Thermal plasticity in the invasive South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract

South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating invasive global insect pest of tomato, *Solanum lycopersicum* (Solanaceae). In nature, pests face multiple overlapping environmental stressors, which may significantly influence survival. To cope with rapidly changing environments, insects often employ a suite of mechanisms at both acute and chronic time-scales, thereby improving fitness at sub-optimal thermal environments. For *T. absoluta*, physiological responses to transient thermal variability remain under explored. Moreso, environmental effects and physiological responses may differ across insect life stages and this can have implications for population dynamics. Against this background, we investigated short and long term plastic responses to temperature of *T. absoluta* larvae (4^{th} instar) and adults (24–48 h old) field populations. We measured traits of temperature tolerance *vis* critical thermal limits [critical thermal minima (CT_{min}) and maxima (CT_{max})], heat knockdown time (HKDT), chill coma recovery time (CCRT) and supercooling points (SCP). Our results showed that at the larval stage, Rapid Cold Hardening (RCH) significantly improved CT_{min} and HKDT but impaired SCP and CCRT. Heat hardening in larvae impaired CT_{min}, CCRT, SCP, CT_{max} but not HKDT. In adults, both heat and cold hardening generally impaired CT_{min} and CT_{max}, but had no effects on HKDT, SCP and CCRT. Low temperature acclimation significantly improved CT_{min} and HKDT while marginally compromising CCRT and CT_{max}, whereas high temperature acclimation had no significant effects on any traits except for HKDT in larvae. Similarly, low and high temperature acclimation had no effects on CT_{min}, SCPs and CT_{max}, while high temperature acclimation significantly compromised adult CCRT. Our results show that larvae are more thermally plastic than adults and can shift their thermal tolerance in short and long timescales. Larval plasticity advantage over adults reported here suggest asymmetrical ecological role of the larva relative to adults in facilitating *T. absoluta* invasion.

Keywords: Acclimation, environmental stress; hardening; invasive species; Pinworm, thermal tolerance

1. Introduction

Global climate change characterised by increased magnitude and frequency of extreme climatic conditions such as heat waves, cold snaps, severe droughts and floods poses a great threat to biodiversity (Bozinovic et al., 2013; IPCC, 2014; Colinet et al., 2015). Such changes to the bio-physical environments may result in changes in the abundance and geographic distribution of invasive species (Dukes and Mooney, 1999; Walther et al., 2009; Hulme, 2017). Owing to this is the notion that invasive alien species are more eurythermal, or able to maintain physiological functionality across variable temperatures. This is in contrast to stenothermy in non-invasive or indigenous species, which tolerate a narrower thermal range, and thus experience compromised fitness under changing thermal environments (Boher et al., 2016). Furthermore, climate change may also alter the spatio-temporal availability of biotic resources, creating bottlenecks in survival of organisms (Huey and Kingsolver, 2019). Overcoming these environmental challenges is the first of several potential barriers determining whether a species may become established, naturalised and, ultimately, invasive (Richardson and Pysek, 2006; Nyamukondiwa et al., 2010; Mbande et al., 2019). Given that most insects are ectotherms and their body temperature closely matches environmental temperature, dramatic changes in climate and more specifically weather, may affect their survival (Gray, 2013).

Indeed, temperature is one of the key abiotic factors known to directly influence reproduction, development, activity, fitness, survival and spatio-temporal distribution in insects (Bowler and Terblanche, 2008; Klepsatel et al., 2019). Thus, the potential to tolerate environmental temperature stress is important for fitness, survival and adaptation under global change (Karl et al., 2014; Boher et al., 2016). For example, insects have been reported to employ short and long term morphological, behavioural, physiological and molecular mechanisms to withstand extreme environmental conditions (Lachenicht et al., 2010). Physiologically, insects compensate through genetic adaptation which involves allele frequency changes (Karl et al.,

2014) as well as phenotypic plasticity, the ability of a single genotype to change morphological, biochemical and physiological characteristics of an organism under heterogeneous environments (Whitman and Ananthakrishnan, 2009; Sgrò et al., 2016; Griffith et al., 2019). Phenotypic plasticity regarding thermal tolerance encompasses a suite of mechanisms such as rapid cold- and heat-hardening (RCH and RHH respectively) (in the short term) (Chidawanyika and Terblanche, 2011a), acclimation (under managed laboratory conditions) (Mutamiswa et al., 2018a) or acclimatisation (in the long term under field conditions) (Sgrò et al., 2016). The underling basis of these mechanisms is the pre-exposure to sub-lethal temperatures (Chidawanyika and Terblanche, 2011a; Chidawanyika et al., 2017) which may lead to molecular responses such as the expression of heat shock proteins that act as chaperones against protein denaturation, in the case of high temperature exposure (Hoffmann et al., 2003; Nyamukondiwa et al., 2010). For cold tolerance, exposure to sub-lethal temperatures has been associated with increased polyols and lipid content, which protects the insects against extracellular freezing (see review Overgaard and MacMillan, 2017).

Phenotypic plasticity can be adaptive and has been reported to improve survival in Lepidoptera (Stotter and Terblanche, 2009; Chidawanyika and Terblanche, 2011a; Fischer et al., 2010; Mutamiswa et al., 2018a), Diptera (Overgaard and Sørensen, 2008; Kalosaka et al., 2009; Nyamukondiwa et al., 2010), Coleoptera (Chidawanyika et al., 2017; Nyamukondiwa et al., 2018) and related taxon e.g. Collembola (see Chown et al., 2007; Sengupta et al., 2017). Studies have also shown that phenotypic plasticity varies across ontogeny (Marais and Chown, 2008) and that immobile stages generally have inherent higher plasticity to compensate for their inability to behaviorally adapt through seeking benign microhabitats. During insect development, different life stages respond differently to abiotic stress due to the variable microenvironments they experience during their development. This creates differential

adaptations across ontogeny, including differences in behavioral compensation abilities (Mutamiswa et al., 2019).

Different hypotheses have been postulated to explain how phenotypic plasticity facilitates adaptation under changing environments. The beneficial acclimation hypothesis has received considerable interest among insect physiologists and ecologists (Klepsatel et al., 2019) although it is debatable (Woods and Harrison, 2002b). This hypothesis posits that acclimation to a particular environment confers performance and fitness advantage to an organism in that environment over another organism that has not been exposed to that particular environment (Leori et al., 1994; Wilson and Franklin, 2002; Woods and Harrison, 2002a). The 'hotter-is-better' hypothesis states that organisms thermally adapted to warmer environments may have a better performance and fitness advantage than those adapted to colder or benign environments (Klepsatel et al., 2019). Similarly, the optimal developmental temperature hypothesis postulates that organisms raised at optimal temperature have greater survival advantage across many thermal environments (Woods and Harrison 2002b; Klepsatel et al., 2019). In particular, beneficial acclimation and thermal plasticity may play an important role in facilitating the success of invasive pests, although the generality of these effects are not known.

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) native to South America is one of the key global destructive insect pests of tomatoes causing yield losses of 80–100% (Desneux et al., 2010; Brévault et al., 2014). Following its invasion into North Africa around 2008, the past decade has been marked by its rapid spread and establishment in East, West and Southern Africa threatening food security and livelihoods (Desneux et al., 2010; Biondi et al., 2018; Mansour et al. 2018). The successful spread and establishment of *T. absoluta* is a result of a number of reasons. These include a wide host range (cultivated and wild) (Machekano et al., 2018), high fecundity (Uchôa-Fernandes et al., 1995; Tropea Garzia et al., 2012; Biondi et al., 2018), trade globalisation and increased human travel

(Williamson, 1996; Pimentel et al., 2001; Simberloff and Rejmanek, 2001) and tolerance to diverse environmental conditions including temperature (Tropea Garzia et al., 2012; Ponti et al., 2015; Santana et al., 2019).

Projected thermal variability under climate change may have contrasting effects on species adaptation, compromising plastic responses in some species thereby limiting their potential to spread and establish, while enhancing such responses in other species (Sgrò et al., 2016). One of the major points to emerge from recent work on thermal tolerance is that there may be a link between susceptibility to climate change and the magnitude of phenotypic plasticity (e.g. Kellett et al., 2005; Balanya et al., 2006; Calosi et al., 2008; Nyamukondiwa et al., 2011; though see Gunderson and Stillman, 2015; Gunderson et al., 2017). Given the dramatic increase in the risk and invasion rate globally in the past decades, an understanding of plastic responses of invasive species to thermal stress is of fundamental importance in predicting climate change effects on their population dynamics, spatio-temporal distribution (Pimentel et al., 2001; Hance et al., 2007; Ma et al., 2014) and potential establishment in new environments (Nyamukondiwa et al., 2010; Buckley and Huey, 2016).

While several reports have documented significant ecological effects of RCH (Stotter and Terblanche, 2009; Basson et al., 2011; Mutamiswa et al., 2018b), RHH (Chidawanyika and Terblanche, 2011a; 2011b; Mutamiswa et al., 2018b) and acclimation (Lagerspetz, 2006; Fischer et al., 2010; Chidawanyika and Terblanche, 2011a; Sgrò et al., 2016), little is known on phenotypic plasticity of *T. absoluta* and its thermal tolerance (Han et al. 2018). Previous studies on *T. absoluta* have only focused on thermal requirements for development (Krechemer and Foerster, 2015; but see van Damme et al., 2015). To the best of our knowledge, no study has been done to date with focus on its plastic responses to temperature, and likely contributory effects on invasion success. In addition, studies on acclimation responses to temperature treatment, a major mechanism used by insects to cope with temperature variation both in

medium-term timescales (Overgaard and Sørensen, 2008; Nyamukondiwa and Terblanche, 2010; Nyamukondiwa et al., 2010) and over the longer term (e.g. seasonal timescales, Nyamukondiwa et al., 2013) also remain underexplored. In the present study, we investigated the thermal tolerance of *T. absoluta* larvae (4^{th} instar) and adults (24–48 h old) through determining their short- (RCH and RHH) and long -term (acclimation) plastic responses of fitness and survival. We hypothesize that *T. absoluta* is thermally plastic at variable thermal regimes. Understanding such factors associated with successful invasion provides a basis for a predictive framework important for mitigating the introduction, establishment or spread of potentially invasive pest species, and informing suitable control measures (e.g. when and where to focus efforts).

2. Materials and Methods

2.1 Insect culture

Wild populations of *T. absoluta* larvae were collected from infested tomatoes plants at Genesis Farm (S21°8'54.239"; E027°38'48.42"), Matshelagabedi village in North East District of Botswana. All specimens were collected in austral summer (December-February), so that all test organisms have the same environmental history. Larvae were reared in the laboratory on organically produced and pesticide-free excised fresh tomato ("Rodade") leaves inside Bugdorm rearing cages (240mm^3; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under optimum conditions, i.e., 28˚C; 65±10% relative humidity (RH) (Guimapi et al., 2016) and 12L:12D photocycle in climate chambers (HPP 260; Memmert GmbH + Co.KG, Germany) until adult emergence (for adult experiments). For all the experiments, 4^{th} instar wild larvae (field collected) and 24–48h old adults (eclosed from field collected larvae) were used.

2.2 Low temperature plasticity

166 *Tuta absoluta* hardening experiments were performed using established experimental protocols
167 for Diptera and Lepidoptera (e.g., Nyamukondiwa et al., 2010; Mutamiswa et al., 2018b). To
168 assess the effects of RCH and RHH on *T. absoluta* physiological traits, hardening temperatures
169 for the two developmental treatment stages (larvae and adults) were established through
170 preliminary assays. Cold hardening temperature for larvae was derived from critical thermal
171 minima (CT_{min}) (Hoffmann et al., 2003; Lee and Denlinger, 2010) and was defined as 6˚C
172 below CT_{min} (Mutamiswa et al., 2018b) (Table 1). For adults, low hardening temperature was
173 defined as 10˚C above the lower discriminating temperature, consistent with other studies (see
174 Nyamukondiwa et al., 2010) (Table 1). Lower discriminating temperature (-10˚C), defined as
175 the temperature that causes 80–100% mortality upon 2 h exposure to a stressful low
176 temperature was derived from Machekano et al. (2018). These hardening temperatures suffice
177 to elicit RCH responses in similar organisms under laboratory experimental conditions
178 (Hoffmann et al., 2003; Lee and Denlinger, 2010). Larval and adult *T. absoluta* specimens were
179 first placed in a climate chamber at 28˚C and 65±10% RH for 30 min to allow for equilibration.
180 Thereafter, the insects were placed in a zip lock bag and submerged in a programmed water
181 bath (Systronix Scientific, Industria, South Africa) with 1:1 water: propylene glycol and
182 hardened for 2 h at low hardening temperature (-2˚C (larvae) and 1˚C (adults)). Following
183 treatment, insects were allowed to recover under benign conditions (28˚C; 65±10% RH) in a
184 climate chamber for 30 min before measuring physiological traits. Control insects were kept at
185 benign conditions during the same treatment before measuring thermal fitness traits.
186 As for RHH effects, hardening high temperature for larvae was derived from critical thermal
187 maxima (CT_{max}) (Hoffmann et al., 2003; Lee and Denlinger, 2010) and defined as 7˚C below
188 CT_{max}, (see e.g. Mutamiswa et al., 2018b). For the adults, hardening high temperature was
189 defined as 5˚C below the discriminating high temperature (42˚C), derived from Machekano et
190 al. (2018). This hardening temperature is generally adequate to elicit an RHH response in

similar insects (Hoffmann et al., 2003). Organisms were first temperature equilibrated in a climate chamber at 28˚C and 65±10% RH for 30 min before being hardened for 2 h at high temperature (see table 1). Following hardening, organisms were given 30 min recovery time to allow the *de novo* synthesis of heat shock proteins (HSPs) (Nyamukondiwa et al., 2010; Mutamiswa et al., 2018a) before measuring physiological traits. Control organisms were maintained at optimum conditions (28˚C; 65% RH) before measuring physiological traits. Traits measured include critical thermal minima (CT_{min}), chill coma recovery time (CCRT) and supercooling points (SCPs).

Long term acclimation effects on low thermal tolerance were tested using standardised protocols (see Nyamukondiwa and Terblanche, 2010; Mutamiswa et al. 2018b). Acclimation temperatures were defined as 5˚C above and below the optimal temperature (28˚C), for low (23˚C) and high (33˚C) temperature pre-conditioning respectively. The control organisms were acclimated to an optimum temperature of 28˚C and 65% RH. Following three-day acclimation, physiological traits were measured. Such temperature duration is also sufficient to elicit acclimation responses in similar insect taxa (Nyamukondiwa and Terblanche, 2010; Weldon et al., 2011; Mutamiswa et al. 2018a).

2.2.1 Critical thermal minima (CT_{min})

Critical thermal minima were measured on *T. absoluta* larvae and adults using standardized protocols as outlined by Nyamukondiwa and Terblanche (2009). Ten replicate organisms were individually placed in a series of numbered borosilicate glass tubes (‘organ pipes’) connected to an insulated double-jacketed chamber which was linked to a programmable water bath before decreasing the temperature at a rate of 0.25˚C/min until their CT_{min} were recorded. This was repeated twice to yield sample sizes of n = 20 per treatment. CT_{min} was defined as the

temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

2.2.2 Chill coma recovery time (CCRT)

Chill coma recovery time experiments were conducted following established protocols (e.g. Weldon et al., 2011; Nyamukondiwa et al., 2018). Ten organisms from each temperature treatment i.e., larvae and adults were individually placed in 2ml Eppendorf tubes and then loaded into a zip-lock bag which was then submerged in a water bath (1:1 water: propylene glycol) set at 0˚C (larvae) and -2 ˚C (adults) for 1 h. After 1 h under chill coma temperature, the vials were removed from the water bath and placed in a climate chamber set at 28˚C, 65±10% RH for recovery. The chamber was connected to a video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20; Hikvision Digital Technology Co. Ltd, Hangzhou, China) which was linked to a computer for recording observations. This was repeated twice to yield a sample size of n = 20. Chill coma recovery time was defined as the time (in min) required for the insects to regain consciousness, e.g., movement for larvae and standing upright on legs (for adults) following recovery from chill-coma.

2.2.3 Supercooling points (SCPs)

Supercooling points were assayed as outlined by Nyamukondiwa et al., (2013). Twenty organisms from each developmental stage (larvae and adults) and temperature treatment were individually placed into 2 ml Eppendorf tubes. These organisms were ‘fasted’ for 24 hours to clear gut contents and circumvent confounding effects the food gut particles may have on SCPs. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple, inserted through the gauzed lid of the vial and both the insect and thermocouple were secured in contact by a cotton wool. Thermocouples were connected to one of two 8-channel Picotech

TC-08 thermocouple interfaces and temperatures were recorded at 1s intervals using PicoLog software for Windows. In all treatments the insects were first exposed to a temperature of 15˚C for 10 min before decreasing the temperature at a rate of 0.5˚C/ min until SCPs were recorded. SCP for each organism was determined as the lowest temperature recorded before a spike in temperature due to latent heat of crystallization (Nyamukondiwa et al., 2013).

2.3 High temperature plasticity

Hardening and acclimation experiments were conducted using standardised protocols, consistent with low temperature plasticity experiments (see section 2.2) (e.g. Nyamukondiwa et al., 2010; Nyamukondiwa and Terblanche, 2010; Mutamiswa et al., 2018b). Physiological traits were measured following hardening (RCH and RHH) and three-day acclimation at low (23˚C), optimum (28˚C) and high (33˚C) temperature for both larvae and adults. Control organisms were also maintained at optimum temperature (28˚C; 65% RH) before measuring physiological traits. Fitness traits measured following acclimation treatments include CT_{max} and HKDT.

2.3.1 Critical thermal maxima (CT_{max})

Critical thermal maxima were measured using the same protocol as that for CT_{min} except that temperature was ramped up at 0.25˚C/min until the endpoint. Critical thermal maximum was defined as the temperature at which each individual insect lost coordinated muscle function and ability to respond to mild stimuli using a thermally inert object.

2.3.2 Heat knock down time (HKDT)

Heat knockdown experiments were assayed as outlined by Mutamiswa et al. (2018a). Ten replicate individuals in both larva and adult stage were individually placed in 0.65ml

265 microcentrifuge tubes and placed in a climate chamber connected to a camera linked to a
266 computer. The tubes carrying the insects were then exposed to an acute knockdown
267 temperature (50˚C) in the climate chamber. This knockdown temperature was selected basing
268 on preliminary investigations of CT_{max} for larvae and adults ranging 49±0.13˚C and 48±0.2˚C
269 respectively. This was repeated twice to yield sample sizes of n = 20. All observations were
270 made from the climate chamber video recording system. Heat knockdown time was defined as
271 the time (in minutes) at which insects lost activity following exposure to knockdown
272 temperature in the climate chamber.
273
274 ***2.4 Statistical Analyses***
275 Data analyses were done using STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma)
276 and R version 3.3.0 (R Development Core Team, 2019). Critical thermal limits (CT_{max}, CT_{min}),
277 SCP, CCRT and HKDT data were first checked for normality and equality of variances using
278 the Shapiro-Wilk and Hartley-Bartlett tests and met assumptions of ANOVA in all traits except
279 for HKDT. Therefore CT_{max}, CT_{min}, SCP and CCRT results were analysed using factorial
280 ANOVA in STATISTICA. Tukey-Kramer's *post-hoc* tests were used to separate statistically
281 heterogeneous groups. Heat knockdown time results were analysed using generalized linear
282 models (GLZ) assuming a Gaussian distribution and an identity link function in R. Kruskal–
283 Wallis *post hoc* tests were used to separate statistically homogeneous groups for HKDT data.
284
285 **3. Results**
286 ***3.1 Low temperature plasticity***
287 *3.1.1 Critical thermal minima*
288 Hardening (RCH and RHH) significantly affected CT_{min} in both larvae and adults, but the
289 direction of effect differed between the life stages (treatment x life stage interaction, $p<0.001$,

290 Table 2). Rapid cold hardening significantly improved CT_{min} in larvae while impairing it in
291 adults (Fig. 1A). However, RHH significantly compromised CT_{min} in adults (Fig 1A), and
292 marginally improved in larvae. Acclimation also significantly influenced cold tolerance
293 (CT_{min}) (P<0.001) such that low temperature acclimation (23˚C) significantly improved CT_{min}
294 in larvae while it had no effects on adults. Similarly, high temperature acclimation (33˚C) had
295 no significant effects for CT_{min} for both larval and adult *T. absoluta* (Table 2; Fig. 1B). As
296 such, life stage × acclimation interaction effect was not significant (P>0.05) (Table 2).
297
298 *3.1.2 Chill coma recovery time*
299 Hardening significantly influenced CCRT in larvae and adults (P<0.001) (Table 3; Fig. 2A).
300 Rapid heat hardening significantly compromised CCRT in adults, and marginally so in larvae,
301 albeit the latter was non-significant (Fig. 2A). There were no significant differences in CCRT
302 between larvae and adults following RCH, between the controls and RHH larval treatment
303 (P>0.05) (Fig. 2A). Nevertheless, larval CCRT did not significantly differ from the controls.
304 There was a higher magnitude of difference in cold tolerance (CCRT) between larvae and
305 adults following RHH (14.63 min) than RCH (0.23 min) (Fig. 2A). In addition, life stage ×
306 treatment interaction effect was significant (P<0.001) (Table 3). Acclimation also significantly
307 affected CCRT of *T absoluta* larvae and adults (P<0.001) (Table 3; Fig. 2B). Low temperature
308 acclimation (23˚C) marginally improved CCRT in larvae and adults (recovered faster from
309 chill coma) whereas high temperature acclimation (33˚C) compromised it (took more time to
310 recover) in both developmental stages (Fig. 2B). Nevertheless, life stage × acclimation
311 interaction effect was not significant (P>0.05) (Table 3).
312
313 *3.1.3 Supercooling points*

Supercooling points for larvae and adults were significantly affected by hardening (P<0.001; Fig. 3A). For larval *T. absoluta,* both RCH and RHH significantly impaired supercooling ability (more positive SCPs), while for adults, both hardening treatments had no significant effects (Table 4; Fig. 3A). There was a significant life stage × treatment interaction effect (P<0.001) following hardening (Table 4). There were no significant acclimation effects on larval and adult SCPs (Table 4; Fig. 3B). For all developmental stages, there was no significant difference between the controls and that of the two treatments (23 and 33˚C) (Fig. 3B). However, there was developmental stage related difference in SCPs following acclimation to high temperature (33˚C), with larvae having more negative SCPs than the adults (Fig. 3B). In addition, life stage × acclimation interaction effect was significant (P<0.05) (Table 4).

3.2 High temperature plasticity

3.2.1 Critical thermal maxima

Heat tolerance (CT_{max}) in larvae and adults was significantly affected by hardening (P<0.001) (Table 5; Fig. 4A). Hardening significantly reduced CT_{max} in adults, and more significantly so for the RHH treatment (Fig. 4A). However, there was no significant difference in CT_{max} for larvae following both RCH and RHH (Fig. 4A). Similarly, life stage × treatment interaction effect was significant (P0.001, Table 5). In addition, acclimation effects were not significant for larvae and adults CT_{max} (Table 5; Fig 4B). For both developmental stages, low (23˚C) and high (33˚C) temperature treatments did not significantly affect CT_{max} from the control level (Fig. 4B). Nevertheless, it appeared larvae had higher CT_{max} than adults across all the treatments (Fig. 4B). The interaction between life stage and acclimation was also not significant (Table 5).

3.2.2 Heat knockdown time

As in CT_{max}, hardening significantly affected HKDT of larvae and adults (P<0.001) (Table 6; Fig 5A). Rapid cold hardening significantly compromised HKDT for larvae, while it showed no significant effects for the adults (Fig. 5A). Similarly, no significant differences in HKDT were noted in adults following both hardening treatments, and between controls and RHH treatments for larvae (Fig. 5A). In addition, there was a significant life stage × treatment interaction effect (P<0.001) (Table 6). Generally, larvae had higher HKDT (took longer to be knocked down following acute heat stress) than adults across all treatments (Fig. 5A). Acclimation also significantly influenced larvae and adults HKDT (P<0.001) (Table 6; Fig 5B). Low temperature acclimation (23˚C) significantly improved HKDT in both larvae and adults whereas high temperature acclimation (33˚C) impaired it in both life stages (Fig. 5B). Similarly, there was a significant life stage x acclimation interaction effect (Table 6). Consistent with other traits, it also appeared larvae had significantly higher heat tolerance (higher HKDT) across all treatments (Fig. 5B).

4. Discussion

The success of invasive species may stem from their ability to withstand stressful ambient novel environments. As such, basal and phenotypic plasticity traits play a pivotal role in invasive species' successful spread and establishment in new environments. Indeed, fitness and survival may be dependent on adjusting to novel environmental conditions through physiological acclimatization/acclimation and genetic adaptation (Webster et al., 2017; Castañeda et al., 2019; Griffith et al., 2019). It is increasingly being documented that invasive species may particularly have flexible life history traits (Agosta et al., 2018), and this potentially explains their success under heterogeneous environments.

The present study showed that rapid hardening and acclimation affects plasticity of thermal tolerance in *T. absoluta*, albeit asymmetrically for larvae and adults. Larvae appeared to be

364 more thermally plastic than adults, suggesting that *T. absoluta* invasion propagules may be
365 carried in the form of larvae under thermally extreme environments. Hence, this enhanced
366 larval plasticity coupled with the limited one in adults may aid each other to enhance invasion
367 success (see discussions in Nyamukondiwa et al., 2010).
368 It appeared there was higher plasticity for low temperature compared to high temperature
369 following both short- and long-term hardening. Changes in thermal limits as a result of
370 acclimation are thought to be more pronounced at the lower- than upper -thermal thresholds
371 (Klok and Chown, 2003; Weldon et al., 2011; Hoffmann et al., 2013). This is in agreement
372 with our current results where both RCH and low temperature acclimation had a positive effect
373 on CT_{min} of *T. absoluta* larvae. Coello Alvarado et al. (2015) reported improved CT_{min} in
374 *Gryllus pennsylvanicus* adults following long- and -short term acclimation. Similar results have
375 also been reported for *C. partellus* larvae, adult parasitoid, *Cotesia flavipes* (Mutamiswa et al.
376 (2018a) and *Busseola fusca* larvae (Mutamiswa et al., 2018b).
377 In terms of low temperature tolerance (CCRT and CT_{min}), we found that adults had higher basal
378 tolerance than larvae, corroborating a recent study by Machekano et al. (2018) who reported
379 superior basal cold tolerance in adults. Interestingly, larvae showed strikingly higher plasticity
380 of low temperature tolerance than adults. Insects often trade off basal low temperature tolerance
381 for phenotypic plasticity (Nyamukondiwa et al., 2011), consistent with the current results
382 which showed compromised basal low temperature tolerance and improved plasticity of cold
383 tolerance (CCRT and CT_{min}) for larvae. In addition, this also affirms the notion that insects
384 with high basal thermal tolerance may be constrained in phenotypic plasticity of thermal traits
385 (Stillman, 2003; Coello Alvarado et al., 2015). Studies have also shown that less or non-mobile
386 life-stages are generally considered to have evolved inherent high phenotypic plasticity to
387 abiotic stress, to compensate for limited ability of behavioural compensation (Vernon and

388 Vannier, 1996; Klok and Chown, 2001; Jensen et al., 2007; Marais et al., 2009; Mutamiswa et
389 al., 2019).
390 Although Coello Alvarado et al. (2015) reported improved CCRT in *G. pennsylvanicus*
391 following cold acclimation and RCH, our results report no effects for both *T. absoluta*
392 developmental stages for both RCH and low temperature acclimation. The reason for this
393 absence is unknown but may, to a lesser extent be explained by the developmental stages we
394 tested, and choice of acclimation treatments (see discussions in Mutamiswa et al., 2019).
395 Nevertheless, this result indicates that plasticity of thermal tolerance in insects may be species
396 or trait dependent (Nyamukondiwa et al., 2018). It also supports the notion that differences in
397 responses to hardening and acclimation depend on duration of acclimation exposure hence this
398 may consequently determine the direction of induced plasticity and any associated costs and/or
399 benefits (Bowler and Terblanche, 2008). In addition, RHH and high temperature acclimation
400 (33˚C) generally impaired CT_{min} (more positive) in both developmental stages. This result is
401 in keeping with Mutamiswa et al. (2018a; 2018b) who reported compromised cold tolerance
402 (CT_{min}) in larvae and adults of *C. partellus* and *B. fusca* larvae following high temperature
403 acclimation and RHH respectively. Nevertheless, impaired CCRT in adults following high
404 temperature acclimation corroborates a previous study on *C. flavipes* adults (Mutamiswa et al.,
405 2018a). A comparison of larvae and adults showed that the former recovered faster than latter
406 from chill coma following RHH. This likely means that mechanisms associated with tolerance
407 to low and high temperature for *T. absoluta* are decoupled (Hoffmann et al., 2003;
408 Nyamukondiwa et al., 2011). Rapid cold hardening has also been shown to improve thermal
409 fitness in other insect species e.g., *Drosophila melanogaster* (Overgaard and Sørensen, 2008);
410 *Ceratitis rosa* (Nyamukondiwa et al., 2010) and *Cydia pomonella* (Chidawanyika and
411 Terblanche, 2011a).

412 The thermal physiology of any organism is related to the range of environmental temperature
413 where it evolved (Bryant et al., 2002; Hoffmann et al., 2003; Weldon et al., 2011; Ismail and
414 Brookes, 2016; Griffith et al., 2019). *Tuta absoluta* larvae dwell in tomato leaves and feed by
415 mining the leaf mesophyll (Biondi et al., 2018), where it is somewhat buffered from
416 environmental heterogeneity. Thus, the higher larval plasticity to low temperature reported here
417 may be an evolutionary adaptation to cope with changing ambient environments. Faced with
418 stressful environments, adults on the other hand, can adjust their behaviour through e.g. flight
419 (Bowler and Terblanche, 2008). This may help explain the limited plasticity reported here for
420 the adults.
421 Both short- and long-term acclimation did not have any significant effects on SCPs in adults
422 and larvae of *T. absoluta*. The results are in keeping with Mutamiswa et al. (2018a) who
423 reported compromised SCPs for *C. partellus* pupae and adults following low and high
424 temperature acclimation. Interesting, a comparison of larvae and adults showed an enhanced
425 SCPs (more negative temperature) for the larvae relative to adults across all treatments,
426 suggesting higher cold hardiness for the larvae. Boardman et al., 2012 reported that 24 h fasting
427 removes all gut contents in *Thaumatotibia leucotreta* larvae resulting in a decrease in SCPs.
428 While clearance of gut contents was not tested after 24 h treatment in this study, we hypothesise
429 this may likely not have affected our SCPs results here. Some studies have reported that *T.*
430 *absoluta* may struggle to survive in low temperatures (e.g. Cuthbertson et al., 2013; Biondi et
431 al., 2018), and that it may not be able to overwinter under sub-zero environmental conditions.
432 Nevertheless, few empirical studies have tested its cold hardiness under laboratory or field
433 conditions (but see van Damme et al., 2015; Han et al., 2018). This therefore calls for imminent
434 studies looking at *T. absoluta* cold hardiness and overwintering physiology, including
435 ontogenic freeze strategy to help explain how this may affect its current and future geographic

436 distribution as well as population dynamics with a view to developing sustainable pest
437 management programs (Tonnang et al., 2015; Santana et al., 2019).
438 Our study demonstrates effects of short- and long-term acclimation on *T. absoluta* high
439 temperature tolerance. While RHH, RCH, low and high temperature acclimation did not have
440 a significant effect on *T. absoluta* larvae, it appeared to come at a significant cost for adults
441 (see Fig. 4A). Our results are in keeping with Mutamiswa et al. (2018b) who reported impaired
442 heat tolerance (CT_{max}) in *B. fusca* and *Sesamia calamistis* larvae following RCH as well as low
443 and high temperature acclimation. Some insects have been reported to trade off plasticity for
444 other physiological or fitness traits (Liefting and Ellers, 2008; Angilletta, 2009; Basson et al.,
445 2011; Murren et al., 2015).
446 Thermal conditioning to a single environmental stress often has deleterious consequences upon
447 exposure to a different or same stress (reduced fitness). This may partially be accounted for by
448 the cumulative effects of the stress combinations (see discussions in Gotcha et al., 2017).
449 Fitness maladaptations owing to cumulative abiotic stresses reported here somewhat vary and
450 depend on methodology employed e.g. short versus long term acclimation and the magnitude
451 of stress severity (see Mittler, 2006). Similarly, RCH also significantly reduced HKDT,
452 suggesting similar heat tolerance costs of short term low temperature acclimation (Fig. 5A).
453 Conversely, acclimation to low temperature improved HKDT for both adult and larval *T.*
454 *absoluta* larvae suggesting significant cross-tolerance effects. Phenotypic plasticity to one
455 stress factor often confers fitness advantages to a different stressor, a phenomenon called
456 'cross-tolerance' (Sinclair et al., 2015). Such a phenomenon shows that shared co-evolutionary
457 physiological stress resistance mechanisms exist across seemingly heterogeneous stress factors
458 and may help species survive divergent environments.
459 In conclusion, we document larval and adult *T. absoluta* plasticity of thermal tolerance, and
460 their likely contributions to invasion success. To our knowledge, this is the first study to

describe short- and long -term plastic responses to temperature in this species, using field collected populations. Determining the invasion pathway and pest risk assessment is significant for management of invasive pest species. As such, determining risk factors and mechanisms facilitating invasive species upon introduction to novel environments is critical in focusing control efforts. Although our results show the effects of acclimation on *T. absoluta* are complex and may vary considerably depending on the traits and life stage examined, it appears larvae are generally more plastic under short- and long- term acclimation than adults. Therefore, we suggest that larval propagules may be contributing more to the ongoing invasion of *T. absoluta,* owing to their better ability to shift their thermal phenotypes under heterogeneous environments. More studies could explore the role of parental history including testing invasive versus non-invasive species or endemic versus invasive environments to tease apart the exact role of plasticity for invasion success (as in e.g. Jarošík et al., 2015). Furthermore, the limited plasticity in adults ought to be further explored, including the role of other temperature, time and RH combinations in eliciting acclimation (see Mutamiswa et al., 2019). These results are important for pest risk analysis and for informing decision making in management this invasive species.

Declaration of interest

The authors declare no conflict of interest

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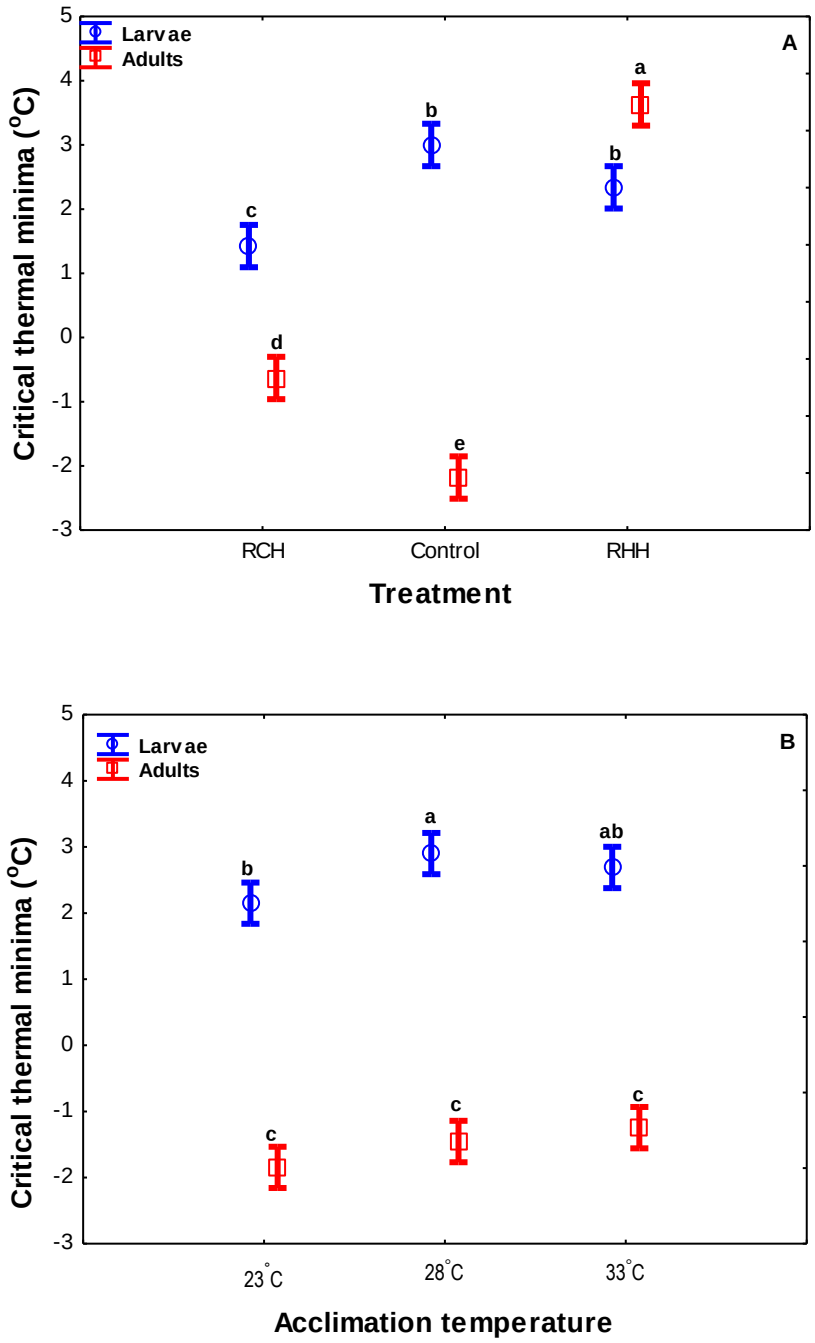
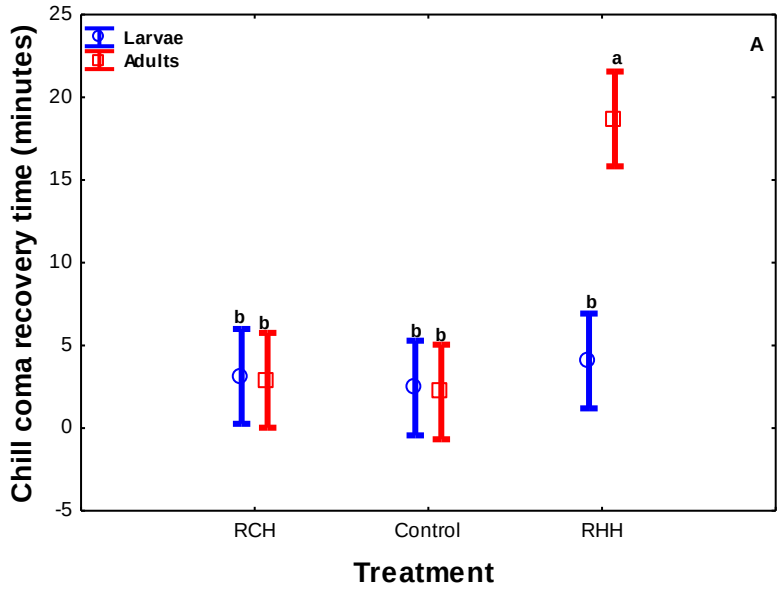


751 **Figure 1:** Effects of short-term acclimation- (A) 2-h pre-treatments/hardening of *Tuta absoluta*
752 larvae (42˚C for RHH; -2˚C for RCH) and adults (37˚C for RHH; 1˚C for RCH) and long-term
753 acclimation (B)- three-day acclimation (23˚C; 28˚C and 33˚C) on critical thermal minima
754 ($CT_{min.}$) Error bars represent 95% confidence limits (CLs) (*n*=20 per group). Means with the
755 same letter are not significantly different from each other. RHH and RCH represents rapid heat-
756 and -cold hardening respectively.



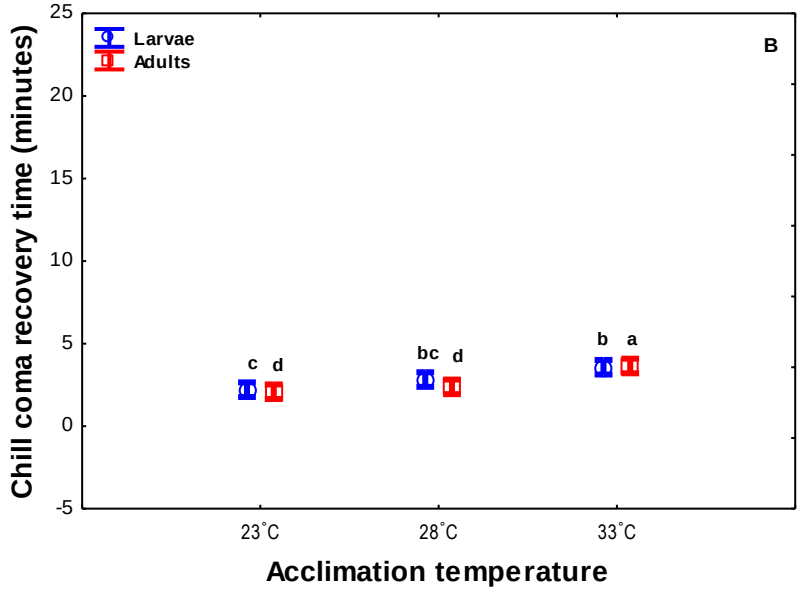
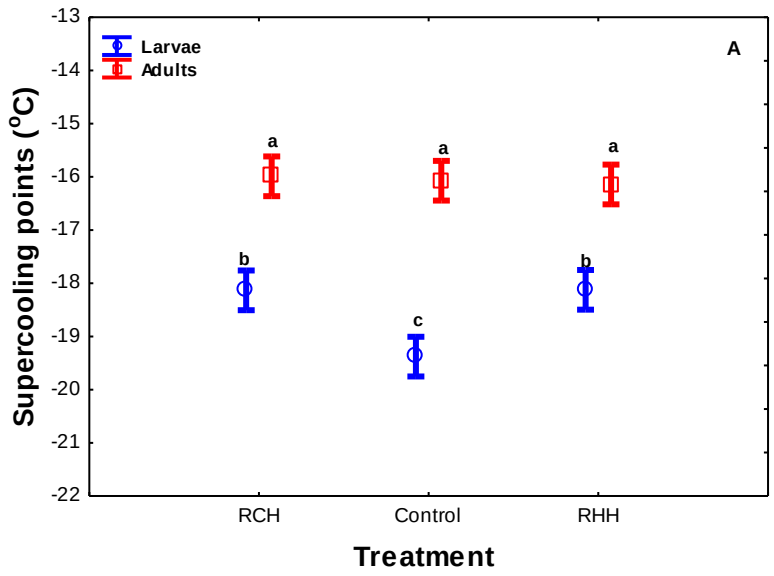


Figure 2: Effects of short-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42ºC for RHH; -2ºC for RCH) and adults (37˚C for RHH; 1˚C for RCH) and long-term acclimation (B)- three-day acclimation (23 ˚C; 28 ˚C and 33 ˚C) on chill coma recovery time (CCRT). Error bars represent 95% confidence limits (CLs) (*n*=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.



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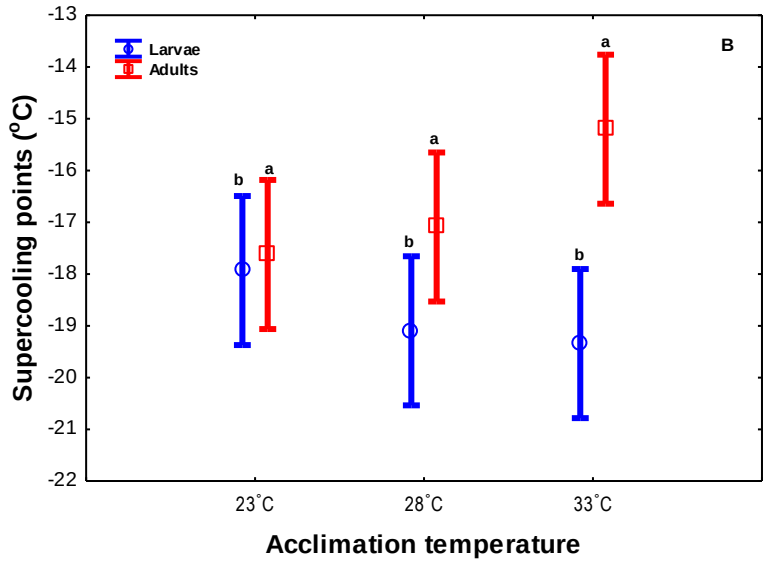

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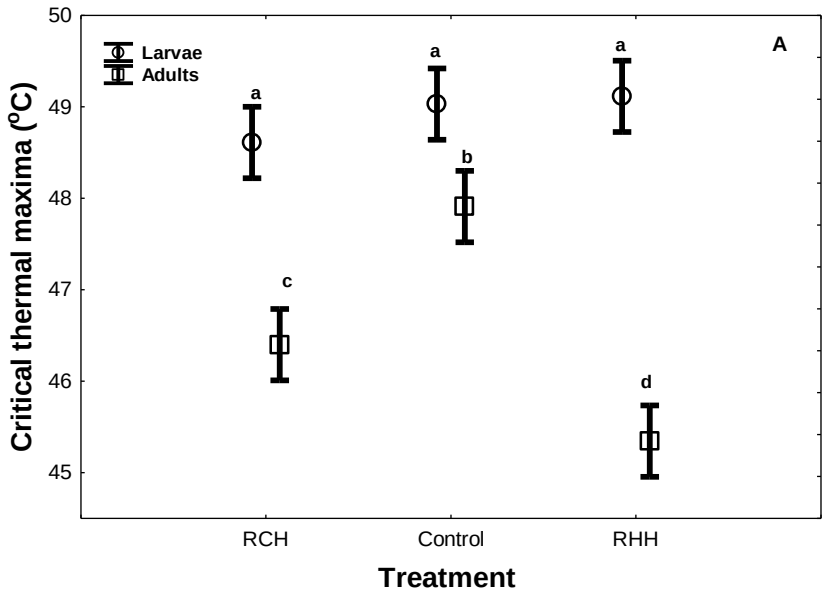
769 **Figure 3:** Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta*
770 larvae (42˚C for RHH; -2˚C for RCH) and adults (37˚C for RHH; 1˚C for RCH) and long-term
771 acclimation (B)- three-day acclimation (23˚C; 28˚C and 33˚C) on supercooling points (SCPs).
772 Error bars represent 95% confidence limits (CLs) (*n*=20 per group). Means with the same letter
773 are not significantly different from each other. RHH and RCH represents rapid heat- and -cold
774 hardening respectively.



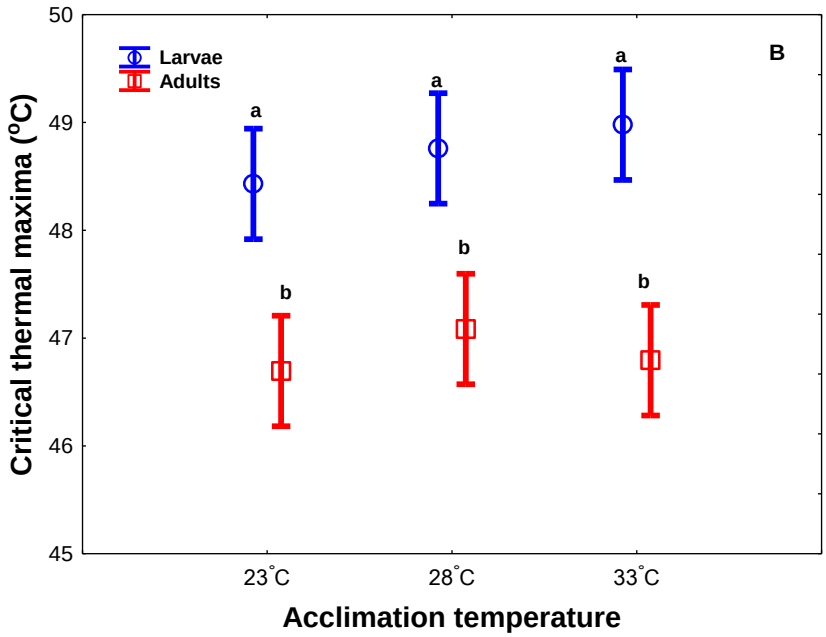
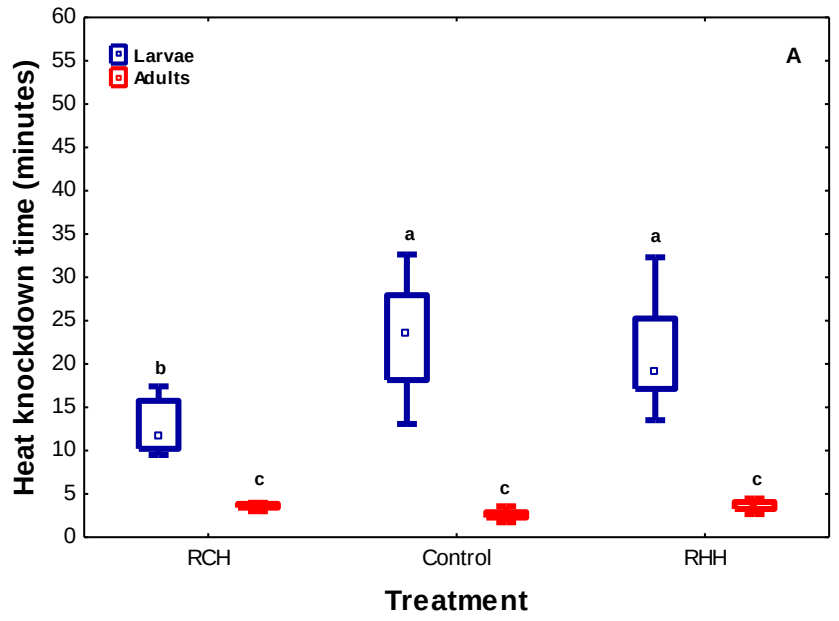


Figure 4: Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42˚C for RHH; -2˚C for RCH) and adults (37˚C for RHH; 1˚C for RCH) and long-term acclimation (B)- three-day acclimation (23˚C; 28˚C and 33˚C) on critical thermal maxima (CT_{max}.) Error bars represent 95% confidence limits (CLs) (*n*=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.



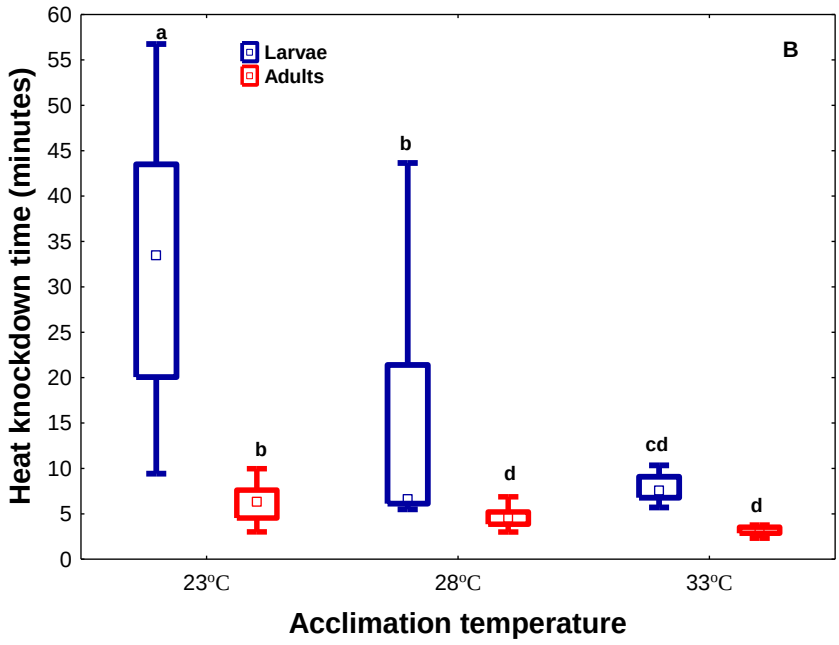


Figure 5: Effects of long-term acclimation (A)- 2-h pre-treatments/hardening of *Tuta absoluta* larvae (42˚C for RHH; -2˚C for RCH) and adults (37˚C for RHH; 1˚C for RCH) and long-term acclimation (B)- three-day acclimation (23˚C; 28˚C and 33˚C) on heat knockdown time (HKDT). Error bars represent 95% confidence limits (CLs) (*n*=20 per group). Means with the same letter are not significantly different from each other. RHH and RCH represents rapid heat- and -cold hardening respectively.

Table 1. Summary table showing hardening temperatures and duration for *T. absoluta* larvae and adults

Developmental stage	Hardening high temperature (˚C)	Hardening low temperature (˚C)	Control temperature(˚C)
Larvae	42 (2h)	-2 (2h)	28
Adults	37 (2h)	1 (2h)	28

Table 2. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42˚C; -2˚C) and adults (37˚C; 1˚C) and three-day acclimation (23˚C; 28˚C and 33˚C) on CT_{min}. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	p
CT_{min}	*Hardening*					
	Intercept	191.77	1	191.77	345.80	< 0.001
	Life stage	117.81	1	117.81	212.43	< 0.001
	Treatment	177.68	2	88.84	160.19	< 0.001
	Life stage×Treatment	209.38	2	104.69	188.78	< 0.001
	Error	63.22	114	0.55		
	Acclimation					
	Intercept	34.03	1	34.03	68.47	< 0.001
	Life stage	503.07	1	503.07	1012.24	< 0.001
	Acclimation	8.66	2	4.33	8.72	< 0.001
	Life stage×Acclimation	1.03	2	0.52	1.04	0.36
	Error	56.66	114	0.5		

805 **Table 3**. Summary table on short term responses to high temperature after 2-h pre-
806 treatments/hardening of *T. absoluta* larvae (42˚C; -2˚C) and adults (37˚C; 1˚C) and three-day
807 acclimation (23˚C; 28˚C and 33˚C) on CCRT. SS = sum of squares, df = degrees of freedom,
808 MS = means of squares.

Trait	Effect	SS	df	MS	F	p
CCRT	*Hardening*					
	Intercept	3714.64	1	3714.64	88.82	< 0.001
	Life stage	668.59	1	668.59	15.99	< 0.001
	Treatment	2037.07	2	1018.54	24.35	< 0.001
	Life stage×Treatment	1473.12	2	736.56	17.61	< 0.001
	Error	4767.79	114	41.82		
	Acclimation					
	Intercept	931.3969	1	931.4	932.89	< 0.001
	Life stage	0.8031	1	0.80	0.80	0.37
	Acclimation	44.4514	2	22.22	22.26	< 0.001
	Life stage×Acclimation	1.3589	2	0.68	0.68	0.51
	Error	113.82	114	0.99		

809

810 **Table 4**. Summary table on short term responses to high temperature after 2-h pre-
811 treatments/hardening of *T. absoluta* larvae (42˚C; -2˚C) and adults (37˚C; 1˚C) and three-day
812 acclimation (23˚C; 28˚C and 33˚C) on SCPs. SS = sum of squares, df = degrees of freedom,
813 MS = means of squares.

Trait	Effect	SS	df	MS	F	p
SCP	*Hardening*					
	Intercept	35942.14	1	35942.14	50592.06	< 0.001
	Life stage	184.09	1	184.09	259.13	< 0.001
	Treatment	10.62	2	5.31	7.48	< 0.001
	Life stage×Treatment	10.45	2	5.22	7.35	< 0.001
	Error	80.99	114	0.71		
	Acclimation					
	Intercept	37655.36	1	37655.36	3564.70	< 0.001
	Life stage	139.04	1	139.04	13.16	< 0.001
	Acclimation	13.75	2	6.87	0.65	0.52
	Life stage×Acclimation	73.76	2	36.88	3.49	0.03
	Error	1204.23	114	10.56		

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Table 5. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42˚C; -2˚C) and adults (37˚C; 1˚C) and three-day acclimation (23˚C; 28˚C and 33˚C) on CT_{max}. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	p
CT_{max}	*Hardening*					
	Intercept	273435.6	1	273435.6	352409.3	< 0.001
	Life stage	168	1	168	216.6	< 0.001
	Treatment	33.9	2	17	21.9	< 0.001
	Life stage×Treatment	35.5	2	17.7	22.9	< 0.001
	Error	88.5	114	0.8		
	Acclimation					
	Intercept	274075.7	1	274075.7	204793.5	< 0.001
	Life stage	104.3	1	104.3	78	< 0.001
	Acclimation	3.2	2	1.6	1.2	0.31
	Life stage×Acclimation	1.6	2	0.8	0.6	0.56
	Error	152.6	114	1.3		

Table 6. Summary table on short term responses to high temperature after 2-h pre-treatments/hardening of *T. absoluta* larvae (42˚C; -2˚C) and adults (37˚C; 1˚C) and three-day acclimation (23˚C; 28˚C and 33˚C) on HKDT. SS = sum of squares, df = degrees of freedom, MS = means of squares.

Trait	Effect	SS	df	MS	F	p
HKDT	*Hardening*					
	Treatment	380	2	190	11.44	< 0.001
	Life stage	7635	1	7635	459.94	< 0.001
	Treatment×Life stage	523	2	261	15.75	< 0.001
	Residuals	1892	114	17		
	Acclimation					
	Acclimation	3775	2	1888	28.1	< 0.001
	Life stage	5754	1	5754	85.67	< 0.001
	Acclimation×Life stage	2268	2	1134	16.88	< 0.001
	Residuals	7657	114	67		