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Thermal fitness costs and benefits of developmental acclimation in fall armyworm

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Abstract

Global increases in mean temperatures and changes in precipitation patterns due to climate change, coupled with anthropogenic pathways, have intensified biological invasions of pest insects. Continuous exposure to bouts of acute and chronic heat and fasting stresses (during e.g., droughts) might improve performance under recurring stresses, therefore enhancing/reducing fitness within- or across- life stages (i.e., ‘carry-over’ effects). Here, we examined developmental acclimation effects in the invasive fall army worm *Spodoptera frugiperda* — a highly invasive economic insect pest of cereal crops, particularly maize — using standardized heat tolerance metrics. Specifically, we assessed the effects of acute (3h) and chronic (3 days) heat treatments (at 32 °C, 35 °C, 38 °C), as well as fasting (48h), on 3rd instar larvae, and tested fitness traits (critical thermal maxima [CT\text{max}] and heat knockdown time [HKDT]) at a later life stage (4\text{th}/5\text{th} larval instar). Acclimation to heat stress and fasting had significant fitness costs (lower CT\text{max}) across majority of treatments. However, both heat and fasting acclimation improved HKDT (except for 35 and 38°C [acute acclimation] and 35°C [chronic acclimation]). Our results suggest context-specific developmental acclimation costs and benefits in *S. frugiperda*. In particular, heat and fasting acclimation potentially have fitness costs and benefits for subsequent developmental stages facing high temperature stress. These results are important for estimating the effects of prior stressful events on future survival of invasive insect species and may be significant in predicting pest population dynamics under changing environmental conditions.

**Keywords:** global change biology; invasive alien species; phenotypic plasticity; *Spodoptera frugiperda*; thermal adaptation.
1.1 Introduction

Biological invasions are a growing ecological and economic threat worldwide (Bellard et al., 2016; Diagne et al., 2021). The success and impact of invasive alien species may interact synergistically with environmental changes, such as increasing temperature for improved survival chances of invasive species, but potential synergies between these processes remain largely unknown (Ricciardi et al., 2021). Insects are among the most impactful taxa worldwide when they invade, owing to a myriad of introduction pathways and rapid human-mediated dispersal, driving some of the highest economic costs worldwide among invasive species (Cuthbert et al., 2021; Venette & Hutchinson, 2021). The fall armyworm (FAW) Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is an economic invasive insect pest of South America origin (Sparks, 1979) that attacks cereal crops, particularly maize. It first invaded the African region through west Africa in 2016 (Goergen et al., 2016; Stokstad, 2017), and by 2017, it had spread to the whole of eastern Africa and parts of southern Africa including Botswana (Day et al., 2017; Stokstad 2017), before spreading to the Middle East and Asia (India) in 2018 (EPPO, 2020; Sharanabasappa et al., 2019).

FAW is a highly multivoltine polyphagous pest, feeding on over 350 plant species across different families (Midega et al., 2018; Montezano et al., 2018; 2019), with a high preference for maize. Larvae feed on leaves, stems, and economic parts of plants e.g., maize cobs, thereby causing economic damage (Rwomushana, 2019). To reach the adult stage, they go through six instar stages, that may take as little as ~11 days (at 32 °C) (Du Plessis et al., 2020). However, FAW larva has striking host plasticity, varying from five to ten instar stages depending on host plant (Ali et al., 1990; Murúa et al., 2003); reportedly having more ‘instars on sub-optimal hosts. This host plasticity facilitates development on less favourable hosts (Esperk et al., 2007) or droughts. Given this host plasticity, African invasion by the FAW represent a substantial biosecurity threat. For example, FAW has threatened maize production
across the world, e.g., the pest accounted for ~17.7 million tones in maize losses across 12 African countries to date (FAO, 2021). Maize remains nevertheless, a staple food for over 200 millions of people globally (Nuss & Tanumihardjo, 2010) and accounts for 40% of the cereal production in Sub-Saharan Africa (FAOSTAT, 2016) with economic, social and political significance. Thus, FAW continental invasion and associated crop losses exacerbates the food security crises in Africa (see e.g., Sasson, 2012).

Bio-physical environmental conditions experienced during early life stages of an organism are significant determinants of many key fitness life history traits (Chown & Nicolson, 2004). In particular, factors such as individual diet, feeding frequency, temperature and relative humidity (RH) environments during early-life stages have significant effects on subsequent developmental stages (Mutamiswa et al., 2019; Sasmita et al., 29; Sgro et al., 2016). Determining the effects of within-developmental stage phenotypic plasticity has been the focus of research for decades (West-Eberhard, 2003; Chown & Nicolson, 2004; Sgro et al., 2016). However, the effects across developmental stages within-generation remain scarce (but see Terblanche & Chown, 2006; Zeilstra & Fischer, 2005), despite their ecological significance. For example, insect developmental stages may be spatially separated, raising questions about how environmental history may differentially shape fitness of subsequent developmental stages within the same generation, but across new environments (e.g., Nyamukondiwa & Terblanche 2010). Indeed, FAW developmental stages may also occupy ≥2 spatially distinct bio-physical environments (Melo et al., 2014) resulting in likely different fitness consequences in subsequent instars. Through adult flight migrations (Nagoshi et al., 2012) and larval silking (FAO, 2018), FAW uses this behavioral adaptation to extend its geographical range into novel areas to circumvent inter- and intra-specific competition. However, it is not known whether prior environment affects fitness of subsequent developmental stages, or aids invasiveness of FAW. Previous studies have nevertheless
documented that overcoming environmental barriers, e.g., of temperature and desiccation tolerance, are critical for invasion success (Richardson & Pysek 2006). For this reason, invasive insects often have high basal stress tolerance, phenotypic plasticity (Nyamukondiwa et al., 2010; Kelley, 2014; Wan & Yang, 2016; Machekano et al., 2018) and metabolic flexibility (Smit et al., 2021).

Other external stressors, such as lack of food, have also been documented to influence insect thermal tolerance in complex, often in unpredictable ways (Nyamukondiwa & Terblanche, 2009). Fasting can occur when there is a lack of adequate food (due to various environmental perturbations) to meet the energy requirements for biological processes in the insect’s body (McCue., 2010). This food deprivation stress is presumed to result in a trade-off in insect thermal tolerance (Scharf et al., 2016) due to cross talk or cross tolerance (Sinclair et al., 2013). Several studies have supported this notion, as fasting pre-treatment often impairs cold tolerance in insects (Gotcha et al., 2018; Kenny et al., 2008). However, fasting acclimation appears not to have any effects on heat tolerance (see Gotcha et al., 2018; Scharf et al., 2016). Nevertheless, how stressful traits in one developmental stage interact with the subsequent developmental stages and environments remains unknown in FAW, despite evidence for phenotypic plasticity within and across ontogeny (see e.g., West-Eberhard, 2003; Sgrò et al., 2016) and across different developmental stages and seasons/environments (i.e., ‘carry-over’ effects) (Norris, 2005; Harrison et al., 2011; Fayet et al., 2016; Ezeakacha & Yee, 2019).

Despite overwhelming evidence of the effects of prior environment on insect fitness (Nyamukondiwa & Terblanche, 2010; Fayet et al., 2016; Ezeakacha & Yee, 2019), few studies have investigated developmental acclimation effects for invasive insects with a view of making inferences for pest invasiveness. Nevertheless, investigation of the effect of environmental history has large ecological implications for organismal fitness under changing
environments. Unravelling developmental acclimation is important in determining how species may react to changes in environment across developmental stages and seasons, and how this may shape their fitness and by extension, their population dynamics. This has downstream implications on designing pest control strategies e.g., through development of early warning systems. Here, we thus examined innate within-generation developmental temperature and fasting acclimation effects on heat tolerance in *S. frugiperda* following high temperature acute and chronic acclimation, as well as fasting. Given its tropical origin, heat tolerance may remain a key trait that facilitates invasion, and more-so in arid and semi-arid environments such as Botswana. We hypothesise that temperature and food deprivation stress in one instar may have heat tolerance fitness costs or benefits across other subsequent non-acclimated developmental stages (developmental acclimation). Confirmation of positive adaptive developmental acclimation effects may have implications on *S. frugiperda* invasiveness under heterogeneous stressful environments associated with changing climate. This knowledge is important in informing spatially-dependant *S. frugiperda* pest management strategies.

2.1 Materials and Methods

2.1.1 Insect rearing and maintenance

Field populations of *S. frugiperda* were collected as 2<sup>nd</sup>-5<sup>th</sup> instar larvae from infested maize crops in two commercial farms; Talana farms (S22°.13467; E28°.59468) and Motloutse River farm, Bobonong Village, Central district of Botswana, and placed in 50 ml vials containing artificial diet, adopted from Tefera *et al.* (2010). Both collection areas are within the same region and experience similar climatic environments. Specimens were reared in Memmert climate chambers (Memmert GmbH + Co. KG, Schwabach, Germany) in the laboratory at optimal conditions (28±1 °C, 65±10% RH) and fed on the same artificial insect diet
Both pupae and moths were kept in Bugdorm cages (Megaview Science Co., Ltd, Taichung, Taiwan) in climate chambers. All adult moths were fed on 10% sucrose solution \textit{ad libitum}. To obtain the next generation of FAW for experimental use, moths were mated in oviposition cages containing a 4-week-old maize plant (as oviposition substrate). Following oviposition, eggs were allowed to incubate and hatch on the host plant. After hatching, 1\textsuperscript{st} instar neonates were subsequently transferred to vials containing the artificial diet (Tefera et al., 2010). Each vial comprised three larvae, reared up to 3\textsuperscript{rd} instar after hatching in the laboratory. However, following moulting to the 3\textsuperscript{rd} instar stage, all larvae were transferred into individual vials in preparation for the experiments and simultaneously to circumvent larval cannibalism, which is usually more apparent from the 3\textsuperscript{rd} instar onwards (see Rwomushana, 2019). Experiments were run using these lab-reared 3\textsuperscript{rd} instar specimens from F\textsubscript{1} to F\textsubscript{4} generations, randomised across the treatments. We assumed that laboratory adaptation has insignificant effects on thermal fitness across the three tested generations, as has been observed in similar experiments (Opperman 2018; but see Hoffman et al., 2001). Acclimation treatments were done following moulting of 3\textsuperscript{rd} instar larvae, and heat tolerance traits (i.e., critical thermal maxima \([\text{CT}_{\text{max}}]\) and heat knockdown time \([\text{HKDT}]\)) were tested on 4\textsuperscript{th} instar larvae following acute acclimation and 5\textsuperscript{th} instar larvae following chronic acclimation (as majority of the larvae moulted twice during the 3-day chronic acclimation plus one day recovery period).

2.1.2 Acclimation experiments

Experimental treatments (acclimation) comprised acute and chronic sub-lethal high temperature acclimation and fasting. This was undertaken in 3\textsuperscript{rd} instar larvae by exposing insects to temperatures of 28.0 (control) 32.0, 35.0 and 38.0 ± 1.0 °C (each under 65 ±10% RH) for 3 hours (acute) and 3 days (chronic) acclimation treatments (see Table 1) in
Memmert climate chambers. Temperatures selected for acclimation were ecologically relevant and based on a previous study that showed temperatures across Botswana to reach up to 42 °C during heat waves (Moses, 2017; see Fig. 1), and considering that the optimal temperature range of *S. frugiperda* is 26-30 °C (Du Plessis *et al.*, 2020). From the optimum temperature range, 28 °C was selected as the control temperature and 3-4 °C was added to establish mild high temperatures for acclimation, based on modified protocols from Mutamiswa *et al.* (2019). Control insects were kept at optimal environmental conditions of 28±1°C and 65±10% RH during experimental treatments before measuring thermal fitness traits (Fig. 1). Following both acute and chronic acclimation, insects were allowed to recover at optimal conditions (28±1°C and 65±10% RH) for 24 hours before measuring physiological traits.

To determine the effects of feeding status on the thermal fitness of *S. frugiperda*, 3rd instar larvae were deprived of food (fasted) for 48 hours. All fasting acclimations were done using a constant time period (48 hours), and results were directly compared to those of acute and chronic temperature treatments. Larvae were removed from artificial diet at 3rd instar and individually placed into empty 50 ml vials without any food, but with a water source (cotton wad, to prevent desiccation associated mortality) for 48 hrs. The larvae were kept under benign conditions (28±1 °C, 65 ± 10% RH; 12L:12D) to ensure that food deprivation was the only limiting factor. Post 48 hrs, larvae were returned to individual vials with access to food (artificial diet) and water *ad libitum* for 24 hours to allow recovery. Measurement of thermal traits was conducted 24 hours post-recovery following methods by Gotcha *et al.* (2018). Control larvae were provided with artificial diet and kept at optimal temperatures and RH (28±1 °C and 65±10%) throughout prior to running experiments.

### 2.1.3 Heat tolerance metrics
To test the effects of heat and fasting acclimation on heat tolerance, (i) $CT_{\text{max}}$ – the maximum temperature allowing insect activity, and (ii) HKDT – the time taken to knock down an insect following acute heat stress, were measured (Chown & Nicolson, 2004). Both traits are ecologically sound heat tolerance indices (Lutterschmidt & Hutchison, 1997; Huey & Kearney, 2020) and correlate well with insect biogeographical patterns. For $CT_{\text{max}}$, individual 4th instar (for acute acclimation) and 5th instar (for chronic acclimation) larvae were placed into an insulated double jacketed chamber with ten ‘organ pipes’ connected to a programmable bath filled with 1:1 water: propylene glycol, which regulates the flow of liquid around the chamber (Grant GP200-R4, Grant Instruments, UK) (Nyamukondiwa & Terblanche, 2009). Critical thermal maxima experiments started at 28 °C (FAW optimum temperature) from which temperature was gradually increased using a ramping rate of 0.25 °C/min until the larvae reached upper temperature limit of activity ($CT_{\text{max}}$) (Nyamukondiwa & Terblanche, 2009) (Table 1). This ramping rate is faster than the natural diel increase in temperature, but nevertheless slower and thus ecologically more relevant than other ramping rates used in literature e.g., 0.5 °C/minute (reviewed in Chown & Nicolson, 2004). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05 °C) was inserted into the organ pipe to record the chamber temperature. The experimental procedure was repeated 3 times to yield $n \approx 30$ larvae per treatment (30 replications). In this study, $CT_{\text{max}}$ was defined as the temperature at which an individual larva lost co-ordinated muscle function (self-righting) and ability to respond to mild prodding using a thermally inert object.

Heat knockdown time was assessed on 4th instar larvae (following acute acclimation) and 5th instar larvae (following chronic acclimation) using standardized protocols (Nyamukondiwa & Terblanche, 2009). We used treatment-specific heat knockdown temperatures, derived from each treatment’s $CT_{\text{max}}$ value plus 2 °C. This heat knockdown temperature is ecologically
sufficient to elicit heat knockdown effects in insects (see e.g., Hoffman et al., 2003; Mutamiswa et al., 2019). Thus, knockdown temperatures of 53.0, 50.0, 51.4, and 50.7 °C were used as acute knockdown temperatures for 28 (control) 32, 35, and 38 °C acclimation pre-treatments respectively, whereas 53.0, 51.6, 51.9 and 50.8 °C were used as chronic knockdown temperatures for 28 (control) 32, 35, and 38 °C acclimation pre-treatments, respectively. Ten individual larvae were placed in 1.5 ml microcentrifuge tubes and placed in a Memmert climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at various temperatures as indicated above. Temperatures above $\text{CT}_{\text{max}}$ cause heat coma in insects and are often used in HKDT assays (see Nyamukondiwa et al., 2011). 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 and timing. Heat knockdown time was defined as the time (in minutes) at which an individual larva lost activity following acute heat stress.

2.1.4 Data analysis

Data analyses were all performed using R, version 4.1.1 (R Development Core Team, 2021). The residuals were first checked for normality and variance homogeneity using Shapiro–Wilks and Levene’s tests, respectively, and were found to violate normality and homogeneity of variance assumptions. Kruskal-Wallis tests were thus used to examine $\text{CT}_{\text{max}}$ and HKDT as a function of treatment for each respective acute and chronic exposure treatment (i.e., four separate models). Dunn tests were used post-hoc for pairwise comparisons, with p-values adjusted via the Holm method (Ogle et al., 2021).

We summarized the magnitude of both $\text{CT}_{\text{max}}$ and HKDT following acclimation using methods by Tarusikirwa et al. (2020) and Mutamiswa et al. (2019). Specifically, we calculated the magnitude of change in thermal fitness conferred by the acclimation treatment.
using the formula below, where the mean heat tolerance (CT<sub>max</sub> / HKDT) after each treatment was divided by the mean control CT<sub>max</sub> or HKDT results of which were tabulated into Table 1.

**Magnitude of change** = \( \frac{\text{Final CT}_{\text{max}} (\text{or HKDT}) \text{ for each treatment}}{\text{Control CT}_{\text{max}} (\text{or HKDT})} \)

where a value > 1 denotes beneficial acclimation effects, whereas a value < 1 denotes potentially deleterious acclimation effect (see Leori *et al*., 1994; Marais & Chown, 2008)

### 3.1 Results

For acute exposures, CT<sub>max</sub> differed significantly among control group, 32 °C, 35 °C and fasting acclimation treatments (\( \chi^2 = 58.397, \text{df} = 4, p < 0.001 \)). Control group 4<sup>th</sup> instar CT<sub>max</sub> was significantly higher than 32 °C, 35 °C and fasting acclimation treatments (all \( p < 0.01 \)), but not 38 °C (\( p > 0.05 \)). In turn, 38 °C treatment CT<sub>max</sub> was significantly greater than 32 °C, 35 °C and fasting acclimation groups (all \( p < 0.05 \)). CT<sub>max</sub> following fasting acclimation significantly exceeded the 32 °C treatment (\( p < 0.05 \)) (Fig. 2a). In addition, heat tolerance (CT<sub>max</sub>) for acute treatments was significantly lower than chronic exposure treatments (\( \chi^2 = 35.070, \text{df} = 4, p < 0.001 \)). For chronic acclimation, Control group CT<sub>max</sub> was again highest, and significantly greater than 32 °C, 38 °C and fasting acclimation treatments (all \( p < 0.05 \)), but not 35 °C (\( p > 0.05 \)). CT<sub>max</sub> at the 35 °C treatment was also significantly greater than 38 °C and following fasting acclimation (both \( p < 0.05 \)) (Fig. 2b).

Following acute 3<sup>rd</sup> instar acclimation exposures, HKDT differed significantly among treatment groups (\( \chi^2 = 51.220, \text{df} = 4, p < 0.001 \)). Control HKDT was significantly lower than 32 °C and fasting acclimation groups (both \( p < 0.05 \)), but not 35 °C or 38 °C (both \( p > 0.05 \)). Fasting acclimation and 32 °C treatment groups had the highest HKDT, which significantly exceeded 35 °C and 38 °C (all \( p < 0.001 \)) (Fig. 2c). For chronic exposures, HKDT again significantly differed among treatments (\( \chi^2 = 32.332, \text{df} = 4, p < 0.001 \)). Control group HKDT
was significantly lower than 32 °C, 38 °C and fasting acclimation groups (all p < 0.01), but not 35 °C (p > 0.05), whereas 38 °C significantly exceeded 35 °C (p < 0.05) (Fig. 2d).

Chronic and acute acclimation had contrasting results on the direction of both CT\textsubscript{max} and HKDT plasticity (Table 1). Both acute and chronic acclimation, as well as fasting, yielded negative deleterious plastic effects (i.e., negative magnitude; see Table 1) for CT\textsubscript{max}, whereas positive effects on HKDT were exhibited following chronic exposure and fasting, but not acute exposures beyond 32 °C.

4.0 Discussion

Population dynamics of individuals that may occupy multiple spatially-distinct habitat environments can be highly complex (Webster et al., 2002). As such, sub-lethal stressful conditions experienced during early developmental stages e.g., early instars of the larvae, may be important in determining key life history traits, either manifesting as beneficial through e.g., developmental acclimation effects (see Chown & Nicolson, 2004), or as deleterious (see e.g., Marais & Chown, 2008). Our results showed that fasting and high temperature acclimation at the 3\textsuperscript{rd} instar larval stage had mixed effects on the heat tolerance of subsequent developmental stages (4\textsuperscript{th} instar larval stage) in FAW, tested as CT\textsubscript{max} and HKDT, and following both acute and chronic acclimation. In particular, fasting, acute and chronic high temperature acclimation of 3\textsuperscript{rd} instars reduced CT\textsubscript{max} of 4\textsuperscript{th} instar larval stage of \textit{S. frugiperda}. This may point to a deleterious effect of developmental acclimation conditions tested here on \textit{S. frugiperda} heat tolerance. Thus, the prior exposure to sub-lethal environmental stressors early in life may have negative consequences for subsequent developmental stages, synonymous to deleterious acclimation (Marais & Chown, 2008), and suggest that both the acclimation treatment and CT\textsubscript{max} stress test effects are additive (Jorgensen et al., 2021). Although chronic acclimation was lower than control, it generally
had higher $C_{T_{\text{max}}}$ than the acute acclimation treatments at the same temperatures. However, HKDT assays yielded contrasting results from those of $C_{T_{\text{max}}}$, with both fasting and specific high temperature acclimation treatments increasing heat tolerance (HKDT) for both acutely and chronically acclimated 3$^{\text{rd}}$ instar $S. \text{frugiperda}$. Our results, thus, confirm that acclimatory exposure of one developmental stage may have fitness costs and benefits to subsequent developmental stages, and that these costs and benefits depend on the context of the fitness metric being tested (e.g., $C_{T_{\text{max}}}$ vs HKDT). Thus, exposure to sub-lethal stress during development may have fitness and survival consequences on later life stages (Klockmann et al., 2017), and by inference, could affect the propensity of invasive species establishment success in novel environments.

While acclimatory conditions experienced during one life stage of an organism can have fitness costs and benefits within that life stage, acclimatory effects can also manifest across developmental stages. Developmental acclimation effects are adaptive and may presumably facilitate ‘carry-over’ stress resistance under novel stressful environments. $Spodoptera \text{frugiperda}$ is a highly invasive economic insect pest of cereal crops, particularly maize. Given the mobility of its life stages e.g., larva (through silking) and adults (through flight), it is largely unknown how previous environments may shape fitness of the same and/or subsequent life stages, and by inference, invasion propensity thereof. Our results showed that most acute and chronic heat acclimation treatments, as well as fasting, significantly depressed $C_{T_{\text{max}}}$. This result means that sub-lethal food deprivation and heat stress at the 3$^{\text{rd}}$ instar stage larvae may have negative $C_{T_{\text{max}}}$ fitness consequences on 4$^{\text{th}}$ and 5$^{\text{th}}$ instar larvae and probably by extension, other subsequent developmental stages. Climate change is often associated with episodes of acute and chronic heat stress, and prolonged droughts that may limit food resources (IPCC, 2014; Stillman, 2019). Thus, the deleterious effects of acclimation treatments recorded here may mean that frequent episodes of environmental heat
and food deprivation stress faced in nature may offset heat tolerance of subsequent developmental stages, affecting population dynamics of invasive species. The reason for the negative effects of heat acclimation reported here are largely unknown. However, it may point to the notion that the stress faced during acclimation treatment and $CT_{\text{max}}$ assays is additive (see Jorgensen et al., 2021). Moreover, we also speculate that we may have missed certain acclimatory cues or specific treatment combinations that specifically elicits $CT_{\text{max}}$ acclimation responses. Thus, future studies may need to explore differential temperature and time combinations at all higher instar stages of the larvae that may elicit acclimation. One more interesting result observed here is that a treatment to one stress may also have negative effects on a divergent stress. For example, $3^{rd}$ instar acute and chronic fasting acclimation had deleterious consequences on $5^{th}$ instar larval $CT_{\text{max}}$. This may point to the notion that injury associated with diverging environmental stresses may be the same (see e.g., Shen et al., 2015; Farahani et al., 2020), and that divergent stress effects may thus be additive. Nevertheless, the lack of beneficial acclimation effects for heat acclimation reported here is consistent with studies on *Tuta absoluta*, that reported no beneficial acclimation effects following chronic high temperature acclimation (Tarusikirwa et al., 2020).

By contrast, both acute and chronic $3^{rd}$ instar larvae acclimation had beneficial effects on subsequent larval HKDT, albeit for specific treatments ($32 \, ^\circ \text{C}$ and fasting for acute acclimation, and $32, 38 \, ^\circ \text{C}$ and fasting for chronic acclimation). Conditions eliciting acclimation responses are highly complex and often context-dependent (Chown & Nicolson, 2004; Sgro et al., 2016; Mutamiswa et al., 2019). This agrees with our results, that observed positive acclimation responses were specific to certain heat acclimation groups and corroborates with previous reports suggesting that conditions conferring acclimation responses are highly context-dependent (see Mutamiswa et al., 2019). Similarly, acute heat acclimation at 35 and 38 $^\circ \text{C}$, and chronic heat acclimation at 35 $^\circ \text{C}$, had no significant effects
on HKDT. This result means that heat wave episodes associated with climate change on 3rd instar larvae of *S. frugiperda* may have positive or neutral effects on subsequent developmental stages in the context of HKDT but not CT\textsubscript{max}. Thus, *S. frugiperda* may have fitness benefits under projected heat stress in terms of enduring long durations of mild to high temperature stress associated with changing climates, potentially translating into greater invasiveness and resilience in high temperature tropical habitats.

Comparisons for HKDT experimental traits have often been investigated using a more constant temperature (see Chown & Nicolson 2004). However, here, we used different heat knockdown temperatures to investigate HKDT across different acute and chronic temperature treatments. Thus, differences in results reported here may also be partly due to the treatment-specific heat knockdown temperature methodology used in our study. Furthermore, the results also showed the positive beneficial effects of acclimation to a divergent stress trait (fasting) on a different stressor (heat tolerance [HKDT]). Such cross tolerance represents shared co-evolutionary response mechanisms to stress traits involved (see Gotcha et al., 2018), and represent another facet that may help invasive species survive highly variable stressful environments, e.g., through integrated stress resistance (see discussions in Renault et al., 2015). Cross tolerance results reported here are nevertheless in contrast with reports on *Ceratitis rosa*, where fasting resulted in increased CT\textsubscript{max} and reduced HKDT (Gotcha et al., 2018). These results indicate that fasting has dissimilar effects on different traits used to measure heat tolerance, and that elicitation of acclimation responses are trait dependant.

Results obtained here thus mean that food deprivation (i.e., temporary absence of host plants) during mid-season droughts, and other plant-damaging natural disasters such as hailstorms, may provide a benefit in periods of rapid heat shock, such as heat waves, through cross tolerance developmental acclimation effects. Increased heat tolerance (HKDT) reported here for certain treatments may represent context-specific expression of heat shock proteins and
other hormonal heat stress regulators (Hoffmann et al., 2003; King & Macrae, 2015). Heat shock proteins and hormonal activation due to heat stress usually occur over a certain temperature threshold, which are context-dependent but usually range from 39 – 41 °C (Qazi et al., 2019). Similar results were observed in Drosophila mojavensis (Patterson & Crow, 1940), where high temperature acclimation at one developmental stage increased HKDT in subsequent developmental stages (Diaz et al., 2021). Indeed, sublethal high temperature stress may influence physiological traits in addition to other life history traits e.g., fecundity and longevity (Nguyen & Amano, 2010). A study on Plutella xylostella (L.) showed that high temperature acclimation on larval stages had effects on adult stages, affecting oviposition patterns and adult life span (Zhang et al., 2015). Our results thus indicate that timing of sublethal high temperature stress such as heat waves may enhance thermal fitness and survival of other life stages of invasive pest insects. Therefore, survival of S. frugiperda may increase in hot tropical areas even during high temperature incidences. Inclusion of effects of extreme high temperature incidences in pest management forecasts will increase accuracy of invasive species biogeographical patterns (Skendžić et al., 2021). Likewise, processes and events at one developmental stage and in a particular season may have far-reaching consequences for other developmental stages in a different season; a phenomenon called ‘carry-over’ effects (Harrison et al., 2011). While this phenomenon has largely been explored in migratory birds, little is known on how this affects the fitness of migratory insects in general (but see Galarza et al., 2019), and invasive insects in particular. Future work should accordingly examine carry-over effects in S. frugiperda and assess how they may shape population dynamics and pest invasiveness. We also observed that acclimation responses were trait-dependant and observed such positive effects only for HKDT; a more acute heat stress metric compared to CT<sub>max</sub>. We thus speculate, with caveats, that the differences in chronic exposure to stress during CT<sub>max</sub> and HKDT assays may also have affected differential acclimation responses
reported here. Future studies should thus look at more acute $CT_{\text{max}}$ ramping methodologies e.g., 0.5 or 1 °C/minute (see e.g., Terblanche et al., 2007) to try and capture potential acclimation cues that we may have missed.

Costs and benefits of plastic acclimation are difficult to predict and are not uniform across species, metrics tested and acclimation treatments. A study done by Alemu et al., (2017) showed that age, increasing rate of temperature change and duration of heat hardening resulted in a benefit or increase in $CT_{\text{max}}$. Factors such as body mass have also been shown to have a cost on HKDT (Nyamukondiwa et al., 2018). Our results thus show mixed but context (trait)-dependent effects of both acute and chronic developmental acclimation. Specifically, we first show that while both heat and fasting acclimation at one developmental stage negatively affected heat tolerance measured as $CT_{\text{max}}$, it contrastingly improved another heat tolerance trait (HKDT), suggesting differential trait related acclimation responses. The positive beneficial acclimation responses for HKDT were, however, treatment dependant, suggesting specific cues eliciting acclimation for the HKDT trait. Second, we show that while heat acclimation had negative effects (additive stress effects [see Jøgensen et al., 2021]) for $CT_{\text{max}}$ (same stressor), acclimation to a divergent stress (food deprivation) may also have additive stress effects on a different stress (heat tolerance [$CT_{\text{max}}$]). These results support our hypothesis that abiotic stress in one developmental stage may have fitness costs and benefits in the subsequent developmental stages (e.g., Ezeakacha & Yee, 2019). We could, however, not find acclimation responses for $CT_{\text{max}}$ and other treatments for HKDT. We speculate that we may have missed exact treatment combinations that elicit acclimation response for $CT_{\text{max}}$ and these specific treatments for HKDT. Thus, future work should consider more comprehensive acclimation treatment combinations. Forecasting of insect pest invasions are usually done through prediction of how changes in mean temperature affect insect pest population dynamics (Zhang et al., 2015). Our results provide a step into understanding costs
and benefits of developmental acclimation in *S. frugiperda* and how it may affect pest population dynamics. Future pest forecasting models should thus incorporate developmental acclimation responses and by extension, ‘carry-over’ and integrated stress resistance effects (see e.g., Norris & Taylor, 2006; Nyamukondiwa *et al.*, 2022) to potentially improve accuracy of model outputs and pest early warning systems.

**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.

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**Authors' contributions**

Project conceptualization and management: BS HM CN.

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Formal analysis: RC CN.

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Visualization and validation: BS HM RC CN.

Writing – original draft: BS HM CN.

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Table legends

**Table 1**: Magnitude and direction of change of critical thermal maxima and heat knockdown time. Magnitude < 1 indicates deleterious (negative) effects of acclimation, whereas magnitude > 1 indicates beneficial (positive) effects of acclimation.

Figure legends

**Figure 1**: Experimental flow diagram indicating acclimated developmental stages, type of acclimation (acute versus chronic), acclimation temperatures (28 [control]; 32; 35 and 38 °C) and the traits tested (HKDT and CT$_{\text{max}}$). Traits were tested on instar 4 in acute acclimation and instar 5 in chronic acclimation pre-treatments. Heat knockdown temperatures were acclimation treatment temperature-specific, and were derived from average CT$_{\text{max}}$ values, following methods by Hoffman et al., (2003), CT$_{\text{max}}$ = critical thermal maximum; HKDT = heat knockdown time.

**Figure 2**: Critical thermal maxima (CT$_{\text{max}}$ [a, b]) and heat knockdown time (HKDT [c, d]) following acute (a, c) and chronic (b, d) temperature treatments. For all fasting pre-treatments, organisms were deprived of food for 48h. 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 × interquartile range. Points are raw data. Acute and chronic represents acute and chronic acclimations.
Tables

Table 1: Magnitude and direction of change of critical thermal maxima and heat knockdown time.

| Type of acclimation | Acclimation temperature (°C) | *Magnitude of change (CT$_{max}$) (folds) | *Magnitude of change (HKDT) (folds) |
|---------------------|-----------------------------|------------------------------------------|-----------------------------------|
| Acute               | 32                          | 0.968                                    | 1.175                             |
|                     | 35                          | 0.974                                    | 0.840                             |
|                     | 38                          | 0.995                                    | 0.832                             |
| Chronic             | 32                          | 0.991                                    | 1.290                             |
|                     | 35                          | 0.099                                    | 1.240                             |
|                     | 38                          | 0.995                                    | 1.572                             |
| Fasting             | 28                          | 0.981                                    | 1.130                             |

*Magnitude < 1 indicates deleterious (negative) effects of acclimation, whereas magnitude > 1 indicates beneficial (positive) effects of acclimation
Figures

Figure 1
Figure 2.