

# Developmental diet, life stage and thermal acclimation affect thermal tolerance of the fall armyworm, *Spodoptera frugiperda*

Siphephelo M. Phungula<sup>1</sup>  | Kerstin Krüger<sup>1</sup>  | Robert S. Nofemela<sup>2</sup>  | Christopher W. Weldon<sup>1</sup> 

<sup>1</sup>Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

<sup>2</sup>Insect Ecology Division, ARC-Plant Health and Protection, Roodeplaat, South Africa

## Correspondence

Christopher W. Weldon, Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.  
Email: [cwweldon@zoology.up.ac.za](mailto:cwweldon@zoology.up.ac.za)

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## Abstract

Insect thermal tolerance affects survival and distribution of species but can vary within and between individuals due to the environmental conditions they experience. The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), recently invaded Africa and its local hotspots are associated with weather and crop phenology. We investigated the effects of larval diet (maize plants, wheat plants or chickpea-based artificial diet), life stages and acclimation temperature on thermal tolerance traits of FAW to explore how these variables might contribute to its presence in the field. First and sixth instar larvae and adults reared on each diet at 25°C were acclimated for 24 h at 20, 25 or 30°C. We then recorded the critical thermal minimum (CT<sub>min</sub>) and critical thermal maximum (CT<sub>max</sub>) of individuals. Sixth instars had the highest CT<sub>min</sub> but the effects of acclimation and diet on this trait depended on the life stage being tested. CT<sub>min</sub> of first instars increased with acclimation temperature when fed on an artificial diet, but sixth instars and adults were not affected by acclimation or larval diet. CT<sub>max</sub> was lowest among adults but acclimation and diet again had effects that differed between life stages. CT<sub>max</sub> of first instars and adults increased with acclimation temperature but not in sixth instars. Sixth instars and adults reared on the artificial diet had the highest CT<sub>max</sub> but diet had no effect on first instar CT<sub>max</sub>. Our results show the complexity of thermal tolerance across FAW life stages and suggest the need to consider local temperature variation and available dietary resources when predicting their potential distribution.

## KEYWORDS

acclimation, CT<sub>max</sub>, CT<sub>min</sub>, fall armyworm, life stages, thermal tolerance

## INTRODUCTION

Insect thermal tolerance is a trait not purely determined by genetic architecture, but also by factors that affect insect fitness such as thermal history, host nutritional quality and life stage (Huey et al., 1992; Nyamukondiwa & Terblanche, 2009; Terblanche et al., 2011). For example, acclimation temperature, a laboratory mimic of thermal

acclimatization in the field, has been widely studied for different insect species. In some cases, acclimation temperature is positively correlated with thermal tolerance (referred to as beneficial acclimation; Deere & Chown, 2006), although negative correlations and no acclimation can also be observed depending on species, and environmental and methodological conditions (Chown et al., 2009). The effects of host plant quality (Abarca et al., 2018; Kleynhans et al., 2014; Villanueva

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et al., 2011) and that of life stage (Marais et al., 2009) on thermal tolerance have been reported, but are understudied in unison and thus poorly understood. When comparing maize and sorghum as hosts, resilience to heat and cold was higher in *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) when reared on sorghum compared to maize (Mutamiswa et al., 2020). In *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), supplementing the diet with high sugar concentrations resulted in metabolic imbalances and reduced tolerance to cold stress (Colinet et al., 2013). In contrast, a diet rich in carbohydrates improved high temperature tolerance in canopy ants, *Azteca chartifex* Forel (Hymenoptera: Formicidae) (Bujan & Kaspari, 2017). A carbohydrate rich diet not only increases body glycogen content and thus acts as an energy source (adenosine triphosphate (ATP)), but it is also a water source that can be used for evaporative cooling (Gibbs et al., 1997). Thermal sensitivity and other important traits needed to respond to varied environmental conditions and may also differ between life stages of holometabolous insects, as they usually inhabit different microhabitats (Kingsolver et al., 2011). The life stages also exhibit differences in their mobility to avoid potentially stressful conditions. For example, adults may fly to suitable conditions while larvae and pupae are restricted in their range, which leads to ontogenic differences in survival.

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous migratory pest that is native to the Americas (Early et al., 2018). Until 2016, this devastating pest of many agricultural crops was restricted to its native range, apart from occasional interceptions in Europe that did not lead to population establishment (European Union, 2018). However, both the rice and maize strains (Pashley, 1986, 1988) of FAW are now established outside the Americas: populations were reported from North and West African countries in 2016 (Goergen et al., 2016), South Africa in 2017 (DAFF, 2017), India in 2018 (Kalleshwaraswamy et al., 2018) and by January 2019, the pest had been reported from Yemen (FAO, 2019a), Thailand (FAO, 2018), China (FAO, 2019b) and Australia (FAO, 2020). The relatively short generation time (Castro & Pitre, 1988), high migration potential (Johnson, 1987; Nagoshi et al., 2018; Nagoshi & Meagher, 2008) and polyphagous nature (Barros et al., 2010; Montezano et al., 2018) of FAW make it a successful invasive species.

Maize (*Zea mays* L.) is one of the host plants preferred by FAW (Montezano et al., 2018). The cultivation of this crop is strongly associated with FAW abundance in Africa (Niassy et al., 2021). In South Africa, maize is a staple crop grown in all the nine provinces (DALRRD, 2021). In some areas of the country, wheat (*Triticum aestivum* L.) is grown in the same regions as maize as a dryland crop when soil moisture is still high after seasonal rainfall or where irrigation is available (Shew et al., 2020). However, it is apparent that the continuous availability of host plants, together with overwintering conditions favourable for survival, support FAW 'hotspots' where adults can fly from one host to another (Niassy et al., 2021).

To protect crops from FAW, it is imperative to determine the ways in which environmental conditions shape FAW persistence, which includes its physiology, to underpin field observations. In this regard, temperature-dependent development of FAW in South Africa was studied by Du Plessis et al. (2020) at constant temperatures between

18 and 32°C, with egg to adult development being optimal between 26 and 30°C. Using extrapolation methods, the authors estimated the developmental threshold for eggs, larvae and pupae to be 13.01, 12.12 and 12.57°C, respectively. However, Keosentse et al. (2021) reported that third to sixth instar larvae are able to remain active below 8°C with increasing developmental stages surviving lower thermal minima. The same study reported a lack of plastic response to low thermal stress in FAW larvae. The adults had an even lower critical thermal limit of  $1.9 \pm 0.9^\circ\text{C}$ . Although the lower thermal limits for development of FAW have been studied (Foster & Cherry, 1987; Keosentse et al., 2021), upper thermal limits and other factors that affect thermal tolerance, such as developmental diet and acclimation, have not yet been reported. Furthermore, studies did not indicate which FAW strain, maize or rice, was evaluated. The strains differ in their host use and some aspects of their physiology (Ingber et al., 2021; Nagoshi et al., 2018).

In the present study, we aimed to expand current knowledge of the drivers of FAW presence by determining its tolerance to stressful temperatures. Specifically, we determined how the critical thermal limits of various life stages of FAW differ when: (a) feeding on different diets (either maize, wheat or chickpea-based artificial diet), and (b) exposed to different acclimation temperatures of 20, 25 and 30°C. Most FAW reports in sub-Saharan Africa are on maize and sorghum (Njuguna et al., 2021). Our FAW culture was established with individuals collected from maize. Based on this, we predicted that individuals developing on maize would perform better than those on wheat. We further predicted that FAW thermal tolerance would be affected by acclimation temperatures, and that effects would vary with life stage and diet. We expected thermal limits of adults to be less plastic in response to acclimation temperatures than the immature stages as flight can be used to avoid thermal stress. This study has important implications, particularly for the prediction of the potential distribution of FAW in regions where narrow grass (wheat) rather than broad grass (maize) hosts are available.

## MATERIALS AND METHODS

### Source and rearing of insects

The FAW culture used for the experiments was established with pupae sourced from the Agricultural Research Council—Plant Health and Protection, Roodeplaat campus, Pretoria, South Africa in June 2018. The original specimens for the culture were collected from an infested maize field in the Onderstepoort area of Pretoria, South Africa (latitude:  $-25.6495$ , longitude:  $28.1854$ ) in January 2017. Molecular analysis of a mitochondrial genetic marker, *CO1*, showed that the FAW culture that we used for this study contained both rice- and maize-strains, and the *Tpi* gene marker confirmed that both strains were present as well as an interstrain hybrid (Janse van Vuuren, 2021).

Rearing of FAW was done at the insectary of the Department of Zoology and Entomology at the University of Pretoria, Hatfield, South Africa. The culture was maintained in square glass cages (30 × 30 cm) kept in growth chambers under a 14 L: 10D photoperiod (cool white L18W/640) at a temperature of  $25 \pm 2^\circ\text{C}$  and

75 ± 10% relative humidity. The rearing cages had muslin cloth on the half of two opposing sides to facilitate ventilation. Adults were supplied with water and honey to feed on. Adult females were provided with wheat plants on which to lay eggs in glass cages containing pelleted vermiculite (c.1.5 cm depth) on the floor. These oviposition cages were monitored daily for egg hatch. One day after hatching, neonates were moved to new cages and provided with diets of either maize plants, wheat plants or chickpea-based artificial diet, hereafter referred to as 'artificial diet', in separate cages. The cages with maize or wheat plants were stocked with 30 neonates, whereas 25 neonates were transferred to cages with the artificial diet. Larvae that had attained a larger size were assumed to have fed on conspecifics and were excluded from experiments. Each cage contained pelleted vermiculite on the floor with a depth of 1.5 cm. Moistened cotton wool was placed on the bottom of a Petri dish to maintain humidity in cages with artificial diet. The humidity in cages with plants was 80 ± 10% relative humidity and did not require moistened cotton wool.

The maize diet consisted of yellow grain maize plants, *Zea mays* L. (hybrid KKS 4520), that were not treated with any pesticides or genetically modified. For the wheat diet, *Triticum aestivum* L. var. Duzi plants, not treated with pesticides, were used. Seeds were planted in a potting soil mixture consisting of composted bark, compost and topsoil (3:1:1; Bark Unlimited Outdoor Potting Soil) in plant pots. The plants were grown in an environment-controlled room at 24°C, 12 L: 12D photoperiod (Cool white L30W/640 and Fluora L30W/77, Osram, Germany) and 65 ± 10% relative humidity and watered three times a week. Maize seedlings at BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) growth stage 13 and wheat seedlings at BBCH growth stage 12 (Meier, 2018) were used for oviposition by females or as larval diet. Da Silva and Parra (2013) showed that cannibalism in FAW is not obligatory but happens when there is food scarcity. Consequently, maize and wheat plants were replaced regularly to avoid cannibalism among larvae reared on these diets.

The artificial diet was prepared following a modified diet from Kfir (1994). The diet included bacteriological agar (2 g) dissolved in 73 mL warm distilled water with chickpea flour (52 g), yeast (7 g), ascorbic acid (700 mg), sorbic acid (300 mg), nipagin M (methyl-4-hydroxybenzoate) (440 mg) and a phosphoric acid/propionic acid solution (48 mL). The artificial diet was prepared in a laminar flow cabinet with autoclaved and UV-treated equipment. The diet was cut into cubes (2 × 2 × 2 cm) and placed on a UV-sterilized Petri dish bottom which was then placed inside the cages. The diet was kept at 5 ± 2°C in a refrigerator until use within 3 days of preparation.

## Acclimation procedure

First and sixth instar larvae used for experiments were acclimated at either 20, 25 or 30°C for 24 h on their developmental diets within 1 day of hatching or moulting. Adults obtained from larvae reared on the three diets were also acclimated at either 20, 25 or 30°C for 24 h. The insects acclimated at 25°C were used as a control group for

ageing effects (Nyamukondiwa & Terblanche, 2010). The acclimation of insects was standardized and commenced at 12h00 ± 20 min each day so that they completed the 24 h acclimation duration at a similar time on the days of experiments. An acclimation duration of 24 h is often sufficient to acclimate insects to novel constant temperatures (Weldon et al., 2011) and longer periods of acclimation were not feasible due to the first instar being completed in approximately 3 days (Du Plessis et al., 2020). Individuals from each acclimated cohort were loaded into 10 mL glass test tubes stoppered with cotton wool and used immediately for experiments.

## Critical thermal limits

Critical thermal limit tests were used to determine the temperature at which an individual insect lost its coordinated muscle function, thereby failing to carry out basic physiological activities and behaviours. This behavioural endpoint was marked by the failure of the insect to respond to a gentle stimulation by a fine paintbrush. Each individual insect in a labelled glass test tube with no diet added was placed into a test tube rack and submerged into a programmable water bath (CC-K25, Huber Kältemaschinenbau, Offenburg, Germany). This was repeated until 10 insects per diet, life stage and acclimation temperature were tested to determine critical thermal minimum (CT<sub>min</sub>) and critical thermal maximum (CT<sub>max</sub>). One test tube without insects contained a type T (copper/constantan) thermocouple connected to a temperature data logger (0.05°C accuracy; TC-08, Pico Technology, St. Neots, UK) to measure the temperature in the test tubes. The temperature data logger was connected to a personal computer to record the temperature change during tests. Both CT<sub>min</sub> and CT<sub>max</sub> tests were started at a setpoint temperature of 25°C that was maintained for 5 min before the temperatures were ramped down (CT<sub>min</sub>) or ramped up (CT<sub>max</sub>) at a rate of 0.1°C/min. This rate of temperature change is similar to those measured in the field, as recommended by Chown et al. (2009). The temperature at which each insect lost its coordinated muscle function was recorded as its CT<sub>min</sub>/CT<sub>max</sub>. Each individual insect was used only once for each test.

## Data analyses

Data were first subjected to Shapiro–Wilk tests to check for normality. As the data were not normally distributed, we used aligned rank transformation analysis of variance (ANOVA) (Wobbrock et al., 2011) to assess the effects of life stage, diet and acclimation temperature on CT<sub>max</sub>. Where there was a significant effect, pairwise aligned rank transform contrasts were used to establish which treatments differed from each other. For all procedures, we used a statistical significance threshold of  $\alpha = 0.05$  and a medium-to-large effect size (partial eta squared;  $\eta^2 > 0.06$ ). These statistical analyses were carried out using R (version 4.2.3).

We matched our laboratory-derived measures of CT<sub>min</sub> and CT<sub>max</sub> with field temperatures. The critical thermal limits that we used were

for the most susceptible FAW life stage to cold (sixth instar larvae) and heat (adults) when reared on maize. We selected three localities in South Africa where maize and wheat are routinely cultivated on a rotational basis. Hourly records of estimated temperatures at 2 m above ground level were obtained from the National Aeronautics and Space Administration (NASA) Prediction of Worldwide Energy Resource (POWER) Project (<https://power.larc.nasa.gov>, last accessed 14 March 2022). Specifically, we obtained records between 1 January

2019 and 31 December 2021 from the following locations: Cedara Agricultural Research Station, KwaZulu-Natal province (−29.5412, 30.268); Christiana, North West province (−27.9135, 25.1668); and Groblersdal, Limpopo province (−25.1834, 29.4002). The timing of temperatures more extreme than the critical thermal limits was assessed visually to explore the potential for critical thermal limits to influence susceptibility of maize and wheat grown in each region to infestation by FAW.

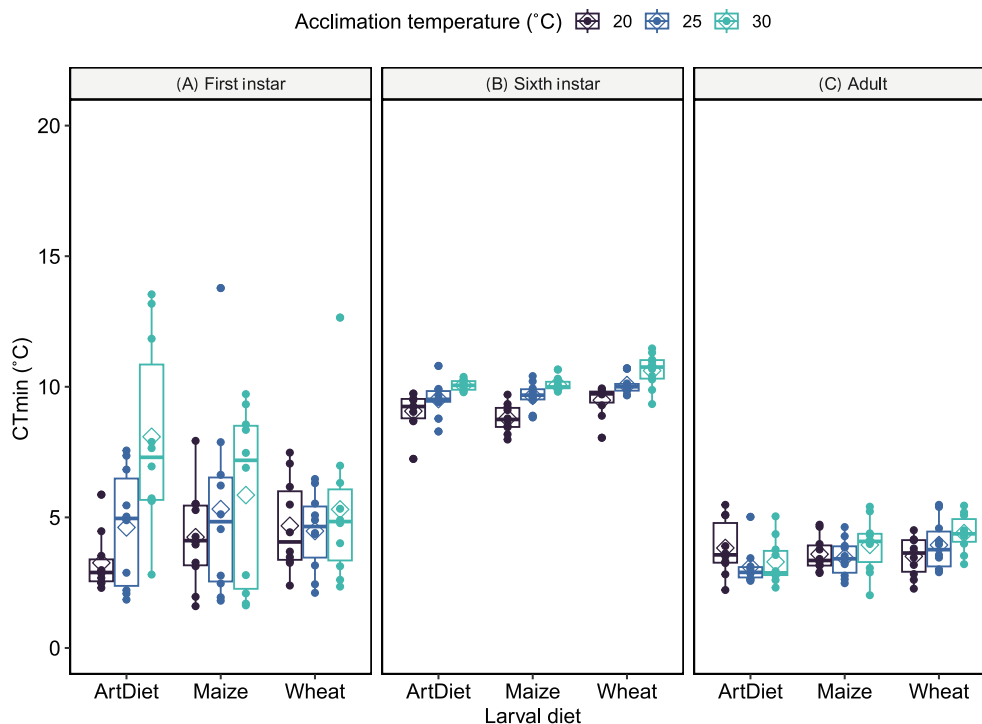
**TABLE 1** Results of aligned rank transformation analysis of variance (ANOVA) for the effects of life stage, diet, acclimation and their interactions on  $CT_{min}$  of *Spodoptera frugiperda*.

Factors	d.f.	F	p	$\eta^2$
$CT_{min}$				
Life stage	2	225.171	<0.001	<b>0.650</b>
Diet	2	1.094	0.334	0.009
Acclimation	2	25.452	<0.001	<b>0.173</b>
Life stage × Diet	4	2.243	0.065	0.036
Life stage × Acclimation	4	4.520	<b>0.002</b>	<b>0.069</b>
Diet × Acclimation	4	1.638	0.165	0.026
Life stage × Diet × Acclimation	8	3.111	<b>0.002</b>	<b>0.093</b>
Residual	243			

Note: Statistically significant  $p$ -values (<0.05) and medium-to-large effect sizes (partial eta squared;  $\eta^2$ ) are in bold type.

## RESULTS

The three-way interaction between life stage, diet and acclimation had a significant effect on FAW cold stress tolerance ( $CT_{min}$ ) (Table 1).  $CT_{min}$  differed significantly between the life stages, with sixth instar larvae being the least tolerant to cold stress overall regardless of diet and acclimation (Table 1, Figure 1). The average  $CT_{min}$  for first instars, sixth instars and adults was  $5.1 \pm 0.3$ ,  $9.7 \pm 0.1$  and  $3.7 \pm 0.1^\circ\text{C}$  (mean  $\pm$  SE), respectively. The mean difference between  $CT_{min}$  of first instars and adults was also significant. Overall,  $CT_{min}$  of FAW increased significantly with each elevation in acclimation temperature. However, when taking the significant three-way interaction into account, acclimation only influenced  $CT_{min}$  in first instar larvae that were fed an artificial diet (Figure 1a). In these larvae, there was no significant difference in  $CT_{min}$  when acclimated at 20 and  $25^\circ\text{C}$ , but those acclimated at  $30^\circ\text{C}$  had significantly higher average  $CT_{min}$ .



**FIGURE 1** Critical thermal minimum ( $CT_{min}$ ) for (a) first instar larvae, (b) sixth instar larvae and (c) adults of *Spodoptera frugiperda* developing on different diets and acclimated at different temperatures [AcclimTemp ( $^\circ\text{C}$ )]. The figures show box plots with individual data points and the mean overlaid (diamonds) for each group.

FAW tolerance to heat stress ( $CT_{max}$ ) was significantly affected by all main effects, and the interactions of life stage by diet, and life stage by acclimation (Table 2). Life stage had a particularly pronounced effect, with adults ( $44.2 \pm 0.1^\circ\text{C}$ ) being significantly less tolerant to heat stress than both first ( $48.0 \pm 0.3^\circ\text{C}$ ) and sixth instars ( $46.6 \pm 0.1^\circ\text{C}$ ). Acclimation also significantly affected  $CT_{max}$ . Individuals acclimated at  $30^\circ\text{C}$  ( $46.7 \pm 0.3^\circ\text{C}$ ) tolerated significantly higher temperatures than those acclimated at  $20^\circ\text{C}$  ( $45.7 \pm 0.3^\circ\text{C}$ ). However, life stage had a significant interaction with acclimation (Table 2; Figure 2). First instar larvae and

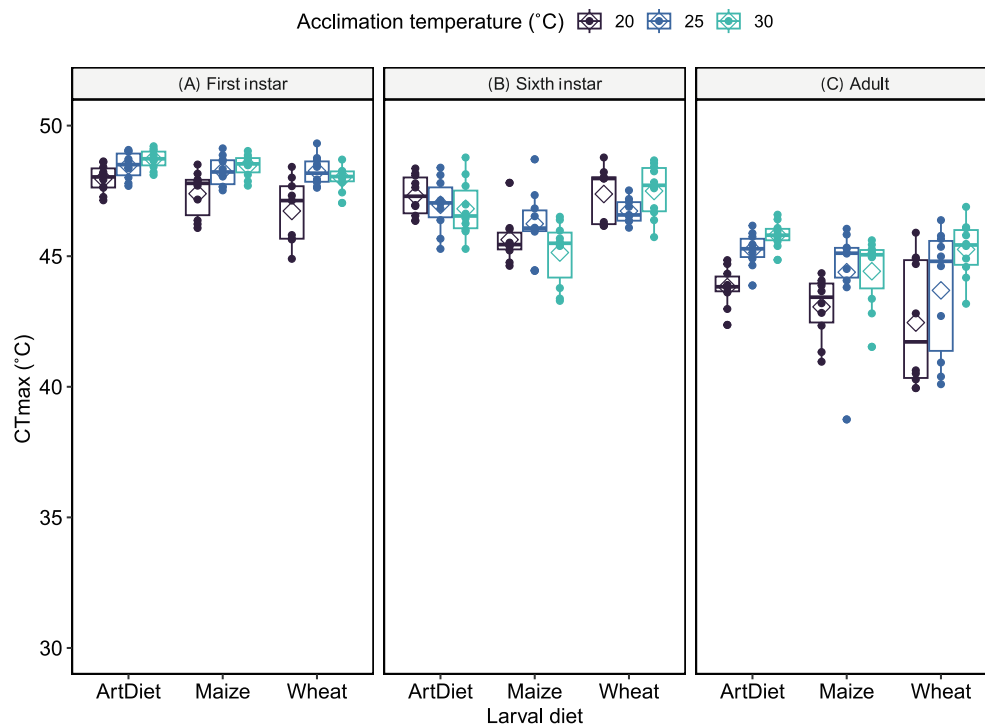
adults acclimated at  $20^\circ\text{C}$  had lower heat tolerance than those acclimated at  $30^\circ\text{C}$  but did not differ significantly with those acclimated at  $25^\circ\text{C}$ . No significant difference was observed between sixth instar larvae acclimated at the three temperatures. In addition, there was a significant interaction between life stage and diet on  $CT_{max}$ . First instar larvae fed wheat had a lower  $CT_{max}$  than those fed the artificial diet, with maize-fed larvae at this stage being intermediate (Figure 2a). Sixth instar larvae fed on maize had the poorest tolerance to heat stress compared to both artificial diet and wheat, but there was no difference between artificial diet and wheat (Figure 2b). Adults exhibited larger variation in  $CT_{max}$  than other life stages, particularly those that developed on wheat (Figure 2c).

Over the 3 years for which temperature data were obtained, there was no risk of the  $CT_{max}$  for FAW being exceeded in Cedara, Christiana or Groblersdal (Figure 3). In contrast, temperatures regularly dropped below the  $CT_{min}$  of sixth instar larvae (reared on maize) in the cooler months of the year. Temperatures were lower than the  $CT_{min}$  most often at Cedara, where the average frequency of temperatures below the threshold was  $165 \pm 9$  days (Christiana:  $137 \pm 5$  days; Groblersdal:  $86 \pm 9$  days). At Cedara and Christiana, temperatures regularly dropped below the  $CT_{min}$  in April for an average duration of  $6 \pm 1$  and  $3 \pm 1$  h, and only returned to similar conditions by October. At these sites, temperatures sometimes dropped below the  $CT_{min}$  during maize growing and harvesting. In contrast, at Groblersdal temperatures only regularly dropped below the  $CT_{min}$  between May and September, a time period that aligns with the harvesting of maize, and planting and growth of wheat.

**TABLE 2** Results of aligned rank transformation analysis of variance (ANOVA) for the effects of life stage, diet, acclimation and their interactions on  $CT_{max}$  of *Spodoptera frugiperda*.

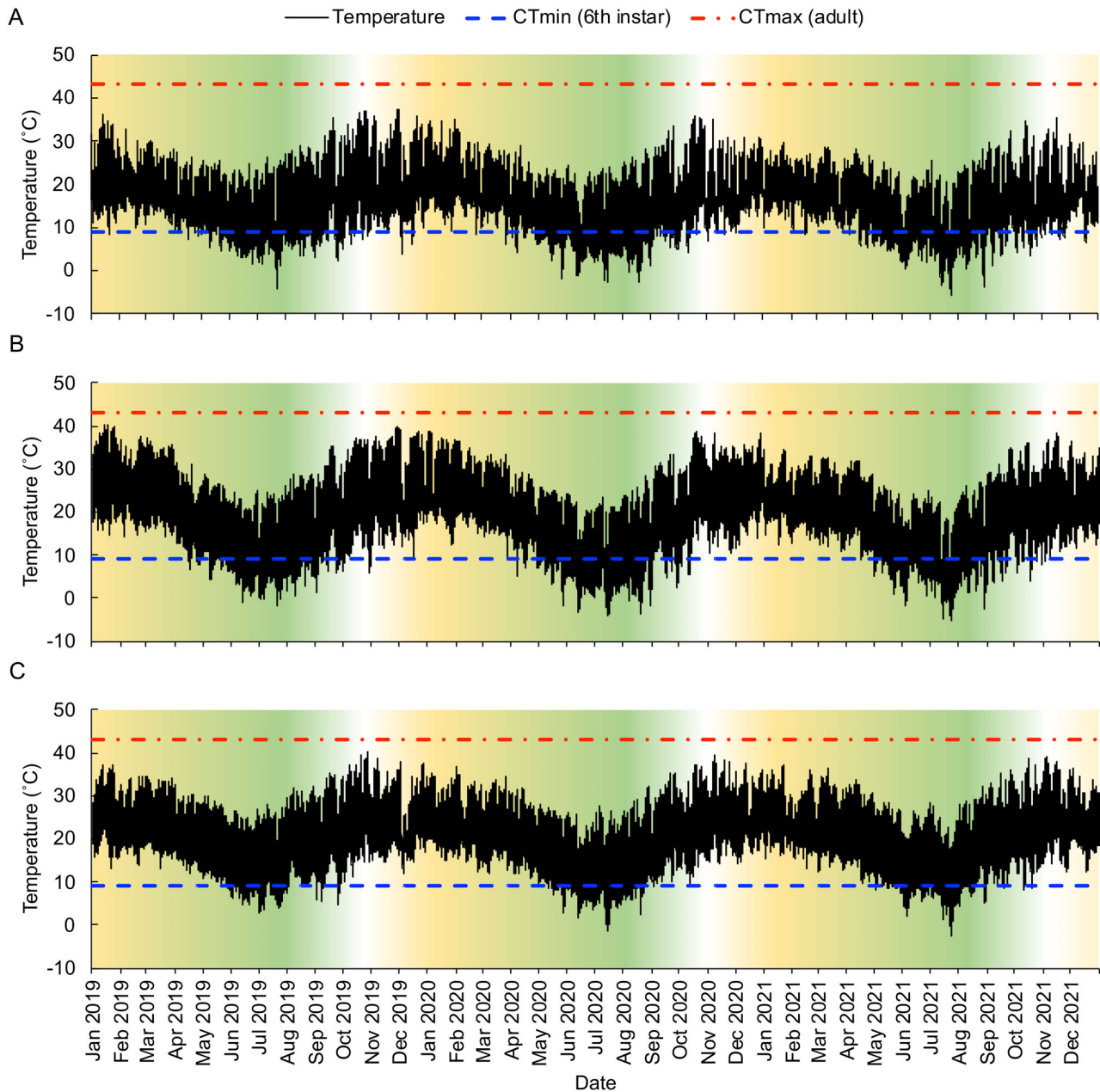
Factors	d.f.	F	p	$\eta^2$
$CT_{max}$				
Life stage	2	336.319	<0.001	<b>0.736</b>
Diet	2	18.999	<0.001	<b>0.136</b>
Acclimation	2	22.517	<0.001	<b>0.157</b>
Life stage $\times$ Diet	4	8.859	<0.001	<b>0.128</b>
Life stage $\times$ Acclimation	4	9.576	<0.001	<b>0.137</b>
Diet $\times$ Acclimation	4	2.333	0.056	0.037
Life stage $\times$ Diet $\times$ Acclimation	8	1.713	0.096	0.054
Residual	241			

Note: Statistically significant  $p$ -values (<0.05) and medium-to-large effect sizes (partial eta squared;  $\eta^2$ ) are in bold type.



**FIGURE 2** Critical thermal maximum ( $CT_{max}$ ) for (a) first instar larvae, (b) sixth instar larvae and (c) adults of *Spodoptera frugiperda* developing on different diets and acclimated at different temperatures [AcclimTemp ( $^\circ\text{C}$ )]. The figures show box plots with individual data points and the mean overlaid (diamonds) for each group.





**FIGURE 3** Hourly temperature range from (a) Cedara, (b) Christiana and (c) Groblersdal over 3 years (January 2019–December 2021). Horizontal lines represent critical thermal limits of the most sensitive life stages of *Spodoptera frugiperda* when reared on wheat:  $CT_{min}$  of sixth instar larvae (blue);  $CT_{max}$  of adults (red). Background shading represents the growing season of maize (yellow) and wheat (green) with overlap between harvesting of maize and planting of wheat tending to occur between April and June. Temperatures for each location were extracted from the National Aeronautics and Space Administration (NASA) Prediction of Worldwide Energy Resource (POWER) Project (<https://power.larc.nasa.gov>, last accessed 14 March 2022).

## DISCUSSION

The results of our study suggest that the thermal tolerance of FAW is influenced by life stage, acclimation temperature and diet. Life stage had a large effect on both  $CT_{min}$  and  $CT_{max}$ . Sixth instar larvae were the most sensitive life stage to cold stress followed by first instar larvae with the adults being the most tolerant. The opposite is true for  $CT_{max}$ , with adults being the most sensitive life stage followed by the sixth instar larvae, and the first instars being the most tolerant of high temperature stress.

Life-stage effects on thermal tolerance have been reported in FAW by Keosentse et al. (2021). These authors investigated cold stress of four larval instars (3rd, 4th, 5th and 6th) and adults. The authors reported that adults had higher tolerance to cold stress compared to all the tested larval instars. Our results support these findings. However, Keosentse et al. (2021) also reported an increase in cold tolerance in later instars. Their results contrast with those from our study. Not knowing on which FAW strain the study of Keosentse et al. (2021) was based, we suggest that the poor cold tolerance in

sixth instars found in our study with the inter-strain population could be due to the energetic costs needed for pupation, as this is the last life stage just before the non-feeding pupal stage begins. Other studies with species from a range of insect orders have shown, like ours, that cold tolerance decreases with later life stages. For example, Klokk and Chown (2001) observed that adult *Paractora dreuxi* Seguy (Diptera: Helcomyzidae) had significantly higher  $CT_{min}$  (lower tolerance to cold stress) than the larval stages. They also showed that adults had lower  $CT_{max}$  (lower tolerance to heat stress) than the larval stages. In mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), adult beetles were more tolerant to high temperature stress than either larvae or pupae (Vorhees & Bradley, 2012).

Variation in thermal tolerance between life stages can be attributed to multiple factors, including the cost of mechanisms to avoid extreme temperatures, morphological and physiological distinctness of each life stage and the microhabitat each life stage inhabits (reviewed by Bowler & Terblanche, 2008; Kingsolver et al., 2011). In our study, high tolerance to cold stress exhibited by adult *Spodoptera frugiperda* could be because they have evolved mechanism/s to tolerate cold stress during migration from areas experiencing cold winter conditions to milder climates to start a new population (Johnson, 1987; Nagoshi et al., 2012). It may also be that the adults are mainly nocturnal and so have evolved to tolerate cooler conditions experienced at night. Smith (1957) proposed that two types of acclimations exist in adult insects. Firstly, physiological acclimation attained during the adult stage and attributed to temperature regimes experienced during this stage. Physiological acclimation is not innate and lasts only for as long as the new thermal conditions persist (Chown & Terblanche, 2006). It is unlikely that adult *S. frugiperda* use this mechanism for  $CT_{min}$  as they showed little plasticity in response to acclimation temperatures. Secondly, there is a more fixed form of acclimation, termed developmental acclimation, which is influenced by the temperatures experienced during the pre-adult stages. Determining the form of acclimation was beyond the scope of this study, but it needs to be investigated in the future, especially for migratory insects like the FAW as the adults, as well as the different strains, may inhabit geographically different habitats to juvenile stages.

Several studies attributed the often-observed trend of decline of heat tolerance in later stages to increased mobility across ontogeny (Bowler & Terblanche, 2008; Marais et al., 2009; Vorhees & Bradley, 2012). This allows the winged adults to behaviourally compensate for stressful temperatures by escaping to avoid heat stress with ease. For  $CT_{max}$  in the current study, adults were the most sensitive to heat stress perhaps because they may avoid heat stress behaviourally by flying to less stressful environments. However, adult FAW showed plasticity in response to acclimation as the  $CT_{max}$  of adults acclimated at 30°C was 2.1°C higher than those acclimated at 20°C. This indicates that adults use different stress responses to heat and cold stress (e.g., behavioural for  $CT_{min}$  and both physiological and behavioural for  $CT_{max}$ ).

The high tolerance of first instar larvae to heat stress could be attributed to physiological characteristics of this life stage. The first instar larvae feed on the surface of the leaves where temperatures are high (Kingsolver et al., 2011). Although first instars are able to escape unfavourable conditions through silking to some extent, they

may have evolved physiological mechanisms to mitigate heat damage better than sixth instars and adults. It is also important for first instars to acclimatize to variation in temperatures as the leaf may experience larger fluctuations in temperature within and among days than other plant parts. This could explain the observed plasticity in thermal tolerance of first instars: acclimation at 30°C led to an average  $CT_{min}$  of 6.9°C compared to an average  $CT_{min}$  of 5.6°C for 20°C acclimation and 6.0°C for 25°C acclimation. Similar plasticity to thermal acclimation was observed for the  $CT_{max}$  of first instars; acclimation at 20, 25 and 30°C led to an average  $CT_{max}$  of 47.4, 48.3 and 48.4°C, respectively. Unlike first instars, which feed on the plant surface, sixth instars burrow into plant parts. Sixth instars normally feed in the maize whorl or under the covering sheath of cobs, which have a high moisture content. Because water has a high heat capacity, the temperatures in these plant structures seldom reach temperatures equal to that of the leaf surfaces and therefore the late instars are usually protected from high temperatures (Kingsolver et al., 2011). This could explain the lack of significant thermal acclimation in heat tolerance in sixth instars observed in this study. Moreover, the sixth instars are close to metamorphosis, and energy may be invested in metamorphosis rather than in acclimating to varying temperatures.

Diet may influence stress tolerance mechanisms by functioning as a source of nutrients needed in biosynthesis of protective products. For instance, carbohydrates and proteins have been reported to affect thermal stress tolerance in some species. In *D. melanogaster*, adults feeding on carbohydrate-enriched diet were more tolerant to cold stress compared to those on a protein-enriched diet (Andersen et al., 2010). In the same study, *D. melanogaster* fed with the protein-enriched diet had improved heat stress tolerance compared to those on the carbohydrate-enriched diet. However, carbohydrates improved  $CT_{max}$  in canopy ants, *A. chartifex* (Bujan & Kaspari, 2017). It is therefore possible that the observed heat stress tolerance (high  $CT_{max}$ ) in the sixth instar FAW larvae feeding on artificial diet in this study is due to high protein (up to 25%) and carbohydrate (up to 49%) contents of the chickpea flour (Rachwa-Rosaik et al., 2015), which was the main ingredient of the diet. It also appears that this effect continues to the adult stage. A high protein larval diet has also been observed to improve adult thermal tolerance in *D. melanogaster* (Andersen et al., 2010) and newly emerged *Ceratitidis cosyra* (Pullock et al., 2023). In the case of first instar larvae, where  $CT_{max}$  was high but not affected by diet, it may be that high heat stress tolerance is aided by the emerging neonates consuming the protein-rich egg shells (Capinera, 2017; Sparks, 1979), thereby adding protein to the diet. The large variation in  $CT_{min}$  of first instar larvae (range of 12.2°C, compared to 4.2°C for sixth instars, and 3.5°C for adults) may also be because neonates consume different quantities of eggshells (Luginbill, 1928).

There is a relationship between physiological limits, like critical thermal limits, and the ability of insect species to survive and reproduce in an area. For example, Alruiz et al. (2022) found that heat tolerant *Drosophila* species sampled along a latitudinal gradient in Chile have a wider distribution range than cold adapted ones, and population growth estimates generally aligned with species thermal tolerance. While their definition of  $CT_{max}$  differs from ours, with an emphasis on thermal death rather than cessation of activity, the high  $CT_{max}$  measured in this

study means that FAW is unlikely to encounter acute stressful high temperatures in the maize and wheat producing areas of South Africa. Given the availability of maize in summer, wheat in cooler months, as well as other cereal grasses and the wide dietary breadth of FAW, the pest could achieve a wide distribution. Cold temperatures are more likely to limit FAW in South Africa; the  $CT_{min}$  of FAW is not low enough to remain active during winter temperatures in the maize and wheat growing regions of Cedara, Christiana and Groblersdal. However, in other areas at lower altitude or latitude with warmer winters where maize can be grown, FAW populations may persist and make use of available hosts to breed. For example, the relatively milder conditions in the Groblersdal area, with less frequent and shorter periods below  $CT_{min}$ , may provide an opportunity for some life stages to take advantage of wheat and other cereal grasses as hosts to survive to the next season. These areas with milder winter temperatures could act as a reservoir for the pest to re-establish, as has been found in East Africa (Niassy et al., 2021). These predictions need to be tested by pairing field observations of FAW presence and abundance on cereals with the incidence and duration of temperatures more extreme than the critical thermal limits that we report here.

## CONCLUSIONS

The current study highlights that the evaluation of thermal tolerance should be done across life stages and researchers should be critical of using thermal biology data from one life stage and assume its relevance for all individuals and life stages. Even though acclimation did not affect critical thermal limits of all life stages, there was a general pattern of beneficial acclimation in most cohorts. The mechanism for this thermal plasticity is not fully known but points to differences among life stages and, to some extent, capacity to respond to cold and hot conditions. The study further suggests that the diet is an important factor that can confound thermal biology studies if not considered, even for a polyphagous pest like FAW. Such information aids in understanding ecological and evolutionary responses of FAW to varying and extreme climatic conditions, and how the pest may perform in different regions or times of the year when the host plants cultivated differ.

## AUTHOR CONTRIBUTIONS

**Siphephelo M. Phungula:** Formal analysis; investigation; writing – original draft; data curation. **Kerstin Krüger:** Conceptualization; funding acquisition; writing – review and editing; supervision; resources. **Robert S. Nofemela:** Conceptualization; investigation; writing – review and editing; supervision. **Christopher W. Weldon:** Conceptualization; formal analysis; writing – review and editing; visualization; methodology; supervision; project administration; resources.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.25403/UPresearchdata.19615191>, reference number 19615191.

## ETHICS STATEMENT

None.

## ORCID

Siphephelo M. Phungula  <https://orcid.org/0000-0002-2486-9080>

Kerstin Krüger  <https://orcid.org/0000-0001-6357-1589>

Robert S. Nofemela  <https://orcid.org/0000-0002-6935-9519>

Christopher W. Weldon  <https://orcid.org/0000-0002-9897-2689>

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