

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

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Abstract

Transgenerational plasticity (TGP) is a physiological mechanism where environmental experiences of parents affect phenotypes of the offspring, and potentially facilitates organisms' survival in stressful environments. The Angoumois grain moth, *Sitotroga cerealella* (Olivier), is a dominant primary grain pest in Africa, particularly affecting sorghum and maize staples. Despite its widely known economic significance, little is known on its thermal responses, particularly how acclimation in one generation impacts fitness in successive generations. We tested how heat (acute and dynamic) as well as fasting acclimation in one generation affects physiological tolerance (i.e., critical thermal maxima [CT_{max}] and heat knock down time [HKDT]) using laboratory-reared *S. cerealella* populations. Specifically, we assessed whether pre-exposure to heat or fasting stress confers fitness costs or benefits in subsequent generations (i.e., through TGP). Results showed acclimation had significant effects in interaction with temperature ramping rates on CT_{max} . Moderate acute heat (34°C) and dynamic temperature (28-38°C) acclimations reduced F₁ CT_{max} and HKDT, particularly at low ramping rates (for CT_{max}). We did not find fasting acclimation to significantly affect CT_{max} , indicating a lack of evidence for cross tolerance and/or susceptibility. For HKDT, all acclimation treatments (including fasting) showed significantly lower HKDT compared to controls, and mostly so, for the 38°C and dynamic acclimation (28-38°C) treatments, indicating a transgenerational fitness cost of acclimation. This is the first report of the costs of transgenerational plasticity in a stored product pest of economic significance and has implications in designing heat-based phytosanitary grain treatments.

Key words

critical thermal maxima; epigenetic inheritance; heat knockdown time; life history; ramping rate; postharvest pests; *Sitotroga cerealella*

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 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 within generation effects (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 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 in Africa (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 (Angelovič *et al.*, 2018). 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, we 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. We 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, we 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.

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 Bugdom 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 the optimal rearing conditions (28 ± 1°C, 65 ± 10% RH, 24D photoperiod) in the Memmert climate chamber until the F₂ generation adults emerged. Moths that remained at optimal rearing conditions were used as controls.

Fasting acclimation treatments

For fasting acclimation, 100 F₁ moths were placed in 240 cm³ 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 ± 1°C, 65 ± 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 F₂ generation. The F₂ ±2 days old, unsexed moths were used for the TGP experiments.

Variable acclimation treatments (dynamic acclimation)

For the variable temperature acclimation experiment, 300 moths were placed in Bugdorm cages (240 cm³) 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°C/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.

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 ($0.06^\circ\text{C}/\text{min}$, $0.12^\circ\text{C}/\text{min}$, $0.25^\circ\text{C}/\text{min}$ and $0.5^\circ\text{C}/\text{min}$) until CT_{max} was recorded for all 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). Ten adults were placed in $15\ \mu\text{l}$ 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).

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. 1**). For the control and fasted groups, ramping rates 0.5°C/min had a significantly higher CT_{max} than the lower rates (0.06, 0.12 and 0.25°C/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°C/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°C/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°C/min was significantly lowest (all $p < 0.001$), while 0.25°C/min was significantly greater than 0.12 and 0.5 (both $p < 0.05$), and 0.12 and 0.5°C/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. 2**). 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°C/min ramping rate was significantly greater than the other rates (all $p < 0.001$), whereas 0.25 was significantly greater than 0.06 and 0.12°C/min (both $p < 0.05$), which in turn did not significantly differ ($p > 0.05$).

Considering HKDT, acclimation treatments caused significant differences ($F_{(4,145)} = 22.744$, $p < 0.001$) (**Fig. 3**). 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

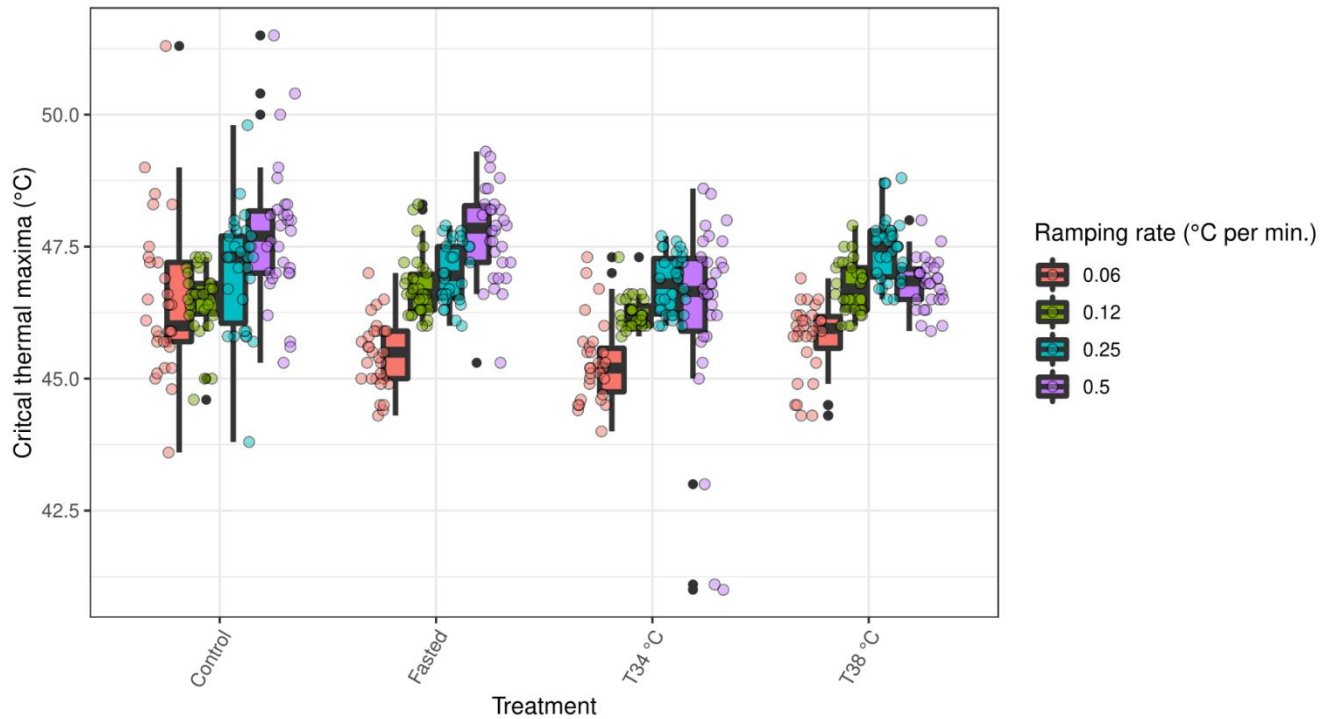


Figure 1. Boxplots showing CT_{max} across treatment groups and ramping rates following acute acclimation. Acute acclimation included 72 hours of food resource deprivation (fasted) and 2-hour exposure to sublethal high temperature (T34 °C and T38 °C). In the boxplots, the horizontal bars display the 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.

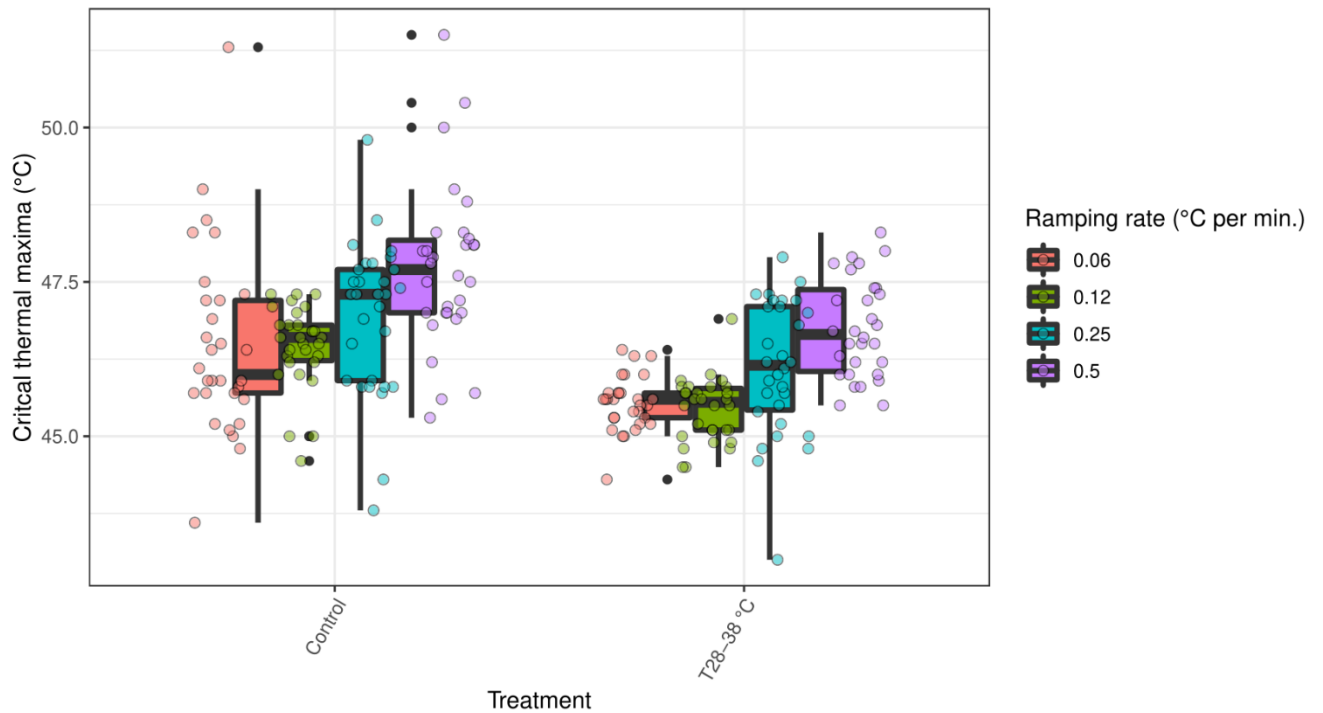


Figure 2. Boxplots showing CT_{max} across treatment groups and ramping rates after variable temperature acclimation. The dynamic temperature acclimation was defined as diel variation between 28-38 °C at 0.5 °C per min. for 72 hours. In the boxplots, the horizontal bars display the median, the box gives 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.

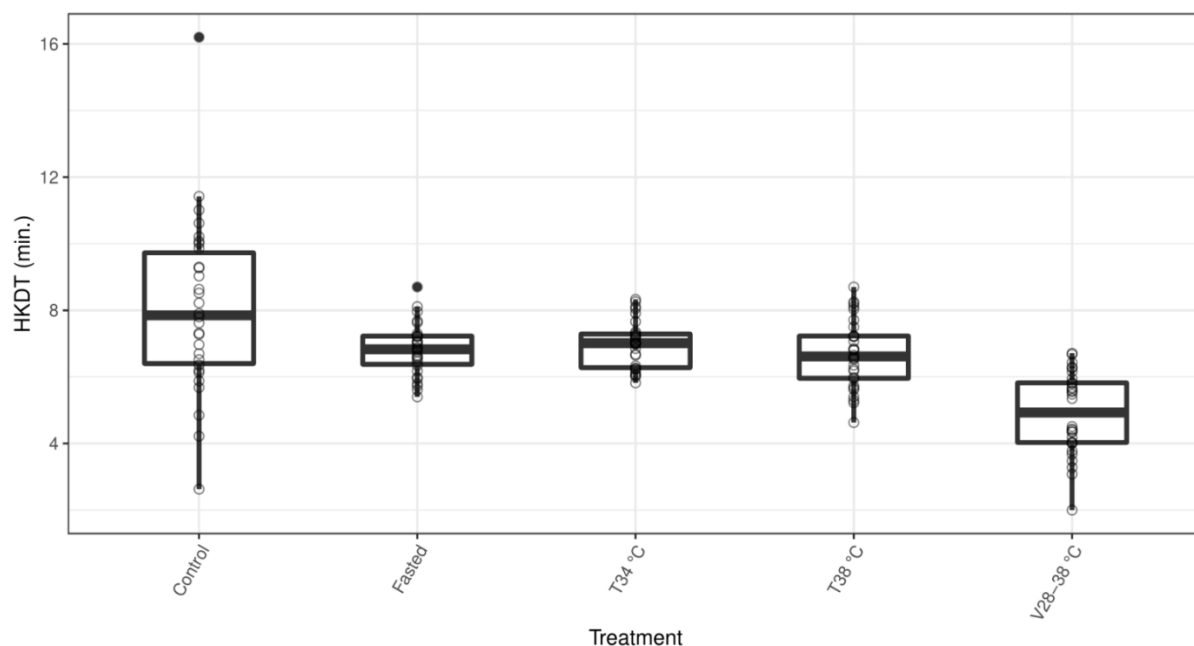


Figure 3. Boxplots showing heat knockdown time (HKDT) across treatment groups. Treatment groups were deprivation of food for 72 hours (fasted), acute sub-lethal temperature pre-treatments (T34 and T38 °C), variable temperature fluctuations around 28-38 °C (V28-38°C) and a control (28 °C). In the boxplots, 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.

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).

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. Our results showed firstly that high temperature acclimation had significant effects on heat tolerance of F₂ offspring. Second, we 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). Our 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 (Liefing & 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. Our results are contrary to the notion that within-generation beneficial acclimation is near ubiquitous in insects (Angiletta 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, we speculate that we may 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 our 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, our 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.

Our 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 our 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 that 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). Our 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. Our 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 Machezano *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 F₂ offspring, (ii) higher ramping rates (0.5°C/min) had beneficial effects on heat tolerance (higher CT_{max}) of F₂ offspring while lower ramping rates (0.06°C/min) had an opposite effect,

and (iii) both parental fasting and heat acclimation were maladaptive for F₂ 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.

Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Submission declaration and conflict of interest statement

This work has not been previously published. Authors declare no conflict of interests.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

All authors declare no conflict of interests.

Funding

PM, HM, and CN acknowledge funding from the Botswana International University of Science and Technology (BIUST). RC acknowledges funding from the Alexander von Humboldt Foundation and Leverhulme Trust (ECF-2021-001) fellowships.

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Acknowledgements

We acknowledge the Botswana International University of Science and Technology for financially supporting this project as well as the Botswana Ministry of Agriculture extension officers and respective farmers who assisted in the sourcing of insects used in the project. We are also grateful for the support and advice provided by our colleagues that improved the research.

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