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Testing the thermal limits of Eccritotarsus catarinensis: A case of thermal plasticity

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Short Title: Eccritotarsus catarinensis thermal plasticity

Abstract

Water hyacinth is considered the most damaging aquatic weed in South Africa. The success of biological control initiatives against the weed varies nation-wide, but control remains generally unattainable in higher altitude, temperate regions characterised by cold winters, where establishment often requires multiple agent introductions. Eccritotarsus catarinensis (Hemiptera: Miridae) is a biological control agent of water hyacinth that was first released in South Africa in 1996. By 2011, it was established at over 30 sites across the country. These include the Kubusi River, a site with a temperate climate where agent establishment and persistence was unexpected. This study compared the critical thermal limits of the Kubusi River insect population with a laboratory-reared culture to determine whether any physiological plasticity was evident that could account for its unexpected establishment. There were no significant differences in critical thermal maxima (CT_{max}) or minima (CT_{min}) between sexes, while the effect of rate of temperature change on the thermal parameters in the experiments had a significant impact in some trials. Interestingly, both CT_{max} and CT_{min} differed significantly between the two populations, with the field individuals tolerating significantly lower temperatures (CT_{min}: -0.3°C ± 0.063 [SE], CT_{max}: 42.8°C ± 0.155 [SE]) than those maintained in the laboratory (CT_{min}: 1.1°C ± 0.054 [SE], CT_{max}: 44.9°C ± 0.196 [SE]). Acclimation of each population to the environmental conditions typical of the other for a five-day period illustrated that short-term acclimation accounted for some, but not all of the variation between their lower thermal limits. This study provides evidence for the first cold-adapted strain of E. catarinensis in the field, with potential value for introduction into other colder regions where water hyacinth control is currently unattainable.

Keywords: Water Hyacinth, Biological Control, CT_{min}, CT_{max}, Temperature Ramp Rate

Word count: 4168 words

Introduction

Classical biological control (biocontrol) of invasive plant species is generally regarded as a safe, cost-effective method to maintain alien populations below an economically significant threshold (Wittenberg & Cock, 2005). Unfortunately, the extent of control attained by released agents is often unpredictable (Wittenberg & Cock, 2005). This is particularly true for the control of water hyacinth, Eichhornia crassipes (Mart.) Solms-Laub. (Pontederiaceae), a global weed in sub-tropical and temperate regions (Charudattan, 2001; Julien & Griffiths, 1998). In South Africa, complete control has been attained in some areas, such as at the New Year's Dam (Eastern Cape, South Africa) (Hill, Cilliers, & Neser, 1999), whereas plant populations in the cooler high altitude 'Highveld' regions continue to persist despite repeated agent releases (Coetzee, Hill, Byrne, & Bownes, 2011). By 2013, a total of eight arthropod biocontrol agents and one fungal pathogen (Cercospora rodmanii Conway [Mycosphaerellaceae]) had been released for the control of water hyacinth in South Africa, more than anywhere in the world (Coetzee et al., 2011; Sutton, Compton, & Coetzee, 2016). This high number is indicative of the effort and amount of resources used in an attempt to combat the weed, but results still remain far below those achieved in tropical countries (Hill & Coetzee, 2017).

Several factors contribute to the reduced effectiveness of water hyacinth biocontrol programmes in South Africa and other temperate regions. These include eutrophication of water bodies (Hill et al., 1999), hydrology in smaller systems (Julien, 2001), and, relevant to this study, climatic conditions (Hill et al., 1999; Hill & Olckers, 2001). A number of water hyacinth infestations in the higher-elevation interior of South Africa are affected by winter frosts, which often cause extensive plant dieback that result in population crashes of released

agents (Hill & Olckers, 2001). During springtime, the water hyacinth recovers quickly, but due to cold-induced mortality and retarded reproductive output, the insect populations have been shown in many cases to only reach sizes that cause sufficient damage levels by mid-summer (Hill et al., 1999; King, 2011). This seasonal lag period between the host and agent populations allows for virtually unregulated plant growth for several months, particularly when combined with nutrient rich waters. As water hyacinth is known to be able to double its population size every 11 to 18 days (Edwards & Musil, 1975), the lag is capable of nullifying any previous agent damage.

Biocontrol agents that are released into areas with a climate similar to that of their origin are generally more successful (Samways, Osborn, Hastings, & Hattingh, 1999), and mismatching of climates can lead to agent failure. Suitability of an agent from a thermal standpoint, and thus an element of predictability about the extent of control that is achievable, can be determined through pre-release physiology testing (Byrne, Currin, & Hill, 2002). These often-simple tests can prevent the release of ill-matched species for biocontrol purposes, thereby saving much effort and funding that would be wasted by mass rearing and releasing unsuitable candidates, but this is seldom done. Of the eight arthropods released against water hyacinth in South Africa, the thermal physiology of only three have been completely tested and mapped for potential post-release distribution patterns at the time of this study, namely Eccritotarsus catarinensis Carvalho (Hemiptera: Miridae) (Coetzee et al., 2011), Megamelus scutellaris Berg (Hemiptera: Delphacidae) and Niphograpta albiguttalis Warren (Lepidoptera: Pyralidae) (May & Coetzee, 2013).

Eccritotarsus catarinensis is a leaf-sucking bug first released as a biocontrol agent in South Africa in 1996 (Coetzee, Byrne, & Hill, 2007). By 2011, the insect had established at more than 30 sites around the country (Taylor, Downie, & Paterson 2011). It damages water hyacinth via the gregarious feeding of adults and four nymphal instars on the underside of leaves, with death of the leaf ensuing as a result of chlorophyll loss (Hill et al., 1999). As is expected based on the theory above, the impact, and therefore success, of E. catarinensis is more pronounced at sites that do not experience winter frosts, particularly in the more sub-tropical KwaZulu-Natal coastal plain (Coetzee et al., 2007). Establishment has failed after several release attempts at certain sites in the Western Cape and Gauteng provinces, which all differ in frost occurrence, altitudes and climates (Coetzee et al., 2007). These failures are in contrast with the predicted distributions for E. catarinensis mapped by Coetzee et al. (2007). This post-release study was conducted on a laboratory culture of the agent (Coetzee et al., 2007), however, which was most likely kept at relatively stable temperatures that are not adequately reflective of field conditions. The coldest site that E. catarinensis occurs at in South Africa is on the Kubusi River (Stutterheim, Eastern Cape, South Africa). The site falls within a temperate climate with frequent frosting events during winter, yet the agent has persisted there since its release in 1997 (Coetzee et al., 2007; Coetzee et al., 2011), and its impact on the weed has been shown to be highly dependent on seasonal changes with adequate control achieved towards the end of summer (Maseko, unpubl.). According to the modelling work of Coetzee et al. (2007) and Coetzee et al. (2011), the population should have completed approximately 110 generations in the 20 years since its release.

This study assessed whether a shift in the thermal tolerance of the population of E. catarinensis on the Kubusi River has occurred as a response to long-term (between-generations) exposure to unfavourable conditions, thereby developing a more cold-tolerant population of the mirid.

Furthermore, variation in thermal tolerances of field- and laboratory-maintained populations were assessed to determine the effect of short-term (within-generation) acclimation, as has been shown in other insect species (Terblanche, Klok, Krafsur, & Chown, 2006). These factors were assessed using critical thermal (CT) limit determination. We hypothesized that the CTs of field and laboratory populations of E. catarinensis would be significantly different as a result of thermal adaptation, CT limits would differ significantly according to temperature ramp rates, and differences recorded between populations would not solely be the result of short-term acclimation effects.

Materials and methods

Eccritotarsus catarinensis populations

All of the E. catarinensis populations used here were originally introduced into quarantine in Pretoria, South Africa, as biological control agents from Florianopolis, Brazil, in 1992, and released into the field in 1996 (Hill et al., 1999). The 'quarantine' E. catarinensis individuals used in this study were sourced from a single culture held at Rhodes University (Grahamstown, South Africa). This population was started with individuals from the Pretoria quarantine population in 2007 (8 years prior to this experimentation), and was maintained on laboratory-grown water hyacinth (supplemented with the slow release fertilizer Multicote™ 8 [15 N: 3 P: 12 K] [Haifa Chemicals Ltd., Cape Town, South Africa] to ensure plant quality). The insects were maintained at a constant temperature of 26°C, with an approximate 50/50 sex ratio, under glass house conditions, which ensured a natural diurnal light cycle in the facility. These insects were chosen to be compared to the field population because of this long, multi-generational exposure time to constant conditions.

The ‘field’ population used in this study consisted of individuals collected from a water hyacinth infestation on the Kubusi River (32°35’33” S, 27°25’19” E; Stutterheim, Eastern Cape, South Africa; altitude: 840m asl). Eccritotarsus catarinensis was released on the River in 1997, and at the time of this study had been established at the site for 18 years without any need for further releases. Data on the daily maximum and minimum temperatures recorded in the vicinity of the Kubusi River (Dohne weather station: 32°31′46″S, 27°27′03″E) were supplied by the South African Weather Service for the period following the release of E. catarinensis until 2015 to determine the climate conditions to which the mirids were exposed. Mean monthly temperatures for January to December in each year were calculated using Equation 1. Months with fewer than 21 days of data were excluded from calculations.

Equation 1: $\left(\frac{Sum\ Daily\ Maximum\ Temperatures + Sum\ Daily\ Minimum\ Temperatures}{Days\ in\ month}\right) / 2$

Collection of specimens for this study took place over two days in May 2015 at different sites along the River. The collected population was then maintained in a constant environment chamber at Rhodes University at a temperature of 16 ± 2°C (the annual mean environmental temperature at the Kubusi site, as determined from South African Weather Service data) on water hyacinth collected from the field site during the duration of testing, which was completed as quickly as possible.

Critical thermal limits

Methods for determining the CT range used here follow Coetzee et al. (2007) and Klok and Chown (2003), where the limits were defined as the point at which co-ordinated muscle

function was lost, and locomotion was impaired (as indicated by the inability to self-right when inverted). Twenty individuals were used in single trials, resulting in a total of 720 individuals used in CT_{min} and CT_{max} testing of specimens collected from the field and quarantine populations: with 70 male and 110 female field insects used to determine CT_{min}; 91 male and 89 female quarantine insects used to determine CT_{min}; 69 male and 111 female field insects used to determine CT_{max}; 118 male and 62 female quarantine insects used to determine CT_{max}. These were placed individually into 1.5ml air-filled vials sealed with moistened cotton wool. All experimentation was completed in a Grant Optima TX150-R4 programmable water bath with a Grant GP2000 thermostat unit temperature control unit, and an external Tenmars YFE-160A thermocouple thermometer to confirm that accurate temperatures were attained and maintained within the experimental vials.

Insects in CT trials were first exposed to a 10°C change in temperature at a rate of 0.5°C/min for determination of both the lower (CT_{min}; starting at 20°C down to 10°C) and upper (CT_{max}; starting at 30°C up to 40°C) critical thermal limits. Starting temperatures were chosen to reflect those used by Coetzee et al. (2007) for comparability. Following five minutes at the latter temperatures, the specimens were then exposed to a temperature ramp (either down for CT_{min} or up for CT_{max}). Much debate has ensued over the amount of experimental noise that arises as a result of methodological factors during CT testing, particularly with reference to acclimation temperatures and the rate of temperature ramps during trials (Terblanche, 2014; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007). Thus, three different rates of temperature change were used in different trials, 0.5°C/min, 0.25°C/min and 0.125°C/min (after Terblanche et al., 2006), to test the possible effects they could have on the CTs. At each 1°C change in temperature, the vials containing the insects were removed from the water bath momentarily and each insect's ability to self-right briefly checked before returning them into the bath. The

temperature at which individuals lost the ability to self-right was recorded and the specimen removed from the trial to confirm recovery at room temperature. Sex identification took place after the completion of each trial. The CT_{min} and CT_{max} of individuals from the field and quarantine populations were determined in separate trials, conducted on the same day, with three replicates of 10 individuals, using new individuals each time.

Thermal acclimation

To assess the differences in the CT_{min} between the quarantine and field populations of E. catarinensis, and how much could be accounted for through the effects of short term acclimation, each population was exposed to environmental conditions similar to those experienced by the other population at the same time for a five day acclimation period (after Chown, Jumbam, Sørensen, & Terblanche (2009) and Terblanche et al. (2006)). A total of 360 (96 male and 84 female standard quarantine; and 73 male and 107 female standard field individuals) insects were exposed to acclimatizing environments. Field-collected specimens were placed into the quarantine glass house and fed laboratory-grown plants, as per normal quarantine procedure. Quarantine-based insects were moved to a controlled environment chamber set at 10°C ± 2°C, the mean minimum monthly temperature for June and July 2015, at Kubusi (as supplied by the South African Weather Service) to mimic field conditions. This population was maintained on whole field-collected insect-free plants taken from the Kubusi River site. CT_{min} trials were then conducted on both populations with limited time difference between them following the methods described above. As the lower thermal limit is cited as the main factor responsible for the patchy distribution of E. catarinensis throughout South Africa (Coetzee et al., 2007), only CT_{min} values were assessed for these experiments, with three replicates of 10 new individuals each time.

Statistical analysis

All statistical analyses were performed in Statistica Version 13.2 (TIBCO Software Inc., 2017). General linear model factorial ANOVAs determined whether there were differences in the critical thermal limits of E. catarinensis from the original quarantine population, the Kubusi River field population, the acclimated quarantine population and the acclimated field population, followed by Tukey's HSD post hoc comparisons. Population, sex, and temperature ramp rate of the experiment were used as categorical independent factors in all models.

Results

Standard field and quarantine climatic conditions

Mean monthly temperatures recorded by the South African Weather Service at a weather station close to the Kubusi River over the 1997 to 2015 period since the insects were introduced ranged from a minimum of 10.6°C (July, 2011) to a maximum of 22.85°C (February, 2003). Mean values for each month throughout this period had a minimum of 13.03°C (July) and a maximum of 20.64°C (February) (Figure 1). These temperatures reflect seasonal changes in the region, with summer considered to run from 1 December to 28/29 February; autumn from 1 March to 31 May; winter from 1 June to 31 August; and spring from 1 September to 31 November (South African Weather Service). In contrast, the temperature to which the quarantine population was exposed has remained constant at around 26°C throughout the culture's existence.

Critical thermal limits

Critical thermal limits of the Kubusi River field population of E. catarinensis were significantly lower than the quarantine population for both the CT_{min} (Field CT_{min} = -0.3°C ± 0.063 [SE], Quarantine CT_{min} = 1.1°C ± 0.054 [SE], $F_{1, 354}$ = 78.76, P < 0.001) and the CT_{max} (Field CT_{max} = 42.8°C ± 0.155 [SE], Quarantine CT_{max} = 44.9°C ± 0.196 [SE], $F_{1, 354}$ = 88, P < 0.001) (Figures 2 and 3), indicating a degree of cold hardening over time. There was no significant difference in CT_{min} between the sexes from both populations (Male CT_{min} = 0.4°C ± 0.082 [SE], Female CT_{min} = 0.4°C ± 0.074 [SE], $F_{1, 354}$ = 5.4, P = 0.777), but males had a significantly higher CT_{max} than females (Male CT_{max} = 44.3°C ± 0.192 [SE], Female CT_{max} = 43.2°C ± 0.186 [SE], $F_{1, 354}$ = 5.4, P = 0.021). There was no significant interaction between population and sex for either the CT_{min} ($F_{1, 354}$ = 0.7, P = 0.419) or CT_{max} ($F_{1, 354}$ = 1.0, P = 0.308).

Acclimation

The E. catarinensis population collected from the field that was acclimated to quarantine conditions (i.e. 26ºC for 5 days) had a significantly higher CT_{min} (0.2°C ± 0.068 [SE]) than the CT_{min} of the field population (-0.3°C ± 0.063 [SE]) (Figure 2). In turn, this acclimated field population had a significantly lower CT_{min} (0.2°C ± 0.068 [SE]) than the acclimated quarantine population of E. catarinensis (i.e. 10ºC for 5 days) (0.4°C ± 0.075 [SE]), which was significantly lower than the CT_{min} of the quarantine population (1.1°C ± 0.054 [SE], $F_{1, 708}$ = 78.760, P < 0.001) (Figure 2). These results suggest that the thermal limits of E. catarinensis are plastic, and change over a short period of time, allowing the insect to withstand colder or warmer temperatures.

Duration of exposure significantly affected the CT_{mins} from all of the populations, where the CT_{min} of the mirids exposed to colder temperatures for the longest times (i.e. ramp rate =

$0.5^{o}C.min^{-1}$) was significantly lower (0.2°C ± 0.060 [SE]) than those exposed to the colder temperatures for the shortest times (i.e. ramp rate = $0.125^{o}C.min^{-1}$) (0.5°C ± 0.071 [SE], $F_{2, 708} = 5.922$, $P = 0.003$). An intermediate ramp rate ($0.25°C.min^{-1}$) resulted in an equally intermediate CT_{min} of 0.3°C ± 0.062 (SE). There was no significant interaction between population and ramp rate ($F_{6, 708} = 0.684$, $P = 0.488$). There was also no significant difference between sexes or any interactions including sex, so this variable was excluded from the final model.

Discussion

It has been estimated that approximately 44% of all biocontrol agents released to control invasive plants worldwide had failed to establish as a result of climatic incompatibility (McEvoy & Coombs, 2000). This is thought to be directly linked specifically to the effect of cold-tolerance on distribution patterns in insects (Kleynhans, Mitchell, Conlong, & Terblanche, 2014).

Critical thermal limits

The thermal tolerances of E. catarinensis collected from the field population on the Kubusi River have changed from the figures described by Coetzee et al. (2007), who used specimens from a laboratory population of insects at the University of the Witwatersrand. Coetzee et al. (2007) reported a CT_{min} of 1.2 ± 1.17 °C (SD) for the species. Consequently, the local population now appears to be better suited to the climatic conditions experienced on the Kubusi River. These results emulate those found in a variety of other studies that assessed cold-acclimated insect populations (e.g. Chidawanyika & Terblanche, 2011 and Kristensen, Hoffmann, Overgaard, Sørensen, Hallas, & Loeschcke, 2008).

In contrast to the persistence of E. catarinensis in the Kubusi area, numerous introductions of this agent into other regions with similar climatic conditions have failed as an apparent result of an intolerance to cold winter periods (Hill & Olckers, 2001). The cold climate adaptation shown by the Kubusi insects suggests that future releases into colder sites should come from cultures of this specific population. However, cold-tolerant strains in some other insect species have shown that physiological costs may be incurred when they move into regions outside of the environment in which they have become adapted (Kleynhans et al., 2014; Kristensen et al., 2008). These costs may take the form of slower developmental rates or reduced foraging rates (Kristensen et al., 2008). These two factors can have significant impact on the effectiveness of biocontrol organisms in controlling their target weed, but if E. catarinensis sourced from the Kubusi River are moved into a region with similarly cool conditions this should not prevent establishment.

Acclimated critical thermal limits

The adapted CT_{min} of both insect populations following five day acclimation periods provides support for the beneficial acclimation hypothesis, which is defined as follows: 'animals that are acclimated to a particular temperature range will exhibit enhanced fitness within that range compared to animals acclimated to differing thermal ranges' (Leroi, Bennett, & Lenski, 1994). This ability to acclimate in a relatively short period of time illustrates a degree of phenotypic plasticity in E. catarinensis.

The CT_{min} of males and females did not differ significantly in any of the experiments. Female E. catarinensis are generally larger than their male counterparts (Hill et al., 1999). In several other studies on a variety of insects, body size was shown to be an important contributor to heat tolerance and desiccation resistance (Chown, Scholtz, Klok, Joubert, & Coles, 1995; Kaspari, 1993; Lighton & Quinlan, 1994), as well as showing a positive correlation with survival in cold conditions (Renault, Hance, Vannier, & Vernon, 2003). The similar CT limits of male and female E. catarinensis suggests that the two sexes have similar temperature tolerances, a result that may reflect the relatively small extent of their sexual size dimorphism (Hill et al., 1999).

The effect of the rate of temperature change on CT values was variable, but where differences were detected, it was observed that the slower the ramp rate, the smaller the CT range (higher CT_{min} and lower CT_{max}). This result provides further support for the theory that thermal plasticity is present in E. catarinensis. Powell and Bale (2006) found that insects generally exhibit a response to declines in temperature called cold hardening. This means that the more frequently the insect is exposed to cold temperatures, the more it is able to tolerate these temperatures (Powell & Bale, 2006). Powell and Bale (2006) found that cold hardening generally becomes more prevalent with a slower rate of temperature change, leading to a decrease in CT_{min} and an increase in CT_{max}, and thus a broadened thermal tolerance. The results recorded in this study do not support their findings, but rather align with those of Chown et al. (2009) and Terblanche et al. (2006) in their studies on the Tsetse fly (Glossina pallidipes Austen [Diptera: Glossinidae]) and Argentine ant (Linepithema humile Mayr [Hymenoptera: Formicidae]) respectively. While the results reported in these papers, as well as in this study, were variably significant in different trials, they suggest that tolerance increases with slower rates of temperature change (Chown et al., 2009; Terblanche et al., 2006), which are also known to be more ecologically relevant (Sinclair, 2001). The basis of the inter- and intra-specific

variation displayed in the thermal tolerance response to the rate of temperature change remains an area of debate, but the lack of a cold hardening response in insects originating from stable tropical regions (as in the cases of E. catarinensis and the Tsetse fly) is likely to have an influence (Terblanche, Clusella-Trullas, Deere, & Chown, 2008), as are the presence of behavioural adaptations for avoidance of cold conditions (Block, Baust, Franks, Johnston, & Bale, 1990). Through personal observation, the authors can confirm here that E. catarinensis exhibits such behaviour by congregating in unfurled new leaves around plant stems during cold temperatures.

The effect of the rate of temperature change on thermal profiles is important to consider in both pre- and post-release analyses of insects used for biocontrol in order to reduce experimental noise and clarify interpretations. This is particularly true for pre-release CT analyses that are used to predict an agent's success and potential post-release distribution. It is also important that the ramping rates used replicates as much as possible what the insect would be exposed to under field conditions. For example, Nyamukondiwa and Terblanche (2010) found that a rate of temperature change of 0.06°C/min most closely mimicked the natural diurnal environmental conditions that the insects were exposed to in their experiments.

The alteration of CT_{min} values as a result of acclimation provides evidence of phenotypic plasticity in E. catarinensis, but the lack of convergence between the CT_{min} of the standard populations and acclimated populations indicates that there are other factors unaccounted for in this study that affect the thermal physiology of the insects. This means that while short-term acclimation takes place, the average CT_{min} values observed in a population is also the product of other factors. The interaction between short, within-generation, and long, between-

generation, acclimation is likely to be important (Terblanche et al., 2006). Between-generation acclimation implicates genetic variability in associated traits. While there are several hypotheses with case study evidence about the mechanisms of heritable variation resulting from unfavourable environmental conditions (see Hartl, Dykhuizen, & Dean, 1985; Hoffmann & Parsons, 1991; Pal, 1998), the review by Hoffmann and Merilä (1999) concluded that there were no consistent effects of unfavourable conditions on whether or not a trait reaches subsequent generations. The perseverance of E. catarinensis on the Kubusi River for over 90 generations (Coetzee et al., 2011) suggests that the opposite may hold true, however, where cold-tolerance appears to have been retained, and adapted throughout the generations. Further genetic testing is required now to confirm that this population has become a genetically cold-adapted population based on inherited traits.

Conclusions

The putative cold-adapted strain of E. catarinensis from the Kubusi River needs further physiological and genetic testing before it can be concluded to be distinct from laboratory cultures and populations released in warmer areas of South Africa. This further post-release evaluation deserves prioritization because of the benefits of having a cold-adapted agent available for control of water hyacinth. In particular, the potential range of this agent could be expanded to include the higher altitude, colder areas where other agents are providing ineffective control. The primary cause of failure in these regions was identified by Hill et al. (1999) and King (2011) as the lag period between plant growth and insect population recovery post-winter. A cold-adapted strain of the agent may reduce this lag period, and thereby afford better control, but to confirm a link between the physiological tolerances we have examined

with overwinter survival and population expansion in the spring will also require field-based studies.

This study serves to highlight the importance of post-release evaluations of biocontrol agents, a topic that remains a neglected area of study (May & Coetzee, 2013). Such evaluations need to not only monitor population sizes, but also be aware of possible environmental adaptations being displayed as a result of any novel selection pressures that they have encountered. The phenotypic plasticity exhibited by E. catarinensis over short time scales also indicates that in-laboratory environmental and rearing conditions can be utilized to significantly improve agent performance post-release. This will result in a reduction in the need for multiple releases, as well as better performance in the field, all of which will result in a greater likelihood of successful control, as well as lowering financial expenditure (Terblanche, 2014).

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Declaration of interests

There are no conflicts of interest to report for any of the authors on this paper.

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Figure legends

Figure 1: Mean monthly temperatures (± SE) recorded by the South African Weather Service at the Dohne weather station on the Kubusi River for the period 1997 to 2015.

Figure 2: The mean CT_{min} ± SE (°C) of Eccritotarsus catarinensis from all of the standard and acclimated populations.

Figure 3: The mean CT_{max} ± SE (°C) of Eccritotarsus catarinensis from the standard field and quarantine populations.