

**Fig. 3** Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media three months following infestation (n=4).

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## *Sitotroga cerealella* (Olivier) resilience to extreme temperature and desiccation may explain its increasing pest status in changing climates

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DOI 10.5073/jka.2018.463.040

## Abstract

The mechanisms underlying *Sitotroga cerealella* survival under variable and increasing mean thermal and desiccation environments typical under global change is currently unknown. To understand how *S. cerealella* survives extreme abiotic stressors typical of stored-grain environments, we measured *S. cerealella* tolerance temperature and desiccation. The results showed that to survive desiccating grain storage environments, *S. cerealella* relied more on high body water content (BWC) ( $70.2 \pm 3.72\%$ ) compared to lipid reserves ( $9.8 \pm 0.81\%$ ). In desiccating environment, *S. cerealella* showed a reduced water loss rate ( $0.056\text{mg/h}$ ) (equivalent of  $1.81\%$  of body water/hour) which would require 19.31 h to reduce the insect body water to its critical minimum ( $35.23\%$  body water content at death), which is  $50.20\%$  of normal initial body water. Similarly *S. cerealella* exhibited high basal heat tolerance with critical thermal maximum of  $46.09 \pm 1.042^\circ\text{C}$  and a heat knockdown time of  $7.97 \pm 1.64$  minutes. Basal cold tolerance was relatively compromised (critical thermal minima of  $4.52 \pm 1.06^\circ\text{C}$  and chill

coma recovery time of  $5.80 \pm 1.17$  minutes), following 1h at 0°C. We found no significant correlation ( $P > 0.001$ ) between BWC and the measured thermal tolerance traits. Low water loss rates reported here may be an evolutionary resistance mechanism for desiccation tolerance. Observed abiotic stress tolerance may explain the ubiquitous distribution of *S. cerealella* in Africa which is likely to enhance its survival and increase its pest status under global change.

**Keywords:** storage insect pests, abiotic stress, thermal tolerance, desiccation tolerance, stored cereal grain, stress tolerance mechanisms.

## 1. Introduction

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) is a cosmopolitan primary coloniser of cereal grains in warm regions of the world (Hansen et al., 2004; Bushra and Aslam, 2014, Demissie et al., 2014). It is a dominant component of the cereal grain pest complex typical in small-scale farmers' stores along with *Sitophilus* species, *Prostephanus truncatus* (Horn.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) (Hansen et al., 2004). It is one of the most problematic pests of sorghum (Mvumi et al., 2003; Mubayiwa et al., 2018) which is the second most important cereal in sub-Saharan Africa (SSA) (Mubayiwa et al., 2018). The high pest status of *S. cerealella* stems from its larval internal kernel feeding habit which minimises contact with insecticides (Bushra and Aslam, 2014). Moreover, this internal feeding has been reported to contribute to insecticide resistance (Bushra and Aslam, 2014). Tunnels made by the larvae result in grain quantitative and qualitative losses, exposure to secondary pests and microbial attack (Akter et al., 2013). In addition, the feeding frass and scales from dead moths reduce the aesthetic value and hence economic value of the grain. In consequence, it is imperative to understand its ecological characteristics specifically abiotic stress responses to enable the development of alternative non-chemical control methods. The current cereal grain storage methods by small-scale farmers in developing countries (Nukenine, 2010; Nyagwaya et al., 2010) promote the unabated proliferation of *S. cerealella*, making it one of the most abundant problem pests especially in small grains (Mvumi et al., 2003; Hansen et al., 2004). However, to-date no study has focussed on the abiotic stress tolerance of *S. cerealella*, especially temperature and desiccation, two environmental stressors mainly used as proxy to determining insects' survival (Kelley 2014) and potential pest status.

Most terrestrial arthropods including insects are vulnerable to extreme temperature stress (Chown and Nicholson, 2004) and water loss due to their high surface area to volume ratios (Gibbs, 1997; Weldon et al., 2013, 2016). Different species have developed different mechanisms to enhance dehydration tolerance to survive low relative humidity environments (Weldon et al., 2013) such as stored grain habitats. We hypothesize that *S. cerealella*'s ability to withstand desiccation and extreme temperatures typical in tropical climates where it is dominant, likely contributes to its enhanced survival and hence, continued increase in pest populations and grain damage. The objectives of the current study were therefore to determine (1) *S. cerealella* heat and cold tolerance in comparison to like species and in relation to prevailing tropical temperatures; (2) determine whether *S. cerealella* desiccation survival is due to high lipid or body water storage or both and how water loss control may contribute to the moth's desiccation survival.

## 2. Materials and Methods

### 2.1 Insect rearing and handling

Insects were reared in the Eco-physiological Entomology Laboratory at Botswana International University of Science and Technology. Field-collected moths were placed in sterilized maize grain (-15°C for 14 days followed 7 days of preconditioning at room temperature of  $25 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$  RH) in  $35 \times 35\text{cm}^2$  bugdorm insect cages (BugDorm®, MegaView Science Co., Ltd. Taiwan). The bugdorms were placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) maintained at  $25 \pm 1^\circ\text{C}$ , 10:14 day and night photoperiod, and  $65 \pm 10\%$  RH. The moths were fed on 10% sugar solution through the dental-wick method (Shelton et al., 2012) and supplied with randomly folded black-dyed filter paper for egg laying and resting. After 7 days, the moths were

removed from the grain leaving grain and filter papers with eggs. F<sub>1</sub> hatched larvae were allowed to feed on the grain until adult stage. Unsexed 2-3 day old F<sub>1</sub> adult moths were used for the experiments. To reduce mortality, moths damage and excessive loss of scales, resting moths were carefully handled by individual trapping into 0.6 ml polypropylene eppendorf tubes with minimum direct contact.

## 2.2 Body water and lipid content (BWC and BLC)

To determine Body water content (BWC), 50 moths were collected from the bugdorms and weighed (RADWAG<sup>®</sup>, Wagi Elektroniczine, Model As220.R2, Radom, Poland) to 0.0001 g in 0.6 ml numbered and uniformly perforated polypropylene eppendorf tubes. These were placed on eppendorf-tube-holders and transferred to a laboratory oven (UF160, Memmert GmbH + Co.KG, Germany) set at 60°C for 48 h. Moths were re-weighed after 48 h and the BWC was calculated as the difference between the initial and the final body mass. (Lease and Wolf, 2010)

For Body lipid content (BLC), 50 moths were individually placed in uniformly perforated, pre-weighed and numbered 0.6 ml polypropylene eppendorf tubes. These were dried in an oven (UF160, Memmert GmbH + Co.KG, Germany) at 60°C for 48 h. The moths were immediately weighed after drying to determine dry weight and transferred to unperforated 2ml eppendorf tubes containing 1.5ml diethyl ether. The tubes were gently agitated at 37°C for 24 h before the insects were removed and re-dried in the oven (60°C) for 24 h (Lease and Wolf, 2010). After drying, the moths were weighed and the BLC was calculated as the difference between the initial insect dry mass and the final (lipid-free) dry mass.

## 2.3. Water loss rates (WLR)

Desiccation tolerance experiments were conducted following standard protocols (Gibbs, 1997; Weldon et al., 2013). After initial mass of the moths were recorded, 50 moths were individually placed in numbered 0.6 ml uniformly perforated polypropylene eppendorf tubes. The tubes were placed in small loose granules of desiccant (Drierite, W.A. Hammond Drierite Co. Ltd, Xenia, USA) with < 10% RH at 25°C in a large airtight laboratory glass bowl. Vials were inspected every 3 h and mortality was recorded.

## 2.4. Critical thermal limits (CTLs)

Critical thermal limits (CT<sub>min</sub> and CT<sub>max</sub>) were measured using a protocol developed by Nyamukondiwa and Terblanche (2010). Ten individual moths were placed in a series of test tubes floating in insulated double-jacketed chambers or "organ pipes" connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol and subjected to a constant cooling or heating rate. Moths were first given 10 minutes to equilibrate at 25°C before the temperature was ramped up or down for CT<sub>max</sub> or CT<sub>min</sub> respectively at a three different rates; 0.12, 0.25 and 0.5°C/min. This was repeated three times to yield sample sizes of n = 30 individuals per treatment. A thermocouple (type T 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the middle (control) test tube to monitor temperature. In the current study, CT<sub>max</sub> and CT<sub>min</sub> were defined as the maximum or minimum temperature, respectively; at which each individual moth lost coordinated muscle function, following mild prodding with a thermally inert object (see Nyamukondiwa and Terblanche, 2010).

## 2.5. Chill coma recovery (CCRT) and heat knockdown time (HKDT)

CCRT and HKDTs were conducted following a method developed by Weldon et al. (2011). For CCRT, moths were individually placed in 0.6 ml eppendorf tubes and then loaded into a large zip-lock bag which was subsequently submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol mixture, was set at 0°C for 1 hour. After 1 hour of exposure, the tubes

were removed from the water bath and transferred to a Memmert climate chamber set at 25°C, 65% RH for moth recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer where observations were recorded. In this study, CCRT was defined as the time (in minutes) taken by an individual moth to recover and stand upright on its legs. For HKDT, ten moths were individually placed in 0.6 ml eppendorf tubes and placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at  $48 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  RH. Observations were recorded as explained for CCRT. In this study, HKDT was defined as the time (in minutes) at which moths lost activity following exposure to high (48°C) temperature. Both CCRT and HKDT were repeated three times ( $n = 30$  individuals moths).

## 2.6. Data analysis

Data analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma). For CTLs, ramping rate was used as a factor in one-way Analysis of Variance (ANOVA) and statistically significant means were separated using Tukey-Kramer's post-hoc test. However, for BWC, WLR, LC, HKDT and CCRT mean values were presented and compared to like species, e.g *Plutella xylostella* (L.) and *Tuta absoluta* (Meyrick).

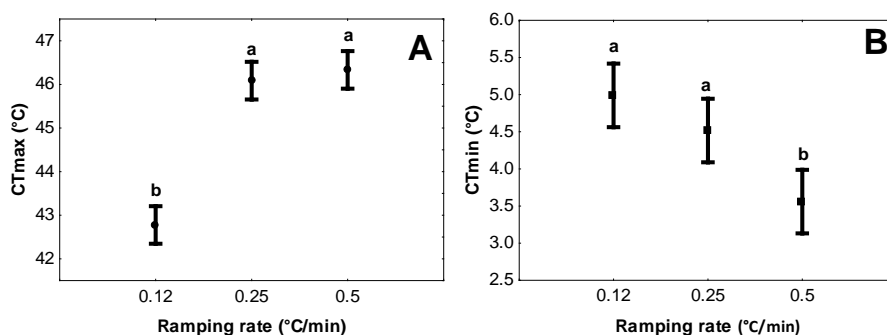
## 3. Results

### 3.1. Body water content, water loss rates, water loss at death and body lipid content

*Sitotroga cerealella* had a mean body water content (BWC) of  $1.80 \pm 0.517$  mg with an average body mass (BM) of  $2.61 \pm 0.691$  mg resulting in 70.2% BWC. The water loss rates of 0.056 mg/h (1.81% BW/h) were low, showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment ( $\leq 10\%$  RH) to reach its critical body water at death, 35.23% of initial body water. There were no significant correlation ( $P > 0.001$ ) between BWC and thermal traits (CTLs, HKDT and CCRT). The BLC of ( $9.8 \pm 0.81\%$ ), was relatively low compared to BMC indicating that lipids were unlikely used as a source of metabolic water.

### 3.2 Thermal tolerance (CTmax, CTmin, HKDT and CCRT)

At a benign ramping rate of  $0.25^\circ\text{C}/\text{min}$ , *S. cerealella* had a CTmax of  $46.1 \pm 1.04^\circ\text{C}$  which was significantly higher ( $F_{(2, 87)} = 83.921$ ,  $P < 0.0001$ ) than the CTmax at lower ramping rate ( $0.12^\circ\text{C}/\text{min}$ ),  $42.8 \pm 1.19^\circ\text{C}$  (Fig 1A). However, higher ramping rate ( $0.5^\circ\text{C}/\text{min}$ ) did not have a significant effect on CTmax (Fig. 1A).



**Fig. 1** The critical thermal limits (CTLs) for *S. cerealella* at different ramping rates: (A) critical thermal maxima (CTmax); and (B) critical thermal minima (CTmin).

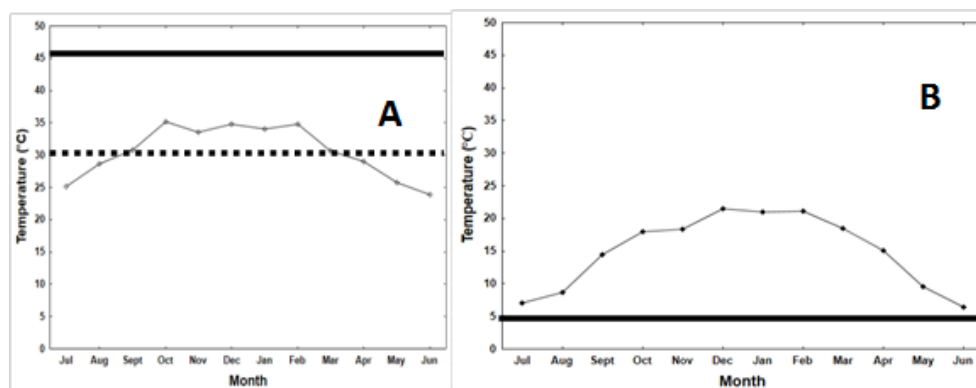
Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. At the benign ramping rate of 0.25°C/min, *S. cerealella* showed a CTmin of 4.5±1.06°C, which was significantly higher ( $F_{(2,87)} = 11.443, P = 0.0004$ ) than the CTmin at a higher (0.5°C/min) ramping rate (3.6±0.98°C) (Fig. 1B). However, lower ramping rate (0.12°C/min) did not have a significant effect on CTmin (Fig. 1B).

**Tab. 1** Thermal traits of *S. cerealella* compared to like species.

Insect species	CTmax (°C)	CTmin (°C)	HKDT (min)	CCRT (min)	Reference
<i>S. cerealella</i>	46.1±1.04	4.5±1.06	7.9±1.64	5.8±1.17	Current study
<i>Plutella xylostella</i>	46.6±0.52	-3.2±0.41	3.8±0.65	2.48±0.40	Machekano et al., 2018a
<i>Tuta absoluta</i>	44.1±0.43	-5.2±0.23	*	*	Machekano et al., 2018b

\*Denotes that data on that thermal trait were not available.

Table 1 shows the comparison of thermal traits between *S. cerealella*, *P. xylostella* and *T. absoluta*. The CTmax is comparable to *P. xylostella* and *T. absoluta* (Machekano et al., 2018a & b, upcoming). Compared to other economic Lepidopterans *S. cerealella* shows almost double the time (7.9±1.64 minutes) needed to be knocked down by heat stress (HKDT) compared to *Plutella xylostella* (3.8±0.65 minutes). Its CTmin (4.5±1.06°C) however, was higher (4.5±1.06) than *P. xylostella* (-3.2±0.41°C) and *T. absoluta* (-5.2±0.23°C) (Table 1). In addition, the chill-coma recovery time (CCRT), was almost two-fold that of *P. xylostella* (Table 1). To understand whether *S. cerealella* was likely to cope with prevailing field temperatures, we compared its critical thermal limits to field meteorological data for the 2015-2016 seasons (Fig. 2).



**Fig. 2** (A) The mean maximum field temperature data in Botswana (2015-16) (black curved line) compared to *S. cerealella* CTmax (black solid line) and optimum temperatures (black dotted line), (B) the mean minimum field temperatures (black curved line) compared to *S. cerealella* CTmin (black solid line).

The difference between the highest recorded temperature (35.2°C) (October, 2015) and *S. cerealella* CTmax was ~10°C (Fig. 2A), evidence of a very high thermal safety margin (Deutsch et al., 2008) for *S. cerealella* considering a 30.0°C optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella*'s CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

#### 4. Discussion

*Sitotroga cerealella*'s high BMC (70.2%) of total body mass, was comparable to desiccation-resistant *Drosophila* species observed by Gibbs and Matzkin (2001). This entailed that *S. cerealella* likely used high body water storage to tolerate low relative humidity, typical of grain environments to survive desiccation. This is so because, high levels of internal body water content extends the time required for dehydration to critical levels that would induce mortality. The observed low water loss rates showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment to reach its critical body water at death. This suggests that, *S. cerealella* likely uses two mechanisms; high body water and low water loss rates to tolerate desiccation in dry stored-grain habitats. The exact physiological mechanisms used by *S. cerealella* to reduce water loss need further investigation. Lack of significant correlation ( $P > 0.001$ ) between BWC and thermal traits (CTLs, HKDT and CCRT) further suggests that high body water content solely played a significant role in desiccation tolerance. This is supported by the relatively low body lipids ( $9.8 \pm 0.81\%$ ), which explains that lipids were unlikely to be a source of metabolic water but probably energy for this species (Arrese and Soulages, 2010). Demissie et al. (2014) reported that low relative humidity did not have a significant effect on the growth and development of *S. cerealella* except egg hatching, suggesting that apart from the egg stage, all stages of *S. cerealella* are capable of tolerating desiccation.

Our data and previous reports suggest that *S. cerealella* is heat-tolerant. Its CTmax is comparable to the thermally resilient *P. xylostella* (Machekano et al., 2018a) and the invasive *Tuta absoluta* (Machekano et al., 2018b, upcoming). However, on the low temperature scale, *S. cerealella* showed compromised CTmin ( $4.5 \pm 1.06^\circ\text{C}$ ) compared to like species. The time taken by the moths to recover from chill-coma was almost two-fold that of *P. xylostella*. Both CTmin and CCRT responses suggest limited low temperature tolerance for *S. cerealella*. High temperature tolerance likely explains why it is a major pest in the warm tropical climates especially SSA (Hansen et al., 2014; Bushra and Aslam, 2014) as similarly reported for fruitflies (Nyamukondiwa et al., 2010) and stemborer species (Mutamiswa et al., 2017).

Only low ramping rate had an effect on CTmax, but higher ramping rate ( $0.5^\circ\text{C}/\text{min}$ ) did not significantly shift the CTmax. This result suggests the inability of *S. cerealella* to adjust its CTmax in the short-term or rapid heat hardening but only in the long term. Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. On the low side of the temperature scale, only the higher ramping rate ( $0.5^\circ\text{C}/\text{min}$ ) significantly depressed the CTmin. This result suggests faster ramping rates enhanced low temperature tolerance, measured as CTmin. This plastic effect has been observed in similar Lepidopterans (Machekano et al., 2018a and b) and likely facilitates adaptation to novel but stressful environments (Nyamukondiwa et al., 2010). Compared to other economic Lepidopterans, *S. cerealella* showed two-fold time needed to be knocked down by heat stress compared to *Plutella xylostella* further attesting its thermal resilience on the higher side of the temperature scale.

The difference of  $\sim 10^\circ\text{C}$  between the highest recorded field temperature and *S. cerealella* CTmax, is evidence of a very high thermal safety margin (Deutsch et al., 2008) from its  $30.0^\circ\text{C}$  optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella*'s CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

We conclude that *S. cerealella* uses high body water storage and low water loss rates to tolerate desiccation in low humidity stored grain habitats. *Sitotroga cerealella* shows high basal heat but not cold tolerance, and coupled with potential plasticity and behavioural regulation, this may aid its survival under abiotic stressful environments. Grain cold treatment may be used as an effective pest control method against *S. cerealella*.

## Acknowledgements

We would like to acknowledge Botswana International University of Science and Technology (BIUST) for funding this work

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