SHORT, NON-REFEREED PAPER

UNDERSTANDING THE RECENT INVASION OF *CACOSCELES NEWMANNII* (COLEOPTERA: CERAMBYCIDAE) INTO SUGARCANE FROM A THERMAL PERSPECTIVE

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Abstract

The recent invasion of the indigenous longhorn beetle *Cacosceles newmannii* (Coleoptera: Cerambycidae) into sugarcane of KwaZulu-Natal has resulted in widespread concern surrounding its pest status and further potential for spread and damage in surrounding sugarcane crops.

Its dramatic host shift from indigenous vegetation can be attributed to several potential mechanisms, including abiotic and biotic factors. The thermal limits to activity and survival of larvae and adults in a set of laboratory experiments are examined, and a predominant hypothesis about the cause of high temperature death (i.e. oxygen limitation theory) is tested. We also report on mortality rate across a range of temperatures of *C. newmannii* on sugarcane and artificial diet. Thermal trait information is then integrated with what is known about sugarcane environments in KwaZulu-Natal and supply the first step in forecasting the potential spread of this longhorn beetle.

Keywords: Cacosceles newmannii, thermolimit respirometry, critical temperature, OCLTT theory, crop pest, stress tolerance

Introduction

Temperature is a key environmental driver of insect population dynamics and distributions, since it directly affects survival, developmental rate and reproduction (Angilletta, 2009), as well as locomotor activity and access to resources. Characterising thermal tolerance is therefore crucial to understand emerging pests' biology and to adapt management measures. Moreover, survival to stressful temperature may be a limiting factor of invasion into novel environments. One hypothesis that aims to explain ectotherm's thermal tolerance is the theory of oxygen- and capacity-limited thermal tolerance (OCLTT; Pörtner, 2001) that states that the capacity of an animal to supply oxygen to tissues is constrained by the mismatch between oxygen supply and demand at temperatures approaching limiting values (e.g. Giomi *et al.*, 2014; Pörtner and Giomi, 2013). If this hypothesis is correct, its primary prediction is a reduction in thermal tolerance when oxygen supply is reduced, but also a decrease in the aerobic scope when the temperatures approach the critical limit (Verberk *et al.*, 2016).

We used a previously unexamined Cerambycid species belonging to a group expected to have hypoxia-adaptations owing to its saproxylic nature, *Cacosceles* (*Zelogenes*) *newmannii* (Thomson, 1877). This longhorned beetle species native to South Africa has recently undergone a host shift into sugarcane, which causes important economic losses for the growers. It was investigated whether oxygen availability influenced the maximum temperature withstood by adults and larvae under normoxia and hypoxia. Also investigated was larval survival on different diets and across different temperatures.

Materials and Methods

Thermolimit respirometry

C. newmannii larvae and adults were collected in the Entumeni area of KwaZulu-Natal sugarcane farms and kept individually at 25°C in a 16 L: 8 D regime with food provision.

Specimens were placed in flow-through chambers to perform thermolimit respirometry in order to assess thermal limits under normoxia (21 $kPaO_2$) and hypoxia (2.5 $kPaO_2$, balance N_2) following methods adapted from Boardman and Terblanche (2015). All individuals were weighed pre- and post-respirometry. The CO_2 production was recorded differentially ($\dot{V}_{CO2}$) in ppm. Animals were given a 15-minute period of equilibrium temperature of 25°C to settle, after which the temperature was ramped up at a rate of 0.06°C.min^{-1} to a maximum of 50°C. The critical maximum temperature, $\dot{V}_{CO2}$ CT$_{max}$, was defined as the point at which spiracles switched from high to low variability (Boardman and Terblanche, 2015). $\dot{V}_{CO2}$ CT$_{max}$ and absolute and factorial aerobic scopes were computed according to Boardman and Terblanche (2015).

To test the differences in supply and demand, ANOVAs were made with the CT$_{max}$ predictions of larvae versus adults under hypoxic and normoxic conditions and a general linear models were used to test the effect of mass on $\dot{V}_{CO2}$ CT$_{max}$.

Larval survival

Field-collected larvae were kept in the lab at 15, 25 and 30°C on two different diets in order to investigate a potential effect of diet on survival rate. Larvae were kept either on surgarcane, or on artificial diet adapted from the recipe used for other Cerambycids. In parallel, other larvae were kept individually in jars at 30°C for 45 days to test for their tolerance to desiccation and starvation. They were either deprived of water, using silica gel, or of food. Survival was checked daily in all cases.

Results

Absolute and factorial aerobic scopes as well as maximum metabolism were significantly higher for adults than for larvae (Table 1) even after mass adjustment.

Table 1. Summary data (mean and s.e.m) for adults and larvae under hypoxia and normoxia and results of statistical comparison between adults and larvae. Sample sizes are given between brackets. Significant comparisons between adults and larvae appear in bold.

		Mean mass (g)	Metabolism Max (ml.h-1)	$\dot{V}CO2$ CTmax (C°)	Absolute aerobic scope (ml h-1 g-1)	Factorial aerobic scope (ml h-1 g-1)
Larvae	Normoxia	1.99 ±1.01 (9)	1.71 ± 1.57 (8)	46.00 ± 0.85 (8)	1.19 ± 1.23 (8)	38.12 ± 18.92 (8)
	Hypoxia	2.61 ± 1.10 (13)	1.70 ± 1.32 (11)	42.21 ± 1.57 (11)	0.94 ± 0.70 (11)	2.38 ± 1.02 (11)
Adult	Normoxia	1.32 ± 0.51 (14)	5.95 ± 2.65 (11)	45.55 ± 1.32 (10)	16.12 ± 34.64 (12)	167.18 ± 165.85 (12)
	Hypoxia	1.13 ± 0.46 (8)	1.15 ± 0.64 (7)	43.77 ± 1.90 (7)	2.38 ± 1.48 (7)	49.64 ± 57.18 (7)
Comparison between adults and larvae		**F(1,37)= 14.888, p<0.001**	**F(1,34)=10.478, p=0.00269**	Z=-1.067, p=0.286	**F(1,34)=25.040, p<0.001**	**F(1,33)=10.503, p=0.00272**

$\dot{V}_{CO2}$ CT$_{max}$ under different conditions is summarised in Table 1. Modifications of CT$_{max}$ between normoxia and hypoxia depended on the life stage (F(1,32)=4.224, p=0.040). However, hypoxia significantly decreased $\dot{V}_{CO2}$ CT$_{max}$ for both adults and larvae (F(1,17)=38.033, p<0.001 for larvae; F(1,15)=5.2419, p=0.037 for adults) but did not alter the

maximum metabolism. CT_{max} mean values were always higher than average temperature values in the Entumeni region (Figure 1).
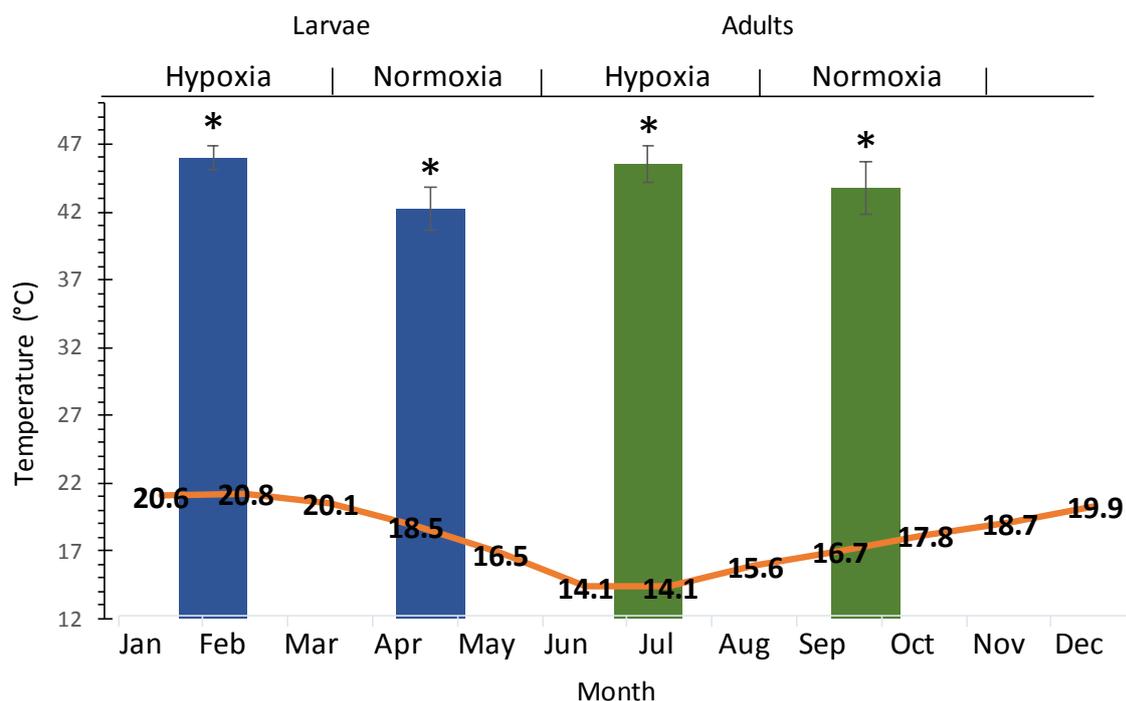

Figure 1. Mean plot of CT_{max} for larvae (blue) and adults (green) and mean monthly temperatures in Entumeni (orange, collected from https://en.climate-data.org/).

Comparisons of diet revealed that larvae survived equally well on artificial diet and sugarcane at 15 and 30°C (even if slightly better on artificial diet). At 25°C, however, 65% of the larvae were still alive after 232 days on sugarcane, and only 18% were alive on the artificial diet. After 45 days of experiment, 70% of the starved larvae and 74% of the desiccated were still alive.

Discussion

Understanding pest population dynamics is crucial to set up appropriate integrated pest-management programs. This study provides essential baseline data on *C. newmannii* tolerance to its environment. Indeed, despite some pilot studies *C. newmannii* biology is still mainly unknown (Way *et al.*, 2017).

Adults and larvae live in potentially contrasted environments, notably in terms of temperature and oxygen availability. Adults can benefit from mobility to avoid thermal stress, whereas larvae seem more constrained by their environment, which could have increased their thermal tolerance (i.e. Marais and Chown (2008) for flies). However, larvae and adults did not show significant differences in terms of CT_{max}, unlike many other insect species (Boardman and Terblanche, 2015; Javal *et al.*, 2016; Jensen *et al.*, 2007; Bowler and Terblanche, 2008). Similarly, larvae had a lower aerobic scope (i.e. an excess capacity to deliver oxygen to tissues, Verberk *et al.*, 2016). This may suggest that the larval environment is not stressful enough to trigger physiological adaptations that would enhance thermal and hypoxia tolerance.

Observed CT_{max} revealed that heat tolerance of *C. newmannii* was consistent with values commonly observed on other Coleoptera species (e.g. Chidawanyika *et al.*, 2017; García-Robledo *et al.*, 2016, Vorhees and Bradley, 2012). CT_{max} values and mean monthly temperatures in Entumeni revealed that *C. newmannii* would probably not be limited in its

expansion by high temperatures. Starvation and desiccation result also pointed out that the larvae and adults were considerably resistant to environmental stress.

Finally, comparison of diets seem to indicate that larvae survive equally well on sugarcane and on artificial diet. The notable exception observed at 25°C is most probably due to diet contamination that was not observed at other temperatures. The use of artificial diet will eventually considerably facilitate the maintenance of colonies in the laboratory and will therefore improve experiments planning.

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