



Thermal tolerance in *Spodoptera frugiperda*: Influence of age, sex, and mating status

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ABSTRACT

Temperature plays a key role in shaping the physiology and behavior of ectotherms where responses can depend on age, sex and ontogeny. We investigated the effects of age, sex and mating status on thermal tolerance of *Spodoptera frugiperda* 3-, 6- and 9-days old adults heat shocked at adult, pupal and larval stages, at 40 °C for 2 h. Our results showed varied significant critical thermal maxima across mating status, age, sex and life stage in tested insects after heat shock. The virgin females CT_{max} increased with age while it fluctuated in males and 6 days old virgin females had higher CT_{max} than males. The 3 day old mated females had the highest heat tolerance while 6 days old virgin males the lowest heat tolerance. There was no significant difference in CT_{max} recorded between virgin males and females aged 3 and 9 days as well as 3, 6 and 9 day mated males and females. There was also no significant interaction between mating status and sex but there was a significant interaction effect between life stage and age. Mated males had higher CT_{max} than females across all heat shocked developmental stages. The results show a high level of fitness and survival in *S. frugiperda* females, which may be advantageous in invasion. This study also showed “carry over” effects in adults heat shocked at juvenile stage, with aged adults showing decline in CT_{max} . This study suggests that plasticity of thermal tolerance may be an important approach for *S. frugiperda* invasion.

Introduction

Invasive insect pests are a major threat to global agricultural crop production [1]. With warming climates due to climate change, the scourge of such pests is projected to increase [2,3]. This leads to increased costs of crop production due to investment in pest management strategies and outright food insecurity in the cases where crop damage goes unabated (Shrestha, 2019; [3,4]. Extreme weather events associated with climate change are increasing and these may influence not only insect biogeography but individual physiological performance and their population dynamics at various temporal scales [5]. For example, heatwaves which are usually acute in nature, may disrupt biological functions of organisms with cascading effects to populations through limited activity and survival [6]. Such rapid extreme climatic events may even have profound impact on insects than gradual temperature changes [6–8].

The physiological adjustments that are employed by insects to buffer against thermal stress improve fitness through rapid and

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pliable non-heritable phenotypically plastic responses [9,10]. This ability to buffer extreme temperatures is important for invasion success and is reportedly prevalent in pests with high dispersal propensity [11,12]. Indeed, plasticity is very beneficial to insects as it increases their thermal safety margins, thereby enabling activity in otherwise unfavorable environments [7,12–16]. Understanding of heat tolerance and its plasticity is of great importance in the prediction of invasion risk and success under climate change.

The extent to which phenotypic plasticity buffer climate change-associated effects is a subject for debate and its effects on the maintenance of ecosystem function is still not clear [17,18] but ontogeny is known as the major factor that influences the magnitude of phenotypic plasticity responses to abiotic stressors [19–21] where the less mobile life-stages exhibit most plasticity [9].

Thermal tolerance varies between populations, among species, individuals and within individuals [22,23]. Among other factors that affect insect biochemical and physiological process, including thermal tolerance are the age, sex, mating status, body size, feeding or nutritious status [14,23–25]. Ontogeny, age and sex specific variations within individuals have received relatively little attention even though they are important in understanding thermal adaptations of organisms [23]. Thermal tolerance varies and is not expected to be constant throughout an individual's lifetime especially for organisms such as insects whose life cycles are complex and different life stages inhabit different microhabitats [20,26,27]. The extent to which species cope with extreme temperature conditions at different life stages and how exposure of juvenile stages to such extremes may affect survival and reproduction at a later stage [22,23]. For example, in *Drosophila*, short term exposure to extreme temperatures at larval and pupal stages influenced development and hatching success, and resistance to extreme temperatures was uncoupled across developmental stages [27]. This shows that different physiological pathways are used during ontogeny and these may or not be mediated by the production of heat shock proteins (HSPs) [23,28]. Acclimation at larval stage improved physiological response to thermal tolerance in *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), which were carried over to each stage of development until adult stage [20]. This suggests that plastic physiological responses acquired during development may improve adult fitness and survival at a later stage [14].

The reproductive function of organisms is known to be sensitive to heat (e.g. [12,29]) especially that of males [30,31]. Sexes may differ in various ways in their sensitivity to heat stress with females being less sensitive than males [32,33]. Sperm and egg production are both affected by temperatures. Exposure to extreme conditions (low or high) may not only reduce temperature sensitive performance but may cause irreversible damage to fertility such as deformed sperm, and sterility (permanent such as egg or sperm mortality or temporal) [6,31]. Heat shock may also reduce male reproductive success by reducing sexual attractiveness to females [34]. In *Drosophila mojavensis* (Diptera: Drosophilidae), heat stress caused males to be less attractive to females due to change in the epicuticular hydrocarbons production [35]. From the foregoing, it is apparent that temperature plays a key role in mediating the population dynamics of ectotherms through life-stage and sex-linked carry over effects.

Spodoptera frugiperda (J.E Smith) (Lepidoptera: Noctuidae), commonly known as the fall armyworm, is a devastating polyphagous insect pest of cereals. It is known to feed on over 186 plant species from 42 families [36], and is a major pest of *Zea mays* L. (maize) and *Sorghum bicolor* (L) (Moench) [37]. Being major staple food crops of sub-Saharan Africa (SSA), yield losses due to pest damage result in significant poverty and food insecurity [38]. In Africa, the maize losses due to *S. frugiperda* are estimated at US\$2.5 to 6 million annually [39]. Although, *S. frugiperda* is native to tropics and sub tropics of the Americas [40], it has since rapidly spread across much of SSA since first being reported in West Africa in 2016 [37].

Spodoptera frugiperda is multivoltine and adults have high dispersal propensity, flying up to 100 km per night [41–46]. Apart from these population level predisposing factors, thermal tolerance is likely to play a key role in the invasion ecology of the *S. frugiperda*. It is therefore important to understand the role of climate and specifically extreme hot conditions on the population dynamics and distribution of *S. frugiperda* given that global temperatures are projected to increase due to global warming [3]. This study aimed to determine the role of heat shock at juvenile and adult stages on the thermal tolerance of *S. frugiperda* of variable age, sex and mating status. Specifically, we investigate the effects of adult and juvenile heat shock on heat tolerance measured as CT_{max} across age, sex and mating status. We hypothesised that basal thermal tolerance of *S. frugiperda* adults varies across age, sex and mating status following heat shock. The results are significant in pest risk assessments and informing pest management options under shifting abiotic environments.

Materials and methods

Insect culture

The initial colony of *S. frugiperda* was obtained as larvae and pupae from North West University, Potchefstroom, South Africa. Pupae were maintained in wide mouth plastic tubs (1 liter) containing moist soil until adult eclosion. Larvae were maintained in vials containing artificial diet from Southland product INC, USA. Insects were kept at an optimum temperature of 28 °C, 65± 5 relative humidity and 12L: 12D photoperiod [47] in the insectarium at the University of the Free State, Bloemfontein, South Africa. Adults were sexed at pupal stage following Kalleshwaraswamy et al. [48]. This process involved observing the distance between genital opening and anal slot of pupa under the dissecting microscope (Leica 10,445,538, MZ8, Leica Corporation, Switzerland), with the females exhibiting longer distance than males. In addition, males have two pairs of rounded tubercles, one pair around the genital opening and the other around the anal opening while females have one pair around the anal opening only. Following eclosion, individual male and female adults were paired, and each pair released into a cage (40 × 40 × 60 cm) with potted maize plants (2 weeks old) for oviposition for gravid females. The plants were monitored daily for the presence of eggs. Adult moths were provided with 10 % sucrose solution soaked in moistened cotton wool placed in a Petri dish for feeding ad libitum [47].

Effects of adult heat shock on age, sex, and mating status

To evaluate the effects of age, sex (female and male) and mating status on basal thermal tolerance of *S. frugiperda*, 3-, 6- and 9-day old virgin and mated adults were heat shocked at 40 °C for 2 h in propylene vials with a small piece of moist cotton wool placed on the bottom of each vial to prevent desiccation-related mortality (Fig. 1). These hardening regimes were based on similar previous studies that elicited hardening responses [49] and based on microclimate recordings from fields with *S. frugiperda* activity [50,51]. Following heat shock, critical thermal maxima (CT_{max}) was measured following dynamic protocols outlined by Nyamukondiwa & Terblanche [52] and Chidawanyika & Terblanche [7]. Ten adult *S. frugiperda* from heat shock treatments were individually placed into a double jacketed “organ pipe” chamber comprising 11 separate 200 mm tubes, connected to a programmable water bath (Grant model Tx150; Grant Instruments, UK) filled with 1:1 water: propylene glycol and was subjected to a constant heating. In the ‘organ pipe’, insects were given 10 min first to equilibrate at 28 °C (which is their optimal developmental temperature) before ramping temperature up (CT_{max}) at a rate of 0.25 °C min⁻¹. This was repeated twice to yield $n = 20$ per treatment (20 replications). A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the control chamber to monitor chamber temperatures. Critical thermal maximum was regarded as the upper temperature at which the adult lost coordinated movement or ability to self-right after mild proding with a soft hair camel brush.

Effects of juvenile heat shock on adult thermal tolerance

To determine the effects of age and developmental heat shock on *S. frugiperda*, 4th and 6th instar larvae and 24–48 h old pupae were heat shocked at 40 °C for 2 h (Fig. 1). Thereafter, they were returned to the insectarium where they were kept at 28 °C, 65 ± 5 % relative humidity until adult eclosion and were grouped according to mated or virgin, age (3, 6 and 9 days old) and sex (female and male). Following heat shock, CT_{max} was measured as specified in Section 2.2. (Fig. 1). Eggs and 1st to 3rd instar were excluded from the experiment as they could not withstand the discerning heat shock temperature for 2 h.

Data analysis

Data were first checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests, respectively. In all cases, linear model assumptions of analysis of variance (ANOVA) were met (Shapiro–Wilk and Hartley–Bartlett tests, $p > 0.05$). As a result, CT_{max} data were analysed in STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma) using full factorial ANOVA with life stage, age sex and mating status being the categorical factors while CT_{max} was the dependent variable. Tukey-Kramer’s *post-hoc* tests were used to separate statistically heterogeneous groups.

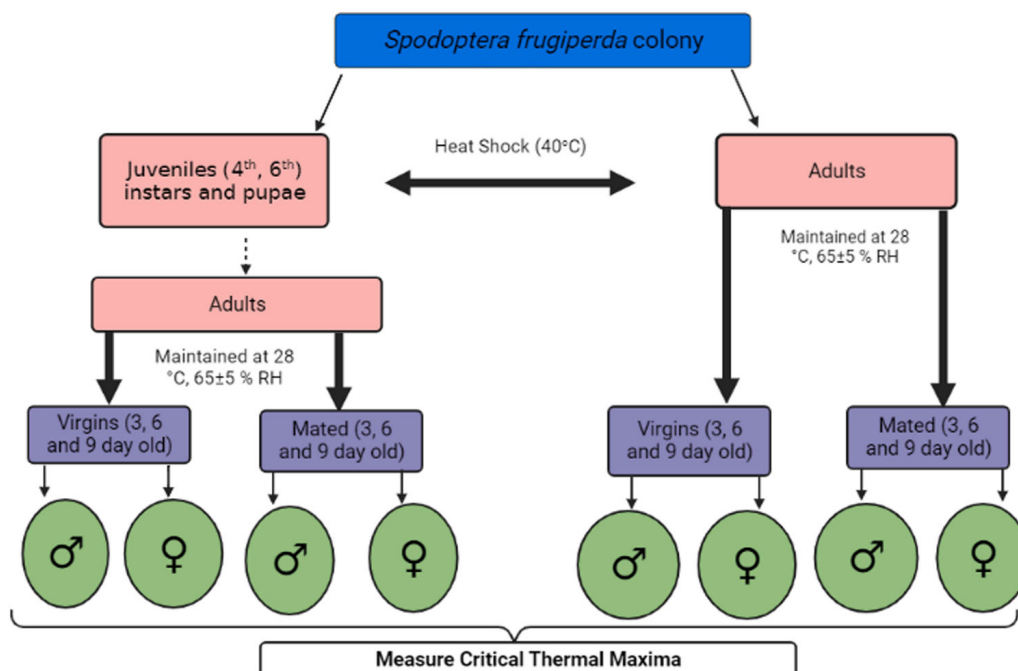


Fig. 1. Schematic representation of *Spodoptera frugiperda* heat shock assays. Juveniles (4th and 6th instar larvae and pupae) were heat shocked (40 °C for 2 h and thereafter developmentally acclimated under optimum conditions until adult stage before measuring heat tolerance (critical thermal maxima). Virgin and mated adults were also heat shocked, maintained under optimum conditions before measuring critical thermal maxima.

Results

Adult heat shock

Critical thermal maxima significantly varied across mating status, age and sex in tested insects following heat shock ($P < 0.01$) (Table 1). There was a significant interaction effect in CT_{max} between mating status and age, age and sex as well as mating status, age and sex ($P < 0.001$) (Table 1). In virgin females, CT_{max} increased with age while fluctuating in males. However, in mated females CT_{max} decreased with age and fluctuated in males (Fig. 2). There was a significant difference in CT_{max} between virgin 6 day old females and males with females recording higher heat tolerance (Mean \pm SE) (46.8 ± 0.16 °C) than males (Mean \pm SE) (44 ± 0.66 °C) (Fig. 2). However, no significant difference in CT_{max} was recorded between virgin males and females aged 9 (Mean \pm SE) (46.45 ± 1.22 °C; 47.5 ± 0.5 °C) days as well as 3, (48 ± 0.5 °C; 47.5 ± 0.33 °C) 6 (45 ± 1.5 °C; 46.5 ± 0.28 °C) and 9 (46.5 ± 0.5 °C; 46.7 ± 0.33 °C) day mated males and females (Fig. 2). The 3 day old mated females recorded the highest heat tolerance (Mean \pm SE) (46.5 ± 0.5 °C) while the 6 day old virgin males recorded the lowest heat tolerance (Mean \pm SE) (44 ± 0.66 °C) (Fig. 2). Nevertheless, the interaction between mating status and sex was not significant ($P = 0.69$) (Table 1).

Developmental acclimated adults

Critical thermal maxima significantly varied across life stages, sex and age of tested virgin insects ($P < 0.001$) (Table 2; Fig. 3). The two-way interaction between life stage and age of virgin adults had a significant difference ($P < 0.001$). However, no significant differences were recorded in the two-way interaction between life stage and sex ($P = 0.59$), age and sex ($P = 0.11$) as well as three-way interaction between life stage, age and sex of developmentally acclimated virgin adults ($P = 0.12$) (Table 2) showing no variation in thermal sensitivity between life stages. Virgin females recorded higher CT_{max} than males across all heat shocked developmental stages (Fig. 3). Critical thermal maxima of adult males and females decreased with age following developmental heat shock at 6th instar stage (Fig. 3). However, fluctuations in CT_{max} in both adult males and females were recorded following developmental shock at 4th instar and pupal stages.

In mated insects, CT_{max} varied significantly across age and sex ($P < 0.001$) (Table 3; Fig. 4). There was a significant interaction effect between life stage and age ($P < 0.001$) (Table 3). Like in virgin insects, mated females also recorded higher CT_{max} than males across all heat shocked developmental stages (Fig. 4). Critical thermal maxima of adult males and females also decreased with age following developmental heat shock at 4th and 6th instar stages with fluctuations recorded following heat shock at pupal stage (Fig. 4).

Discussion

In the present study, heat tolerance of *S. frugiperda* was influenced by age, sex and mating status in agreement with other studies, which attributed the factors as key for mediating thermal tolerance [25]. Females recorded higher CT_{max} than males following adult heat shock. This indicates that females are more heat tolerant and exhibit higher level of plasticity than males consistent with Cui et al. [53] who reported high temperature tolerance levels in *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B females than males. Our results therefore suggest that *S. frugiperda* females have a fitness and possibly survival advantage when encountering transient heat stress in nature.

An improvement in insect thermal tolerance can come at a negative cost of other physiological parameters and can be dependent on somatic condition or age [54,55]. In our study, heat tolerance in virgin females increased with age while decreasing with age in mated females suggesting a link between reproductive fitness and tolerance to heat stress but mediated by age. While the reason is currently unknown, our results underlie some trade-off between thermal tolerance and reproduction. It is however plausible that variation in energy reserves and resource allocation after mating could have influenced the tolerance to heat [56,57]. This warrants further investigation to fully elucidate this variation. Our results indicate that mating may significantly affect heat tolerance of adult *S. frugiperda* during aging. Males and females responded differently to high temperatures such that mated females had a high basal heat tolerance than virgin males. A similar result in different taxon has been reported, where mating in *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) influenced thermal tolerance such that mated females had higher basal thermal tolerance (CT_{max}) than virgins

Table 1

Summary statistical results from full factorial analysis of variance (ANOVA) showing effects of adult heat shock on thermal tolerance of virgin and mated adult *Spodoptera frugiperda*. SS = sums of squares, DF = degrees of freedom.

Effect	SS	DF	MS	F	P
Intercept	256,106.9	1	256,106.9	136,395.7	<0.001
Mating status	12	1	12	6.4	<0.01
Age	41	2	20.5	10.9	<0.001
Sex	27.3	1	27.3	14.6	<0.001
Mating status x Age	30.4	2	15.2	8.1	<0.001
Mating status x Sex	0.3	1	0.3	0.2	0.69
Age x Sex	66.5	2	33.3	17.7	<0.001
Mating status x Age x Sex	41.6	2	20.8	11.1	<0.001
Error	202.8	108	1.9		

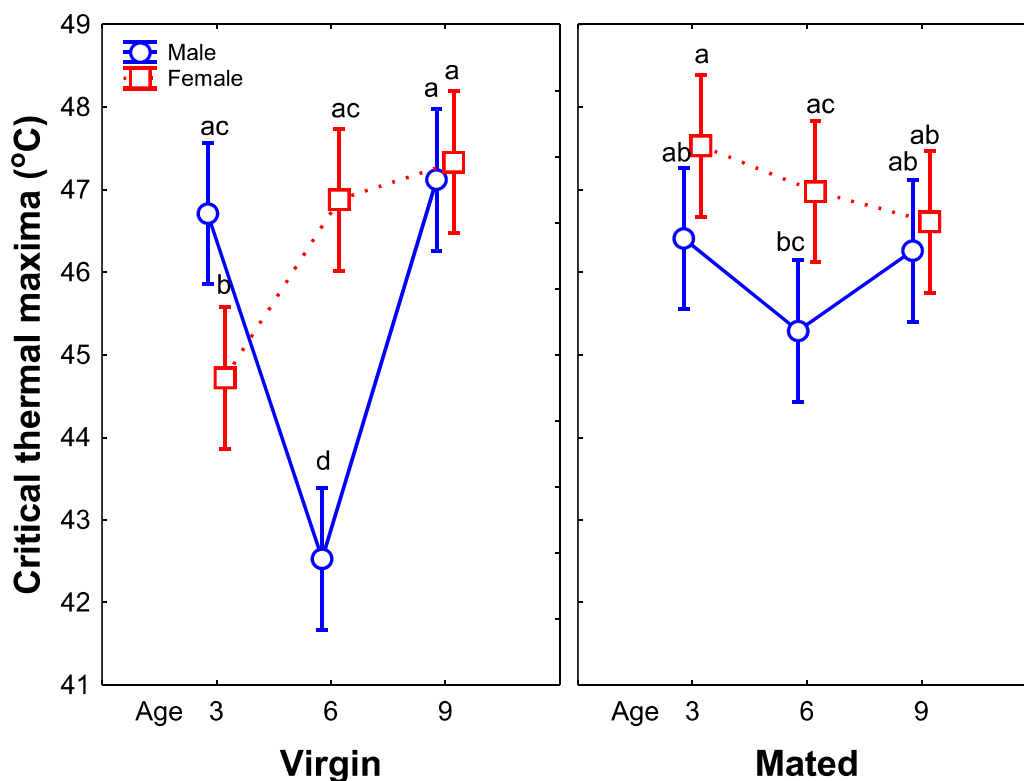


Fig. 2. Effects of heat shock (40 °C for 2 h) on basal heat tolerance (CT_{max}) of virgin and mated *Spodoptera frugiperda* adults. Error bars represent 95 % confidence limits (N = 20) and means with the same letter are not significantly different. CT_{max} = critical thermal maxima.

Table 2

Summary statistical results from full factorial analysis of variance (ANOVA) of juvenile heat shock (2 h) on thermal tolerance of virgin *Spodoptera frugiperda* adults (N = 20). SS = sums of squares, DF = degrees of freedom.

Effect	SS	DF	MS	F	P
Intercept	378,051.7	1	378,051.7	257,113.2	<0.001
Life stage	154.9	2	77.5	52.7	<0.001
Age	95.8	2	47.9	32.6	<0.001
Sex	33.	1	33.8	23.0	<0.001
Life stage x Age	125.6	4	31.4	21.4	<0.001
Life stage x Sex	1.6	2	0.8	0.5	0.59
Age x Sex	6.7	2	3.3	2.3	0.11
Life stage x Age x Sex	10.9	4	2.7	1.9	0.12
Error	238.2	162	1.5		

[12]. These results suggest that mated female adults may have evolved adaptive strategies to cope with high temperatures.

Previous studies have reported that heat stress experienced in juvenile life-stages may have variable effects on other later developmental stages [33,58–60]. In heat shocked juveniles (4th and 6th instars), CT_{max} of adult males and females decreased with age such that older adults recorded lower CT_{max} than younger ones (Figs. 3 and 4). Such trends may be associated with developmental ‘carry-over’ effects [23]. According to evolutionary theories of aging, strength of natural selection declines as organisms age since reproduction occurs at young stages [61]. In addition, as insects age, they often accumulate abnormal proteins over time which may become less functional resulting in increased susceptibility to heat stress [25,62]. Conversely, Li et al. [25] reported otherwise in older *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) adults recording higher CT_{max} than younger ones. This suggests that evolutionary responses to thermal tolerance are highly genetically conserved and species-specific. Further comparison of larval and pupal heat shocked insects in our study showed females from pupal heat shock recording higher CT_{max} than those from larval heat shock (Fig 3). This supports the notion that immobile life-stages (eggs and pupae) are more phenotypically plastic in their thermal tolerance due to limited capacity to behaviourally thermoregulate [9,63]). As a result, the pupal fitness advantage was passed to the emerging adults through cross over effects.

In virgin and mated insects, females recorded higher heat tolerance than males following larval and pupal heat shock. However, Mutamiswa et al. [58] recently reported otherwise in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) males and females following pupal heat stress. This indicates that thermal tolerance following juvenile stress may be species dependent. In addition, it indicates that females are more heat tolerant than males which, also gives them a survival advantage and population perpetuation under changing

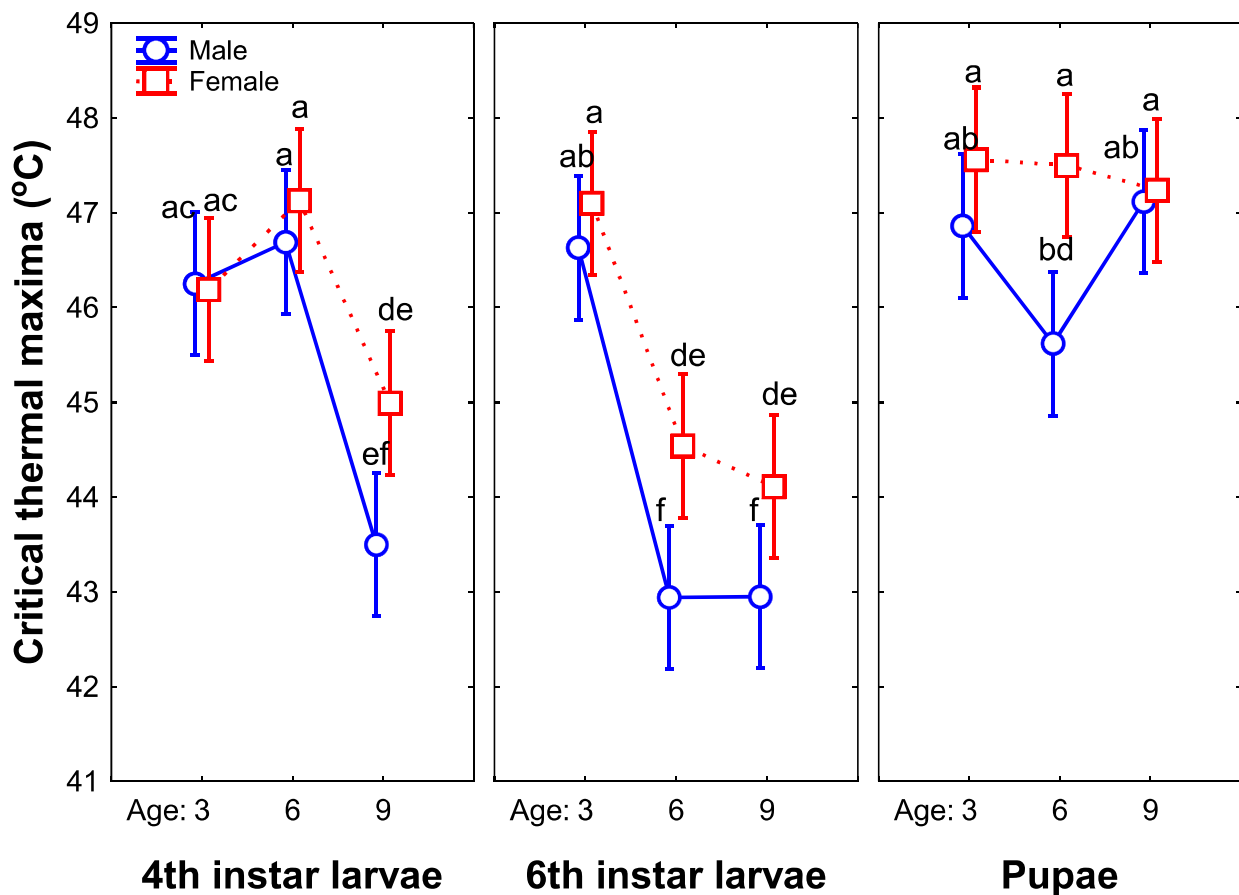


Fig. 3. Effects of juvenile heat shock (40 °C for 2 h) on basal heat tolerance (CT_{max}) of adult virgin *Spodoptera frugiperda* (N = 20). Error bars represents 95 % confidence limits. Means with the same letter are not significantly different. CT_{max} = critical thermal maxima.

Table 3

Summary statistical results from full factorial analysis of variance (ANOVA) of juvenile heat shock (2 h) on thermal tolerance of mated adult *Spodoptera frugiperda*. SS = sums of squares, DF = degrees of freedom.

Effect	SS	DF	MS	F	P
Life stage	1.6	2	0.8	0.44	0.64
Age	312.6	2	156.3	88.02	<0.001
Sex	47.2	1	47.2	26.6	<0.001
Life stage x Age	60.4	4	15.1	8.51	<0.001
Life stage x Sex	4.8	2	2.4	1.36	0.26
Age x Sex	13.6	2	6.8	3.83	0.024
Life stage x Age x Sex	7.5	4	1.9	1.06	0.38

climate.

Microclimatic field temperature data recorded in an agroecosystem where *S. frugiperda* inhabits indicate that hot days are regularly observed under field conditions (see [49–51]). These climatic conditions may be worsened by ongoing climate variabilities [54]. However, the duration of these suboptimal temperature conditions is currently unknown. As a species with overlapping and short generation, *S. frugiperda* life stages may likely experience these stressful conditions in agroecosystems. Given the differential responses of *S. frugiperda* following juvenile and adult heat shock and its capacity to tolerate high temperature conditions, it indicates that this invasive insect pest has a potential to spread and establish in most agroecosystems in sub Saharan Africa through population perpetuation under changing environment. As a result, this may negatively affect crop production and household food security. While this study focused on fixed/ static heat shock temperature and duration of exposure, future studies should also focus on dynamic fluctuating temperatures at relatively longer time scales mimicking diurnal temperature fluctuations (e.g. [24]) to investigate reversible and non-reversible physiological responses of this invasive insect pest following thermal exposure. This will aid in elucidating the fitness costs of these responses.

In conclusion, this study shows that age and mating status plays a crucial role in thermal tolerance and persistence of *S. frugiperda* under climate change. Juvenile heat shock demonstrated adult “carry over” over effects with aged adults showing a decline in CT_{max}.

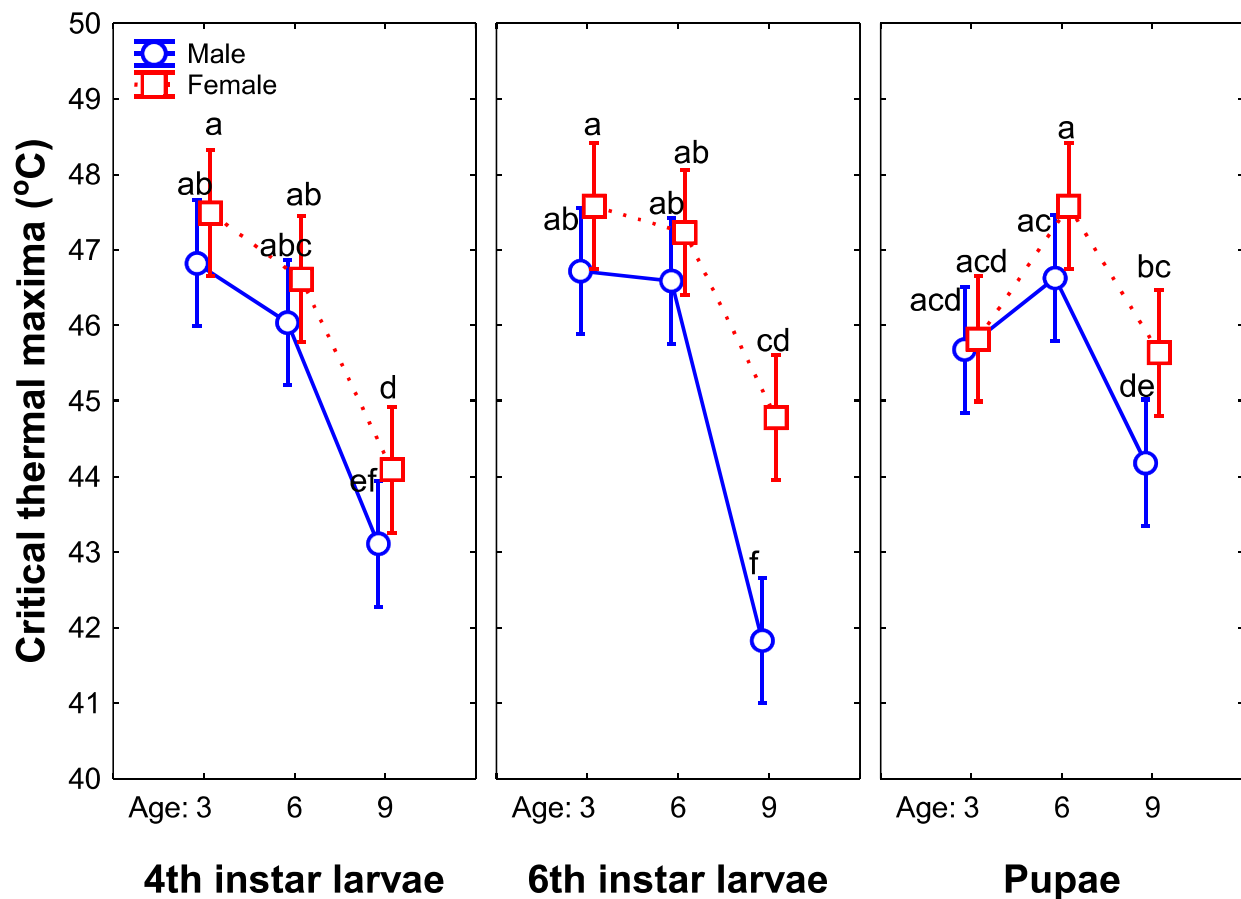


Fig. 4. Effects of juvenile heat shock (40 °C for 2 h) on basal heat tolerance (CT_{max}) of mated adult *Spodoptera frugiperda* ($N = 20$). Error bars represents 95 % confidence limits and means with the same letter are not significantly different. CT_{max} = critical thermal maxima.

ectothermic. Heat shock at pupal stage was advantageous in thermal tolerance at adult stage and females showed fitness and survival advantage when exposed to heat stress. Females fitness may show population persistence since females may continue to oviposit when males are long dead. This study suggests that plasticity of thermal tolerance may be an important mechanism for species *S. frugiperda* population persistence in novel environments

CRediT authorship contribution statement

Abongile Mbande: Data curation, Formal analysis, Methodology, Investigation, Writing – review & editing. **Reyard Mutamiswa:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Frank Chidawanyika:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition, Validation.

Declaration of Competing Interest

The authors Abongile Mbande, Reyard Mutamiswa and Frank Chidawanyika declare no conflict of interest.

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