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Trait-dependent plasticity erodes rapidly with repeated intergenerational acclimation in an invasive agricultural pest

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Abstract

Climate change is associated with increased mean temperatures and amplitudes manifesting both acutely and chronically, triggering organism stress responses that confer fitness costs and/or benefits. The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is an invasive postharvest agricultural pest. While host shift is its well-known potential invasive mechanism, how repeated intergenerational stress environments may influence offspring phenotypes is largely unknown. We thus evaluated physiological and ecological performance of LGB following repeated intergenerational acute heat acclimation to insinuate its likely responses to projected increased bouts of heat stress associated with climate change. Parental colonies were acutely heat-acclimated separately at 35°C and 38°C; 80% RH for 2 h in climate chambers and released onto sterilized maize grain at optimal conditions (32°C, 80% RH). The F_1 progenies were, respectively, acclimated at the same conditions and incubated to F_2 generation. We then evaluated physiological and ecological performance under optimal conditions across parental, F_1 and F_2 generations. Our results showed that plasticity was highly trait dependent, and that acclimation did not affect F_1 and F_2 critical thermal maxima, but did improve critical thermal minima. However, while acclimation improved heat knockdown time at F_1, repeated acclimation significantly reduced heat knockdown times at F_2, suggesting plasticity erosion with generational repeated acclimations. Acute acclimation negatively affected ecological performance of F_1 generations although this was restored with repeated acclimation in F_2 populations. Our results suggest that the LGB may inflict more economic damage with repeated heat stress due to generational adaptation to temperature stress. The results contribute to knowledge on pest forecasting modelling under changing climates and provides a framework for phytosanitary adjustments in heat treatment protocols for international grain trade.

KEYWORDS
climate change, ecological performance, fitness costs, heat treatment, intergenerational thermal plasticity, physiological performance, *Prostephanus truncatus*

INTRODUCTION

Temperature is an important environmental factor that primarily affects the ecology of ectothermic organisms (Dillon et al., 2010). While climate is generally warming (Wong, 2023), fine-scale daily and seasonal fluctuations are concomitantly increasing and becoming more frequent and prolonged (Davis-Reddy & Vincent, 2017; Smith & Lancaster, 2020; Wong, 2023). These temperature fluctuations expose ectothermic organisms to constantly changing levels of thermal stress (IPCC, 2021; Sørensen et al., 2019; Ventura et al., 2023), which may inflict fitness costs (Heidi & Sorte, 2022; Klockmann et al., 2017; Mpofu et al., 2022), but seldom benefits (Roeder et al., 2021). Projections suggest that high and extreme temperatures are expected in tropical climates, for example, in sub-Saharan Africa (SSA; Davis-Reddy & Vincent, 2017), where invasions by polyphagous agricultural and forest insect pests have increased food losses and burdened ecosystem services (Singano et al., 2020; Stathers et al., 2013). Interactions between climatic stressors and biological invasions could alter ecological and socio-economic impacts of insect pest species (Mlambo et al., 2024), leading to possible development of environmental stress adaption mechanisms to maintain ecological function. Therefore, ability to shift phenotypes in the short to long timescales when exposed to stressful environments may shape impacts and ecosystem function in the face of environmental change (Drown et al., 2021). This adaptation creates resilience in insect pests in a changing environment scenario where most recommended pest management methods are increasingly becoming climate strained (Andrew & Hill, 2017; Chidawanyika et al., 2012; Ma, Zhang, et al., 2021; Muatinte & Van Den Berg, 2019).

Heat stress responses in arthropods are mediated by behavioural microhabitat selection (Pincebourde et al., 2016), genetic adaptation and physiological changes (e.g., through phenotypic plasticity; Sgrò et al., 2016; Tarusikirwa et al., 2020; Walzer et al., 2020). While genetic adaptation is important for long-term survival, behavioural changes and phenotypic plasticity are important for short-term responses to heat stress that present increased chances of surviving extremely high-temperature environments (Horowitz, 2001; Malmendal et al., 2006). Some stress responses are rapidly triggered within-generation in minutes to hours or days (i.e., rapid and long-term acclimation), while some responses are gradual, extending and/or manifesting in successive generations (transgenerational plasticity [TGP]) (Cavieres et al., 2020; Kristensen et al., 2018; Lann et al., 2021). Thermal tolerance adaptation, however, often results in fitness costs (Driessen et al., 2011; Segaiso et al., 2022) and trade-offs in biological performance of arthropod species (Cavieres et al., 2020; Dixon et al., 2009). Fitness costs/benefits manifest either through plasticity and/or phenotypic costs/benefits (Driessen et al., 2011).

Plasticity costs comprise of all investment costs of eliciting plastic responses compared to a fixed phenotype. For example, costs of acquiring information regarding the changing thermal conditions and maintaining the physiological mechanism for plasticity (Smith & Lancaster, 2020). However, phenotypic costs encompass investments made in terms of energy and resource expenditure to produce an inducible phenotype and is of significance especially in the expression of reversible traits such as body size (Driessen et al., 2011). Phenotypic adaptation is a recurrent process due to seasonal thermal fluctuations, and hence the costs of producing an induced phenotype are paid for repeatedly throughout the life cycle of ectotherms (Driessen et al., 2011). Despite the understanding that thermal acclimation induces both adaptation and fitness costs, to our knowledge, little is known about (i) the effects of acclimation, either single or repeated, on the direction of heat tolerance traits; and (ii) the subsequent effects on ecological performance of individual insects, particularly commodity/plant damage in agricultural pests, which thus, require species-specific data for accurate estimations (Cavieres et al., 2020; Donelson et al., 2016).

Stored grain insect pests experience temperature rises in storage environments due to the enclosed nature of grain facilities as well as grain cell respiration, especially at high grain moisture content. This constant exposure further induces inevitable acclimation to heat stress (Panigrahi et al., 2020; Wang et al., 2020). The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera; Bostrichidae) is a devastating pest of stored maize grain and dried cassava roots originally from Central America and spread into Africa through grain trade as first reported in Tanzania in 1970 (Hodges et al., 1983). Maize grain damage as high as 100% and weight losses as high as 50% have been reportedly caused by *P. truncatus* as the range of synthetic pesticides commonly used by farmers are not effective against the pest (Cugala et al., 2007; Mlambo et al., 2017; Mutambuki et al., 2019); particularly those based on pyrethroids and organophosphates (Machekano et al., 2017). Full details on the current global pest status of *P. truncatus* and its biological traits are provided in Quellhorst et al. (2021). As an ectotherm, *P. truncatus* relies on thermal tolerance mechanisms like acclimation capacity (Mutamiswa et al., 2021) as well as heat-shock responses to survive heat stress (Harvey et al., 2023; Jian, 2019; Zhu et al., 2022). However, the effects of heat stress tolerance and/or plasticity on biological performance of *P. truncatus* are unknown.

Here we thus demonstrate the effects of single and repeated acute heat stress on the thermal fitness and ecological performance of *P. truncatus*, an invasive pest of significant biosecurity threat to stored maize grain and dried cassava roots as well as alternative hosts, for example, forest timber products (Hodges et al., 1983; Muatinte & van Den Berg, 2019). We chose *P. truncatus* because of its high pest status and economic significance, invasive nature (Arthur et al., 2019; Quellhorst et al., 2021) and high heat tolerance (Fields et al., 1992; Machekano et al., 2020; Mahroof et al., 2005), coupled with rapid responses to acclimation (Mutamiswa et al., 2021). Critical thermal limits to activity (critical thermal maxima [CT_{max}], critical thermal minima [CT_{min}]), heat knockdown time (HKDT) and upper lethal temperature (ULT) limits are standard metrics used to measure insect physiological thermal responses (Terblanche et al., 2011) and are reasonable proxies for estimating species ecological niches (Chown & Nicolson, 2004). These traits (CT_{max}, CT_{min}, HKDT and ULTs) are important measures of insect thermal tolerance and are measured using ecologically relevant static and/or dynamic standard protocols synonymous with pest experiences under natural environments (Gunderson & Stillman, 2015).

We thus aimed to determine the effect of single and repeated acute heat acclimation on the intergenerational responses of *P. truncatus* in terms of physiological and ecological performance. Such investigation can form the basis for managing the pest, as it provides quantitative information on the direction and magnitude of thermal tolerance traits, fitness and/or benefits thereof and the likely impacts of resultant ecological performance from repeated stressful environmental exposure associated with climate change. Specifically, we measured physiological (CT_{max}, CT_{min}, HKDT and ULTs) and ecological traits (feeding rates, fecundity and body weight) following single (at F_1 generation) and repeated (at F_2 generation) acute heat acclimation. We hypothesized that acute heat acclimation would improve these physiological and ecological traits in *P. truncatus* at both F_1 (with single acclimation) and F_2 (repeated acclimation).

MATERIALS AND METHODS

Insect rearing

A colony of wild *P. truncatus* was established through aggregated pheromone trapping from surrounding forests in Gaborone, Botswana (24°35′01″ S; 25°56′49″ E). An aggregation pheromone (active ingredients: 1-Methylethyl (E)-2-pentenoate and 1-Methylethyl (E, E)-2,4-dimethyl-2,4-heptadienoate) was procured from NovAgrica (Hellas, South Africa) through the Ministry of Agricultural Development and Food Security in Gaborone, Botswana. This formed the parent culture, which was reared in 1 L Consol jars with gauzed lids on cobbed yellow maize (SeedCo 608 variety, SeedCo Group, Gaborone, Botswana) at 32 ± 1°C and 80 ± 5% relative humidity (RH; the optimum conditions for *P. truncatus*; Shires, 1980; Subramanyam & Hagstrum, 1991; Quellhorst et al., 2021) in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Schwabach, Germany) under a LD 12 : 12 h photocycle. Maize grain was sterilized by freezing at −18°C for 2 weeks and then equilibrated by holding at 32°C, 80% RH for 7 days before being used for the trials (Hodges & Dobson, 1998; Mutamiswa et al., 2021). Grain moisture content was measured using a digital Unimeter (Agri-Enviro Solutions) (Centurion, South Africa) and confirmed at 12.2% after equilibration. To get a uniformly aged batch of insects, adults were removed from the grain after 21 days and the first laboratory generation of uniformly aged adult insects that emerged thereafter was defined as the F_1. This F_1 generation was then exposed to acute heat acclimation treatments as previously described in Section 2.3 for subsequent intergenerational plasticity experiments.

Bioassays

We tested intergenerational plasticity across two generations of adult stages (3–7 days old) of a laboratory-reared colony of *P. truncatus*. Specifically, we determined CT_{max}, CT_{min}, HKDT and ULTs (specific methods for each metric are detailed in Section 2.2.1) for successive F_1 and F_2 generations following acute heat acclimations (for 2 h at 35°C and 38°C; 80% RH). The chosen acclimation temperatures are deemed ecologically relevant and were simulated from conditions experienced by the insects in the natural collection sites (Nkemelang et al., 2018; Walzer et al., 2020). Furthermore, these temperature × time acclimation conditions are sufficient to elicit acclimation conditions in insects (Chown & Nicolson, 2004; Cole et al., 2023; Enriquez & Colinet, 2019; Weldon et al., 2011). We also assessed biological performance of *P. truncatus* following acclimation using adult individual body weight, fecundity, maize grain damage, grain weight loss and amount of dust produced due to *P. truncatus* feeding activity. For comparison with test insects, an adult *P. truncatus* group maintained at 32 ± 1°C; 80 ± 5% RH served as a positive control.

Mixed sex adults in 1 L Consol jars were exposed to acute heat acclimation at static 35°C and 38°C separately for 2 h in Memmert climate chambers. After acclimation, the insects were returned to the climate chamber at 32°C and 80% RH in labelled 1 L Consol jars and were provided with sterilized yellow maize cobs and left to oviposit under LD 12 : 12 h photocycle for 21 days. Adult insects were then removed from each jar to enable emergence of the F_1 generation following methods by Machekano et al. (2020) and Mutamiswa et al. (2021). Adult *P. truncatus* from each group (F_1) was then assayed for physiological (heat stress tolerance) and ecological performance (fecundity, adult body weights, grain damage, grain weight loss and insect feeding dust produced). A sub-culture was retained from the F_1 and re-acclimated at the same conditions to get the next filial generation (F_2) (Walzer et al., 2020; Figure 1). Although the acclimation temperatures differed for the two sub-cultured treatment groups (35°C and 38°C), the rearing conditions for oviposition were the same (32°C and 80% RH). The acclimation temperatures of each F_1 sub-culture matched the F_2 acclimation temperatures (Figure 1). Five treatments, namely, Parent_32°C (T1 the control), F_1_35°C (T2), F_1_38°C (T3), F_2_35°C (T4) and F_2_38°C (T5), were thus evaluated (Figure 1).

Thermal tolerance metrics

Critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min})

Two CTL (CT_{max} and CT_{min}) programmes were run using 0.25°C/min and 0.5°C/min ramping rates. These ramping rates were previously reported to resemble more ecologically relevant temperature changes and hence enable detection of variations in thermal limits to activity and survival of insects (Terblanche & Mitchell, 2017). Most studies on CTLs have used the same ramping rate of 0.25°C/min and 0.5°C/min (Chown & Nicolson, 2004; Mutamiswa et al., 2021) while others, for example, Mpofu et al. (2022) used even much lower rates of 0.06°C/min, all with mixed results. Our ramping rates thus allowed for CTLs comparison with much of the available literature (see discussion in, e.g., Chown & Nicolson, 2004). Ten adult insects were each placed in individual transparent organ pipes of a double-jacketed chamber connected to a programmable water bath (refrigerated and heated circulating bath with a temperature range of −25°C to +200°C; LAUDA

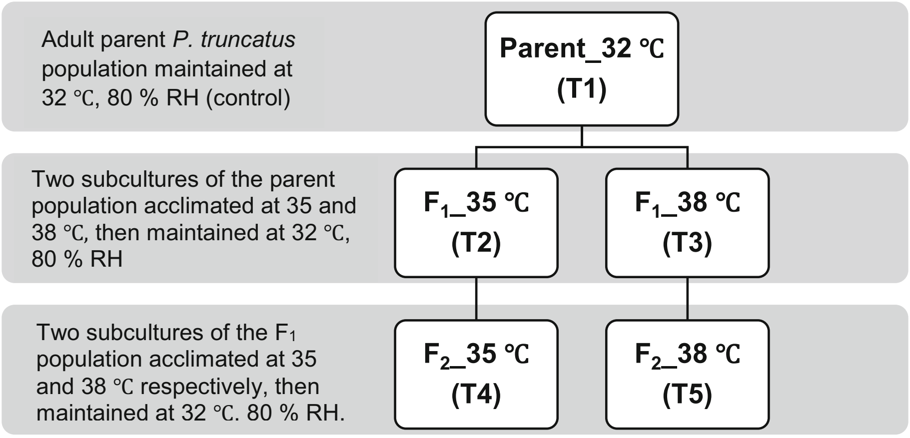

FIGURE 1 Acute heat acclimation protocol (2 h exposure at the respective temperatures) and illustration of the treatments and rearing process from mixed populat adult parent *Prostephanus truncatus* population to F_2 generation. T1-T5 = Treatments 1–5.

Ecogold® RE 2025, Lauda-Königshofen, Germany) with a 1:1 water to propylene glycol solution running through the chamber to control the temperature (Chidawanyika & Terblanche, 2011). These organ pipes are standard instruments used in physiological ecology studies to quickly assess critical thermal limits in small animals (especially ectotherms) but have also been used for small mammals (Chown & Nicolson, 2004).

From a setpoint temperature of 32°C, which represent optimum conditions, temperature was ramped either up (CT_{max}) or down (CT_{min}) at the different ramping rates (0.25 or 0.5°C/min) until CT_{max} and CT_{min} were recorded, respectively. CT_{max} was defined as the maximum temperature at which insects lost coordinated muscle function to self-right and ability to respond to mild stimuli from a thermally inert object (Nyamukondiwa & Terblanche, 2009; Tarusikirwa et al., 2022). On the other hand, CT_{min} was recorded as the minimum exposure temperature at which insects lost coordinated muscle function (Terblanche et al., 2007). Temperature readings were made from a thermocouple (type K 36SWG) connected to a digital thermometer (Fluke 54 series IIB) inserted in the control organ pipe of the chamber. The water bath had 11 organ pipes, thus allowing 10 insects and one control to be assayed at a run time. The procedure was therefore repeated three times to yield 30 replicates ($n = 30$) for each ramping rate, generation time and acclimation temperature.

Heat knockdown time

HKDT was assayed for adult *P. truncatus* by acutely exposing insects to 50°C using the Memmert climate chamber with a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, Hangzhou, China) connected to a computer for recording activity. This acute heat knockdown temperature (50°C) was used following the species basal heat results, that is, CT_{max} data from Machekano et al. (2020). Furthermore, findings from Fields et al. (1992) indicate that temperatures of 50°C or beyond cause 100% mortality in stored product insect pests in less than an hour. Temperature of the climate chamber was allowed to stabilize at 50°C for 10 min before adult *P. truncatus* was placed each in 1.5 mL Eppendorf tubes in the climate chamber. HKDT, captured from the video recording system, was recorded as the time in minutes at which an insect lost activity following exposure to acute heat stress (Machekano et al., 2020; Mutamiswa et al., 2018, 2021). Thirty adult beetles were assayed per generation and acclimation temperature to obtain 30 replicates ($n = 30$).

ULTs survival

Prostephanus truncatus adult insects of mixed sexes were subjected to lethal temperature assays by direct plunge method (Chidawanyika & Terblanche, 2011) in a water bath filled with a 1:1 mixture of water and propylene glycol (Machekano et al., 2020; Terblanche et al., 2008). Ten beetles were loaded in each of three 60 mL plastic vials with perforated lids to get a total of three replicates ($n = 30$). A wet cotton ball was placed in each vial to maintain RH and avoid confounding mortality related to desiccation (Stotter & Terblanche, 2009). The three vials were then placed in a water-tight zip lock bag and plunged into a water bath for each temperature × time treatment. The insects were exposed to ULTs of 38°C, 42°C, 46°C, 50°C and 52°C for 0.5, 1, 2, 3 and 4 h, at varied temperature–time combinations (see Supplementary Figure S1) until a range of insect mortality from 0% to 100% was recorded. The temperatures (38°C and 42°C) partly represent conditions experienced in the insect collection sites (Byakatonda et al., 2018; Mguni, 2020) and are above the optimum temperatures for development of storage insects (Agrafioti et al., 2019; Fields, 1992). After exposure, the insects were transferred to sterilized maize grain stored at fixed conditions of 32°C, 80% RH, that is, optimal conditions for *P. truncatus* for recovery. After 24 h, survival was scored on test adults for each temperature–time treatment. Survival was recorded as a coordinated response to mild stimuli from prodding (with a camel hairbrush) and/or normal insect behaviour such as feeding, self-righting, walking or flying post-treatment (Nyamukondiwa & Terblanche, 2009; Stotter & Terblanche, 2009).

Ecological performance

Body weight, fecundity, maize grain damage, grain weight loss and weight of dust generated due to insect feeding were used to assess ecological intergenerational performance of *P. truncatus* following heat acclimation. Twenty sexed beetles (10 male: 10 female) were placed in 500 mL rearing jars with sterilized maize grain (500 kernels) and left for 21 days to mate and oviposit under LD 12 : 12 h photocycle in a Memmert climate chamber at 32°C, 80% RH. The beetles were sexed to ensure chances of mating in each treatment. However, the results (fecundity and adult individual weight) were not sex-aggregated. Each treatment was replicated three times ($n = 3$). Sex determination was done by examining clypeal tubercles of live beetles under a light microscope (Shires & McCarthy, 1976). The clypeal tubercles are more pronounced and further apart in females than males (Shires & McCarthy, 1976). After 21 days, adult insects were removed from the jars. The total number of emerged progeny recorded after 35 days following the first emergence was recorded as fecundity (Gvozdenac et al., 2018; Masasa et al., 2013). Emerging insects were weighed to 1 mg at 5-day intervals using a precision balance (PGW 453e, Adam Equipment™, Johannesburg, South Africa). At the end of the experiment, that is, after 56 days, grain damage, grain weight loss and weight of insect feeding dust were assessed. The count and weigh assessment method was used for grain damage and grain weight loss determination as follows:

$$\text{Grain damage} = \frac{Nd}{Nd + Nu} \times 100, \tag{1}$$

$$\text{Grain weight loss} = \frac{NdWu - NuWd}{((Nd + Nu) \times Wu)} \times 100, \tag{2}$$

where Nd = number of damaged grains, Nu = number of undamaged grains, Wu = weight of undamaged grains and Wd = weight of damaged grains (Boxall, 2002).

Data analyses

Normality tests were performed on all datasets using Kolmogorov–Smirnov and Lilliefors test. $\mathrm{CT_{max}}$, $\mathrm{CT_{min}}$ and HKDT data did not meet the assumptions of normality and were analysed using Kruskal–Wallis analysis of variance (ANOVA) and median test. All the other datasets except survival, having satisfied the normality tests ($p > 0.05$), were then analysed using one way ANOVA in Statistica 13 to determine if there were any significant differences among treatments (which were the basis of explanatory variables; Table 1). In the case of significant differences, post hoc analysis was done using Fisher's least significant difference (LSD) test to separate treatment means. A generalized linear model with a beta distribution was used to analyse survival rates as a function of two factors: exposure temperature and exposure time, as well as their interaction (Brooks et al., 2017), in R version 4.3.1 (R Core Team, 2023). Survival rates were transformed to remove extreme values (0 and 1) to meet the beta distribution assumptions:

TABLE 1 A summary of response and explanatory variables used in each data analysis model.

Model	Variables Explanatory variable(s)	Response variable(s)
Kruskal–Wallis test	Treatments (×5), ramping rates (×2) Treatments (×5)	$\mathrm{CT_{max}}$, $\mathrm{CT_{min}}$ HKDT
One way ANOVA	Treatments (×5)	HKDT, fecundity, grain damage, grain weight loss, insect feeding dust
Generalized linear model	Exposure temperature (×5), exposure time (×5)	Survival rate

$$s_t = (s_i(n-1) + 0.5)/n, \tag{3}$$

where s_i is the survival rate and n is the total sample size. Analysis of deviance was used to report the main effect coefficients with Type III sums of squares (Fox & Weisberg, 2019). Estimated marginal means were used to compute pairwise comparisons post hoc (Lenth, 2023).

RESULTS

Effects of acclimation on $\mathrm{CT_{max}}$ and $\mathrm{CT_{min}}$

Heat tolerance ($\mathrm{CT_{max}}$) in *P. truncatus* was not significantly influenced by single (at F_1) and repeated (at F_2) heat acclimations (Kruskal–Wallis test: $H_{(4)} = 4.881$; $p = 0.300$). In F_1, the slower ramping rate (0.25°C/min) caused a reduction in heat tolerance while the higher ramping rate (0.5°C/min) improved heat tolerance. The reverse was true in the F_2 generation, where the more gradual (slower) ramping rate improved $\mathrm{CT_{max}}$ and the rapid rate decreased $\mathrm{CT_{max}}$ resulting in convergence of $\mathrm{CT_{max}}$ ranges between 48°C and 52°C for both rates (Figure 2a). This, however, did not significantly improve heat tolerance of acclimation treatments when compared to the parent population.

For $\mathrm{CT_{min}}$, however, heat treatment significantly influenced tolerance (Kruskal–Wallis test: $H_{(4)} = 35.120$; $p < 0.001$). The trend was especially apparent for the slowest ramping rate compared to the higher rate. In the interaction, the 0.25°C/min ramping rate improved cold tolerance in F_1 but the 0.5°C/min rate did not. In the F_2 generation, cold tolerance significantly improved for both ramping rates to a range of 3°C–5°C (Figure 2b).

Effects of acclimation on HKDT

Acute heat acclimation significantly improved HKDT (Kruskal–Wallis test: $H_{(4)} = 104.141$; $p < 0.001$) in *P. truncatus* in the F_1 generation. However, these beneficial HKDT acclimation effects were quickly reversed and/or lost by repeated acclimation in the F_2 generation for

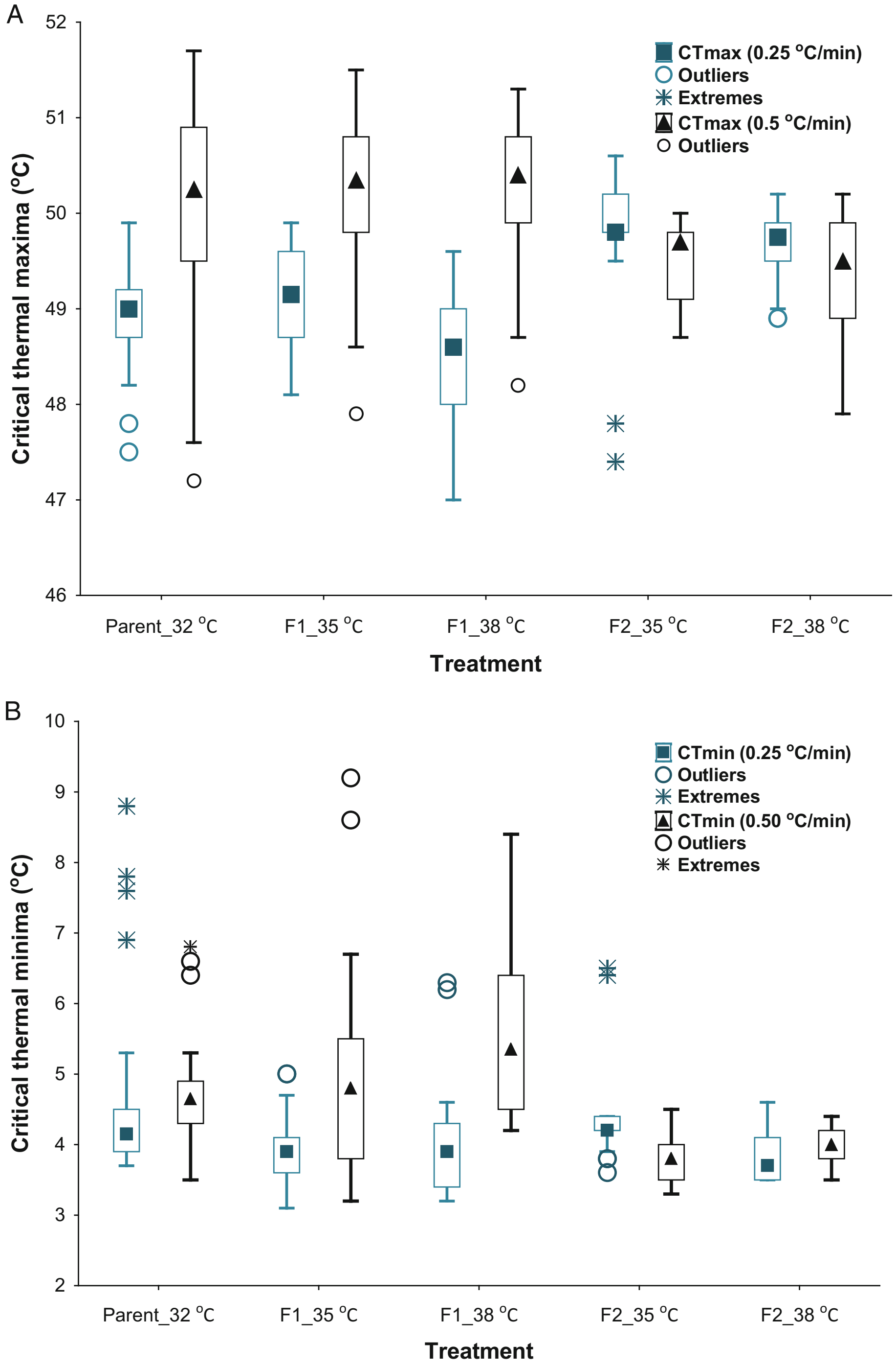

FIGURE 2 Box and whisker plots showing critical thermal limits for adult *Prostephanus truncatus* after acclimation ($n = 30$): (a) median critical thermal maxima (CT_{max}) for treatments across 0.25 and 0.5°C/min ramping rates and (b) median critical thermal minima (CT_{min}) values for treatments across 0.25 and 0.5°C/min ramping rates. Parent_32°C = *P. truncatus* parent control population (32°C, 80% RH); F_1_35°C = *P. truncatus* F_1 generation acclimated at 35°C for 2 h; F_1_38°C = *P. truncatus* F_1 generation acclimated at 38°C for 2 h; F_2_35°C = *P. truncatus* F_2 generation acclimated at 35°C for 2 h; F_2_38°C = *P. truncatus* F_2 generation acclimated at 38°C for 2 h. All treatments were maintained at 32°C, 80% RH after acclimation.

both acclimation temperatures (35°C and 38°C). HKDT significantly improved from a median of 10 min for the parent population to 11.5 minutes in the respective F_1 generations. In the F_2 generations, however, HKDT was significantly reduced to between 6 and 8 min, representing HKDT cost due to repeated acclimation (Figure 3).

Effect of ULTs on survival

Prostephanus truncatus survival rates were mediated by a significant interaction between temperature and exposure time ($\chi^2 = 97.531$, df = 16, $p < 0.001$). This was principally because there was no survival

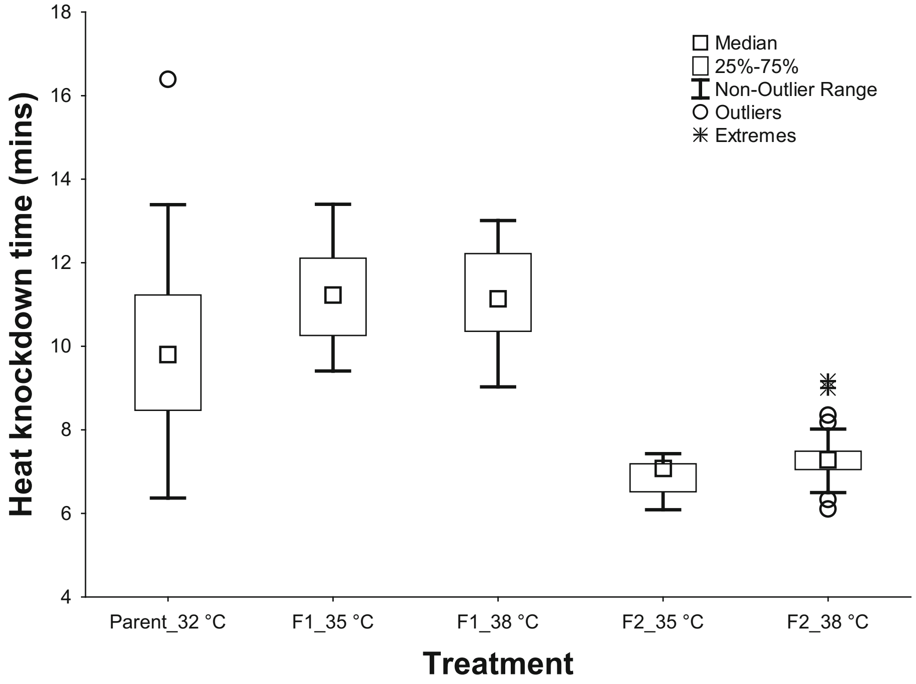

FIGURE 3 Box and whisker plots showing median heat knockdown time (in minutes at 50°C) of adult *Prostephanus truncatus* control group and acclimation treatments ($n = 30$). Parent_32°C = *P. truncatus* parent control population (32°C, 80% RH); F_1_35°C = *P. truncatus* F_1 generation acclimated at 35°C for 2 h; F_1_38°C = *P. truncatus* F_1 generation acclimated at 38°C for 2 h; F_2_35°C = *P. truncatus* F_2 generation acclimated at 35°C for 2 h; F_2_38°C = *P. truncatus* F_2 generation acclimated at 38°C for 2 h. All treatments were maintained at 32°C, 80% RH after acclimation.

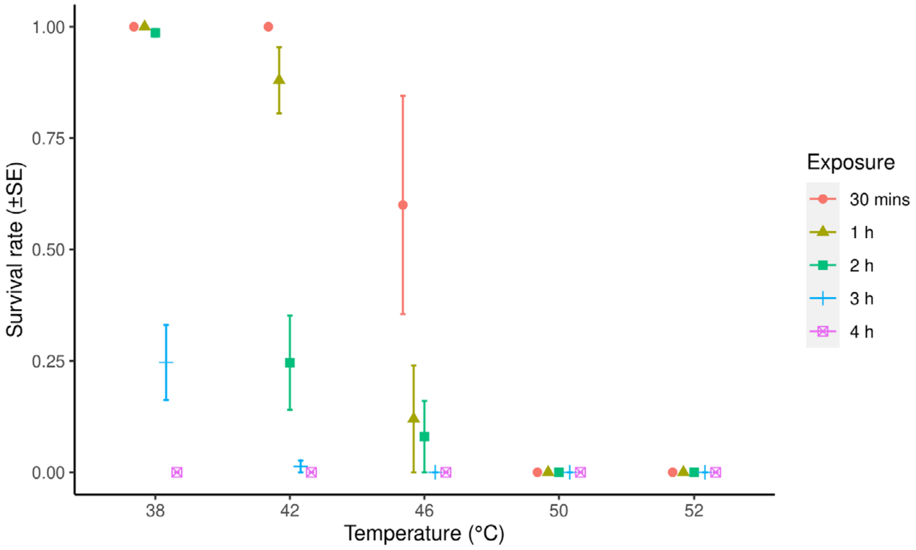

FIGURE 4 Survival rates recorded for 3–7 days old adult *Prostephanus truncatus* ($n = 3$). Treatment combinations were: (exposure temperatures; 38, 42, 46, 50, 52°C) × (Exposure durations; 30 min, 1, 2, 3, 4 h).

above 50°C, which dissolved any influence of exposure time, whereas below 50°C, exposure time always mediated survival. At 38°C, survival after 3 and 4 h was significantly lower than 0.5 and 1 h and 2 h exposures (all $p < 0.001$). On the other hand, at 42°C, survival at 2, 3 and 4 h was significantly lower than 0.5 and 1 h (all $p < 0.001$). At 46°C, survival at 1, 2, 3 and 4 h was significantly lower than 0.5 h (all $p < 0.001$). Therefore, as temperature increased, the threshold for significant survival differences among exposure times shortened, whereby shorter exposure times approached complete mortality (Figure 4).

Effects of acclimation on ecological performance of *P. truncatus*

Adult weights

Body weights of *P. truncatus* adults significantly improved ($F_{4,145} = 5.020$; $p = 0.001$) in the F_1 generation following acclimation at both 35°C and 38°C. With repeated acclimation, however, body weights of F_2 individuals significantly reduced for the 38°C acclimation treatment whereas the 35°C maintained higher adult body weights. Mean individual body weights of Parent_32°C were recorded as 2.3 ± 1 mg and this was significantly lower than F_1_35°C and F_1_38°C weights, which ranged from 2.52 to 2.70 ± 1 mg. In the F_2 generation, adult body weights of F_2_38°C decreased to 2.3 ± 1 mg whereas for F_2_35°C body weights were maintained at 2.7 ± 1 mg (Figure 5).

Fecundity, grain damage, grain weight loss and insect feeding dust

Generally, ecological performance of *P. truncatus* significantly decreased (all $p < 0.05$) in F_1 generation following a single heat acclimation and then improved for some traits in the F_2 generation following repeated acclimation (Table 2). Fecundity ($F_{4,10} = 40.42$; $p < 0.05$), grain damage ($F_{4,10} = 48.74$; $p < 0.05$), grain weight loss ($F_{4,10} = 6.310$; $p = 0.008$) and insect feeding dust ($F_{4,10} = 9.799$; $p < 0.05$) were all significantly lowered by single acclimation in the F_1 generation. Generational repeated acclimation resulted in improved F_2 ecological performance in *P. truncatus* for some traits relative to F_1 but not the control population. For example, F_2_38°C significantly increased fecundity (106. 67 ± 14.62 adults), grain damage (52.28 ± 2.87%) and grain weight loss (10.40 ± 1.97%) compared to F_1 populations and F_2_35°C. Thus, an interaction between repeated acclimation and the degree of acclimation temperature mediated ecological performance in F_2 treatments. For all parameters, parent control populations had the highest ecological performance; fecundity (194.33 ± 16.46 beetles), grain damage (61.30 ± 3.89%), grain weight loss (15.52 ± 1.00%) and insect feeding dust (9.50 ± 0.92%).

DISCUSSION

We aimed to determine the effect of single and repeated acute heat acclimation on the intergenerational responses of *P. truncatus* in terms of physiological and ecological performance. Firstly, we showed that the responses are non-linear, trait- and generation-dependant. For example, acute heat acclimation improved specific traits while it decreased or had no effect on other traits at both F_1 and F_2 levels. Second, we also showed trait-dependant erosion of beneficial as well as detrimental ecological effects following acclimation. For example, F_1 single acclimation significantly improved HKDT but these beneficial

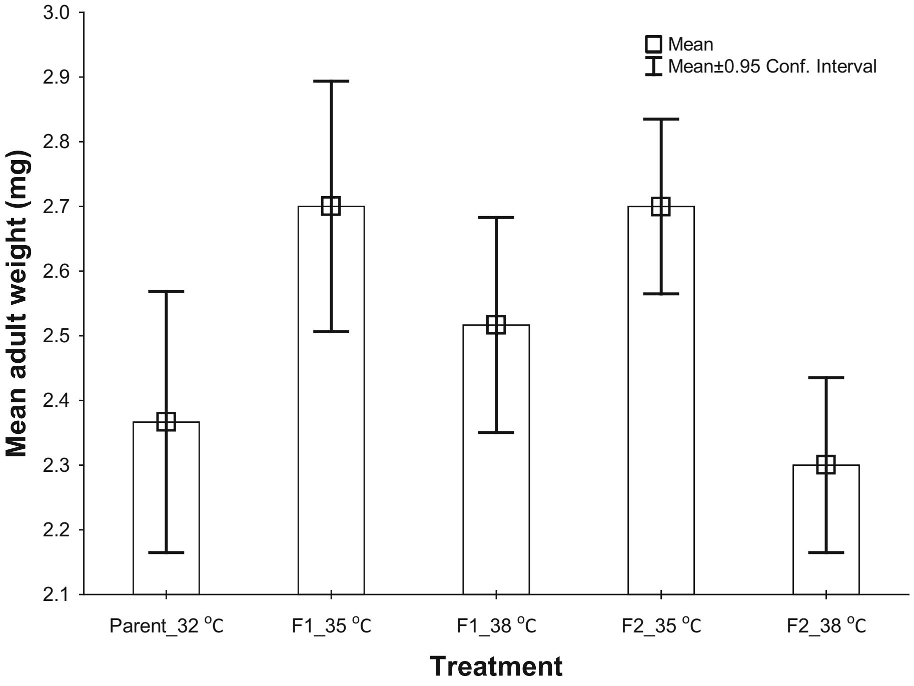

FIGURE 5 Effects of *Prostephanus truncatus* acclimation at 35°C and 38°C on adult weights recorded over two generations. The values presented are means ± SEM at 95% confidence interval ($n = 30$). Parent_32°C = *P. truncatus* parent control population (32°C, 80% RH); F_1_35°C = *P. truncatus* F_1 generation acclimated at 35°C for 2 h; F_1_38°C = *P. truncatus* F_1 generation acclimated at 38°C for 2 h; F_2_35°C = *P. truncatus* F_2 generation acclimated at 35°C for 2 h; F_2_38°C = *P. truncatus* F_2 generation acclimated at 38°C for 2 h. All treatments were maintained at 32°C, 80% RH after acclimation.

TABLE 2 Ecological performance of *Prostephanus truncatus* measured in terms of fecundity, insect feeding dust, grain damage and grain weight loss following acclimation at 35°C and 38°C recorded over a 56 days ($n = 30$).

Treatment	Fecundity	Grain damage (%)	Weight loss (%)	Insect feeding dust (%)
Parent_32°C	194.33 ± 16.46^{c}	61.30 ± 3.89^{c}	15.52 ± 1.00^{c}	9.50 ± 0.92^{b}
F_1_35°C	33.33 ± 3.53^{a}	23.60 ± 1.96^{a}	5.03 ± 1.62^{a}	4.18 ± 0.13^{a}
F_1_38°C	58.33 ± 2.60^{a}	27.52 ± 0.33^{a}	8.25 ± 1.73^{ab}	4.34 ± 0.22^{a}
F_2_35°C	58.67 ± 1.45^{a}	29.21 ± 1.47^{a}	8.25 ± 1.20^{ab}	5.20 ± 0.58^{a}
F_2_38°C	106.67 ± 14.62^{b}	52.28 ± 2.87^{b}	10.40 ± 1.97^{b}	4.38 ± 1.17^{a}
$F_{4,10}$	$p < 0.0001$	$p < 0.0001$	$p = 0.01$	$p < 0.01$

Note: Parent_32°C = *P. truncatus* parent control population (32°C, 80% RH); F_1_35°C = *P. truncatus* F_1 generation acclimated at 35°C for 2 h; F_1_38°C = *P. truncatus* F_1 generation acclimated at 38°C for 2 h; F_2_35°C = *P. truncatus* F_2 generation acclimated at 35°C for 2 h; F_2_38°C = *P. truncatus* F_2 generation acclimated at 38°C for 2 h. All treatments were maintained at 32°C, 80% RH after acclimation.

acclimation effects were eroded by repeated acclimation. On the other hand, ecological costs on fecundity, adult body weight and feeding rates (measured as grain damage) in F_1 were recovered in F_2. Third, ramping rates significantly influenced CT_{max} estimates. Single and repeated acclimations did not significantly affect CT_{max} values. However, ramping rate effects were significant in interaction between generations and temperatures. Slower ramping rate reduced CT_{max} estimates in F_1 generation although repeated acclimation in F_2 generation improved heat tolerance. Similarly, acute heat acclimation improved cold tolerance for the slower ramping rate in F_1 generation and also for the higher ramping rate in F_2 generation following repeated acclimation.

Critical thermal limits are measured during a gradual increase in body temperature and are ecologically important measures of insect thermal tolerance as they represent limits to activity for organisms as experienced with changes in climatic patterns in the natural environment (Gunderson & Stillman, 2015). Although acclimation is intended to improve thermal tolerance traits by broadening the thermoregulation temperatures (Horowitz, 2001), in the current study, single and repeated acclimations were trait-dependant, as some traits (e.g., CT_{max}) were negatively affected by acclimation and ramping rate. This resulted in loss of plasticity, which is consistent with observations by Brennan et al. (2020) who reported that plasticity is lost in the long term (F_2 generation in this case) as animals adapt to changing conditions. Furthermore, Gunderson and Stillman (2015) found that heat acclimation marginally improves heat tolerance because tropical ectotherms like *P. truncatus*, have a narrow thermal window due to their inherent high basal heat tolerance (Machekano et al., 2020). In contrast, Mutamiswa et al. (2021) reported improved heat tolerance in *P. truncatus* following acclimation within generation, but no tests were done for repeated generational acclimations. Ramping rates also had significant effects on CT_{max} and CT_{min} and this is in concurrence with findings on storage pests from other studies (Machekano et al., 2020; Mpofu et al., 2022).

Our results highlight that thermal tolerance in *P. truncatus* is highly influenced by interaction between temperature and ramping rates and the beetle is more susceptible to gradual changes in extreme temperatures (0.25°C/min) compared to rapid changes (0.5°C/min) in extreme temperatures, consistent with the notion that slower rates of stress results in more and cumulative heat stress damage (Jørgensen et al., 2021). Similar observations were noted by Machekano et al. (2020). CT_{max} ranged between 47°C and 52°C, CT_{min} (3°C–10°C), HKTD (5–14 min; rarely exceeding 15 min) and in ULTs *P. truncatus* survival was mediated by exposure duration with 0% survival achieved at 46°C for as little exposure as 3 h. These findings could be applied in grain heat treatment protocols for phytosanitary disinfestation. Previous studies have shown that heat acclimation improved cold tolerance in the fruit fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Bubliy & Loeschcke, 2005), consistent with the current findings. Single acclimation improved HKDT in the F_1 generation and this was lost in the F_2 generation for both acclimation temperature regimes. Improvement in HKDT results in F_1 following single acclimation is consistent with the beneficial acclimation hypothesis (Wilson & Franklin, 2002). However, repeated acclimation at F_2 may result in cumulative or additive stress, which may offset acclimation benefits (Jørgensen et al., 2021). A 100% survival rate was recorded at 38°C up to 2 h exposure. At that same exposure temperature, survival then declined to 25% at 3 h and 0% at 4 h exposure durations. Similar results were reported in Machekano et al. (2020) who then proposed thermal (physical) control of *P. truncatus* at lower ramping rates to moderate exposure temperatures × long exposure durations, that is, 45.5°C for 4 h in stored grain to save energy and reduce grain treatment costs. Lethal temperatures kill insects by mechanisms such as disruption of lipid structure in membranes, rate imbalances and desiccation. In addition, high temperatures cause a decrease in atmospheric RH and a depletion in grain moisture content (Fields et al., 1992) creating unfavourable conditions for insects.

The effects of acute heat acclimation on the ecological traits of *P. truncatus* also varied with acclimation temperature and with single F_1 versus F_2 repeated acclimation. Ecological performance generally declined in F_1 generation following a single acclimation and only improved in F_2_38°C in the F_2 generation. Heat acclimation significantly reduced the *P. truncatus* fecundity in F_1 generation, which then resulted in reduced grain damage, grain weight loss and insect feeding dust generated in the respective treatments. The F_2_38°C treatment recorded significantly higher fecundity, grain damage and grain weight losses compared to F_1_35°C, F_1_38°C and F_2_35°C, which points to a regain in ecological traits following repeated acclimation, leading to improved performance. Cavieres et al. (2020) demonstrated similar results in which a trade-off between thermal tolerance and fecundity was observed in a parental fly population, resulting in 'partial compensatory responses' in the offspring. This is because reproduction is the most heat sensitive life history trait in insects and has a very narrow thermal tolerance range (Ma, Ma, & Pincebourde, 2021). Besides negatively affecting fecundity, Huang et al. (2007) also found that heat hardening depresses adult feeding and this can partly explain the low grain damage and weight losses recorded for acclimation treatments in this study. Bubliy and Loeschcke (2005) postulated that ectotherms generally respond to extreme heat by lowering metabolism to conserve resources, further supporting the low ecological performance seen in the current study. Results of individual weight analysis are inclined to a similar trend, where F_2_38°C recorded weights similar to the parent_32°C which were significantly lower than F_1_35°C, F_1_38°C and F_2_35°C. This observation, as articulated by Walzer et al. (2020), manifests as intergenerational responses speeding up development in F_2 generations and causing larger body sizes compared to initial (parent) generations.

Our results on *P. truncatus* adult individual weights ranged from 2.3 to 2.7 mg following acute heat acclimation treatments. These individual adult weights were in the lower range compared to those (3.05–3.6 mg) reported in Haines (1981) possibly due to a different host (cassava) used in the study by Haines (1981). Further, fecundity ranged 30–194 adults, grain damage (23–61%), grain weight loss (5–15%) and insect feeding dust (4–9%) were recorded over a 56-day period on maize grain. Comparable results of progeny production, maize grain damage and insect feeding dust production in *P. truncatus* were reported by Altunç et al. (2023). However, Altunç et al. (2023) only investigated progeny production without heat acclimation. At optimum conditions of 32°C and 80% RH, *P. truncatus* complete its life cycle in 25–27 days (Hodges et al., 1983; Muatinte et al., 2014; Shires, 1980; Quellhorst et al., 2021). At these conditions, a single female lays around 20 eggs at a time depending also on the food available (Quellhorst et al., 2021) and up to 300 eggs in a lifetime (Suma & Russo, 2005). At 32°C and 80% RH, Quellhorst et al. (2020) demonstrated that *P. truncatus* produces significantly higher progeny, grain damage, grain weight losses and insect feeding dust compared to lower (20°C) or 'excessive' (35°C) temperatures. However, these experiments were done in interaction with the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) to mimic where these two coexist. Hence, the individual effects of *P. truncatus* may be difficult to tease apart using results by Quellhorst et al. (2020). Our study is thus the first report of *P. truncatus* performance as a single species under repeated generational heat hardening and the effects thereof on maize grain. Insect feeding dust was significantly higher in the control treatment F_1_32°C, which also had the highest progeny for all treatments. This may mean that reproduction may be lower under higher stressful temperatures, and with repeated bouts of heat stress. This is likely a result of trade-offs in life history traits, for example, *P. truncatus* may trade off reproduction for heat stress protection mechanisms, which may require large energy reserves (Neven, 2000). Altunç et al. (2023) linked high insect feeding dust production to high reproduction in *P. truncatus* as the feeding dust is meant for protection and development of the larvae (see also Quellhorst et al., 2021).

Various researchers have posed the question of what temperature increases under climate change would mean to insect pests of stored grain (Arthur et al., 2019; Nyabako et al., 2021; Stathers et al., 2013; Singano et al., 2020). Most of the conclusions pointed to increased reproduction, damage and range expansion of stored grain insect pests. The current study demonstrates that acute heat acclimation has negative effects on physiological and ecological functions of *P. truncatus* to a limited number of generations; at least one generation based on our data. This, however, is not a model to interpret what would happen with different other insect pests of stored grain or insect pests from different taxa as is the case in normal stored-grain ecosystems. As such, further studies including transgenerational plastic responses and extending the investigations beyond two generations and in the presence of other different cosmopolitan or invasive insect pests of stored grain are recommended to determine the effects of changing climatic conditions on physiological and ecological performance under competition as is the case in natural habitats. One of the few studies on stored grain thermal responses is Mpofu et al. (2022) who investigated transgenerational responses to heat and fasting acclimation in the Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) and concluded that acclimation resulted in fitness costs on the moth. Mutamiswa et al. (2021) reported improved heat tolerance in *P. truncatus* following heat (and fasting) acclimation. Mpofu et al. (2022) and Mutamiswa et al. (2021), however, did not (i) repeat the acclimations generationally and (ii) did not focus on the effects of acclimation on ecological performance. The current study did not extensively investigate the number of days to progeny emergence and beetles' longevity following heat acclimation, which are important parameters that would otherwise improve our understanding of the effects of changing climatic conditions on the biology of *P. truncatus*. Furthermore, this study indicates potential improvements on existing heat-based phytosanitary disinfestation protocols, especially on pests like *P. truncatus*, that are hard to control with conventional synthetic pesticides. The study points to opportunities for pest physical control options through cooling or heating, for example, by solarization to suppress beetle populations, especially with increased warming (see also discussions in Abdelsamea et al., 2023; Fields et al., 1992).

In conclusion, our study found that: (i) intergenerational responses to single or repeated heat acclimation in *P. truncatus* are trait- and generation-dependent, (ii) while acclimation improved HKDT at F_1, repeated acclimation significantly reduced F_2 HKDT, suggesting plasticity erosion with repeated acclimation, (iii) single acclimation negatively affected ecological performance (fecundity and grain damage potential) of *P. truncatus* in the F_1 generation. However, repeated acclimation led to adaptation in F_2 generation and thus regaining ecological performance back to near parental, (iv) thermoregulation in *P. truncatus* is significantly affected by ramping rates and their interaction with acclimation temperature and/or generation. *Prostephanus truncatus* will therefore continue to be a serious threat under climate change as repeated bouts of extreme heat experienced in the natural environment will result in adaptation to stress, leading to improved intergenerational ecological performance and enhanced survival of this important agricultural and forest invasive pest.

AUTHOR CONTRIBUTIONS

Shaw Mlambo: Investigation; methodology; writing – original draft; writing – review and editing; visualization; validation; data curation; formal analysis. **Honest Machekano:** Conceptualization; methodology; data curation; validation; formal analysis; supervision; writing – review and editing; writing – original draft; visualization; investigation. **Brighton M. Mvumi:** Conceptualization; visualization; writing – review and editing; validation; supervision; methodology. **Ross N. Cuthbert:** Software; formal analysis; writing – review and editing; data curation; methodology. **Casper Nyamukondiwa:** Conceptualization; methodology; funding acquisition; writing – review and editing; validation; visualization; supervision; resources; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests that could have influenced the work reported in this paper.

DATA AVAILABILITY STATEMENT

Datasets analysed for the current study can be obtained from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study used local colonies of an agricultural insect pest. Ethical approval was not applicable.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting information.

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