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Reduced mobility but high survival: thermal tolerance and locomotor response of the specialist herbivore, *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae), to low temperatures

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Abstract

Disentangling the responses of insects to variations in their thermal environment is central to our understanding of the evolution of temperature-dependent performance in these species. Here, we report results of experiments examining the effects of high (upper lethal temperature = ULT) and low (lower lethal temperature = LLT) temperature and exposure time on the survival of larvae and adults of a multivoltine, nocturnal moth species, *Pareuchaetes insulata*, a biological control agent whose impact on an invasive weed, *Chromolaena odorata* has been variable in South Africa. The influence of temperature and acclimation on locomotion performance of the moth was also investigated. Temperature and duration of exposure significantly affected survival of both adults and larvae of *P. insulata* with more extreme temperatures and/or longer durations proving to be more lethal. Third instar larvae and adults are both freeze intolerant and had LT_{50} of −5.9 and −4.7°C, respectively, after a 2 h exposure. Although cold acclimation was beneficial to the nocturnal larvae, temperatures below 10°C significantly reduce their locomotion activities. The average daily minimum temperatures in the coldest months at three locations in South Africa are over 5°C lower than those of Fort Lauderdale, Florida, USA, where *P. insulata* was originally collected. Our results suggest that lethal high or low temperatures at short timescales are trivial in explaining the variable performance of *P. insulata*, but reduced locomotion at sub-lethal temperatures may be an important driver of the population dynamics of the biocontrol agent (especially in winter months) and may consequently explain the low population levels of the moth because of possible reduced feeding by larvae during night-time low temperatures.

Keywords: *Pareuchaetes insulata*, locomotion performance, acclimation, lethal temperatures, biological control agent, *Chromolaena odorata*

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Introduction

Ambient temperature is a key environmental factor influencing a variety of aspects of animal ecology and evolution, especially for ectotherms due to their limited

thermoregulatory capacity. For example, insect performance traits such as development rates, fecundity, longevity, and mobility (Bale *et al*., 2002; Hughes *et al*., 2004; Tamiru *et al*., 2012; Ferrer *et al*., 2014; Watt *et al*., 2016) coupled with physiological and biochemical processes (Hochachka & Somero, 2002; Denlinger & Lee, 2010; Terblanche, 2013) are strongly affected by ambient temperatures. Insect response to temperature extremes is thought to be a key driver of life cycle events, species geographic distribution and population dynamics in time and space (Terblanche, 2013). At longer time scales, temperature not only influences survival but also seasonal and evolutionary processes (Denlinger & Lee, 2010) resulting in variation of life history traits and the development of phenotypic plasticity (Chown *et al*., 2007; Régnière *et al*., 2012; Ferrer *et al*., 2014). The ability of temperature to influence survival and activities (e.g., locomotion, feeding) at short time scales is also of critical importance (e.g., Li *et al*., 2011; Esterhuizen *et al*., 2014). Empirical evidence suggests that insect species have an optimal temperature range (where performance reaches a maximum); outside of this range, performance rapidly decreases (e.g., development is slowed, mobility is reduced, see Wu *et al*., 2013; Esterhuizen *et al*., 2014; Hough-Goldstein *et al*., 2016) and mortality may occur due to alterations in physiological and metabolic activities (Angilletta *et al*., 2002; Martin & Huey, 2008; Angilletta, 2009).

Understanding variation in insect thermal tolerance is important in the applied context (e.g., Terblanche, 2014; Zhao *et al*., 2015). The predictions of biological response to temperature variation are used in population forecasting models (Terblanche *et al*., 2008; Zhao *et al*., 2015; Hough-Goldstein *et al*., 2016), which influence pest management decisions such as whether to release certain biological control agents or to integrate conventional control methods with biological control. About 40% of weed biological control agents (usually insects) fail to establish while a number of others perform poorly in their introduced ranges (McEvoy & Coombs, 2001). Some of these failures can be attributed to climate incompatibility, especially with low temperatures (McClay, 1996; Byrne *et al*., 2002, 2003; May & Coetzee, 2013). Therefore studies on thermal tolerance and locomotion performance of the specialist herbivore, *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae: Arctiinae) [released in South Africa (between 2001 and 2009 for the biological control of the invasive alien weed, *Chromolaena odorata* (L.) King and Robinson (Asteraceae)] are not inconsequential.

Following the release of over 1.9 million individuals of different life stages of the moth (from Florida – USA, Cuba and Jamaica), only the Floridian population is thought to have established (880 000 individuals of different life stages), at only one site, after it was released at some 21 sites in KwaZulu-Natal province (north-eastern South Africa), South Africa (Zachariades *et al*., 2011).

Prior to the release of *P. insulata*, two related species, *Pareuchaetes pseudoinsulata* Rego Barros and *Pareuchaetes aurata aurata* (Butler) failed to establish, while other potential biocontrol agents failed to survive or breed at all (reviewed in Zachariades *et al*., 2011). Following the establishment of *P. insulata* in South Africa, two population outbreaks were recorded in 2005 and 2014 (Strathie & Zachariades, 2014). However, the population levels of the moth in the field remain generally low. Although egg predation by ants was initially thought to be responsible for the establishment failure of *P. pseudoinsulata* (Kluge, 1994), temperature (climate incompatibility) may also have played a role. The low populations of *P. insulata* could also be due to temperature incompatibility (Uyi *et al*., 2016*a*) or their response to lethal or sub-lethal temperatures over long periods or at short time scales. To our knowledge there are no published studies on the thermal tolerance and the effect of temperature on the locomotion ability of *P. insulata.* Therefore, knowledge of the effects of temperature on the activity of *P. insulata* can assist in determining the effects of sub-lethal temperatures on biological activities such as feeding by the larvae of the moth. Such a study may be helpful in terms of (i) understanding the establishment failure of the moth at some release sites, (ii) understanding the poor performance of the moth, (iii) predicting the potential distribution of and the impact of the moth in relation to the South African climate and (iv) predicting more suitable release sites.

Because temperatures that are lethal to insects are a function of both the degree of the temperature variation and the duration of exposure (Chown & Nicholson, 2004; Li *et al*., 2011), this study investigated a range of time-temperature combinations, which may be lethal at short time scales in larvae and adults of *P. insulata*. A further objective of this study was to investigate the effect of acclimation (thermal history) on the locomotion performance of the larvae of *P. insulata*, because the relationship between ambient temperatures and insect performance has been previously reported to be dependent on thermal history at several timescales in some insects (e.g., Deere & Chown, 2006; Esterhuizen *et al*., 2014). Similarities or differences in seasonal temperature patterns between the collection and release sites (introduced range) can help explain the success or poor performance of biological control agents (Hopper & Roush, 1993; Byrne *et al*., 2003). In order to determine whether climate incompatibility is a primary factor responsible for the poor establishment success or variable performance of *P. insulata* in South Africa, we compared climate data (daily minimum and maximum temperatures averaged per month) between Fort Lauderdale, Florida, USA, where *P. insulata* was collected and locations (Durban South, Umkomaas, and Thohoyandou) in South Africa where the host plant of the moth is invasive.

Materials and methods

Study organisms

Chromolaena odorata is an invasive perennial shrub native to the Americas from southern USA to northern Argentina (Gautier, 1992). In its invasive range, *C. odorata* grows in a wide range of vegetation types such as forest margins, grasslands, roadsides, agricultural lands, and disturbed forests posing a significant threat to agriculture, biodiversity and livelihoods (see reviews in Zachariades *et al*., 2009; Uyi *et al*., 2014*a* and references therein). The shrub can grow 2–3 m in height. Prolific flowering peaks in December to January in the northern hemisphere and June to July in the southern hemisphere.

The southern African biotype of *C. odorata*, which originated from Cuba or Jamaica, is morphologically and genetically distinct from the more widespread biotype (Asian/West African biotype) invading Asia, Oceania and West, East and Central Africa (Zachariades *et al*., 2009; Paterson & Zachariades, 2013). The weed was first recognized as naturalized in KwaZulu-Natal (KZN) province, South Africa, in the 1940s from where it spread to other climatically suitable provinces and neighbouring countries (Zachariades *et al*., 2011). The shrub has been declared a weed (category 1b) under the National Environmental Management: Biodiversity Act of

South Africa, because of its invasiveness in the north-eastern parts of the country.

Pareuchaetes insulata is a multivoltine moth that is active all year round with no known period of dormancy. The female adult lays its eggs in batches on the underside of the leaves of *C. odorata*. The eggs hatch after ±4.8 days at 25°C (Uyi *et al.*, 2014*b*). At 25°C in the laboratory, the early instars (1st, 2nd, and 3rd instars) feed during day and night by scraping the epidermal layer of *C. odorata* leaves. The 4th, 5th, and 6th instar larvae are strictly nocturnal. Feeding by 3rd to 6th instar larvae is aggressive and characteristic, often leaving only the mid-rib of the leaf. The older larvae feed on foliage of *C. odorata* during the night, quit the plant foliage at or soon after sunrise and spend the day hidden in leaf litter at the base of the plant. Late 5th and 6th instars of *P. insulata* migrate down the base of the *C. odorata* plants and pupate in a flimsy cocoon in the leaf litter. Development time from larva to adult (at 25°C) usually ranges from 26 to 42 days depending on food quality (Uyi *et al.*, 2014*b*, 2015).

While the moth did establish at a single release site (after it was released in multiple locations in South Africa), its population in the field is usually extremely low (Zachariades *et al.*, 2011) making it a difficult candidate to study in a field situation. Occasional recovery and outbreaks of the moths are thought to be influenced by variation in temperature and quality of *C. odorata* foliage amongst other factors (Uyi, 2014). Here, experiments were conducted to understand aspects of the thermal physiology of the moth.

Origin and maintenance of plant and insect cultures

Pareuchaetes insulata individuals were collected in the Sappi Cannonbrae plantation, Umkomaas, South Africa (30°13′S, 30°46′E), where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.*, 2011). The individuals of the moth were maintained in a temperature-controlled room for about two generations on *C. odorata* bouquets from the field (in and around Durban) at 25 ± 2°C, 65 ± 10% relative humidity (RH), with a photoperiod of L12 : D12. Newly hatched larvae were fed with cut leaves inside 700 ml (1 egg batch per container) aerated plastic containers, while older larvae were reared inside 2 L 'Freezette' rectangular plastic trays (32 × 22 × 6 cm^3) (30 larvae per tray) with *C. odorata* bouquets. Fresh leaves were added as needed. Pupae were placed in 2 L Freezette trays containing vermiculite and monitored for eclosion.

Thermal tolerance (effects of lethal temperatures and exposure time on survival)

The lower lethal temperature (LLT) and the upper lethal temperature (ULT) for adults and 3rd instar larvae were assessed using a standard 'plunge' protocol (e.g., Sinclair *et al.*, 2006; Terblanche *et al.*, 2008). Adults (males and females combined) and larvae of moths used were reared under controlled constant conditions (25 ± 2°C, 70 ± 10% RH; 12L : 12D). Thermal tolerance was measured as a proportion of insects surviving after exposure to a constant temperature for a fixed period of time, over a range of experimental temperatures using a circulating programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany). The water bath was filled with 90% ethanol to allow for sub-zero temperature use without freezing.

For ULT and LLT trials, 60 ml glass vials (sealed at the top with cotton wool) containing either individuals of 2-day old moths (for the adult trials) or 3rd instar larvae (for larval trials) (4 in each vial × 10 vials = 40 individuals per temperature treatment) were placed inside a plastic zip lock bag placed inside the programmable water bath and were subjected to temperature treatments for a fixed time period (0 h = control group, 0.5, 1, 2, and 4 h) (for rationale see Chidawanyika & Terblanche, 2011). The range of conditions tested always encompassed the full range of moth survival from 0 to 100% covering a temperature range of −10 to 0 and 25–43°C for LLT and ULT trials, respectively. For the ULT trials, the vials were lined with moistened filtered papers to maintain humidity. A fine type-T thermocouple connected to a digital thermometer (TECPEL 305, Taiwan, ±0.1°C accuracy) was regularly used to monitor the temperature inside the vials and the water bath to ensure that the desired temperature during treatments was achieved and maintained. Following treatments, the moths (adults or larvae) were removed from the water bath and placed inside a 100 ml plastic container with a circular net screen window (2.5 cm diameter on the top for ventilation) and lined at the bottom with a moistened filter paper to maintain humidity. The moths were either provided with 50% honey solution or excised leaves depending on their life stage. The containers were then placed inside a growth chamber (Labcon, South Africa) set at normal rearing temperature (25°C) and survival was scored after 24 h. Survival was considered as a coordinated response to gentle stimuli (e.g., normal behaviour such as walking, feeding or flying for adults upon gentle prodding).

Locomotion performance trials

Day-old 3rd instar larvae of *P. insulata* were obtained from a colony maintained at 25°C as described above. Some of these larvae were placed in 2 L Freezette rectangular plastic trays (32 × 22 × 6 cm^3) (about 100 larvae per tray) with *C. odorata* bouquets and placed inside a growth chamber set at the rearing temperature (25°C) for the warm acclimation treatment, while for the cool acclimation treatment, larvae inside 2 L Freezette trays were placed in a growth chamber set at 10°C. The actual temperature and humidity experienced by the larvae in each chamber was recorded at hourly intervals using data loggers (Hygrochron iButtons, model DS 1923, Maxim Integrated Products, San José, CA, USA, 0.5°C accuracy) attached to the inside wall of each tray (hourly readings for warm acclimation treatment: range, 24.53–25.47°C; mean ± SE, 24.71 ± 0.01°C; RH: range, 68.3–77.6%; mean ± SE, 71.2 ± 2.1%; hourly readings for cool acclimation treatment: range, 9.68–10.58°C; mean ± SE, 10.43 ± 0.01°C; RH: range, 66.5–75.8%; mean ± SE, 70.1 ± 1.9.1%). The chosen acclimation temperatures represent the range of conditions likely to be encountered by the larvae in the various release sites in KZN province. The two sub-colonies were exposed to their new environment for 48 h (2 days) before the start of the experiment to ensure acclimation. Previous work on insects has indicated that a 1- to 7-day acclimation period is sufficient for the full change of phenotype to be realized (e.g., Hoffmann & Watson, 1993; Weldon *et al.*, 2011).

The influence of temperature and acclimation temperature on locomotion performance of *P insulata* larvae was investigated by recording the proportion of warm-acclimated and cold-acclimated 3rd instar larvae capable of walking when exposed to a range of temperatures (four test temperatures;

6, 11, 15, and 20°C) and the time spent moving during each 30-second exposure (for rationale, see Boiteau & Mackinley, 2012). We did not perform trials at temperatures higher than 20°C because 100% locomotion activity was recorded at 20°C. Also, higher temperatures are less likely to influence poor performance of biological control agents in South Africa (Byrne *et al.*, 2003). To ensure that the test temperature was kept constant, the experiments were done on a 0.60 m^2 temperature controlled stage (with an aluminum floor) connected to a programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany). Larvae were allowed to equilibrate for 2 mins prior to estimating the locomotion parameters of interest. A fine type-T thermocouple connected to a digital thermometer (TECPEL 305, Taiwan, ±0.1°C accuracy) was placed on the floor of the stage to ensure that the desired test temperature was achieved and maintained. Five larvae were individually exposed to a particular test temperature on the stage for 30 s and the number of insects mobile at each temperature, and the time spent walking during each exposure, was recorded with an electronic stop watch. This observation (data from five larvae) constituted one replicate and a total of five replicates were used for each treatment temperature (or exposure) (i.e., 25 larvae were used for each treatment temperature). Relative humidity was at 100% at the surface of the stage because of condensation at the cold surface of the aluminium plate.

Microclimate and macroclimate data

Hygrochron iButtons (Hygrochron iButtons, model DS 1923, Maxim Integrated Products, San José, USA, 0.5°C accuracy) were used to record microclimate temperatures and relative humidity at 1-hour sampling frequencies at three locations in Sappi Cannonbrae Plantation (*P. insulata* established site), near the coastal town of Umkomaas, South Africa over a period of 13 months (between June 2013 and June 2014). At each site, one iButton (suspended 60 cm above ground level within *C. odorata* thickets) was used to record climate data. The iButtons were placed inside 100 ml screw-top plastic containers with circular screen windows and holes around the container. This was done to prevent biased temperature readings and to easily allow air flow. The iButtons were never placed in direct sunlight and were protected from direct rainfall. The reason for suspending the iButtons was to determine or record climate data at the feeding microsite because larvae feed on the leaves of the plant.

Five years (2008–2012) annual weather data (average daily minimum and maximum temperatures within the month) for Durban South, KZN province and Thohoyandou, Limpopo province in South Africa, were obtained from the South African Weather Service. Similarly, 5 years (1996–2000) annual weather data (average daily minimum and maximum temperatures within the month) for Fort Lauderdale, Florida, USA were obtained from the Florida Climate Centre (https://climatecenter.fsu.edu/climate-data-access-tools/downloadable-data).

Statistical analysis

Following arcsine square root transformation of the survival data (Li *et al.*, 2011), the effects of temperatures and time on survival was analyzed using a Generalized Linear Model (GLZ) (assuming normal distribution with an identity link function). Probit regression was used to calculate the temperatures estimated to cause 50% mortality (LT_{50}), the temperature causing 50% of tested individuals to die in a given period (see Li *et al.*, 2011). A Pearson χ^2 test was applied to comparisons of walking frequency and a student's *t*-test to mean duration of movement between warm-acclimated and cold-acclimated larvae. Apart from the GLZ analysis that was performed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA), all other analyses were performed using Genstat 14.0 (VSN International, Hemel Hempstead, UK).

Table 1. Generalized linear model (GLZ) results for effects of temperature, time, life stage and all interactions on higher and lower lethal limits in *Pareuchaetes insulata*. Following arcsine square root transformation, normal distributions with an identity link function were assumed.

Effect	d.f.	Wald $\chi 2$	*P*
Upper lethal temperature (ULT)			
Intercept	1	2140.14	**0.0001**
Temperature	8	412.31	**0.0001**
Time	3	94.31	**0.0001**
Life stage	1	0.08	0.771
Temperature × time	24	122.26	**0.0001**
Temperature × life stage	8	0.21	0.996
Time × life stage	3	0.27	0.964
Temperature × time × life stage	16	1.07	0.998
Lower lethal temperature (LLT)			
Intercept	1	1608.76	**0.0001**
Temperature	8	335.24	**0.0001**
Time	3	47.55	**0.0001**
Life stage	1	5.64	**0.018**
Temperature × time	24	48.84	**0.002**
Temperature × life stage	8	6.14	0.632
Time × life stage	1	0.18	0.894
Temperature × time × life stage	8	1.03	0.998

Statistically significant values are indicated in bold.

Results

Thermal tolerance (effects of lethal temperatures and exposure time on survival)

The temperature, and the time period that *P. insulata* was exposed to it, significantly affected the survival of the insects at either high (ULT) or low (LLT) temperatures (table 1). As expected, an increase in severity (extreme) of exposure at low or high temperatures resulted in increased mortality (figs 1 and 2). Likewise, an increase in the duration of exposure at any given temperature resulted in a reduction in *P. insulata* survival (figs 1 and 2). Life stage significantly affected survival in the LLT trials, but did not in the ULT trials (table 1). The interaction of temperature and the duration of exposure was highly significant, resulting in shorter periods of time required to inflict 100% mortality at extremely severe low or high temperatures, suggesting limited plasticity of survival in these trials (table 1; figs 1 and 2). Based on the results of the LLT and ULT trials, the temperatures estimated to cause 50% mortality (LT_{50}) were calculated. As expected, ULT_{50} for each life stage decreased with extended exposure time (table 2). Exposure to 40.1 and 40.0°C for 2 h caused equal mortality (50%) of adults and larvae. LLT_{50} for each life stage increased as the exposure time increased (table 2). When 3rd instar larvae were exposed for 0.5, 1, 2, and 4 h, the LLT_{50} values were −8.1, −6.5, −5.9, and −4.3°C, respectively. When adults were exposed for 2 h, LLT_{50} value was −4.7°C.

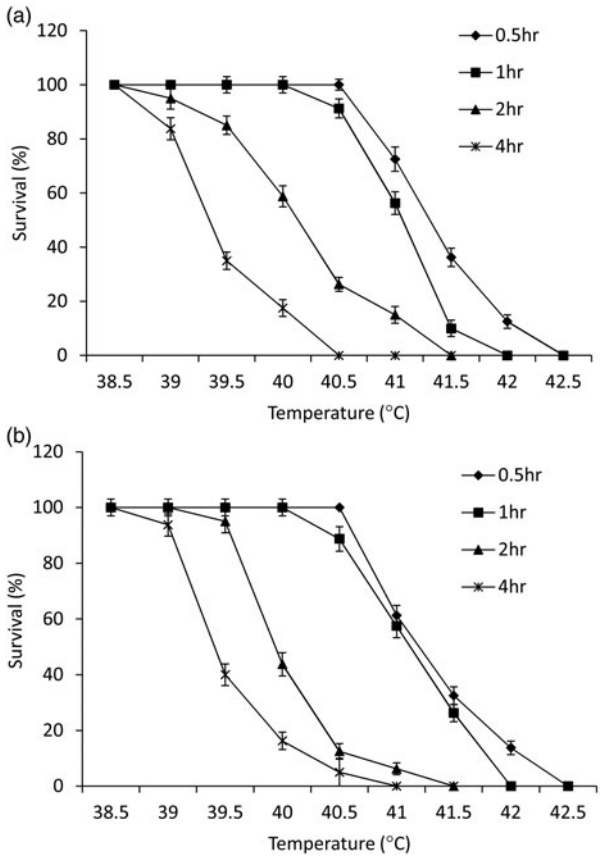

Fig. 1. Mean (±SE) survival of *Pareuchaetes insulata* (adults and larvae [a and b]) exposed to different high temperatures for four exposure durations. Each symbol is a mean of ten replicates of four individuals per replicate ($n = 40$ per symbol). Note that 0.0 h treatments (handling controls) experienced no mortality during these experiments (data not shown).

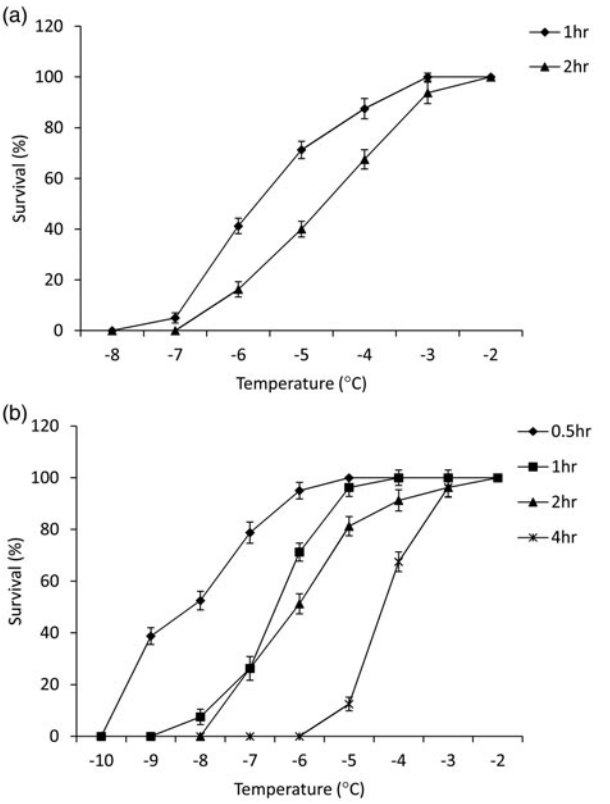

Fig. 2. Mean (±SE) survival of *Pareuchaetes insulata* (adults and larvae [a and b]) exposed to different low temperatures for two (adults) and four (larvae) exposure durations. Each symbol is a mean of ten replicates of four individuals per replicate ($n = 40$ per symbol). Note that 0.0 h treatments (handling controls) experienced no mortality during these experiments (data not shown). Data are not available for adult exposure at 0.5 and 4 h because of the huge numbers of adults needed for these experiments.

Effect of thermal history on locomotion performance

Locomotion performance of *P. insulata* increased with increasing temperatures and cold-acclimated 3rd instar larvae of the moth had a higher level of locomotor activity than warm-acclimated larvae when exposed to low temperatures (fig. 3). When both groups of acclimated larvae were placed on the locomotion stage set at 11°C, only 68.2 ± 4.7% of warm-acclimated larvae dispersed at the set temperature compared with 100.0 ± 0.0% for cold-acclimated larvae (Pearson $\chi^2 = 38.10$, d.f. = 1, $P < 0.001$; fig. 3a). The greater mobility of cold-acclimated larvae was evident not only in the frequency of individuals capable of movement but also in the duration of walking activity for cold-acclimated larvae (fig. 3b). When both groups of acclimated larvae were exposed to 11°C, cold-acclimated larvae spent 70.8% (21.24 ± 1.15 s) of their time moving compared with 43.8% (13.16 ± 1.03 s) for warm-acclimated larvae ($t = 5.30$, d.f. = 8, $P < 0.001$; fig. 3b). Similarly, cold-acclimated larvae spent more time walking when both groups of acclimated larvae were exposed to 15°C; cold-acclimated larvae spent more (95.2%) time moving compared with the warm-acclimated ones (84.6%) ($t = 4.06$, d.f. = 8, $P < 0.004$; fig. 3b).

Microclimate data

The microclimate temperature data recorded at the known *P. insulata* established site in Sappi Cannonbrae Plantation, Umkomaas, South Africa ranged between 6.4 and 34.3°C (table 3). Although there is not a large difference between the maximum summer temperatures between the collected and introduced sites, the winter temperatures (average daily minima) differed substantially (table 3). Fort Lauderdale's coldest months (January and February), experiences a mean daily minimum temperature of 15.9°C, whereas all three of the introduced sites had mean daily minimum temperatures ranging from 8.6 to 12.8°C during winter (June–August) in South Africa. Furthermore, absolute minimum temperatures are well below 10°C (6.4–8.6) for 4 consecutive months at the established site at the Sappi Cannonbrae Plantation. Microclimate data also showed that the nocturnally feeding larvae (3rd to 6th instars) of *P. insulata* may spend up to 32% of their time at temperatures below 10°C during winter (table 4).

Discussion

The effects of time and temperature (ULT and LLT) and thermal history on *Pareuchaetes* species (e.g., *P. insulata*,

Table 2. Higher and lower lethal temperatures (LT_{50}) of *Pareuchaetes insulata* when exposed to a range of temperatures for various durations. Note that data presented here are analyses from upper lethal temperature (ULT) and lower lethal temperature (LLT) trials. For both ULT and LLT trials, insects were exposed to a range of temperatures (38.5–42.5 and −10 to −2 for ULT and LLT, respectively).

Exposure (hours)	Life stage	LT_{50} (°C)	95% confidence interval
Higher lethal temperatures (LT_{50})			
0.5	Adult	41.4	41.1–41.7
	larva	41.2	40.9–41.6
1	Adult	41.3	40.9–41.8
	Larva	41.4	41.0–41.8
2	Adult	40.1	39.7–40.4
	Larva	40.0	39.8–40.6
4	Adult	39.2	39.0–39.5
	Larva	39.5	39.2–39.8
Lower lethal temperatures (LT_{50})			
0.5	Adult	*	*
	Larva	−8.1	−8.8 to −7.5
1	Adult	−5.5	−6.1 to −4.9
	Larva	−6.5	−7.1 to −5.9
2	Adult	−4.7	−5.3 to −4.1
	Larva	−5.9	−6.7 to −5.3
4	Adult	*	*
	Larva	−4.2	−4.8 to −3.8

*Data are not available for adult exposure at 0.5 and 4 h.

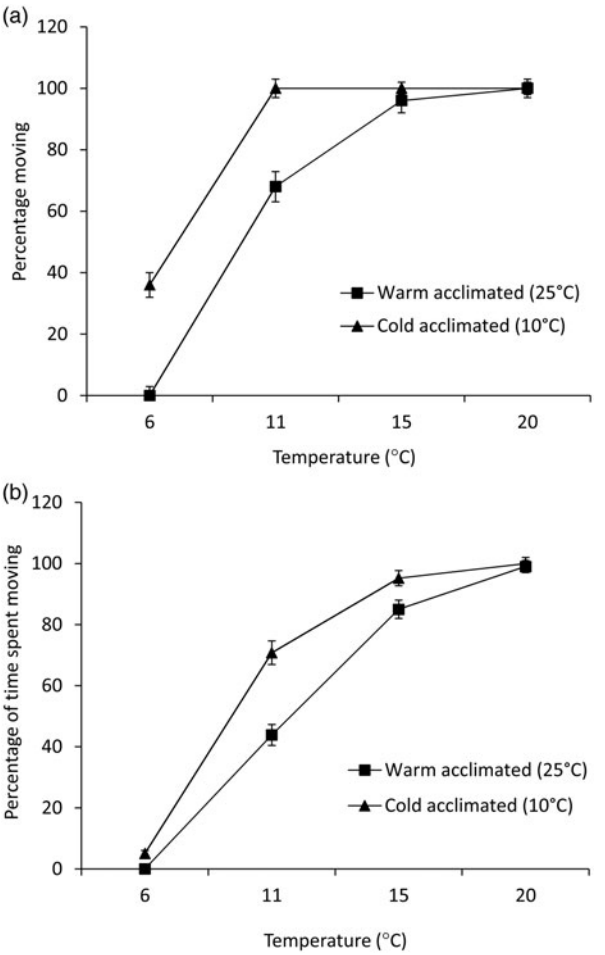

Fig. 3. Comparative mobility of 3rd instar larvae of *Pareuchaetes insulata* acclimated to warm (reared at 25°C) or cold (at 10°C) for 2 days after consecutive 30-second exposure periods to 6, 11, 15, and 20°C on a temperature-controlled locomotion stage. Five individuals were exposed to each temperature and the test was replicated five times. The mean (±SE) percentage of individuals moving (a), and the mean (±SE) percentage of time spent moving (b) for each treatment at each temperature are presented.

P. pseudoinsulata, *P. aurata aurata*) survival have, to our knowledge, not been previously documented. The majority of research on *Pareuchaetes* species to date has focused on taxonomy (Cock & Holloway, 1982), host-specificity testing (Kluge & Caldwell, 1993*a*, *b*) or nutritional ecology (Uyi *et al.*, 2015, 2016*b*). By contrast, relatively little is known about their thermal biology (Uyi *et al.*, 2016*a*). Even though large numbers of several *Pareuchaetes* species have been released in South Africa in an attempt to control *C. odorata*, establishment has been exceptionally difficult (Zachariades *et al.*, 1999, 2011). This study aims to understand whether thermal tolerance of one of these species, *P. insulata*, might be the underlying cause of its poor establishment and low population levels. Therefore, this study documented the thermal tolerance range of *P. insulata*, and showed that low temperatures affect the locomotion performance of 3rd instar larvae, within the context of environmental temperatures measured.

As expected, the survival of adults and larvae of *P. insulata* under varying low or high temperatures of different short durations depends on both the magnitude of the temperature and duration of exposure, as is true for many other insects (e.g., Chidawanyika & Terblanche, 2011; Li *et al.*, 2011). While low temperature is considered the most important abiotic factor that governs the year-to-year abundance and geographic distribution of insects, insects are sensitive to high temperatures because of their variable body temperature and their small bodies (Coulson & Bale, 1991; Hoffmann *et al.*, 2013). Consequently, high extreme temperatures in insects have been linked with water loss, disruption of the structure of membranes (Hochachka & Somero, 2002; Yoder *et al.*, 2009) and denaturation of protein, which restrict enzyme-catalyzed reactions (Chown & Nicholson, 2004). Overall, thermal stress in insects is known to cause neuronal damage (Chown & Terblanche, 2007). Although both the larvae and adults of *P. insulata* demonstrated 100% survival at high temperatures (e.g., 38°C) and low temperatures (−2°C) at short time scales, an increase in temperature or duration of exposure to previously non-lethal temperatures may spell problems for the survival of this insect and its population dynamics in the field. One important question of ecological significance that arises from the findings of this research is whether adults or larvae of *P. insulata* are likely to die from thermal stress in the field. This question was addressed by combining the microclimate temperature field data with thermal tolerance estimates recorded in the laboratory experiments. The recorded microclimate temperature data (mean absolute temperature range: 6.4 and 34.3°C) suggests that temperatures potentially causing low or high temperature mortality never occurred; neither did they approach lethal levels for any duration. From the 5 years of temperature data obtained from the South African Weather Service, minima and maxima recorded at two locations (weather stations) also suggest that

Table 3. Summary of microclimate temperatures (average daily minima, maxima and mean temperatures) recorded using hygrochron iButtons (0.5°C accuracy; 1 h sampling frequency) from June 2013 to June 2014 in Sappi Cannonbrae Plantation, Umkomaas, South Africa, and summary of 5 year annual weather data (average daily minimum temperature within the month and average daily maximum temperature within the month) for Durban South, Thohoyandou, South Africa and Fort Lauderdale, Florida, USA. Monthly absolute maximum and absolute minimum temperatures are given in parentheses.

	Cannonbrae (eastern KZN province) (30.2167 S, 30.7667 E: deg. min) (June 2013– May 2014)		Durban South (eastern KZN province) (29.9650 S, 30.9467 E: deg. min) (2008–2012)		Thohoyandou (Limpopo province) (23.0797 S, 30.3838 E: deg. min) (2008–2012)		Fort Lauderdale, Florida, USA (26.1224 N, 80.1373 W: deg. min) (1996–2000)	
Month	T_{min} (°C)	T_{max} (°C)	T_{min} (°C)	T_{max} (°C)	T_{min} (°C)	T_{max} (°C)	T_{min} (°C)	T_{max} (°C)
January	20.8 (18.6)	30.7 (34.3)	21.3 (20.9)	28.2 (30.4)	19.0 (16.0)	28.7 (29.9)	15.9 (5.0)	24.8 (29.2)
February	20.8 (17.1)	30.2 (33.9)	21.8 (21.0)	29.1 (30.5)	18.4 (16.1)	29.5 (30.6)	15.9 (7.1)	25.3 (29.1)
March	19.9 (14.9)	28.1 (33.3)	20.6 (19.9)	28.5 (30.3)	17.5 (16.4)	29.3 (31.2)	17.4 (10.8)	26.4 (30.8)
April	16.0 (11.3)	27.0 (32.0)	16.9 (16.1)	26.7 (27.6)	14.1 (12.8)	26.3 (27.6)	19.7 (12.9)	28.3 (32.2)
May	14.3 (10.8)	24.6 (28.3)	15.1 (14.3)	25.3 (26.7)	11.8 (11.2)	26.3 (27.0)	22.1 (17.7)	30.2 (33.7)
June	10.5 (6.6)	21.3 (25.1)	11.4 (10.5)	23.3 (24.3)	9.0 (7.3)	24.2 (25.0)	23.6 (20.9)	31.4 (34.3)
July	11.9 (8.6)	22.0 (26.5)	10.6 (9.7)	22.9 (24.1)	8.6 (7.4)	22.8 (25.0)	24.7 (21.8)	32.5 (35.4)
August	11.2 (7.1)	25.7 (30.1)	12.8 (12.2)	23.4 (24.5)	9.8 (9.2)	25.5 (27.2)	24.6 (21.7)	32.5 (34.4)
September	13.1 (6.4)	25.7 (32.6)	14.9 (13.3)	23.8 (25.7)	13.8 (12.5)	28.2 (30.1)	24.2 (21.9)	31.6 (34.3)
October	15.0 (10.1)	25.8 (31.8)	17.0 (16.5)	24.0 (25.1)	16.0 (15.3)	28.9 (30.6)	21.7 (16.6)	29.5 (33.2)
November	17.8 (13.8)	26.8 (33.1)	18.1 (17.5)	25.0 (26.3)	16.9 (15.1)	28.6 (29.2)	19.4 (12.5)	27.2 (30.3)
December	19.0 (16.3)	26.7 (32.8)	20.1 (19.4)	26.9 (29.0)	18.4 (15.4))	29.0 (30.2)	17.2 (8.1)	25.2 (29.2)

Table 4. Percentage of time during the winter months when temperature is below 10°C where hygrochron iButtons were placed to record microclimate data between June 2013 and June 2014 at three locations within *Chromolaena odorata*-infested areas in Sappi Cannonbrae Plantation, Umkomaas, South Africa where *Pareuchaetes insulata* established following its release (between 2001 and 2003). At each location, one iButton was suspended within *Chromolaena odorata* thickets (60 cm above ground level).

Winter months	20 m from the release site (%): 30.2207 S, 30.7816 E	350 m from the release site (%): 30.2220 S, 30.7825 E	650 m from the release site (%): 30.2217 S, 30.7849 E	Mean monthly (mean ± SE)
June 2013	27.27	10.42	1.11	13.08 ± 7.80
July 2013	12.10	3.36	0.0	5.15 ± 3.61
August 2013	13.50	3.49	0.13	5.70 ± 4.01
June 2014	31.81	14.03	0.0	15.28 ± 9.20

temperatures potentially causing low or high temperature mortality never occur (table 3) suggesting that low or high temperatures are not likely to be lethal to *P. insulata* in the field.

The larvae of this moth appeared to be more tolerant to cold temperatures compared with adults. Several other studies have reported the thermal tolerance in insects to vary significantly among life stages (e.g., Mahroof *et al.*, 2003; Jensen *et al.*, 2007; Marais *et al.*, 2009). However, exceptions do exist, such as in the sub-Antarctic *Apetaenus littoralis* Eaton (Diptera: Canacidae) (Klok & Chown, 2000), where no differences among life stages have been recorded.

The locomotion performance of 3rd instar larvae of *P. insulata* is affected not only by the immediate thermal surroundings, but also by recent thermal history (i.e., acclimation temperatures). At low temperatures, we found that *P. insulata* locomotion performance increased with increasing test temperature, largely as might be expected based on previous examinations of insect locomotion or flight performance (e.g., Frazier *et al.*, 2008; Boiteau & Mackinley, 2012; Ferrer *et al.*, 2013). A substantial influence of thermal history (acclimation temperature) on locomotion performance of larvae was also found (e.g., Lachenicht *et al.*, 2010; Boiteau & Mackinley, 2012), with the major result being that cooler acclimation temperatures resulted in improved locomotion ability at cooler test temperatures. This is interesting, given that this is a tropical/sub-tropical specialist herbivore, which originates from stable, warm environments (table 3) in Florida, USA, and shows high dispersal ability (Strathie & Zachariades, 2014). Although the benefits of brief acclimation prior to release are typically very short-lived (e.g., Chidawanyika and Terblanche, 2011), our result suggests that subjecting larvae or adults of *P. insulata* to cool acclimation treatment prior to release might improve performance immediately post release of this biological control agent in the field and might be instrumental in the initial establishment of the moth during cold winter months.

Low temperatures (below 11°C) significantly affected the locomotor abilities of *P. insulata* larvae. For instance, none of the warm-acclimated individuals were able to initiate movement at 6°C, while only 36% of the cold-acclimated individuals dispersed at this temperature, walking for only 2 s of the 30 s' exposure time. This is likely to be of serious ecological significance in the field during winter months – as the mean absolute minimum temperatures in Sappi Cannonbrae Plantation (12 months of microclimate temperature data), Umkomaas during winter ranged between 6.4 and 8.6°C (table 3). Older larvae of this multivoltine species are nocturnal and the nocturnal feeding stages (instars 3–6) last for an average of 12 days at 25°C in the laboratory (Uyi, 2014). This suggests that *P. insulata* larvae might be unable to move or feed in winter (June and August) when the temperature falls below 11°C, thereby preventing escape from indigenous natural enemies (that are more cold-tolerant) or promoting larval starvation. When larvae

are dislodged from the foliage of *C. odorata* plants (either by wind or other factors), the situation may be further exacerbated – as attempts to locate their host plant (to either initiate feeding or seek shelter) might prove unsuccessful. This might lead to an increase in indirect mortality and consequently affect the populations of this biological control agent in field situations.

Most of the time spent (over 30%) below 10°C is usually at night when the older larvae (instars 3–6) should be feeding. Although we do not know the maximum or minimum amount of time required for the nocturnal larvae to feed or acquire sufficient nutrients (on a nightly basis) for growth and development in winter, several studies suggest that temperatures below 10°C affect activities (e.g., locomotion and feeding) as well as growth and development (Lachenicht *et al.*, 2010; Boiteau & Mackinley, 2012; Esterhuizen *et al.*, 2014; Uyi *et al.*, 2016*a*). For example, Lachenicht *et al.* (2010) showed that temperatures below 10°C negatively affected locomotion performance in *Acheta domesticus* L. (Orthoptera: Gryllidae). Poor locomotor abilities in nocturnal herbivores such as *P. insulata* can negatively affect feeding and expose it for a longer period to natural enemies and this may be a key driver of the population dynamics of the biological control agent in the field.

The average daily minimum in Fort Lauderdale during winter ranges between 15.9 and 17.4°C, whereas the daily minimum at three locations in South Africa ranges between 8.6 and 12.8°C. The lower average daily maximum and minimum temperatures at the three locations in South Africa compared to those of Fort Lauderdale (table 3) might cause some differences in the moth's phenology or population dynamics in South Africa compared to its native range. The over 5°C differences in average daily minimum temperatures between locations in South Africa and Fort Lauderdale further substantiate the view that low sub-lethal temperatures will not only affect the locomotor abilities of *P. insulata* during winter months (June–August) in South Africa, but also impact negatively on the establishment and performance (development and reproductive biology) of the moth in the field. The similarity of the average daily minimum temperature range in South Africa to the lower development threshold of *P. insulata* (11.3°C) in a recent study by Uyi *et al.* (2016*a*) represent a further justification for the poor establishment success and performance of this moth because temperatures close to or below the lower development threshold often retard development and/or increase mortality in insects (May & Coetzee, 2013; Uyi *et al.*, 2016*a*). Beyond its effects on locomotion performance of *P. insulata*, our findings support the predictions that low temperatures are likely to restrict the establishment and distribution of some biological control agents (e.g., McClay, 1996; Byrne *et al.*, 2003; May and Coetzee, 2013).

Although this study showed that direct mortality due to low temperature during a short-term exposure (0.5–4 h) might be trivial in explaining the generally poor performance of the moth (low population in the field), it is evident that low temperatures (6 and 11°C) negatively affected locomotion performance of larvae (which are nocturnal and can thus only move to their food source and feed when it is cold). It is not impossible that some of the larvae of *P. insulata* might suffer both direct and indirect mortality due to the low temperatures during winter months but a 'refugium' (a small population of individuals that survives an occasional thermal stress or adverse environmental conditions) might exist. However, the performance (e.g., development, locomotion, mating capacity, flight capacity and fecundity) of such a population under low ambient temperatures in the field remains to be understood and therefore warrants investigation. Further, low winter temperature may also have indirect negative effects (through variation in host plant quality, expressed as increased leaf nitrogen in winter) on the performance (longer development time and reduced pupal mass) of *P. insulata* as has been suggested in Uyi (2014), because there might be a fitness cost associated with excess foliar nitrogen (e.g., Boersma & Elser, 2006; Clissold *et al.*, 2006). Further studies on the effect of temperature on the nutritional ecology (e.g., Clissold *et al.*, 2013) and on mating behaviour of *P. insulata* are needed. Other possibilities regarding the poor performance of *P. insulata* in South Africa are top-down factors such as predation, parasitism, and disease. Although a preliminary study (O Uyi, unpubl.) did not record any egg parasitoids or predators, the role of these top down factors on the different life stages of this insect needs further investigations across a spatio-temporal spectrum.

The failures of weed biological control programmes are frequently attributed to agents not being adapted to the ecological and/or climatic conditions in the areas where the weed is present (McClay, 1996; Byrne *et al.*, 2002, 2003). Therefore, the physiological, ecological and climatic adaptability of introduced natural enemies may play a prominent role in the outcome of any biological control programme. We recommend that weed biological control practitioners conduct thermal tolerance and other temperature-dependent development trials on biological control agents in the laboratory (e.g., May & Coetzee, 2013) as part of the rigorous pre-release studies to avoid wasting resources on agents that would not be climatically adapted (or suitable) to the new environment of their host plants. Further, sub-optimal biological control agents, poorly established or failed biological control agents should be subjected to a number of thermal tolerance trials to decipher whether climate incompatibility (=low temperatures) is culpable for such a poor performance or establishment failure. This study not only contributed to our understanding of the evolution of temperature-dependent performance in *P. insulata*, it also explained the poor performance (at least in parts) of the moth on *C. odorata* in South Africa.

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