or can be passed on across generations- transgenerational effects/plasticity (Sgrò *et al.*, 2016; Tougeron *et al.*, 2020).

2.6. Transgenerational plasticity

While plasticity often develops in response to the environmental conditions within a generation, there are various non-genetic or epigenetic mechanisms that parents might use to change the phenotype of their offspring. Transgenerational plasticity, non-genetic inheritance, anticipatory parental effects, carry-over effects, and intergenerational effects are just a few of the names given to these mechanisms (Bonduriansky *et al.*, 2012; Salinas *et al.*, 2013; Uller *et al.*, 2013). TGP is considered adaptive if parental effects result in an improvement in offspring fitness (Murray *et al.*, 2014; Shama & Wegner, 2014). In some circumstances, parental effects cause offspring to be less fit when parents go through challenging environmental situations (Eriksen *et al.*, 2006; Marshall, 2008). When a parental and offspring environment matches results in negative impacts via negative carryover, this is regarded as nonadaptive TGP (Mousseau & Fox, 1998). A study found that 41% of transgenerational acclimation tests were positive, thus produced beneficial results, while the rest were neutral/ no effects (Donelson *et al.*, 2018). In another study, a thorough meta-analysis of 58 investigations in plants and animals found no overall significant beneficial impact, but a non-significant positive trend, suggesting that TGP is not widely present (Uller *et al.*, 2013).

Given that environmental change would persist through generations for nearly all species, TGP may therefore be especially relevant to understanding the biological effects of climate change (Donelson *et al.*, 2018). Furthermore, TGP can be a mechanism used to protect populations from the immediate consequences of climate change and provide genetic adaptation time to catch up in the long run if it is adaptive, however this may not always be the case (Chevin *et al.*, 2010; Kopp & Matuszewski, 2014). Despite the benefits, to my knowledge, the research on TGP in storage pests is still at infancy (see Mpofu *et al.*, 2022). Most of the work on plastic responses on storage pests mainly considered within-generation acclimation responses, often not within the context of climate change and the modern knowledge of thermal biology (see Fields *et al.*, 1998), and none have considered TGP in storage pests.

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Chapter 3:

Transgenerational responses to heat and fasting acclimation in the Angoumois grain moth

*** Published as** Mpofu, P., Cuthbert, R. N., Machekano, H., & Nyamukondiwa, C. (2022). Transgenerational responses to heat and fasting acclimation in the Angoumois grain moth. *Journal of Stored Products Research*, 97, 101979.

3.1 Introduction

The effects of climate change are impacting biological organisation at different scales (Kane et al., 1992; Haines & Patz, 2004; Araújo & Rahbek 2006; Tol, 2009; Coffel et al., 2017). This has become increasingly important in the 21st century for ecologists, as stressful environmental events (e.g., heat and food deprivation) become the new norm (Stillman, 2019; Lehmann et al., 2020). As climate warming progresses, there is consensus among researchers that heat stress will become more extreme and frequent (Williams et al., 2016; Stillman, 2019) particularly in Africa (Davis-Reddy & Vincent, 2017), raising questions about how organisms will cope with this unprecedented stress and the consequent effects on ecosystems. Warmer climate is expected to generally increase insect pest reproduction, feeding activity and movement potentially worsening losses (Stathers et al, 2013; Singano et al., 2020; reviewed in Lehmann et al., 2020). Climate stress can also exert significant selection pressure in most ectotherms (Zizzari & Ellers, 2014). For example, continuous exposure to extreme heat may result in high risk altered status of pests due to adaptation (Sutherst et al., 2011), e.g., through transgenerational plasticity (TGP) (Harrison et al., 2011). However, this concept has received little attention, especially in insects and thus, early analytical assessments maybe required to evaluate the costs and benefits of TGP responses.

When faced with non-lethal heat stress, insects compensate often adaptively (morphologically, behaviourally, physiologically, or molecular adaptation mechanisms), and through phenotypic plasticity (Somero, 2010; van Heerwaarden et al., 2016). These phenotypic adaptations can either manifest within the same life stages, across developmental life stages (developmental acclimation) within the same generation (as short- [hardening] and long -term [acclimation]), or across different generations (TGP) (reviewed in Harrison et al., 2011; Sgrò et al., 2016). Although within generation plasticity is relatively well-studied (see Chown & Nicolson 2004; Nyamukondiwa & Terblanche, 2009; Sgrò et al., 2016; Mutamiswa et al., 2019), TGP has lacked examination for many taxa. However, recent studies have suggested that TGP may be far more widespread and with far greater ecological effects than only within generation effects as previously reported (Harrison et al., 2011; Liz & Ruiz-Herrera, 2016). These carry-over effects mean that plastic adaptation mechanisms elicited in one generation may be transferred to the next generation (Harrison et al., 2011; O'Connor et al., 2014; O'Connor & Cooke, 2015; Liz & Ruiz-Herrera, 2016). This concept provides context for examining the phenotypic plasticity in tolerance mechanisms acquired during non-lethal stress interactions between distinct life stages of an organism's lifetime (O'connor et al., 2014). However, TGP responses may differ among species and traits, potentially yielding costs and benefits of parental acclimation on subsequent generations (Harrison et al., 2011; O'connor & Cooke., 2015).

Critical thermal maxima (CT_{max}) is a dynamic thermal tolerance metric where an organism is heated from its optimum temperature at a given rate of temperature increase (ramping rate) until loss of righting response (Becker & Genoway, 1979; Terblanche et al., 2007). CT_{max} provides an indication of maximum temperature of activity under acute heat exposure (Vannier, 1994; Becker & Genoway, 1979; Terblanche et al., 2007). Ecologically, this metric shows the highest thermal limit to activity, translating into cessation of key life defining processes, e.g., locomotion, mating, feeding, predator escape and related (Mitchell & Hoffmann 2010). Studies show that organisms can adjust their CT_{max} depending on methodological context, e.g., ramping rate, starting temperature and others (see Becker & Genoway, 1979; Terblanche et al., 2007). As such, ramping rates have been reported to induce plastic responses (see e.g., Kelty & Lee 2001). These plastic responses can be induced through either short-term (minutes to hours) exposure to sublethal hardening treatments or longer term (days to weeks) acclimation treatments, either through static or dynamic protocols (Cossins & Bowler 1987; Kelty & Lee 2001; Sgrò et al., 2016; van Heerwaarden et al., 2016). Heat knockdown time (HKDT) is a static heat tolerance metric that measures the time taken to knockdown organisms following acute heat stress (Becker & Genoway, 1979; Stazione et al., 2020). Given recent increases in heat waves with changing climate (Stillman, 2019), determining how organisms endure acute stress may be significant in explaining ecological consequences of organisms under climate change. Given the ecological relevance of both CT_{max} and HKDT traits, (Chown & Nicolson, 2004; Mitchell & Hoffmann 2010), determining TGP changes using these response metrics thus provide insights on how climate change shapes variation in insect ecophysiology (Somero, 2010; Stazione et al., 2020).

The Angoumois grain moth, *Sitotroga cerealella* is one of the most economically damaging primary storage insect pests, affecting cereal grains worldwide. In sub-Saharan Africa, it has consistently hampered farmers and grain traders in storing their staple cereal grain until the next harvest (Togola et al., 2014), particularly for sorghum (Mvumi et al., 2003). Due to climate change, insects in storage facilities are constantly exposed to sub-lethal high temperature stress due to the enclosed nature and relatively small sizes of grain storage environments. However, the potential for this continuous pre-conditioning to create heat resilient *S. cerealella* phenotypes through beneficial acclimation over generations (TGP) is unknown (Sgrò et al., 2016; Tougeron et al., 2020). In addition, stored product pests like *S. cerealella* may inevitably increase their immediate grain environmental temperature due to their own respiration and constrained space, creating high but stochastic storage temperatures through ‘hot spots’ (Jian & Jayas, 2012; Wang et al., 2021). This exposes the insect pests not only to sustained high constant sub-lethal heat

stress, but also fluctuating (variable) temperatures, conferring potential TGP responses, assuming beneficial acclimation effects (see Angilletta, 2009; Sgrò et al., 2016). Similarly, extended periods of no stored grain expose insects to food deprivation (fasting acclimation), and consequent effects on cross-temperature tolerance remain unknown. Against this background, I thus assessed TGP of a model and economically important storage pest species, *S. cerealella*, post-exposure to acute and dynamic sub-lethal heat stress as well as fasting acclimation. I hypothesized that 1) heat acclimation i.e., exposure to sub-lethal acute or variable temperature and 2) fasting acclimation of parental F₁ populations will improve thermal fitness in F₂ generations through TGP, each assessed via CT_{max} and HKDT traits. Further, I assessed whether acclimation pre-treatment effects would be altered by the speed of thermal ramping rates (as in e.g., Kelty & Lee 2001; Terblanche et al., 2007), whereby slower ramping rates would elicit short-term acclimatory responses leading to higher CT_{max}. These results are important in remodelling grain heat disinfestation treatments through accounting for any positive and/or negative transgenerational plastic acclimatory responses.

3.2 Material and methods

Insect culture

Initial colonies of adult *S. cerealella* were collected from damaged sorghum seeds in the Central District, Botswana from farmers' storage facilities in Malaka village (22°35'35.1" S and 027°07'31.0" E). Malaka village lies in a region that is arid and hot, having a high mean annual temperature of 28.5 °C (Akinyemi & Abiodun, 2019). Insects were thus reared in the laboratory at 28 ± 1 °C, 65 ± 10 % relative humidity (RH) and 24D photoperiod in Memmert climate chambers (HPP 260, Memmert GmbH + Co.KG, Schwabach, Germany) in BugDorm® cages (BugDorm®, MegaView Science Co., Ltd., Taiwan). During this period, *S. cerealella* adults were fed with 10% sucrose solution *ad libitum* using cotton wool dipped in 50ml vials protruding through a perforated lid (the wick method) (Machekano et al., 2018). Moths were provided with sterilized fresh sorghum grain and folded sterile cardboard paper to provide dark sites for resting and oviposition. The first laboratory generation of uniformly aged adults were defined as F₁ and were exposed to heat and fasting acclimation treatments for subsequent TGP experiments.

Acute temperature acclimation treatments

Acute acclimation of *S. cerealella*, was done by placing 100 adults in 240 cm³ Bugdorm cages. The adults were exposed to acute acclimation for 2 hours (as in Kingsolver et al., 2016; Gantz et al., 2020) at static 34°C and 38°C temperatures in Memmert climate chambers. These acclimation temperatures represent frequently recorded temperatures in summer of the study region (Akinyemi

& Abiodun, 2019). After treatment, moths were transferred to cages with fresh grain and allowed to lay eggs for 72 hours in folded heat-sterilized cardboard paper. The moths were then removed, leaving only the eggs on grain and cardboard paper at optimal rearing conditions ($28 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH, 24D photoperiod) in the Memmert climate chamber until the F_2 generation adults emerged. Moths that remained at optimal rearing conditions were used as controls (see **appendix 3**).

Fasting acclimation treatments

For fasting acclimation, 100 F_1 moths were placed in 240 cm^3 bugdorm cages in a Memmert climate chamber. The moths were fasted by withdrawing food (10% sucrose solution and grain) for 72 hours. After 72 hours of fasting acclimation, the moths were then supplied with fresh heat-sterilized sorghum grain, 10% sucrose solution as well as folded heat-sterilized cardboard paper for resting and oviposition and maintained at optimal rearing conditions ($28 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH and 24D photoperiod) in Memmert climate chambers where they were allowed to lay eggs for 72 hours. The moths were then removed, leaving only the eggs which hatched into the F_2 generation. The $F_2 \pm 2$ days old, unsexed moths were used for the TGP experiments (see **appendix 3**).

Variable acclimation treatments (dynamic acclimation)

For the variable temperature acclimation experiment, 300 moths were placed in Bugdorm cages (240 cm^3) and supplied *ad libitum* with 10% sucrose solution and sorghum grain. The cages were then placed in a Memmert climate chamber that was set to oscillate between 28 and 38°C at a ramping rate of $0.25^\circ\text{C}/\text{min}$. The acclimated moths were removed after 72 hours and placed into new cages with fresh sterilized grain, *ad libitum* 10% sucrose solution as well as folded heat-sterilized cardboard paper for resting and oviposition. After 72 hours, the moths were removed and the cages with grain containing eggs were transferred to a Memmert climate chamber set at $28 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH and 24D photoperiod until the eggs hatched into the F_2 generation. The $F_2 \pm 2$ -day old, unsexed adults were used for subsequent TGP experiments (see **appendix 3**).

Critical thermal maxima

A programmable water bath (LAUDA Ecogold® RE 2025, Lauda-Königshofen, Germany) filled with 1:1 water: propylene glycol connected to a transparent double-jacketed chamber was used to assay for CT_{max} (Nyamukondiwa & Terblanche 2009). A thermocouple (type K 36SWG) connected to a digital thermometer (Fluke 54 series IIB) was inserted into the central organ pipe (control chamber) to measure the chamber temperature. Test insects were individually placed into the organ pipes connected to a programmable water bath. Insect body temperatures closely track

their ambient temperatures. As such, the control chamber thermocouple temperature was regarded as being in equilibrium with insect body temperatures in all other chambers (see Stevenson, 1985). All CT_{max} experiments started from an ambient set point temperature of 28 °C (optimal temperature), from which temperature was ramped up at different but ecologically relevant treatment ramping rates (0.06°C/min, 0.12°C/min, 0.25°C/min and 0.5°C/min) until CT_{max} was recorded for all the acclimation treatments. A total of ten test insects, replicated three times ($n = 30$) per treatment (i.e., per acclimation treatment and ramping rate), was used in the experiments. These various ramping rates used were chosen to cover a wide range of possible ecological rates of temperature increases in nature (see discussions in Nyamukondiwa & Terblanche 2009). The CT_{max} was defined as the temperature at which each individual insect lost coordinated muscle function and the ability to self-right or respond to mild stimuli such as gentle prodding with a camel hairbrush due to heat stress.

Heat knockdown time

Heat knockdown time was assayed using standardized protocols (see Nyamukondiwa & Terblanche, 2009). Single ten adults were placed each in 1.5 ml Eppendorf tubes and into a Memmert climate chamber set at 48°C. This heat knockdown temperature was chosen following preliminary assays based on CT_{max} data. A video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) linked to a computer was connected to the climate chamber and used to monitor knockdown activity remotely. The procedure was repeated three times to yield sample sizes of $n = 30$. HKDT was defined as the time (in minutes) at which an individual adult insect lost the ability to self-right.

Statistics

CT_{max} and HKDT were analysed using Gamma-distributed generalized linear models with log links. Three models were built to examine the effects of treatment type and ramping rate (the latter for CT_{max} only) for these response variables [i.e., (i) CT_{max} following acute acclimation; (ii) CT_{max} following variable acclimation; (iii) HKDT]. Deviance residuals and goodness of fit were examined using standard plots. Non-significant terms were removed from the models, with F-tests conducted via analysis of deviance (Fox & Weisberg, 2019). Post-hoc comparisons were performed via Tukey tests (Lenth, 2021). All analyses were conducted in R v4.1.1 (R Core Team, 2021).

3.3 Results

Acute acclimation treatment had a significant effect in interaction with ramping rate on CT_{max} ($F_{(9,464)} = 5.709$, $p < 0.001$) (**Fig. 2**). For the control and fasted groups, ramping rates $0.5^{\circ}\text{C}/\text{min}$ had a significantly higher CT_{max} than the lower rates (0.06 , 0.12 and $0.25^{\circ}\text{C}/\text{min}$) (all $p < 0.05$). Furthermore, for fasted groups, CT_{max} at 0.25 and 0.12 ramping rates were significantly greater than $0.06^{\circ}\text{C}/\text{min}$ (both $p < 0.001$). Other pairwise comparisons lacked statistical clarity in the control and fasted groups (all $p > 0.05$). For the 34°C acclimation treatment, $0.06^{\circ}\text{C}/\text{min}$ exhibited significantly lower CT_{max} than other ramping rates (all $p < 0.05$), which were in turn statistically similar (all $p > 0.05$). Similarly, at 38°C , CT_{max} at $0.06^{\circ}\text{C}/\text{min}$ was significantly lowest (all $p < 0.001$), while $0.25^{\circ}\text{C}/\text{min}$ was significantly greater than 0.12 and 0.5 (both $p < 0.05$), and 0.12 and $0.5^{\circ}\text{C}/\text{min}$ were similar in turn ($p > 0.05$). In general, mean CT_{max} ranged between 46.2°C (34°C) and 46.9°C (control) among treatment groups. CT_{max} did not differ according to treatment group and ramping rate in interaction ($F_{(3,232)} = 0.106$, $p = 0.957$) following dynamic acclimation, but the effects of treatment ($F_{(1,235)} = 53.164$, $p < 0.001$) and ramping rate ($F_{(3,235)} = 20.971$, $p < 0.001$) were significant singularly (**Fig. 3**). CT_{max} was significantly lower following the 28 - 38°C acclimation group as compared to controls ($p < 0.001$), with mean CT_{max} of 46.0 and 46.9°C , respectively. CT_{max} following the $0.5^{\circ}\text{C}/\text{min}$ ramping rate was significantly greater than the other rates (all $p < 0.001$), whereas $0.25^{\circ}\text{C}/\text{min}$ was significantly greater than 0.06 and $0.12^{\circ}\text{C}/\text{min}$ (both $p < 0.05$), which in turn did not significantly differ ($p > 0.05$).

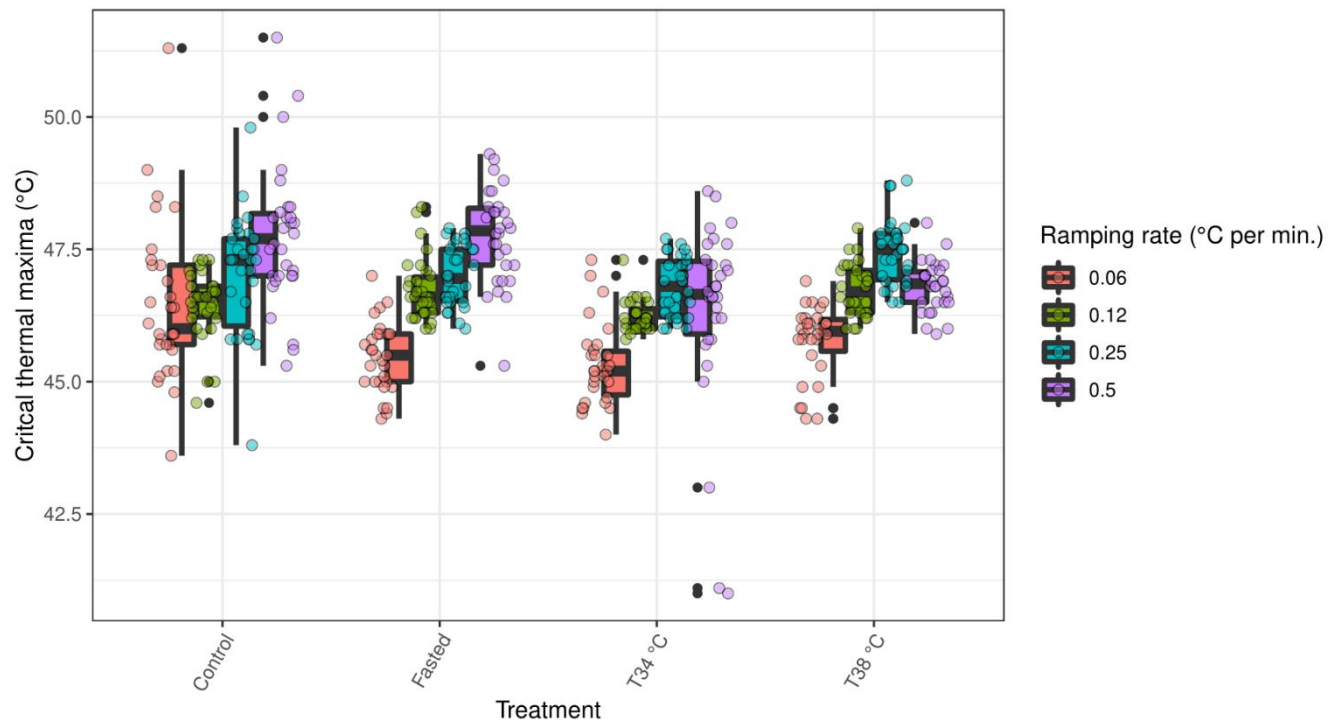


Figure 2. Box plots showing CTmax across treatment groups and ramping rates following acute acclimation. Acute acclimation included 72 hours of food resource deprivation (fasted) and 2-hour exposure to sublethal high temperature (T34 °C and T38 °C). In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges, and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. Colored points are raw data and black points are outliers.

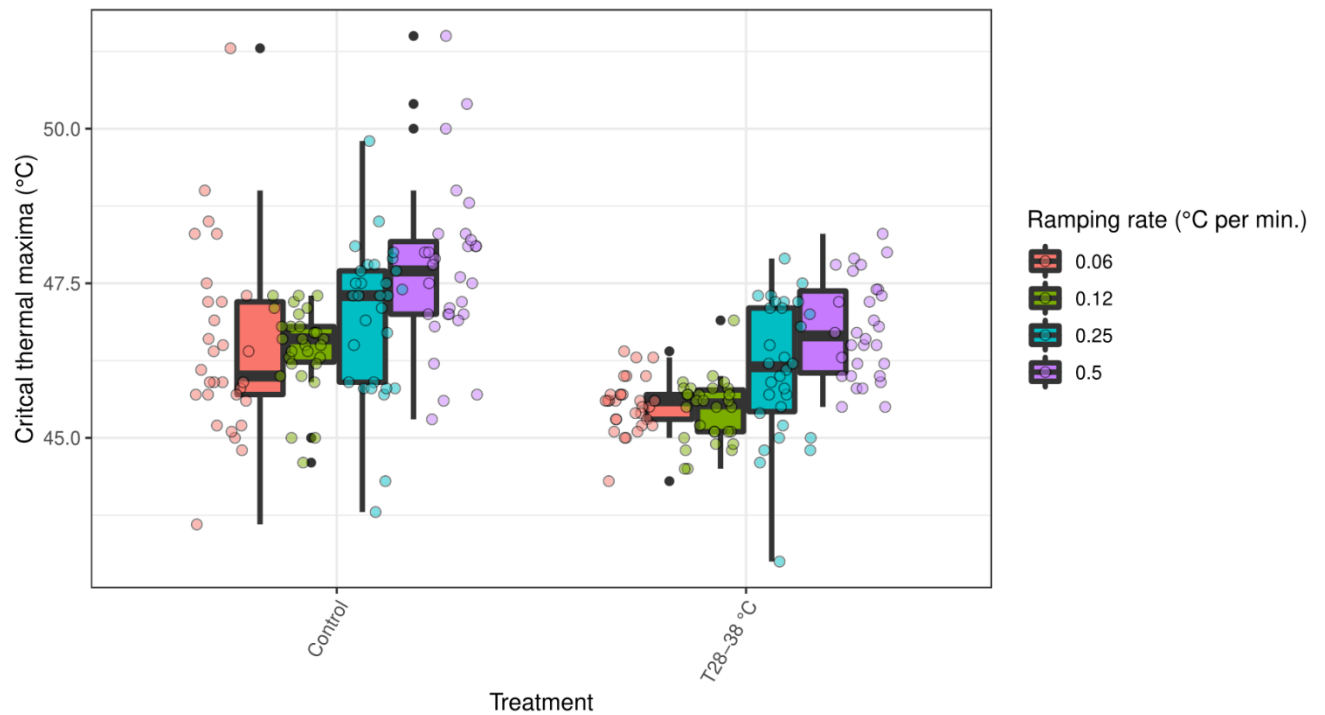


Figure 3. Boxplots showing CTmax across treatment groups and ramping rates after variable temperature acclimation. The dynamic temperature acclimation was defined as diel variation between 28-38 °C at 0.5 °C per min. for 72 hours. In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges, and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. Colored points are raw data and black points are outliers.

Considering HKDT, acclimation treatments caused significant differences ($F_{(4,145)} = 22.744$, $p < 0.001$) (**Fig. 4**). Control groups exhibited significantly highest HKDT (all $p < 0.05$), whereas 28-38 °C exhibited significantly lower HKDT (cost of acclimation) than all other acclimation treatment groups (all $p < 0.001$). However, fasted, 34 °C and 38 °C acclimation treatment groups were statistically similar (all $p > 0.05$), albeit these acclimations also came at a cost of HKDT when compared with the controls. Mean HKDT ranged between 4.9 (28-38 °C) and 8.1 min (control).

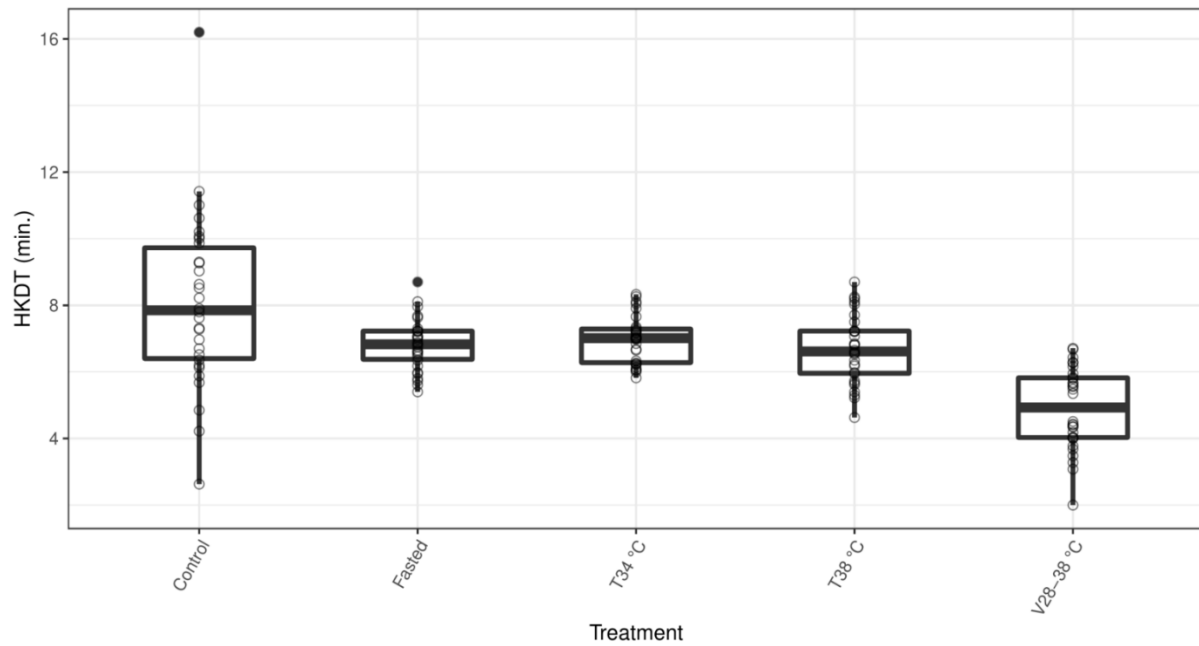


Figure 4. Boxplots showing heat knockdown time (HKDT) across treatment groups. Treatment groups were deprivation of food for 72 hours (fasted), acute sub-lethal temperature pre-treatments (T34 and T38 °C), variable temperature fluctuations around 28-38 °C (V28-38°C) and a control (28 °C). In the boxplots, 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. Unfilled points are raw data and filled points are outliers.

3.4 Discussion

TGP effects are becoming progressively documented across different taxa (Harrison et al., 2011). This mechanism likely governs the fitness and population dynamics of insects amidst changing and stressful environments. My results showed firstly that high temperature acclimation had significant effects on heat tolerance of F₂ offspring. Second, I showed that higher ramping rates (0.5°C/min) had beneficial effects on heat tolerance (higher CT_{max}) of F₂ offspring, whereas lower ramping rates (0.06°C/min) lowered heat tolerance (CT_{max}). Third, we showed that both F₁ fasting, and heat acclimation were maladaptive for F₁ offspring HKDT. Dynamic high temperature acclimation significantly reduced HKDT the most, relative to the control and other treatments. These results show contrasting TGP responses in *S. cerealella*, that are dependent on acclimation treatment and trait context. Unravelling these TGP responses may help explain mechanisms by which insects cope with rapid climate change (Donelson et al., 2018) and may help inform efficacious heat disinfestation pest control protocols for grain storage.

Critical thermal maxima and HKDT are two commonly employed physiological traits, representing ecologically significant fitness proxies (Chown & Nicolson, 2004). CT_{max} represents the maximum temperature that allows organismal locomotory activity, whereas HKDT represents the amount of time taken to paralyze an organism following acute heat stress (Chown & Nicolson 2004; Stazione et al., 2020). Both traits are ecologically relevant (Terblanche et al., 2007; 2011) and can be used as reasonable proxies of other fitness traits (Huey & Stevenson, 1979; Lutterschmidt & Hutchison, 1997; Terblanche et al., 2011). My results showed that both acute and dynamic heat acclimation significantly reduced CT_{max} . Insects may trade off plasticity for higher basal stress tolerance or other physiological or fitness traits (Liefting & Ellers, 2008; Angilletta, 2009; Basson et al., 2012; Murren et al., 2015; Machekano et al., 2021). Thus, the lower CT_{max} values recorded here may explain the lack of plasticity recorded for *S. cerealella* (see discussions in Chown and Nicolson, 2004). Moreover, while plasticity may be near ubiquitous in insects, organisms may often trade-off plasticity for other significant life history traits, owing to the metabolic costs to eliciting plasticity (Mutamiswa et al., 2018). As such, organisms that may not exhibit increased plasticity, may do so to conserve other fitness traits, depending on space and timing of stressful events. My results are contrary to the notion that within-generation beneficial acclimation is near ubiquitous in insects (Angilletta 2009; Leroi et al., 1994). Studies have documented an improvement in within generation CT_{max} following mild heat acclimation (Chown et al., 2009; Nyamukondiwa & Terblanche, 2010; Bublly et al., 2013; Van Heerwaarden et al., 2016; Gotcha et al., 2018). Similarly, Mutamiswa et al. (2021) and Machekano et al., (2021) found improved CT_{max} following static and dynamic heat pre-treatment respectively in beetles. The reasons for the lack of beneficial acclimation reported here are unknown but indicate that responses may be species and/or population specific. Similarly, acclimation responses may also depend on the exact generation acclimated and when the acclimation responses are tested (within versus across generation acclimation). Furthermore, I speculate that I might have missed the exact temperature and time combinations that are required to elicit TGP acclimation responses in the current study. This lack of plasticity may also mean that the stresses acquired during parental acclimation combined with cumulative parental damage may be synergistic in subsequent generations, likely causing greater organismal damage (Jørgensen et al., 2021).

Both acute and variable heat acclimation reduced HKDT, with the dynamic acclimation (T28-38°C) having significantly lower HKDT than all other treatment groups. These results agree with findings from Tarusikirwa et al., (2020) who showed that HKDT was significantly reduced following within-generation heat acclimation in *T. absoluta*. Contrastingly, other studies have reported an improvement in HKDT following mild heat pretreatment (Weldon et al., 2011; Bublly

& Loeschcke, 2005; Sørensen et al., 2019; Tarusikirwa et al., 2020). These plastic responses have mainly been attributed to the expression of heat shock proteins (González-Tokman et al., 2020). Nevertheless, each insect species may have its own specific temperature and time duration combinations required to elicit hardening responses (Sørensen et al., 2019). During HKDT experiments, insects accumulate heat at a fast rate, and may not have enough time to adjust physiologically through Hsps expression. As such, this may limit them from mounting compensatory physiological adjustments (Chidawanyika & Terblanche, 2011). Wang et al., (2008) also documented that continuous heat exposure during dynamic acclimation protocols may limit Hsps expression (Wang et al., 2008). This likely explains the compromised thermal tolerance of F₂ offspring following dynamic acclimation protocols. However, to my knowledge, most studies investigating these plastic responses only considered within-generation acclimation responses, and none have considered TGP in storage pests. This may partly explain the differences in acclimation responses reported here. Furthermore, studies on dynamic acclimation are limited, particularly for storage pests. For such taxa, most studies have only considered static heat acclimation (Bubliy et al., 2012). Thus, my findings suggest that parental heat acclimation is maladaptive for *S. cerealella* F₂ offspring fitness (i.e., reduced CT_{max}), a notably understudied postharvest primary pest. This may therefore mean that dynamic protocols may be more efficacious as pre-treatments over acute ones before exposing insects to static lethal thresholds for efficacious postharvest pest management.

My results further showed that slower ramping rates decreased CT_{max}. Studies have documented that lower ramping rates may result in lower CT_{max} due to insects being exposed to prolonged heat damage (Becker & Genoway, 1979; Manenti et al., 2018; Salachan et al., 2019). However, these results are contrary to other studies which showed higher CT_{max} values following slower ramping rates (Hoffmann et al., 2003; Rezende et al., 2011; Nyamukondiwa & Terblanche, 2010), albeit these studies investigated within-generation rather than TGP. Similarly, slower ramping rates have also reportedly improved low temperature tolerance (Kelty & Lee 2001). Slower ramping rates are generally known to induce a stronger stress response (Hsp expression) than faster ramping rates (Sørensen et al., 2013), thereby likely to result in improved heat tolerance. Similarly, slower heating rates also allow individuals to acclimatize to new temperatures by inducing a hardening response (Becker & Genoway, 1979; Hoffmann et al., 2003; Rezende et al., 2011). In addition, Hsps, are upregulated more at lower than higher ramping rates (Sørensen et al., 2013). In my study, the observed reduction in CT_{max} at lower ramping rates could potentially be due to cellular damage because of prolonged stressful and cumulative heat stress cell damage (Neven, 2000; Sørensen et al., 2013; Lee et al., 2019; Jørgensen et al., 2019; 2021). Insect metabolism increases

in response to temperature (Neven, 2000). Similarly, higher temperatures increase respiration, hypoxic conditions and anaerobic metabolism increases organismal mortality (Neven, 2000). Mortality at high temperatures can also be a result of failure of enzyme catalyzed reactions owing to protein denaturation (El-Shesheny et al., 2016). In addition, energy generated from anaerobic metabolism is insufficient to support insect life processes (Westneat et al., 2003). My results therefore indicate that for *S. cerealella* control, slower heating rates (e.g., 0.06°C/min) can be more effective in grain treatment as it increases insect heat damage through prolonged exposure and thus lower CT_{max} (reduced lethal thresholds) in the next generation. Nevertheless, a significant interaction between ramping rate and pretreatment indicates that the effects of ramping rate are also dependent on prior stress contexts.

During grain storage periods food deprivation occurs naturally when storage facilities are emptied, and insects escape into the environment. Until insects identify and infest another grain source, prolonged periods of inevitable fasting have implications on climatic resilience and plasticity both within and across generations. Organisms may also experience multiple stresses in the environment and may respond to these using signaling pathways (cross talk) or protective mechanisms (cross tolerance) (Sinclair et al., 2013). However, cross tolerance and cross talk may be non-adaptive if the stressors are not experienced simultaneously. My results showed that fasting acclimation had a significant effect in reducing HKDT, but not CT_{max}. This is consistent with similar studies, that showed fasting acclimation had no effects (Bubliy et al., 2012; Gotcha et al., 2018) or significantly compromised heat tolerance (Scharf et al., 2016; Mutamiswa et al., 2018; Mitchell et al., 2017; Mir & Qamar, 2018). This result may indicate the absence of commonly shared physiological mechanisms against the two stressors for *S. cerealella*. Other studies found no effect of fasting on HKDT (Bubliy et al., 2012; Scharf et al., 2016; Gotcha et al., 2018), suggesting a lack of shared common resistance mechanisms between the two traits. Fasting has been shown to weaken insect immune system (Scharf et al., 2016). However, a link between the immune system and thermoregulation remains unexplored (Kutch et al., 2014). In addition, during mild fasting periods, there is accumulation of insect body lipids (Gotcha et al., 2018), which then get depleted during severe fasting (Scharf et al., 2016). Once those reserves are depleted, the insect may have no protection from damage (Sokolova, 2013). In addition, body lipids likely help thermal tolerance through serving as energy reserves that are needed to survive heat stress (Nyamukondiwa & Terblanche, 2009; Klepsatel et al., 2016). Thus, low body lipids, consistent with reports by Machekano et al. (2018), may explain the inability to cope with heat stress reported here following fasting in *S. cerealella*. Contrastingly, other studies found that fasting pretreatment enhanced CT_{max} of various insect taxa (see Nyamukondiwa & Terblanche, 2009; De Vries et al.,

2006; Gotcha et al., 2018; Mutamiswa et al., 2018). However, mechanisms underlying this cross tolerance are unknown. While studies have reported the accumulation of lipids at the onset of fasting (Ziegler, 1991; Arrese et al., 2001; Arrese & Soulagase, 2010), their link with CT_{max} in *S. cerealella* requires further investigation.

This study found that (i) high temperature acclimation had significant effects on heat tolerance of F_2 offspring, (ii) higher ramping rates ($0.5^{\circ}C/min$) had beneficial effects on heat tolerance (higher CT_{max}) of F_2 offspring while lower ramping rates ($0.06^{\circ}C/min$) had an opposite effect, and (iii) both parental fasting and heat acclimation were maladaptive for F_2 offspring HKDT. Continuous dynamic sub-lethal heat stress acclimation is likely to reduce the heat tolerance of *S. cerealella* more than acute static acclimation. Similarly, slower ramping rates also resulted in lower CT_{max} values, presumably due to cumulative heat damage. Thus, dynamic acclimation protocols and slower heating rates likely reduce upper thermal limit thresholds for *S. cerealella*. Incorporating these transgenerational plastic responses to postharvest disinfestation protocols, and accounting for potential acclimation, may help in designing effective pest management protocols, e.g., lower lethal threshold limits and reduced heating time with consequent low energy costs in heat-based grain treatment approaches. Future studies may focus on assessing TGP across ontogeny to better understand how these physiological responses manifest across development stages.

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Chapter 4:
**Transgenerational cross susceptibility to heat stress following cold
and desiccation acclimation in the Angoumois grain moth, *Sitotroga
cereallega* (Olivier)**

4.1. Introduction

The magnitude and frequency of environmental stressors is increasing with climate change (Thuiller, 2007; Schröter et al., 2005; Seneviratne et al., 2014). This poses a threat to the survival and ecological function of many organisms (Jørgensen et al., 2022), including insect pests, as environmental stressors can yield multiple contrasting challenges simultaneously (Gunderson et al., 2016). For example, high temperatures are associated with additional abiotic desiccation stress (see e.g., Hoffmann & Parsons, 1989). In turn, desiccation stress is associated with disruption of food chains resulting in starvation stress (Gotcha et al., 2018). However, predicting organismal responses to multiple simultaneous environmental stressors requires an improved understanding of cross-tolerance mechanisms, especially across generations (Kaunisto et al., 2016).

Numerous environmental stressors may interact in a variety of ways and can manifest additively (Jørgensen et al., 2019; Tarusikirwa et al., 2022). In insects, crosstalk (shared signalling pathways) and cross-tolerance (shared response mechanisms) adaptations facilitate survival in the face of multiple stressors (Sinclair et al., 2013; Pallarés et al., 2017; Gotcha et al., 2018). Crosstalk adaptations occur when signaling reactions to one stressor also triggers resistance to another divergent stressor; while cross-tolerance occurs when common pathways of stress response confer protection against other contrasting stressors once activated (Sinclair et al., 2013). For example, Gotcha et al. (2018) and Kalra et al. (2017) documented cross-tolerance of the flies *Ceratitis rosa* Karsch (Diptera: Tephritidae) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) respectively to starvation or desiccation following heat stress. Cross-tolerance is also reported in storage pests such as the lesser mealworm *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae), which has adapted cross-tolerance between desiccation and cold stress (Renault et al., 2015). It is thought that cross-tolerance aided the successful establishment of *A. diaperinus* in temperate regions including Europe (Klejdysz & Nawrot, 2010). Despite *T. granarium* being native to hot and arid areas of the Indian subcontinent, it is regarded as one of the most cold-hardy insect pests (Wilches et al., 2017; Shivananjappa et al., 2020). Although these insects may only sometimes be exposed to extreme low temperatures in their natural environment, research suggests that the physiological processes that protect diapausing larvae from desiccation may also promote cold tolerance (Shivananjappa et al., 2020).

While cross-tolerance to various environmental stresses is shared across numerous insect species (Bayley et al., 2001; MacMillan et al., 2009; Pallarés et al., 2017; Sinclair, 2000; Sinclair et al., 2013), interaction effects between stressors for a single species cannot be generalized across multiple species. Similarly, tolerance to one stressor does not necessarily confer cross-tolerance

to others, such as observed in *Z. indianus* where no cross-tolerance was detected between desiccation and starvation, but both stressors improve heat tolerance (Kalra et al. 2017). Due to the often-short life spans of insect pests and the temporal nature of stressful environments, tolerance to changing climatic conditions must occur within and across generations (Chown et al., 2011; Harrison et al., 2012; Sgró et al., 2016). However, most studies to date focus on within-generation cross-tolerance effects (Gunderson et al., 2016; Kalra et al., 2017; Gotcha et al., 2018). As such, further investigations are needed to determine whether cross-tolerance acclimation effects can be transferred to the next generation.

The Angoumois grain moth, *Sitotroga cerealella* (Olivier), is a significant primary pest of cereal staples such as sorghum, wheat, rice, barley, and maize (Ghosh & Durbey 2003). It was initially reported in the French province of Angoumois in 1736, but as a cosmopolitan pest of grains, has spread around the globe to various climatic regions (Deshwal et al., 2020). *Sitotroga cerealella* has proven to be a difficult pest to control due to its ability to feed inside grain at larval stage (Bushra & Aslam, 2014), which has been suggested as a mode of insecticide avoidance (Boshra, 2007). Nevertheless, the renewed global drive against pesticides in food, the limited number of registered active ingredients for stored products, and high cost of other control measures such as modified atmospheres, the labour intensity and health risks of pesticides and growing reports of resistance to fumigants (Pimentel et al., 2007; Nayak et al., 2020) provides impetus for alternative control strategies, such as the utilization of temperature disinfestation (Boshra, 2007).

Although *S. cerealella* has established in diverse habitats, there is limited knowledge regarding the mechanism of establishment and persistence in harsh and contrasting environments. Given the overwhelming evidence of co-occurring multiple environmental stressors (see e.g., Gunderson et al., 2016), to my knowledge, few studies have investigated the potential for cross-environmental stress protection in this species (see Mpofu et al., 2022), whether this can be passed on to the next generation, and how this may shape organismal function and ecology. Despite larvae living inside of grain, *S. cerealella* exhibit high basal heat tolerance and is highly resilient to desiccation due to high body water content (Machekano et al., 2018). However, surviving thermal stress or starvation results in reduction of basal heat tolerance in F₂ generations (but see Mpofu et al., 2022). To investigate whether cold acclimation confers transgenerational plasticity in field populations of *S. cerealella* (see Chirgwin et al., 2018; McGuigan et al., 2021), we tested the effects of acute, chronic, and variable low temperature as well as desiccation acclimation of an F₁ parental population on heat tolerance of the F₂ population, measured as critical thermal maxima (CT_{max}) and heat knockdown time (HKDT). These heat tolerance metrics are ecologically relevant

(Terblanche et al., 2011) and can be used as reasonable proxies of insect spatial distribution. However, both traits are affected by a variety of experimental factors, including starting temperature, organismal environmental history, thermal ramping rates and related (Terblanche et al., 2007; Terblanche et al., 2011). Critical thermal limits in particular are affected by ramping rates, often conferring contrasting effects on thermal tolerance estimates (Terblanche et al., 2007). As such, unravelling the effects of ramping rates on upper thermal tolerance limits remains important. Both low static and variable temperature (18-22 °C) acclimation treatments, representing the average winter temperature in the study area (Machekano et al., 2019; Gotcha et al., 2021) and desiccation are ecologically relevant and act as proxies of organismal thermal limits to activity (Huey & Stevenson, 1979; Lutterschmidt & Hutchison, 1997; Terblanche et al., 2011). I hypothesized that low temperature and desiccation acclimation on F₁ improves heat tolerance in F₂ generation through TGP. This cross-tolerance phenomenon, if present, may help explain *S. cerealella* survival in diverse habitats and cosmopolitan distribution and may influence prediction models and pest control techniques.

4.2. Material and methods

Collection of insects and rearing protocols

Damaged sorghum seeds infested with *S. cerealella* were obtained from farmers' storage facilities in Malaka village (22°35'35.1" S and 027°07'31.0" E) in the Central District of Botswana. The site is in semi-arid and hot with a high average annual temperature of approximately 28.5 °C (Akinyemi & Abiodun, 2019). The grain samples with insects were placed in BugDorm® cages (BugDorm®, MegaView Science Co., Ltd., Taiwan) in the Ecophysiological Entomology Research Laboratory at Botswana International University of Science and Technology. Moths were carefully collected manually using vials and/or a pooter into clean BugDorms with sterilised grain where they were reared in Memmert climate chambers (HPP 260, Memmert GmbH + Co.KG, Schwabach, Germany) at 28±1°C, 65±10% relative humidity (RH), and 24D photoperiod. The cages were also furnished with cardboard paper for adults' oviposition and resting refugia, as well as 10 % sucrose solution (*ad libitum*) using the wick technique (see Machekano et al., 2018). The first laboratory generation of uniformly aged adults, (F₁), were subjected to cold and desiccation acclimation procedures in preparation for subsequent intergenerational cross-tolerance experiments (see sections 2.4 and 2.5).

Temperature acclimation treatments

Temperature acclimation comprised both static acute and chronic pre-treatments as well as dynamic (variable) pre-treatments where temperatures were rather more dynamic than static. Both

acclimation protocols confer acclimation responses and have been a subject of immense discussions in literature (see Terblanche et al., 2010). Acute and chronic acclimation treatments were performed as displayed in **Fig 5 and appendix 4**. F₁ generation moths were acclimated to acute (2 hrs) or chronic (72 hrs) low temperatures (18 °C or 22 °C), representative of winter temperatures in the study area, followed by 72 hrs of optimal rearing conditions (28 °C, 65% RH, 24D) (**Fig. 5**). Hundred individual F₁ adults were placed in 240 cm³ Bugdorm cages. For acute acclimation, adults were subjected to 18 °C or 22 °C static temperature acclimation for 2 hrs at 65±10% RH and 24D photoperiod in a climate chamber. This temperature timing is sufficient to elicit acclimatory responses (Weldon et al., 2011). For chronic acclimation, moths were subjected to static temperatures of 18±1 °C or 22±1 °C for 72 hrs in Memmert climate chambers (as in Kingsolver et al., 2016; Gantz et al., 2020) at 65±10% RH and 24D photoperiod. The acclimation temperatures used here were chosen for their ecological relevance since they represent the most frequently recorded temperatures in the study area during winter (Akinyemi & Abiodun, 2019). Following treatment, moths were placed in new cages with fresh grain and allowed to lay eggs in folded heat-sterilized cardboard sheets for 72 hours in total darkness. Moths were then removed, leaving just the eggs on grain in the Memmert climate chamber at optimum rearing conditions (28±1°C, 65±10% RH, 24D photoperiod) until the F₂ generation adults emerged. Control moths were kept at optimum rearing conditions for the duration of the study.

For dynamic pre-treatments, about 100 F₁ moths were placed in Bugdorm cages (240 cm³) and supplied *ad libitum* with 10% sucrose solution and sterilized sorghum grain. The cages were then placed in a Memmert climate chamber that was set to oscillate between 18 and 22 °C (variable temperature acclimation) at a ramping rate of 0.25°C/min at 65±10% RH and 24D photoperiod (**Fig. 5 and appendix 4**). This corresponded to 10 cycles of oscillations between 18-22 °C in 24 h, and 30 in the entire experimental protocol as the acclimation treatment was run for a period of 72 h (3 days). Similar dynamic acclimation protocols elicit acclimation responses in other insect species (Machekano et al., 2021). The acclimated moths were removed after 72 h and placed into new cages with fresh sterilized grain, 10% sucrose solution and oviposition sites at 28±1 °C, 65±10 % RH. After 72 h, the moths were removed and the cages with grain containing eggs were transferred to a Memmert climate chamber set at 28±1 °C, 65±10 % RH and 24D photoperiod until the eggs hatched into the F₂ generation. The F₂ ±2-day old, unsexed adults were used for subsequent intergenerational cross-tolerance experiments.

Desiccation acclimation treatments

For desiccation acclimation, 30 perforated vials, each containing 10 F₁ generation moths to give a total of 300 moths were prepared. The vials containing the insects were then placed into desiccators containing 200 g of new silica gel (see Weldon et al., 2016; Terblanche & Kleynhans, 2009). The desiccators with insects were placed into climate chambers set at ambient conditions (28 ± 1 °C and 65 ± 10 % RH) for 24 h. These sub-lethal desiccation acclimation conditions suffice for the insects to lose between 1-40% of their water before any desiccation related mortality of their body water (Gibbs, 2002; Gotcha et al., 2018), and elicit acclamatory responses (Weldon et al., 2016). During the desiccation period, an i-button was placed in one desiccator to record temperature and RH at 5-minute intervals. The temperature from the i-button readings confirmed temperature and humidity varied between 28 ± 0.1 °C and 0 ± 0.8 % respectively. After acclimation, the hardened moths were transferred into clean cages with fresh sterilised grain and 10 % sugar solution for oviposition in total darkness for 72 h at 28 ± 1 °C and 65 ± 10 % RH. The moths were then removed leaving eggs for the F₂ generation, whose adults were used to run the CT_{max} and HKDT experiments (see **appendix 4**).

Critical thermal maxima (CT_{max})

To determine CT_{max}, a standardised procedure was used following established methods (see Nyamukondiwa & Terblanche, 2009; Nyamukondiwa et al., 2018; Gotcha et al., 2018; Machekano et al., 2022; Tarusikirwa et al., 2022). Briefly individual test insects were placed inside the organ pipes connected to a programmable water bath with a thermocouple connected to a digital thermometer. The temperature of the water bath was then ramped up at three varying ramping rates ($0.06^\circ\text{C}/\text{min}$, $0.25^\circ\text{C}/\text{min}$, and $0.5^\circ\text{C}/\text{min}$) until CT_{max} was recorded for all acclimation treatments (see ramping rate discussions in Terblanche et al., 2007). These ramping rates were chosen to represent a wide range of potential ecological rates of temperature increases in nature (see discussions in Terblanche et al., 2007; Nyamukondiwa & Terblanche 2009). CT_{max} was defined as the temperature at which each individual insect lost coordinated muscular function as well as the ability to self-right or respond to stimuli such as gentle prodding with a camel hairbrush (Gotcha et al., 2018). Ten test insects were replicated three times ($n = 30$) per treatment (i.e., per acclimation treatment and ramping rate).

Heat knockdown time (HKDT)

Heat knockdown time was determined at a temperature of 48 °C and 65% RH in a Memmert climate chamber using established methods (see full descriptions in Nyamukondiwa & Terblanche, 2009). This temperature was chosen following preliminary experiments based on CT_{max} data. Briefly, ten adults were each individually inserted in 1.5 ml Eppendorf tubes and

placed in the climate chamber set at 48 °C and 65% RH. An i-button (Dallas Semiconductors, Model DS1920) (0.5 °C accuracy; 15-min sampling frequency) was placed in the climate chamber to record actual temperatures and RH conditions and confirmed these ambient experimental conditions. The HKDT, defined as the time (in minutes) at which an individual adult lost the ability to self-right was determined based on observations that were made from a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was attached to a computer and was connected to the climatic chamber. The method was repeated three times, yielding n = 30 sample sizes (i.e., 30 replications) for each acclimation experimental treatment.

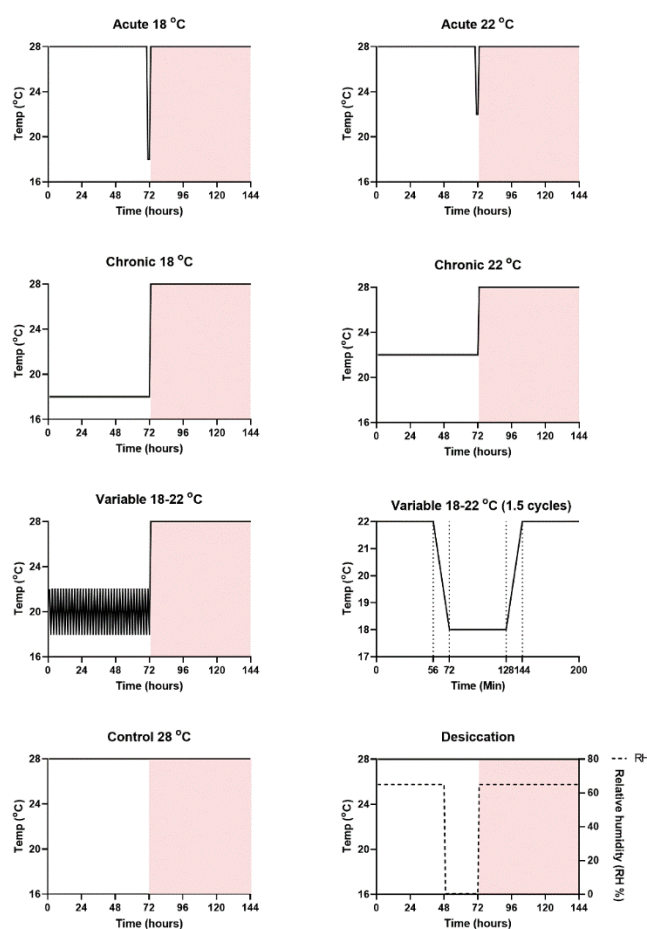


Figure 5. Schematic representation of treatments. Temperature acclimation of *S. cerealella* F₁ generation to acute (2 hr), chronic (72 hr), and variable temperatures as well as desiccation stress. Variable temperature oscillated between 22–18 °C at a ramping rate of 0.25 °C/ min. One complete cycle took 144 minutes making a total of 10 cycles (1440 minutes) in 24hrs. Relative humidity (RH) was 65% unless otherwise specified with 24D photoperiods. Egg laying period (pink shaded area) performed at optimal conditions with access to sucrose ad libitum.

Data analyses

Data analyses were conducted using R v4.1.1 (R Core Team, 2021). Four models were built to examine the effects of treatment and ramping rate (the latter for CT_{max} only) for these response variables following acute, chronic, variable and desiccation acclimation for CT_{max} and HKDT. Deviance residuals i.e., qqplots and goodness of fit i.e., Shapiro Wilk test were examined using standard plots. Non-significant terms were removed from the models, with F-tests conducted via analysis of deviance (Fox and Weisberg, 2019). Post-hoc comparisons were performed via Wilcoxon signed rank tests.

4.3. Results

4.3.1. Transgenerational effects of acute, chronic, and variable (dynamic) low temperature acclimation

F₂ moths from acute and chronic 18 °C acclimated parents demonstrated significantly lower heat tolerance (CT_{max}) compared to optimally reared controls at all ramping rates (**Fig. 6**). However, acute 22 °C acclimation yielded significantly lower CT_{max} compared to the control at 0.06 °C/min and 0.5 °C/min ramping rates but were mildly insignificant at 0.25 °C/min (Wilcoxon Signed Rank Test, $p = 0.003$, adjusted $p = 0.055$) (**Fig. 6**). While acute acclimation at 22 °C resulted in reduced heat tolerance (CT_{max}) of F₂, chronic acclimation at 22 °C yielded no difference from controls at any ramping rate (**Fig. 6**).

Dynamic acclimation of F₁ moths to temperatures between 18-22 °C yielded no difference in CT_{max} at 2 of 3 tested ramping rates (0.06 and 0.5 °C/min) (**Fig. 6**). Comparing static versus dynamic acclimation treatments, results for CT_{max} generally showed that both static and variable low temperature acclimations at the lowest ramping rate (0.06 °C/min) were not statistically significantly different but produced some inconsistent differences at other ramping rates (**Fig. 6**).

Transgenerational effects of desiccation acclimation on thermal tolerance

The F₂ offspring of desiccation acclimated *S. cerealella* parents showed reduced CT_{max} compared to optimally reared controls for all ramping rates, except for 0.25 °C/min, where groups were marginally non-significantly different (Wilcoxon Signed Rank Test, $p = 0.004$, adjusted $p = 0.074$) (**Fig. 6**).

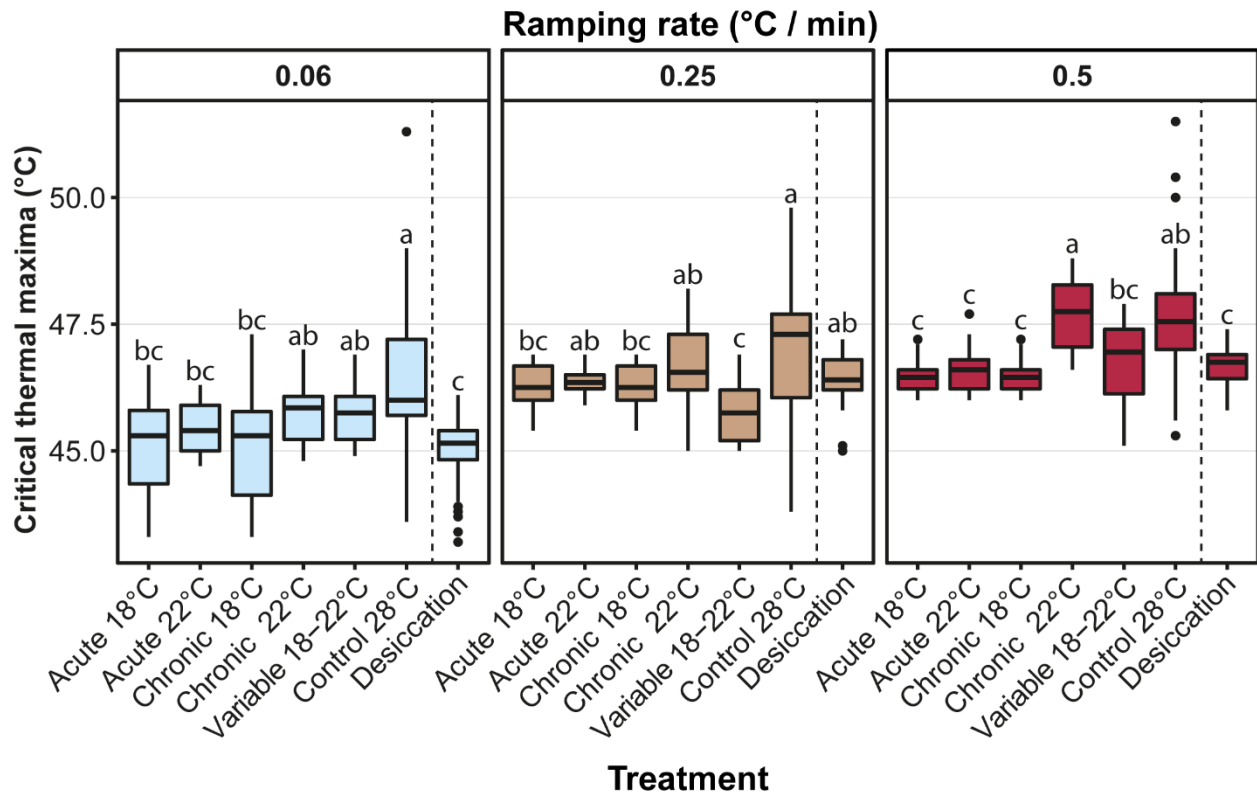


Figure 6. F₁ –generation cold stress and desiccation stress increase F₂ generation susceptibility to heat stress. Critical thermal maxima (CT_{max}) of F₂ moths measured at three ramping rates following F₁ acclimation to acute (2 hr), chronic (72 hr), or variable (~56 min interval over 72 hrs) low temperatures or desiccation stress. Boxplots represent the median with interquartile ranges and whiskers showing the largest and smallest values up to 1.5 interquartile range. Black points are outliers. Statistical letter groupings made by Wilcoxon signed rank tests. Bars with the same letters are not statistically significant from each other.

*Ramping rate alters thermal tolerance scoring in *S. cerealella**

CT_{max} was performed at three ramping rates, with the lowest ramping rate (0.06 °C/min) resulting in the lowest thermal tolerance (**Fig. 6**) and was statistically significant from the 0.25 and 0.5 °C/min ramping rates for all treatment groups (Wilcoxon Signed Rank Test, $p < 0.05$). Increase in ramping rate was correlated to increase in CT_{max} indicating that higher temperatures can be tolerated, but for shorter periods regardless of transgenerational conditioning (**Fig. 7**).

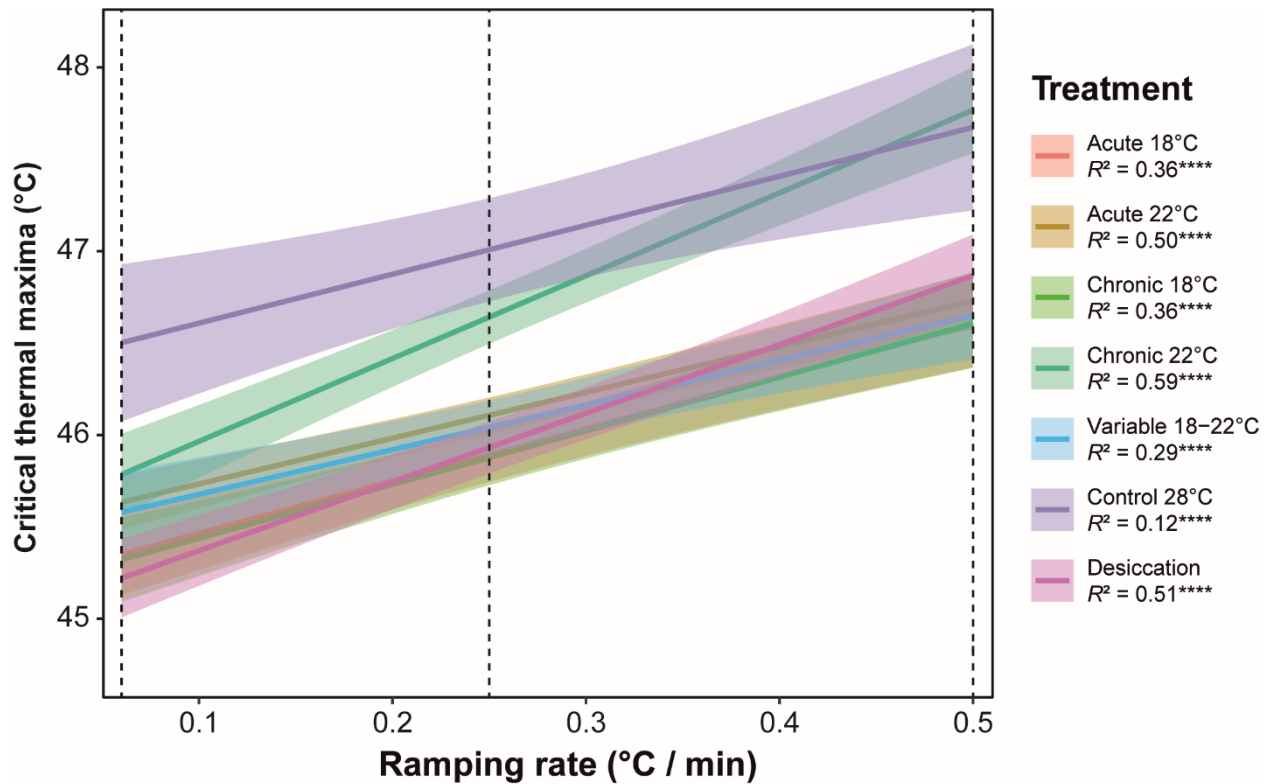


Figure 7. Heat tolerance is dependent on ramping rate for *S. cerealella*. Linear regression of thermal tolerance for all groups where critical thermal maxima (CT_{max}) increases at higher temperature ramping rates. **** = $p < 0.0001$.

Effects of low temperature and desiccation acclimation on heat knockdown time (HKDT)

In addition to CT_{max} , HKDT was measured at static 48 °C. All acclimation treatments either reduced HKDT or had no significant effects on F_2 moths (**Fig. 8**). HKDT produced highly similar statistical groupings compared to CT_{max} at the highest ramping rate of 0.5 °C (compare **Fig. 6** and **Fig. 8**); whereby acute acclimation (at 18 °C and 22 °C) and chronic 18 °C treatments significantly lowered F_2 moths HKDT, but chronic 22 °C and variable (18-22 °C) treatments showed no effects. The only difference between HKDT and CT_{max} at 0.5 °C /min was the desiccation group, which was not significantly different from the control (**Fig. 8**).

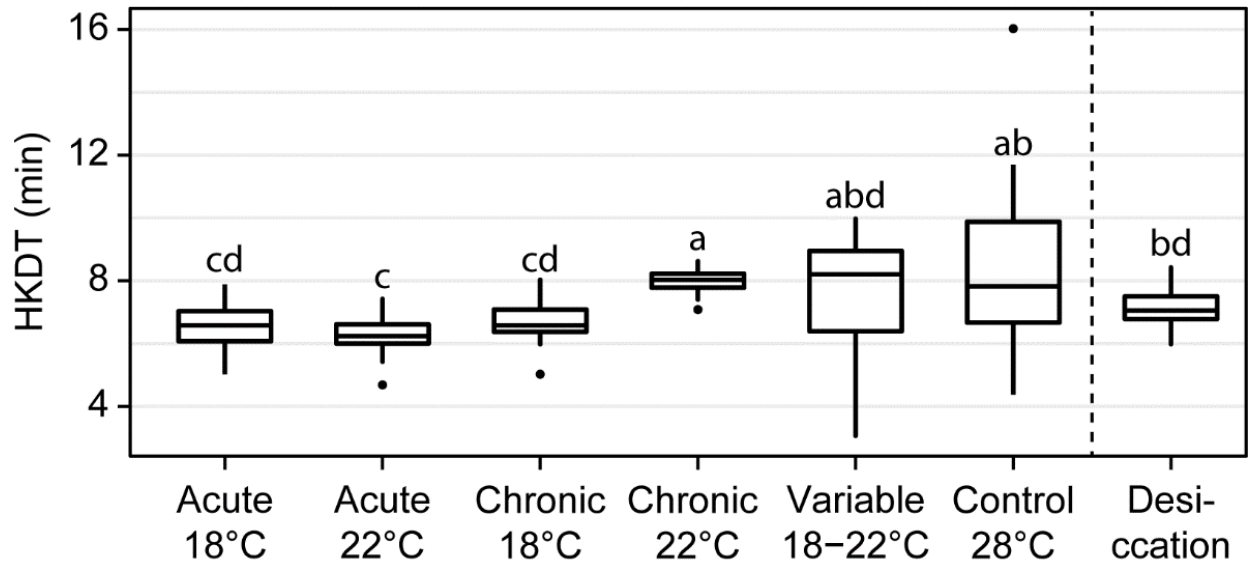


Figure 8. Heat knockdown time (HKDT) of *S. cerealella* F₂ adults following low temperature acclimations or desiccation acclimations. Low temperature acclimations comprised of acute (2hrs), chronic (3 days) and variable (oscillating between 18°C and 22°C for 3 days) treatments. Boxplots represent the median with interquartile ranges and whiskers show the largest and smallest values up to 1.5 interquartile range. Black points are outliers. Statistical letter groupings made by Wilcoxon signed rank tests.

4.4. Discussion

There is a growing body of evidence demonstrating that tropical insects are experiencing increasingly stressful environmental conditions under climate change (Thuiller, 2007; Kingsolver et al., 2013; Seneviratne et al., 2014; Gely et al., 2020; Jørgensen et al., 2022). Tropical insect species must therefore develop adaptive resistance to these varying environmental stressors. However, species from tropical origins are perceived to have limited environmental tolerance ranges and plastic responses when compared to their temperate counterparts (Ghalambor et al. 2006; McCain 2009; Gunderson & Stillman, 2015). My results showed contrasting effects of different acclimations for HKDT and in interaction with temperature ramping rate on CT_{max}. Acute and chronic low temperature acclimation at 18 °C appeared to reduce heat tolerance (CT_{max} and shorten HKDT) in F₂ *S. cerealella* moths. These results indicate lack of inter-generational crosstalk in heat stress following cold acclimation in *S. cerealella*. However dynamic temperature acclimation generally had no effects on both CT_{max} and HKDT. Furthermore, offspring of desiccation acclimated moths showed reduced CT_{max} and no difference in HDKT compared to offspring from non-acclimated moths, indicating no transgenerational cross protection effect between desiccation and heat stress. These results highlight those stresses implemented on F₁

generations can result in transgenerational fitness costs, and therefore may provide novel avenues to disrupt this cosmopolitan storage grain pest. Thus, while *S. cerealella* is highly resilient to thermal and desiccation stress within-generation (see Machekano et al., 2018), this resilience comes at the cost of cross-susceptibility to stresses in future generations. This cross susceptibility to divergent stress traits has been reported in literature (see discussions in e.g., Jørgensen et al., 2019; Tarusikirwa et al., 2022).

Studies on cross-tolerance indicate that exposure of an organism to one stressor often confers resistance to another contrasting stressor, signalling shared co-evolutionary physiological stress resistance mechanisms (Sinclair et al., 2013). However, in this study I found that acute and chronic low temperature acclimation reduced heat tolerance in F₂ populations of the *S. cerealella* indicating lack of transgenerational cross protection effect between these two stressors. Generally, there is a trade-off between low and high temperature tolerance in ectotherms, including insects (Nyamukondiwa et al., 2011). This may also mean that stress effects of both cold and heat are cumulative for *S. cerealella* (as in e.g., Jørgensen et al., 2019) and thus, the species is cross susceptible to these stress traits. To date, most studies have been limited to within-generation cross-tolerance effects (see Nyamukondiwa & Terblanche, 2010; Chidawanyika & Terblanche, 2011a; Kalra et al., 2017; Gotcha et al., 2018). However, my findings are consistent with other studies which found no cross protection effects between traits of heat tolerance and other traits within- (Mutamiswa et al., 2020; Machekano et al., 2021), and across -generations (Mpofu et al., 2022), the latter study specifically for *S. cerealella*. Similarly, some studies have reported lack of within-generation cross-tolerance to heat stress following cold acclimation treatments in other insect species including *Tuta absoluta* (Tarusikirwa et al., 2020) and *Prenolepis imparis* Say (Hymenoptera: Charipidae) (Tonione et al., 2020). Dynamic temperature acclimation on the other hand was found to have no effect on heat tolerance in this species, keeping in line with other studies which have found no effects of cold acclimations on heat tolerance (Gotcha et al., 2018; Tarusikirwa et al 2020; Tonione et al., 2020).

The reason for the lack of inter-generational cross protection effects observed here is unknown. However, previous studies have shown that mechanisms for both heat and cold shock may be overlapping (Hoffman et al., 2003) through e.g., synthesis of HSPs (Chen et al., 2005; Rinehart et al., 2007; Kalosaka et al., 2009; Lencioni et al 2013, Chowanski et al., 2015) which have been documented as a mechanism used by insects against both cold and heat stress (Feder & Hoffmann, 1999). Nevertheless, a handful of studies have also reported mechanisms may be decoupled (reviewed in Angilletta, 2009). For example, related studies have also reported no effects of cold

acclimation on heat tolerance across different insect taxa (Mutamiswa et al., 2017; Gotcha et al., 2018; Tarusikirwa et al., 2020). Some studies have argued that although HSPs can be synthesized during cold shock, they however could not increase cold tolerance (Rinehart et al., 2006; Gbotsyo et al., 2020). Thus, HSPs vary in their response to cold stress because several types of stress factors and genes are activated or deactivated depending on the functional need of the specific gene product (Fulda et al., 2010; Chen et al., 2018). Thus, although HSPs may be synthesized during cold stress, they may however be non-essential for cold stress tolerance, or their expression may not be sufficient to mitigate the negative impacts of low temperature stress. Furthermore, up-regulation of other HSPs may be required for defence and to reduce the accumulation and extent of cold injury (Gbotsyo et al., 2020). Other studies have however argued HSPs are not synthesized during cold shock, but during the recovery phase following cold shock (Colinet et al., 2010; Goto et al., 1998). In a related study, Sorensen et al. (2010) showed that HSPs production is suppressed under non-stressful conditions since they hinder normal cell growth. Therefore, although I align with conclusions from Sorensen et al. (2010) study, I also entertain the possibility of having missed the exact sub-lethal stressful conditions required to elicit HSP expression. My results also show that, heat tolerance was different between acute and chronic acclimation treatments at 22 °C (for HKDT and CT_{max} at 0.5 °C/min), where a 2-hr exposure was more damaging to offspring than a 72-hr acclimation. Further, a 2-hr acute acclimation was equally damaging for both 18 °C and 22 °C treatments (no significant differences found for all measures, see **Fig. 6** and **Fig. 8**). Thus, it appears that short periods at relatively innocuous temperatures have a greater impact on the fitness of offspring than prolonged periods. This finding suggests that the abrupt shift in temperature is the cause of fitness costs, rather than the temperature itself. This mirrors previous findings by Harada & Burton (2019) where a gradual increase in temperature increased survival and improved developmental time of the intertidal copepod *Tigriopus californicus* Baker (Harpacticoida: Harpacticidae) compared to abrupt increases in temperature. Further, they identified that differential expression of HSPs occur between abrupt and gradual temperature changes and argued that the expression of HSPs themselves may not confer tolerance depending on the context of the environmental stress.

In rapid and drastic temperature fluctuations, insects may not be capable of effectively responding, as differential expression of a number of genes including HSPs is energetically costly and will yield no benefit if HSPs are expressed immediately before a switch back in temperature. However, a lack of preparedness to change in temperatures can also have transgenerational impacts, such as in the blow fly *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), where high temperature acclimation of adults resulted in reduced cold tolerance for diapausing progeny and

vice versa (Coleman et al., 2014). As such, cross-tolerance functions may not improve environmental hardiness, but rather prime progeny for specific stresses. However, it is yet to be determined whether gradual exposure to reduced temperatures over a longer time frame provides transgenerational protection to cold stress in *S. cerealella*. Further studies are thus required to identify the relationship between HSP expression and tolerance, as well as exact experimental acclimation treatments to disentangle the lack of transgenerational cross protection observed in *S. cerealella*.

My study reports a lack of cross protection effects between desiccation and heat stress in F₂ *S. cerealella* adults. There was reduced CT_{max} and HKDT in F₂ adults following desiccation acclimation treatment of F₁ moths. This result is in contrast with other within-generation studies that have documented improvements in heat tolerance following desiccation acclimation (Scharf et al., 2015; Bubliy et al., 2012; Bubliy et al., 2013) indicating cross protection effects between the two stressors. However, to my knowledge, there are a limited number of studies on transgenerational cross protection effects to abiotic stressors in insects and none that I know of were done in *S. cerealella*. HSPs have been documented to be expressed at the onset of heat stress and have been reported to be also produced during desiccation stress (Tammariello et al., 1999; Baley et al., 2001; Hayward et al., 2004). The expression of HSPs under desiccation stress has been suggested as the possible reason underlying this cross protection/tolerance effect (Bayley et al., 2001; Gotcha et al., 2018). Nevertheless, although desiccation hardening may seemingly increase heat shock protein expression, which would likely enhance heat tolerance, previous studies have also shown no direct relationship between desiccation acclimation and heat tolerance. For example, desiccation acclimation had no effects on high temperature tolerance of *Sarcophaga crassipalpis* Macquart (Diptera: Sarcophagidae) (Tammariello et al., 1999), in consonance with my results. Nevertheless, whether HSPs were synthesised during my desiccation acclimation treatment, and whether the expression is sufficient to elicit cross protection needs to be further investigated in order to disentangle the observed lack of cross protection effect.

In addition to HSP detection, understanding the role of CT_{max} ramping rates on identified outcomes deserves closer attention, as minor differences in experimental setup can lead to physiologically different responses and differences in results (Terblanche et al., 2007; 2011). In this study, I identify that the slowest ramping rate of 0.06 °C/min resulted in clear differentiation between experimental groups, but 0.25 °C/min did not to the same extent. Whether the ramping rate change induced differences in responses (i.e., desiccation vs heat shock) was not investigated further, but has been suggested previously (Rezend et al. 2011), although to disregard CT_{max} studies based on

physiological response changes has been disputed (Overgaard et al. 2012). However, reduction in CT_{max} following more prolonged experimental protocol at 0.06 °C /minute ramping rate is consistent with the notion of accrued heat stress effects (Jørgensen et al., 2019).

Understanding the independence of short- and long-term plasticity on thermal tolerance is important for understanding an organism's response to changing environments (Gerken et al., 2015). This is also consistent with the current environmental climate change projections, likely to be associated with short- and long-term changes in ambient environments (Murphy & Timbal, 2008; Christensen et al., 2013; Seneviratne et al., 2014). When comparing the effects of short-term (acute) and more long-term (chronic) acclimation effects on heat tolerance, I found that generally there was no significant difference between these two acclimation treatments. Within life stage, the costs and benefits of acclimation responses depend on the duration and intensity of the exposure temperature (Bowler & Terblanche, 2008; Hoffmann et al., 2013; Bahrndorff et al., 2016). This, therefore, suggests that due to the difference in duration between acute and chronic acclimation, there should have been a difference in heat tolerance following the two-acclimation treatments. A previous study reported that long-term pre-exposure either to cold or heat stress is directionally related to survival at thermally stressful conditions later in life (Colinet & Hoffmann, 2012). Nevertheless, previous studies indicate that adult acclimation responses are often reversible compared to developmental acclimation which is often non-reversible (Slotsbo et al., 2016). It is this reversibility of both short- and long-term acclimation that may account for my observed results. Another study reported that the effects of long-term cold acclimation were reversed in less than 24 hours (Slotsbo et al., 2016), suggesting that cold acclimation may be more rapidly reversible in some species. Therefore, it appears that for my study species, both short- term and long-term cold acclimation treatments may not confer lasting benefits in heat tolerance and may explain the lack of transgenerational cross protection observed here. Nevertheless, in other insects, cold acclimation has been reported to improve cold tolerance, but with a trade-off in heat tolerance (Hoffmann et al., 2003; Allen et al., 2012; Chidawanyika & Terblanche, 2011b). However, the trade-off between cold and heat tolerance induced during either developmental or adult acclimation may not be found in all species (see e.g., discussions in Nyamukondiwa et al., 2013), hence there is a need to investigate the trade-off in *S. cerealella*, to be able to better understand the mechanisms of the lack of cross-tolerance in this species.

Empirical evidence suggests that temperature variability will increase as climate change ensues (Field et al., 2012; Thornton et al., 2014). As such, determining the fate of organisms under more dynamic temperature variations is of paramount importance to ecologists. The effects of mean

increases in temperature and variability have been subjects of debate in literature (see Easterling et al., 2000; Terblanche et al., 2010). I found that dynamic temperature acclimation had generally similar effects on heat tolerance with the static acclimation treatments. My results are thus in keeping with previous reports suggesting that dynamic and static acclimation protocols yield consistent results (Rosenheim, 1999; Cooper et al., 2008; Lake et al., 2013; Winther Bak et al., 2020).

Physiological tolerance to one stressor can result in tolerance or resistance to that same environmental stressor or other divergent stressors (Gunderson et al., 2016; Kalra et al., 2017; Gotcha et al., 2018). This cross-tolerance response can provide important insights into the underlying genetic makeup, possible costs and benefits as well as the plasticity of an organism's stress response. My study found that desiccation-, acute and chronic low temperature - acclimations in F₁ reduced heat tolerance (CT_{max} and HKDT) in F₂ populations, suggesting across generation cross susceptibility or lack of cross-tolerance thereof. This cross susceptibility indicates possible fitness costs of exposure to multiple contrasting stressors in the face of climate change (Thuiller, 2007; Gunderson et al., 2016) and may be used in models designing pest management strategies for this cosmopolitan pest species. More research is however needed on transgenerational effects of abiotic stressors, possible cross protection -effects within and across generations for storage pests such as *S. cerealella* including potential mechanisms thereof to inform temperature based phytosanitary disinfestation protocols against this pest.

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Chapter 5:

General discussion

The current study was aimed at identifying non-genetic mechanisms that allows *S. cerealella* to adapt and hence persist with current changing global environments. To achieve this aim, I subjected my study insect, *S. cerealella* to four distinct environmental acclimatory stressors of heat, cold, desiccation and starvation, after which their temperature plastic responses were measured across generations. Positive plastic responses following sub-lethal acclimation of a previous generation would indicate carry over effects of such stimulus across generations. For example, improved heat tolerance in F₂ following thermal, desiccation and starvation acclimations of F₁ was an indication of carry over effects of these stressors across generation in these study insects. These results are as expected since improved heat tolerance following a heat acclimation is something that has been observed in various insect taxa, although most studies were focused within same generation. Nevertheless, this does not rule out the same effect across generations. Similarly, studies have also reported the ability of an acclimation to induce plastic responses when exposed to a different stressor later in what is normally referred to as cross talk. It is therefore for this reason that I had the expectation that other acclimation treatment such as starvation, desiccation and cold acclimation will also improve heat tolerance of F₂ generation, likely showing shared co-evolutionary response mechanisms for the associated stresses.

I found out that, heat acclimation (acute and dynamic) showed reduced heat tolerance measured as both CT_{max} and Heat knockdown time on *S. cerealella* (chapter 3). This is contrary to the BAH postulating that a pre-expose to mild stress confers resistance to a similar more severe stress later in life (Kristensen et al., 2008; Angilletta, 2009). These results are however not surprising as although insects are able to quickly respond to changing environmental conditions through phenotypic plasticity (Whitman & Agrawal, 2009; Moczek, 2010; Somero, 2010; Van Heerwaarden et al., 2016), phenotypic plastic responses have been reported to be reversible (Gabriel, 2005; Whitman & Agrawal, 2009) as well as not fixed (Gibert et al., 2001; Austin, 2013). Thus, phenotypic plasticity is seldom not available in some species and/or developmental stages (Sgrò et al., 2016) and its ability to buffer organisms against climate change has been reported to vary with species (Calosi et al., 2008; Nyamukondiwa et al., 2011; Gunderson & Stillman, 2015; Machekano et al., 2019; Van Heerwaarden & Kellermann, 2020). Furthermore, the potential for phenotypic plasticity to cushion animals against climate change is also a subject of debate in literature, owing to low acclimation response ratios in some species (Gunderson & Stillman, 2015; Van Heerwaarden et al., 2016; Machekano et al., 2021). In this same chapter, contrary to my expectations I also found that fasting (food deprivation) acclimation did not significantly affect CT_{max}, indicating a lack of evidence for cross tolerance and/or susceptibility (chapter 3). This result however is in agreement with numerous studies that have shown lack of cross protection

effect between pre-exposure to starvation stress and heat tolerance (Scharf et al., 2016; Mitchell et al., 2017; Mir & Qamar, 2018). Mutamiswa et al., 2018).

My study went further to also showed a general reduction in heat tolerance measured as both CT_{max} and HKDT following both acute and chronic low temperature acclimation at 18 °C while dynamic temperature acclimation generally had no effects (chapter 4). Desiccation acclimation on the other hand showed only reduced CT_{max} but not HKDT (chapter 4). Generally, these results suggest lack of inter-generational cross tolerance in heat stress following both cold and desiccation acclimation in *S. cerealella*. These results highlight that those stresses implemented on F₁ generations can result in transgenerational fitness costs, and therefore may provide novel avenues to disrupt this cosmopolitan storage grain pest. Thus, while *S. cerealella* is highly resilient to thermal and desiccation stress within-generation (see Machekano et al., 2018), this resilience comes at the cost of cross-susceptibility to stresses in future generations. This cross susceptibility to divergent stress traits has been reported in literature (see discussions in e.g., Jørgensen et al., 2019; Tarusikirwa et al., 2022). Although these results were not as expected, I feel they are great findings to the grain treatment method strategies. Due to public health concerns over the use of chemicals to treat grains, one of the current non-chemical phytosanitary methods for grain pest disinfection is the use of heat and cold temperature treatments (Phillips & Throne, 2010; Beckett et al., 2007; Sirohi et al., 2021). However, there were challenges with these methods due to the high basal heat tolerance of most insect species. This is because the choice of the heating temperatures and their durations is a compromise between the pest thermal tolerance as well as commodity safety (Sørensen et al., 2019). Therefore, any method that can lower the thermal tolerance of the pest will be beneficial as low temperatures does not cause damage or injury to the grains. However, more work is needed to investigate how this can be practically deployed in a real setting.

In conclusion, as climate change increases, there is a high likelihood of increased insect pests because of increased growth and development, species geographic expansions, pest resurgence, invasive species likely increasing insect pest outbreak frequencies and a reduction in food security (Andrew et al., 2013; Thackeray et al., 2016). Nevertheless, insect pest severity may not be uniformly increased by warming temperatures since studies show that insect response to thermal stress is species specific (Nyamukondiwa et al., 2009; Harrison et al., 2011; O'Connor et al., 2015; Gotcha et al., 2021a, b; Mpofu et al., 2022). It is for this reason that my study was able to bring this new information on how pre-exposure to various environmental stressors might affect the heat tolerance of future generations in the study species. This uncertainty about pest responses to rising

temperatures needs to be addressed to develop effective pest management strategies. Policies based on a universally anticipated, generic increase in pest severity will be misguided; rather, a more detailed species-specific understanding of insect pests responses to climate warming is essential to inform effective allocation of public and private resources for pest management efforts in the future. My study was able to document the following knowledge: i), that pre-exposure to sub-lethal divergent environmental stressors in the F₁ generation can lower the thermal tolerance of F₂ *S. cerealella*, ii) that adaptation to high temperatures in *S. cerealella* through phenotypic plastic responses may not be transferred across generations. The information is important as firstly, it shows how the incorporation of acclimation period to the heat treatment strategies may be of benefit if incorporated in grain treatment against *S. cerealella*. Secondly, other biotic stressors such as food deprivation (starvation stress) and desiccation can be coupled with thermal treatment methods during phytosanitary treatments for synergistic efficacy benefits. Therefore, my study is of benefit in that data from this thesis may be used to improve temperature-based phytosanitary treatment protocols or be incorporated and help refine models designing other pest management strategies for this or other economic pest species.

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Appendix 1

Parental acclimation reduces offspring thermal fitness in the postharvest insect species *Sitotroga cerealella* (Olivier)

Published as: **Mpofu, P**, Machekano, H, & Nyamukondiwa, C. (2022). Parental acclimation reduces offspring thermal fitness in the postharvest insect species *Sitotroga cerealella* (Olivier), *Integrated Protection of Stored Products IOBC-WPRS Bulletin Vol. 159*, 2022, pp. 53-58

Introduction

The Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), is a major pest of stored agricultural products across temperate and tropical areas (Trematerra, 2015). Infestations of AGM occur either preharvest or postharvest with larvae burrowing within crop plant kernel grains, rendering them unfit for human consumption (Trematerra, 2015). As a result, continuous protection against the AGM is required to maintain grain quality. Cereal grains such as maize and sorghum are the main staple food commodity for people in Africa (Taylor, 2003). Many farmers store their grain until the next ploughing season to ensure nutritional security and sustainability (Wambugu et al., 2009). This is particularly pertinent due to increasing droughts (Milgroom & Giller, 2013) and production uncertainties around COVID-19 (Muthamilarasan & Prasad, 2021). However, effective, prolonged storage is hindered by storage pests such as AGM, that damage as well as reduce the quantity and quality of stored grain. Although many grain pest protection techniques are available (Fields and White, 2002), the use of extreme temperatures as a grain disinfestation method is gaining traction (Moore & Manrakhan, 2022). Fields (1992) established thermal thresholds for most species, but exposure to sub-lethal high temperature stress, consistent with constrained grain storage environments has not been considered. Stored product pests may also inevitably increase their environmental temperature through respiration, creating temperature ‘hot spots’ and potentially conferring acclimatory responses (see Angilletta, 2009). However, the potential for this acclimation to affect subsequent generations through transgenerational plasticity (TGP) has largely been neglected (see Fields, 1992). That is, despite the potential costs and/or benefits of TGP fitness on stored product pests, little is known about how this will validate or invalidate previously established thermal thresholds for temperature-based disinfestation protocols (see. g., Fields, 1992). TGP occurs when the environment of a parent shapes the phenotype of their offspring. If adaptive, TGP thus protects populations from the effects of extreme temperature while providing time for genetic adaptation to catch up in the long run (Chevin et al., 2010). Several animal and plant taxa have demonstrated TGP in response to abiotic and biotic stressors (Donelson et al., 2018). However, TGP and its implications on pest management remains unknown for postharvest insect pests.

Factors contributing to the global increase of AGM are largely unknown (but see Machekano et al., 2018 a). However, basal and plastic heat tolerance can help insects survive in high temperatures (Nyamukondiwa et al., 2010). Against this background, we thus tested the potential costs and benefits for TGP on heat tolerance in the AGM and make inferences on how this may

validate (or otherwise), existing thermal limits in grain temperature disinfestation protocols. We hypothesized that AGM is resilient to heat stress through natural grain environment acclimation.

Materials and methods

Insect culture

Adult AGM colonies were collected from damaged sorghum seeds from farmers' storage facilities in Malaka village (22°35'35.1" S and 027°07'31.0" E). Insects were reared in the laboratory at 28 ± 1 °C, 65 ± 10 % relative humidity and 24 D photoperiod in Memmert climate chambers. The F1 was exposed to heat and fasting acclimation treatments for subsequent TGP experiments. The moths were provided with 10 % sucrose solution and heat sterilized (80 °C for 48 h) fresh sorghum grain and folded sterile cardboard paper to provide dark sites for resting and oviposition.

Acute temperature acclimation treatments

Acute acclimation of AGM was done by placing 100 adults in 240 cm³ Bugdorm cages. The adults were exposed to acute acclimation for 2 h at static 34 °C and 38 °C temperatures in climate chambers. Following treatment, the moths were transferred to cages with fresh grain and allowed to lay eggs for 72 h. The moths were then removed, leaving only the eggs until the F2 generation adults emerged. Controls were moths that remained under optimal rearing conditions.

Fasting acclimation treatments

In the climate chamber, 100 F1 moths were placed in 240 cm³ Bugdorm cages for fasting acclimation. The moths were fasted for 72 hours by withholding food. After 72 hours of fasting acclimation, the moths were provided with fresh heat-sterilized sorghum grain, and 10 % sucrose solution. They were then kept in climate chambers at optimal rearing conditions and allowed to lay eggs for 72 hours. Emerged F2 moths (± 2 days old) were used for the TGP experiments.

Variable acclimation treatments (dynamic acclimation)

Around 300 moths were placed in cages, fed 10 % sucrose solution *ad libitum* and provided with sorghum grain. The cages were then placed in a climate chamber set to oscillate between 28 and 38 °C at a ramping rate of 0.25 °C/min for 72 h. The acclimated moths were then removed and placed in cages with fresh sterilized grain, 10 % sucrose solution, and folded heat-sterilized cardboard paper for resting and oviposition. The moths were removed after 72 h, and the cages containing eggs were transferred to a climate chamber set at optimal rearing conditions until the eggs hatched into the F2 generation adults, which were then used for the TGP experiments.

Critical thermal maxima

Critical thermal maxima (CT_{max}) were measured using a programmable water bath (filled with a 1:1 water: propylene glycol) connected to a transparent double-jacketed chamber, as described by Nyamukondiwa and Terblanche (2009). A thermocouple connected to a digital thermometer was inserted into the central organ pipe to measure organ pipe temperature. Ten test insects were used, replicated three times ($n = 30$). CT_{max} experiments began at a setpoint temperature of 28 °C and were ramped up at different rate treatments (0.06 °C, 0.12 °C, 0.25 °C and 0.5 °C/min).

Results and discussions

Acclimation treatment had a significant effect on CT_{max} in interaction with ramping rate ($F(6,348) = 2.191$, $p < 0.05$) (**Fig. 9**). Ramping rates of 0.5 °C/min had a significantly higher CT_{max} than lower rates (0.06, 0.12, and 0.25 °C /min) for both the control and acute acclimation (T32 and T36) (all $p < 0.05$). Furthermore, CT_{max} at 0.06 °C/min for acute acclimation was significantly lower than CT_{max} at 0.25 and 0.12 °C/min ramping rates (both $p < 0.001$). Other pairwise comparisons in the control and acute acclimation groups lacked statistical clarity (all $p > 0.05$). For the 32°C acclimation treatment, 0.06 °C/min had a significantly lower CT_{max} than the control and the other ramping rates (all $p < 0.05$). In general, CT_{max} at a given ramping rate did not differ among treatment groups, i. e., T32 and T36 but were generally lower than the control group.

Acclimation treatment had a significant negative effect on CT_{max} using the 0.12 °C/min ramping rate ($p < 0.05$) (**Fig. 10**). The control had a significantly higher CT_{max} than all the low temperature acclimation treatments (all $p < 0.05$). There was however no significant difference between the treatments acute 18 °C, acute 22 °C and chronic 18 °C ($p > 0.05$). Furthermore, CT_{max} at acclimations chronic 22 °C and T18-22 °C were statistically different from all the acclimation groups however they were not statistically different from each other ($p > 0.05$). In general, CT_{max} differed among treatment groups, and they were generally lower than the control group meaning that all low temperature acclimations reduced CT_{max} through costs of TGP. I investigated how different types of acclimations, such as acute, chronic, and variable temperature acclimation stress, affect TGP in the F2 generation after exposing the parent population of adult AGM. My findings showed that both high and low temperature acclimation had a significant TGP effect on F2 offspring heat tolerance. We also demonstrated that higher ramping rates (0.5 °C/min) resulted in higher CT_{max} whereas lower ramping rates (0.06 °C/min) had lower CT_{max} (see **Fig. 9**). These findings show that TGP responses in AGM differ depending on the acclimation treatment. Unraveling these TGP responses could help explain how insects cope with rapid climate change (Donelson et al., 2018) and could help inform effective heat disinfestation pest control protocols for resilient grain pests.

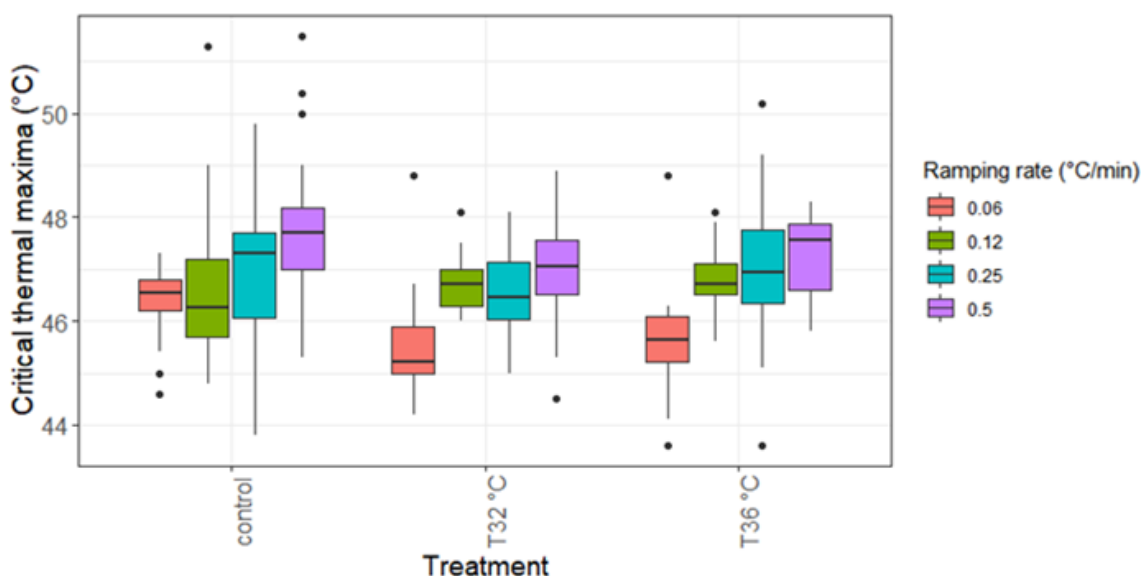


Figure 9. Boxplots showing CTmax across treatment groups and ramping rates after acute temperature acclimation. Acute acclimation was defined as 2h exposure to sublethal high temperature 32 °C (T32) and 36 °C (T36). In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges, and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. Colored points are raw data and black points are outliers.

My findings also suggest that AGM is heat tolerant (Machekano et al., 2018 b). Its high temperature tolerance likely explains why it is a serious pest in warm tropical areas such as sub-Saharan Africa (Machekano et al., 2018 b). Other studies have also documented this heat tolerance for other related species e. g., fruitflies (see Nyamukondiwa et al., 2010) and *Prostephanus truncatus* (see Machekano et al., 2020). Similar findings were also observed in the apple fly maggot, *Rhagoletis pomonella*, which expresses heat shock proteins when exposed to high temperatures (King & MacRae, 2015). These heat shock proteins help insects survive by preventing protein denaturation and cell inactivation during extreme high temperatures (Ma et al., 2020). My findings show that heat and cold acclimation reduced CT_{max} compared to controls, especially at low ramping rates. Based on this information, *S. cerealella* TGP responses are negative, making F_2 generations less heat resistant. Where AGM is the main target pest, farmers who use heat-based grain disinfestation methods implemented through solar radiation, modified passive metal silos or active temperature-controlled bulk storage silos will benefit from low energy requirements for grain treatment (low lethal thresholds and low ramping rates) over time. As a result, enabling low-cost and safer grain storage. Put together, this justifies resource utilization and derive maximum benefits from production resources. AGM had a lower CT_{max} following lower temperature acclimation (**Fig. 9**). This could imply that AGM has limited low

temperature tolerance (Machekano et al., 2018 b). Acute cold exposure has been shown to influence thermal tolerance in other Lepidopterans, such as cereal stemborers (Mutamiswa et al., 2018). The present study indicates that both acute, chronic and variable temperature acclimation significantly affected the CT_{max} of AGM (**Fig. 10**). AGM recorded a reduction in CT_{max} after cold acclimation indicating TGP fitness costs. This agrees with previous studies on *Musica domestica* and *Sarcophaga crassipalpis* which reported TGP reproductive fitness costs following low cold acclimation (Coulson and Bale, 1992; Rinehart and Denlinger, 2000). Cold shock may cause damage to various tissues in a developing insect, many of which can lead to reduced reproductive potential, e. g., delayed development of ovaries (Rinehart and Denlinger, 2000).

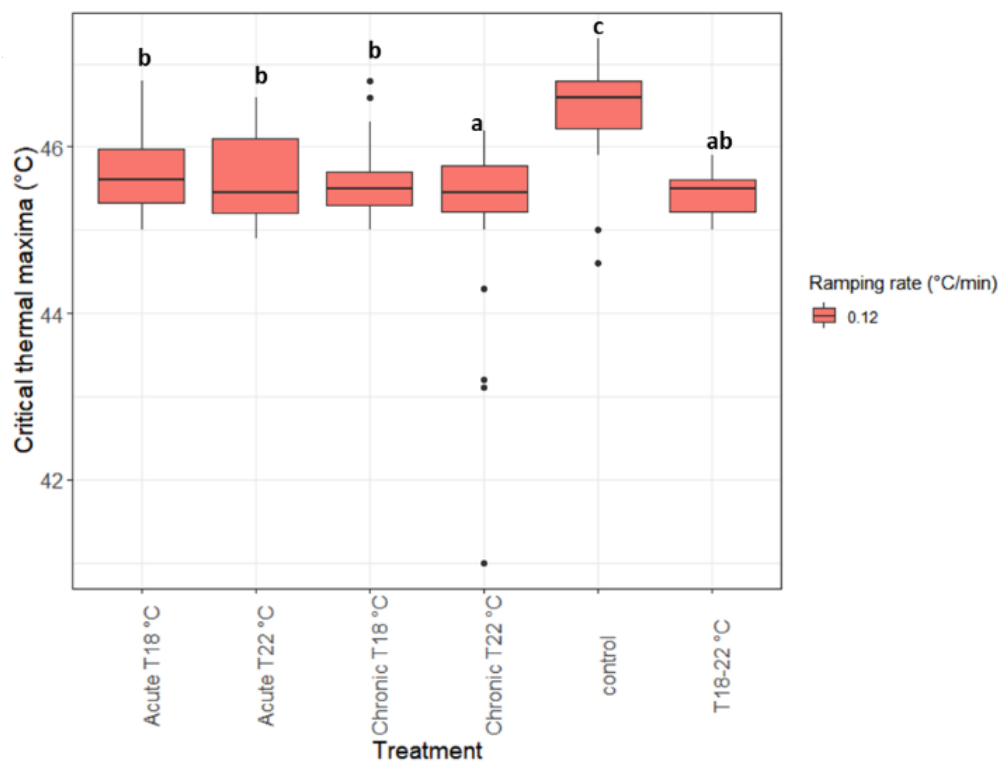


Figure 10. Boxplots showing CT_{max} across treatment groups after acute, chronic (18 and 22 °C) and variable (18-22 °C) low temperature acclimation. In the boxplots, the horizontal bars display the median, the box gives the interquartile ranges, and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. Colored points are raw data and black points are outliers.

My study reveals that past exposure to heat or cold stress can have extreme negative effects on CT_{max} in AGM, particularly at low ramping rates through TGP. Many studies that have been conducted in AGM were within-generation, however the role of TGP in this species was largely neglected, despite playing a critical role in its survival and increasing pest status as shown here.

In this study, TGP was recorded for the first time in the F₂ generation of AGM, indicating evidence of negative carry-over effects that were passed on from one generation to the next. This study is significant in explaining fitness costs and benefits in future insect pest generations through TGP and may help explain the evolution of plastic responses in storage pests for more informed, non-chemical, safer and accurate pest management alternatives.

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Appendix 2

Cold tolerance of stored product beetles; implications on low temperature-based commodity disinfestation

Published as: Machekano, H., Mpofu, P. & Nyamukondiwa, C. (2022). Cold tolerance of stored product beetles; implications on low temperature-based commodity disinfestation. Integrated Protection of Stored Products IOBC-WPRS Bulletin Vol. 159, 2022, pp 53-58.

Introduction

The need for prolonged grain storage has become remarkably important due to production uncertainties e.g., increasing droughts (Milgroom & Giller, 2013) and supply chain disruptions, such as during pandemics and/or conflicts (Muthamilarasan & Prasad, 2021). However, effective, prolonged storage is hindered by storage pests. Various methods have been developed for grain disinfestation during storage (Fields, 1992, Cui et al., 2020). However, issues of cost, fumigant resistance, occupational health and environmental concerns have marred the continued use of most fumigants (Stejskal et al., 2019). With growing consumer consciousness and rapid changes in global trade policies against pesticides, safe and efficacious disinfestation techniques that meet regulatory standards and market demands are required (Flinn et al., 2015). Due to high insulation properties of grain, heat gain takes time once the grain is chilled (David et al., 1977), making extreme low temperatures ideal as a sustainable grain/commodity treatment method (Mullen & Arbogast, 1979; Fields et al., 2012).

Rhyzopertha dominica F. is a globally distributed economic primary pest of small grains with high biotic potential, adaptability and already resistant to some pesticides, including phosphine-based fumigants (Pires & Nogueira, 2018). Secondary economic postharvest pest species, that often co-exist with *R. dominica* include but not limited to *Tribolium castaneum* Herbst, *Oryzaephylus surinamensis* L., *Cryptolestes ferrugineus* (Stephens 1831) (Arbogast & Mullen, 1988).

Previous studies show that *R. dominica* is tolerant to extreme low temperatures (David et al., 1977), with no life stage surviving after exposure to 4.4 °C for 4 - 6 weeks. Similarly, *C. ferrugineus* was observed to have the ability to overwinter at low temperatures, including temperate winters (Fields, 1992; Fields et al., 2012) and depress its supercooling point (SCPs) to -20°C when cold-acclimated (Smith, 1970; Fields, 1992). *Tribolium castaneum* was reported to succumb to -12 to 4°C in flour pallets depending on duration of exposure (Flinn et al., 2015), e.g., -10 °C for 7-9 h could give 100% mortality in both *T. castaneum*, and *O. surinamensis* (Mullen & Arbogast, 1979). Although various metrics are used to measure insect responses to low temperature, the SCPs have been extensively used (Baust & Rojas, 1985) and more recently, along with the chill comma recovery time (CCRT) (Nyamukondiwa & Terblanche, 2011).

The responses of stored product pests to extreme low temperature depend on the insects physiological state, life stage and species (Fields, 1992; Fields et al., 2012). Although previous disinfestation protocols have been estimated (Fields, 1992; Fields et al., 2012; Stejskal et al., 2019), there was no consideration of the plastic responses. Stored product pests high but stochastic storage temperatures through ‘hot spots’ (Angelovič et al., 2018) and external temperature

influence on storage facility peripheries (David et al., 1977). This exposes insect pests to sub-lethal thermal stress, potentially conferring acclimatory responses that could alter both activity thresholds and low lethal limits. In addition, most laboratory experiments that use disinfested grain seem to lack a standard reference for low-temperature lethal thresholds of target species, resulting in excessive variations grain disinfestation methods (see Stejskal et al., 2019). For example, low-temperature disinfestation of different types of grain was reported, ranging from -18°C (Brilinger et al., 2020), to +15°C (Cui et al., 2020) at various chilling durations (Fields et al., 2002; Silva-Aguayo et al., 2004; Flinn et al., 2015).

As no stored product pest was reported to be freeze tolerant (Fields et al., 2012), I used the current dogma on thermal biology, to re-assess the low-temperature limits of four adult stored product beetle pests and ascertain whether the average range around -15 °C mostly reported as the disinfestation temperature (Fields et al., 2012; Stejskal et al., 2019) is still effective on local strains using various low temperature metrics. I hypothesized that ‘blanket’ low temperature treatments may not be efficacious if explicit thresholds for individual species (Fields, 1992) and their strains (Fields, 1998) are not known. My results have implications on safer, cheaper, and more efficacious grain treatment protocols.

Materials and Methods

Insect culture

Insect populations were initially collected from two local farmers in Palapye (S22° 37' 53.9", E027° 02' 59.9" and S22° 34' 31.9", E027° 04' 45.9") in 2019 as laboratory colonies kept in the Ecophysiology Research Laboratory, Department of Biological Sciences & Biotechnology, Botswana International University of Science and Technology. The insects were reared on sorghum grain at 27 ± 1 °C and $60 \pm 5\%$ RH in 2L rearing jars.

Chill comma recovery time (CCRT) and Super cooling points (SCPs)

Beetles in 2 ml Eppendorf tubes were placed into a small zip-lock bag in batches of 10, and then plunged into a water bath set at 0 °C for 1 h. Time to recovery was done at 28 ± 1 °C (ambient) in a climate chamber and CCRT was recorded through observations from the camera. For SCP the same procedure was followed, except that the waterbath was to ramp down to -35 °C at a ramping rate of 0.25 °C/min. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple, inserted through the tube's lid and both the insect and the tip of the thermocouple were held in place with cotton wool. The thermocouple interfaces and temperature were recorded at 1 Hz using PicoLog software for windows.

Low temperature survival

Fifty beetles from each species were placed in 60 mL polypropylene vials with gauzed lids and placed in a 33 × 22cm zip-lock bag, replicated three times. The ziplocks were plunged into a water bath with 99.9 % ethanol for 14 days. The water bath temperature was set at -18 °C to record a vial (insect body temperature) of ~ -15 °C (-15.8±1.07 °C, n = 2980, 10 min frequency). In each vial, insects were provided with ~2 g of sorghum grain to simulate storage conditions. After 14 days, insects were allowed to recover in a climate chamber set at 28 ± 1 °C and 65 ± 5 % RH together with 3 replicates of controls (untreated beetles) of each species. Survival was recorded at 24 h then at 7, 14 and 21 days after treatment (DAT) with access to *ad libitum* food.

Data analysis

All data was analysed in STATISTICA 14.0.0.15 (Statsoft Inc., Tulsa, Oklahoma). A Shapiro-Wilk's normality test was done on SCP, CCRT data showed that it was not normally distributed ($p < 0.05$). Thus, a rank based non-parametric approach was adopted to assess the treatment differences using Kruskal-Wallis nonparametric test at 5% of significance. All the Kruskal-Wallis assumptions on the data were satisfactory. A one-way AVOVA was used to plot survival (%) data. Tukey tests were used for post-hoc comparisons.

Results & Discussion

Cryptolestes ferrugineus and *R. dominica* recovered from chill coma earlier than *O. surinamensis* and *T. castaneum* (indicating higher cold tolerance) following chill coma ($p < 0.001$). The latter took significantly longer time (lesser cold tolerant) ($p < 0.001$) than all the other beetles, whereas *O. surinamensis* was intermediate, i.e., it had CCRT significantly lower than *T. castaneum* ($p = 0.004$) but higher than both *C. ferrugineus* ($p = 0.0036$) and *R. dominica* ($p = 0.0001$). However, *C. ferrugineus* and *R. dominica* cold tolerance did not significantly differ (**Fig. 11A**)

A generally similar trend was observed for the SCP. *Cryptolestes ferrugineus* had the lowest SCP (higher cold tolerance) (-19.72±0.372 °C) ($p < 0.001$), followed by *O. surinamensis* which had significantly higher SCPs (lower cold tolerance) than *C. ferrugineus* ($p = 0.004$) but not *R. dominica* ($p = 0.1463$). *Tribolium castaneum* consistently had the highest SCP (less cold tolerant) than *O. surinamensis* ($p = 0.00002$) and *C. ferrugineus* ($p < 0.0001$), but not *R. dominica* ($p = 0.0889$) (**Fig. 11B**).

When beetles were exposed to -15 °C for 14 days, survival was 0% and was significantly lower than the controls ($p < 0.001$). A mean temperature of ~-15 °C could cause 100% mortality in all

tested beetles. However, *R. dominica* control showed natural mortality that was significantly lower than the other species, but certainly higher than all treatments (Fig. 12).

Comparison between my SCP results and previous are studies summarized in (Fields et al., 2012) for the same species show that my strains generally had more depressed SCPs particularly for *O. surinamensis* and *R. dominica* (see Table1).

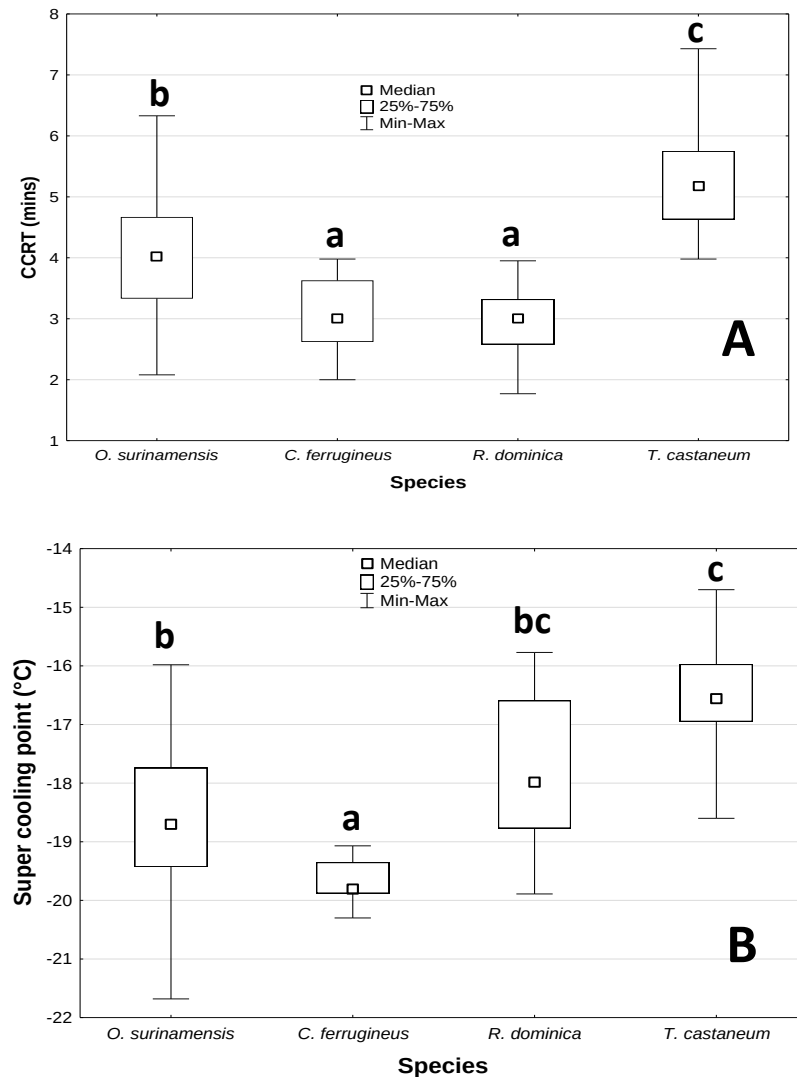


Figure 11. Kruskal-Wallis test for (A) chill coma recovery time (CCRT) for *O. surinamensis*, *C. ferrugineus*, *T. castaneum* and *R. dominica* post exposure to 0 °C for 1 h, and (B) supercooling points (SCP) for *O. surinamensis*, *C. ferrugineus*, *T. castaneum*. Medians with the same letters are not significantly different from each other.

Table 1. Comparison of insect freezing points (SCP) in literature (Fields et al., 2012) and the current (this study) SCP for the adults of the same adult stored product beetles species.

Species name	*Fields et al. 2012	'Machekano et al 2022	Remarks
<i>Oryzaephilus surinamensis</i>	-13.7 °C	-18.7 °C	Lower current SCP
<i>Cryptolestes ferrugineus</i>	-16.7 to -20.4 °C	-19.7 °C	In range, but generally lower current SCP
<i>Tribolium castaneum</i>	-12.3 to -16.0 °C	-16.7 °C	Lower current SCP
<i>Rhyzopertha dominica</i>	-15.2 °C	-17.7 °C	Lower current SCP

*This is a book chapter citation of work carried out in the 1990s (see Fields, 1992). 'Current study

Low temperature lethal thresholds have been put forward for most storage beetles (Fields et al., 2012) but they were not definite, data is scattered in literature and most researchers randomly pick their disinfestation temperature and exposure durations. Low temperature survival also depends on insect strains (Fields, 1998), acclimation and pre-exposure histories, so I set out to determine whether the thresholds reported by Fields et al., (2012) matched my African strains and establish a working low-temperature thresholds benchmarks for laboratory disinfestation of grain through SCP, CCRT and long-term (14 days) exposure to -15 °C. My findings have potential for scaling up to on-farm and warehouse trials, using 'adjusted' thresholds for pre-shipment disinfestations in the grain/flour trade industry. My results showed that the adults for all the four species did not survive -15°C for 14 days.

Lower SCPs did not always follow shorter CCRT. For example, interspecific comparisons showed that *C. ferrugineus* was the coldest tolerant with SCP of -19.7 ± 0.38 °C and a CCRT of 4.1 ± 1.11 , followed by *O. surinamensis* at -18.7 ± 1.34 °C with CCRT of 3.1 ± 0.58 , *R. dominica* at -17.7 ± 1.29 °C with CCRT of 2.9 ± 0.52 min and lastly *T. castaneum* at -16.7 ± 0.95 °C and high CCRT of 5.2 ± 0.76 min. Due to this mismatch between SCP and CCRT, I will base my discussion more on the SCP as a relatively more reliable metric as CCRT was already reported to fail to reveal true variation in thermal performance among other species (Davis et al., 2021).

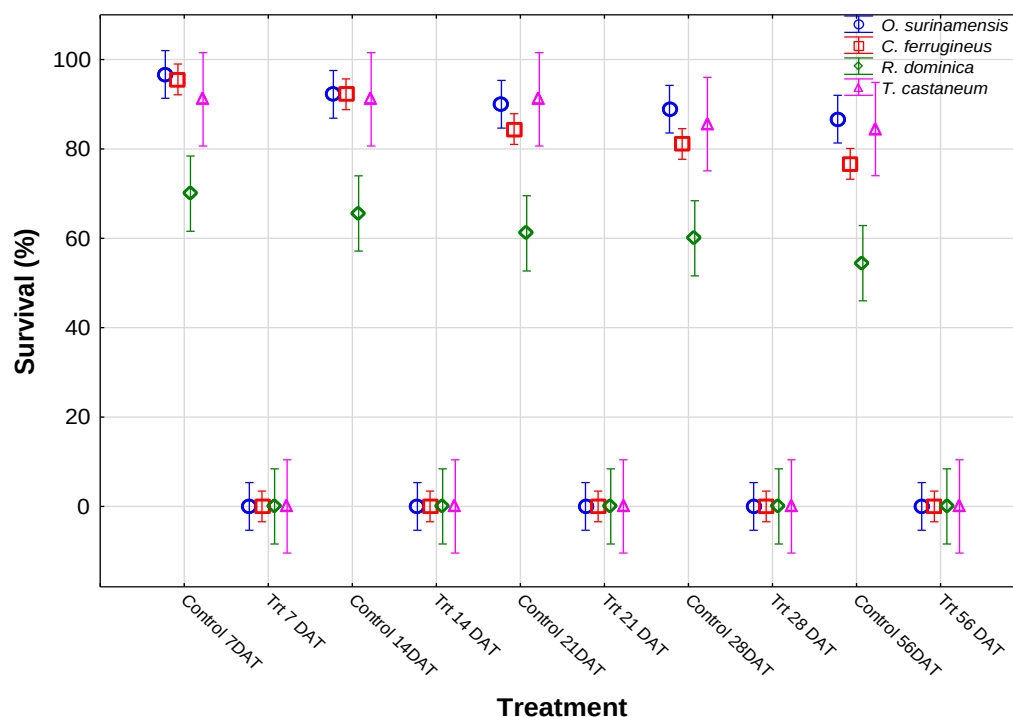


Figure 12. Analysis of Variance (ANOVA) on the survival of *O. surinamensis*, *C. ferrugineus*, *T. castaneum* and *R. dominica* post exposure to -15.84 °C for 14 days. Survival data was recorded at 7, 14, 21 and 56 days after treatment (DAT). Means with the same letters are not significantly different from each other.

My results are consistent with the notion that low temperature lethal thresholds are not static and corroborate with Fields et al., (2012) on *C. ferrugineus* SCPs and to a lesser degree *T. castaneum*. However, the rest have SCPs lower than those suggested by Fields et al., (2012) but not Mullen & Arbogast, (1979) or Flinn et al., (2015) who suggested -12 to 4 °C and -10 °C respectively for *T. castaneum*. This variation maybe attributed to differences in strains used (Fields, 1998), insect adaptations due to ‘natural’ acclimations occurring in storage facilities (Smith, 1970; David et al., 1977; Fields et al., 1998; Angelović et al., 2018) or other factors such as perturbations of bulk water into unfreezable pools (reduction of metabolically active water), or production of cryoprotective compounds, membrane remodeling, and the inhibition of apoptosis (Baust & Rojas, 1985). These results provide a reference benchmark of ‘working’ lower lethal thresholds (and duration) particularly for low-scale disinfestation of seeds and grain e.g., for laboratory experiments where these species are targeted.

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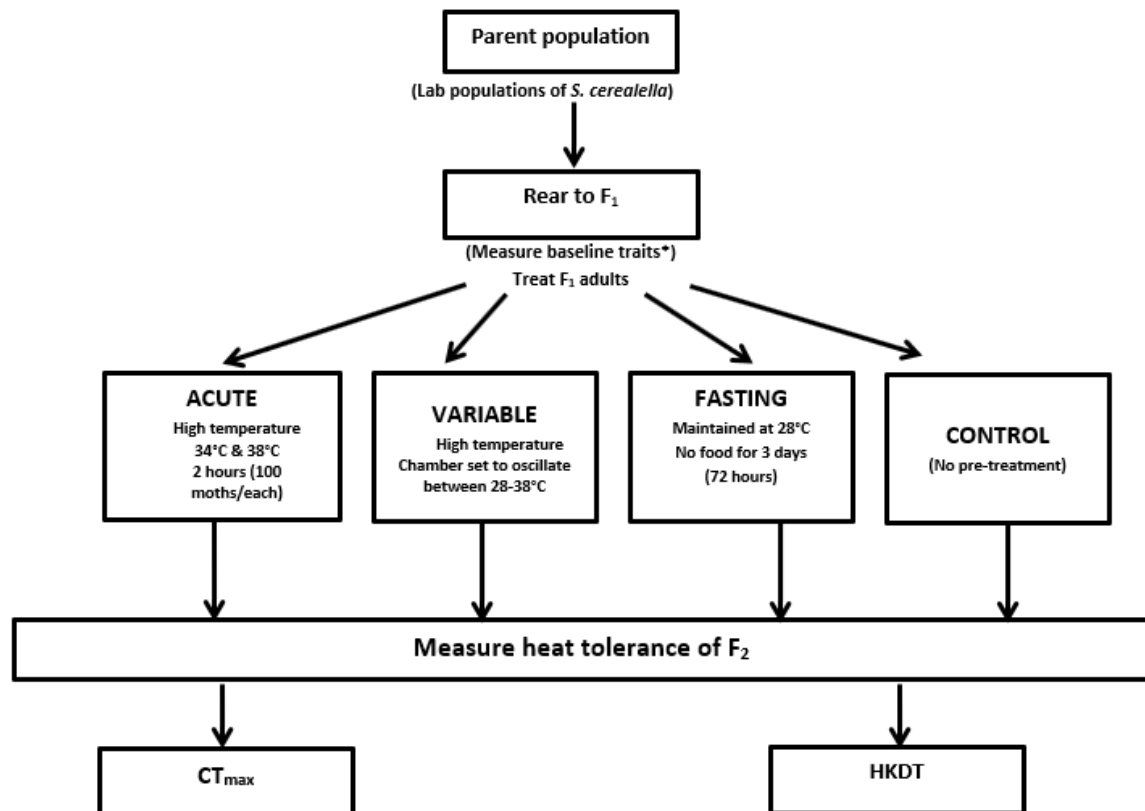
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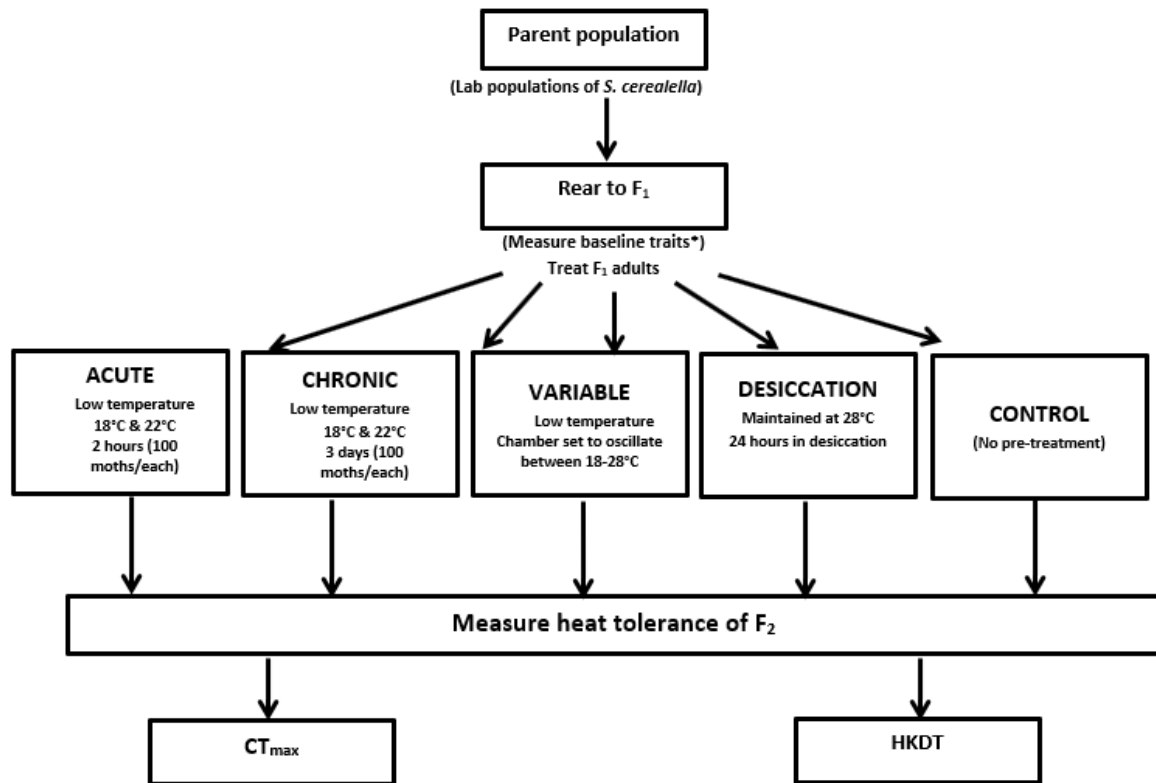
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Appendix 3: Schematic diagram showing temperature acclimation of *S. cerealella* F₂ generation to acute (2 hr) and variable temperatures as well as fasting/starvation stress. Variable temperature oscillated between 28–38 °C at a ramping rate of 0.25 °C/ min. One complete cycle took 144 minutes making a total of 10 cycles (1440 minutes) in 24hrs. Relative humidity (RH) was set at 65% with 24D photoperiods.



Appendix 4: Schematic representation of treatments showing temperature acclimation of *S. cerealella* F₂ generation to acute (2 hr), chronic (72 hr), and variable temperatures as well as desiccation stress. Variable temperature oscillated between 22–18 °C at a ramping rate of 0.25 °C/min. One complete cycle took 144 minutes making a total of 10 cycles (1440 minutes) in 24hrs. Relative humidity (RH) was set at 65% with 24D photoperiods.