1 **Rapid responses are essential: adaptive temporal variation in cold tolerance of the invasive**

2 **Fall Armyworm**

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13 **Running head: Temporal FAW stress adaptation**

Abstract

Overwintering presents an ecological challenge to insects given highly contrasting environments between native versus invaded environments. The capacity for *in situ* adaptation through tracking temporal changes in ambient conditions through phenotypic plasticity is thus ecologically important. The Fall Armyworm is a tropical invasive economic pest of cereals that has become a major biosecurity threat globally. While reports suggest fall armyworm overwinter in tropical environments, little is known on how it survives the environmentally-divergent invaded environments. Here, we tested whether fall armyworm critical low temperature limits to activity adaptively tracked temporal ambient environments. Using field collected populations over 2 years, we show that fall armyworm low temperature responses tracked environmental ambient temperatures. Summer 2020 collected larva had significantly lower cold tolerance (high critical thermal minima [CT_{min}] and chill coma recovery time [CCRT]) than those from winter seasons. However, winter collected larva had the highest cold tolerance (lower CT_{min} and CCRT). These results show adaptive fall armyworm responses to ambient temperature environments suggesting that both long-term and short-term responses may shape pest thermal traits and overall ecology.

Keywords: Invasive species; Overwintering; Pest Management; Phenotypic plasticity; *Spodoptera frugiperda*; Tropical pests

Word count = 8468

Introduction

Temperature plays an important role in ectotherms' growth and development and thus ecological structure of insects (Mellanby, 1939; Taylor, 1981; Sinclair *et al.*, 2015). For example, temperature affects insect phenology (Glazaczow *et al.*, 2016; Peñuelas & Filella, 2001), fecundity, longevity (Ma *et al.*, 2017; Garrad *et al.*, 2016) and activity e.g. mate finding and ecosystem service delivery (Lee & Denlinger, 2010; Gotcha *et al.*, 2021). As such, temperature may singly be the biggest environmental barrier to invasive insects' establishment and success in invaded environments (Richardson & Pyšek, 2006; Nyamukondiwa *et al.*, 2022). Given the increase in extreme temperatures with climate change (Johnson *et al.*, 2018; Stillman 2019); insects are thus continually exposed to sub-optimal low temperatures at different diurnal and seasonal timescales. Low temperatures limit insects' activity, prolong development, and in extreme cases may cause cell and tissue injury and/or death. To survive this, insects living in seasonal habitats have evolved various strategies to overcome these stressors through what is broadly referred to as 'overwintering' (Leather, 2008) while maintaining key functional traits at low temperatures (Block, 1990). Insects may overwinter through using different inherent mechanisms including diapause, migration, depression of supercooling points (SCP) i.e., through directed internal body freezing (Bale & Hayward, 2010; Gullan & Cranston, 2014). Migration and diapause are both behavioral strategies to cope with stress. For example, during migration insects relocate from the stressful environments to more benign ones (Sheikh *et al.*, 2017). Diapause involves *in situ* adaptation through e.g. hibernation (for low temperatures) or aestivation (for high temperatures) where insects lower metabolic and consumption rates until environmental conditions are more optimal (Andrewartha, 1952).

The role of overwintering through diapause or migration has extensively been discussed in literature and helps organisms escape extreme low temperatures *in situ* and *ex situ* respectively (Bale & Hayward, 2010; Chapman *et al*., 2015). The reduction of SCPs allows organisms to depress freezing temperatures and thus prevent bodily fluids from freezing injury (Zachariassen, 1985). Similarly, directed body freezing involves osmotic sequestering of water molecules to the extracellular space thus limiting organ and tissue damage from ice crystals. This strategy is used by a few insects that can withstand very low winter temperatures such as *Pyrrharctia isabella* and *Pterostichus brevicornis* (Kaufmann, 1971; Layne *et al*., 1999; Ramløy, 2000; Bale, 2002). Diapause and migration are widely used by insects and represent two key strategies that explain overwintering survival. For insects overwintering *in situ*, or where conditions are not predictable, tracking environmental ambient temperature and adjusting stress responses concomitant with prevailing ambient environments is critical. Empirical studies have shown that thermal acclimation from exposure to temperatures within thermal limits for minutes to hours (short term) and days to over extended time periods such as seasonally (long term) improves thermal phenotypes in *Drosophila melanogaster*, *Busseola fusca*, *Chilo partellus, Spodoptera frugiperda* and related organisms (Gerken *et al*., 2015; Sgrò *et al*., 2016; Mutamiswa *et al*., 2018b; Segaiso *et al*., 2022). Acclimation is thus important in improving fitness across different timescales. Rapid cold hardening - the ability to improve low temperature tolerance in the short-timescales, a form of acclimation is required to survive unpredictable short bursts of low temperatures (Lee *et al*., 1987). Acclimation may increase survival in thermally variable environments and compensate for more prolonged natural selection (Basson *et al*., 2012; Tarusikirwa *et al*., 2020). For invasive species that end up occupying new habitats with unpredictable changes in climate

82   that may be different from their native seasons hard-wired to their overwintering strategies,

83   rapid, *ad hoc* responses may be required.

84

85   Cold tolerance traits affect multiple organ systems and are thus used to indirectly determine the

86   effects of cold in these systems (Davis *et al*., 2021). Chill coma recovery time does not measure

87   recovery from critical thermal minimum temperature (CT_{min}), but from chill coma, thus may give

88   varying results when compared to other low temperature metrics (Andersen & Overgaard, 2019).

89   Critical thermal minima result in an increase in extracellular potassium concentrations in neurons

90   due to poor regulation of ion pumps which results in neural depolarization in the central nervous

91   system (Andersen & Overgaard, 2019; Armstrong *et al*., 2012; Rodgers *et al*., 2010). Chill coma

92   recovery time however measures the time it takes for insects to recover ion and water

93   homeostasis in muscles when benign temperatures resurface following chill coma onset

94   (MacMillan *et al*., 2014; MacMillan *et al*., 2012). Supercooling point on the other hand measures

95   the lowest temperature at which insects can be cooled down to before internal body fluids freeze

96   (Lee *et al*., 1996).

97

98   Climate change has resulted in more benign to warmer winters over the past few decades

99   (MacDonald, 2010; EPA, 2022). This has caused shifts in seasons, in particular earlier springs,

100   extended growing seasons for plants and changes in phenological syncs (Marshall *et al*., 2020;

101   Liu *et al*., 2018). Despite the warmer winters, there is an increased trend of bursts of acute

102   extreme low temperature events even in areas or seasons that are not characterized by sub-zero

103   temperatures (Kug *et al*., 2015; van Der Walt & Fitchett, 2021; Macquarrie *et al*., 2019)

104   rendering even the existing intrinsic overwintering strategies ineffective in some cases. This

105   means that insects may be subjected to a greater degree of cold stress (Marshall *et al*., 2020) as

106   well as abrupt high seasonal temperature variations (Marshall *et al*., 2020), even in the tropics.

107   How insects cope with these acute episodes of seasonal high and low temperature variations,

108   prolonged or much shortened low temperature (e.g., winter season) or prolonged or shortened

109   high temperature (e.g., summer season) is not exhaustive in literature (Williams *et al*., 2015;

110   González-Tokman *et al*., 2020), particularly for invasive species in new habitats. Insects should

111   thus, adaptively track micro-ambient environmental temperature to safeguard against unexpected

112   rapid temperature fluctuations and prevent mortality associated with acute changes in ambient

113   environments (Halsch *et al*., 2021).

114

115   The Fall armyworm *Spodoptera frugiperda* (J. E. Smith) is an invasive pest of cereal crops

116   including its main host maize which originates from South America (Sparks, 1979; Midega *et*

117   *al*., 2018; Kenis *et al*., 2022; Tay *et al*., 2023). In the past decade, it has successfully invaded

118   most parts of the World because of its high invasiveness, where it continues to cause economic

119   damage to cereal and other crop plants (Johnson, 1987; Nagoshi *et al*., 2012; FAO, 2020; Kenis

120   *et al*., 2022; Tay *et al*., 2023). Unlike other insects, the fall armyworm does not diapause

121   (Sparks, 1986). Because of its low overwintering capacity, it cannot survive the low winter

122   temperatures in cooler parts of the United States (Westbrook *et al*., 2019), southern China and

123   other Asian countries such as Japan and Korea thus, it re-invades these areas post-winter (Early

124   *et al*., 2018; Li *et al*., 2020). Similarly, in South Africa, fall armyworm overwinters in Northern

125   Limpopo and KwaZulu-Natal, where they re-invade other parts of the country, including the

126   Eastern Cape and Western Cape (Van den Berg *et al*., 2020). There are limited studies on low

127   temperature responses of the fall armyworm although that is ecologically significant for

128   explaining overwintering capacity. Most studies to date have focused on high temperature

129   tolerance and plasticity as well as its effects on insect population dynamics (e.g. Keosentse *et al*.,

130   2022; Huang *et al*., 2021; Segaiso *et al*., 2022). To our knowledge, no work has looked at the

131   possible plastic, rapid responses of fall armyworm to changes in cold tolerance traits, despite its

132   importance in rapidly changing environments. A study by Keosentse *et al*., (2022) reported that

133   rapid cold hardening at developmental stage specific temperatures had deleterious effects on

134   CT_{min} of fall armyworm adults and 4th and 6th instar larvae. In another study that measured the

135   survivability of fall armyworm eggs and larvae at low temperatures, older larval instars survived

136   longer that eggs and younger larval instars (1st and 2nd) (Zhang *et al*., 2021). Here, we thus

137   investigated whether fall armyworm critical low temperature activity traits likely tracked the

138   prevailing temperatures as a rapid adaptation mechanism. Specifically, we tested seasonal

139   changes in larval low temperature tolerance traits *vis* critical thermal minima (CT_{min}), chill coma

140   recovery time (CCRT) and SCPs across two seasons (each replicated twice) in Botswana. We

141   hypothesised that fall armyworm cold tolerance will be higher as temperatures plummet though

142   winter and that it will be lower during high summer temperatures. Furthermore, we also

143   hypothesized that short term responses to acute temperature conditions, i.e. acute thermal

144   conditions prevailing just before sampling in the short term may also influence phenotype

145   estimates (as in e.g. Nyamukondiwa & Terblanche 2010).

146

Materials and Methods

Field collection of insects

Field populations of fall armyworm were collected as 3rd instar larvae from commercial and subsistence maize farms within Central District, Botswana: Bobonong (S 22°.13467; E28°.59468"), Machaneng (S 23º 04.320"; E 027º31.874") Dikabeya (S 22°28'37.2"; E 027°13'49.8"), Serowe (S 22°26'00.3" E 026°49'05.6"). All experimental fall armyworm larvae were collected from maize. These three sampling areas are separated by ~214 km (furthest point), are environmentally homogeneous and have similar savanna type of vegetation with average annual rainfall ranging from 321 - 430 mm (Akinyemi, 2021). Summer temperatures range from 32 to 39 °C (Akinyemi, 2017). Sampling was done on the 4th December for summer 2020 and 2nd – 12th December in summer 2021. Winter sampling was done on the 11th of June 2021 and 4th and 9th of May 2022. Summer months in Botswana are between November to March while winter is from May to August (Jain *et al*., 2006). Insects were collected and individually placed in 50 ml vials with gauzed lids containing a meridic sorghum leaf based artificial diet. The diet contains bean and brewer's yeast as protein sources, sorghum leaf which is their natural diet, and other preservatives and antibiotic to prevent bacterial growth, (see full description in Tefera (2010). In the laboratory insects were kept in Memmert climate chambers (Memmert GmbH + Co. KG, Schwabach, Germany) set at 28 °C, 65% relative humidity (RH) and photocycle period of 12 h light and 12 h dark. Insects were kept for a period not exceeding 24 hr before experiments were run to circumvent effects associated with laboratory adaptation. Field populations fed on maize plants before sampling while the laboratory population was fed exclusively on artificial diet saved as control populations. The main fixed factor considered was

170   the season (winter or summer) in which larvae were collected, diet and age were standardised

171   and therefore not considered as factors. Critical thermal minima, chill coma recovery time

172   (CCRT) and supercooling points (SCP) served as the variable factors.

173

174   **Cold tolerance metrics**

175   *Critical thermal minima (CT_{min})*

176   Critical thermal minima were measured using protocols by Nyamukondiwa & Terblanche

177   (2009). Individual 3rd instar fall armyworm larvae were demarcated using head capsule size (as

178   in Montezano *et al.,* 2019). These were placed in organ pipes in an insulated double-jacketed

179   chamber connected to a programmable water bath. The water bath (Lauda Eco Gold, Lauda

180   DR.R. Wobser GMBH and Co. KG) filled with 1:1 water: propylene glycol was set at a ramping

181   rate of 0.25 °C/min at which the insects were subjected to a gradual temperature decrease in the

182   chamber. This ramping rate remains faster than diurnal temperature fluctuations but is more

183   ecologically relevant than most ramping rates in nature, that often include 0.5 and 1 °C/min (see

184   discussions in Terblanche *et al*., 2011). Temperature experienced by the insects was recorded

185   using a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05 °C)

186   inserted in the control organ pipe. The experiment was run twice yielding $n = 20$ (20 replications

187   per treatment). The temperature at which larvae lost coordinated muscle function and did not

188   respond to gentle probing by a thermally inert object or ability to self-right was recorded as the

189   CT_{min} (Mutamiswa *et al*., 2018).

190

191   *Chill coma recovery time (CCRT)*

192 Chill coma recovery time experiments were conducted using established protocols (Mutamiswa

193 *et al*., 2018; Weldon *et al*., 2011). Ten insects were individually placed in 2.0 ml microcentrifuge

194 tubes and placed in two water-tight ziplock bags layered one on top of the other. A thermocouple

195 (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation,

196 China; accuracy: 0.05 °C) was inserted into a separate 2.0 ml microcentrifuge tube and placed

197 together with the insects in the ziplock bags. The Ziplock bag with the insects and thermocouple

198 were submerged into a water bath (Systronix, Scientific, South Africa) filed with absolute

199 alcohol and set at 0 °C for an hour. It has been previously shown that this temperature and time

200 treatment elicits chill coma in fall armyworm and other related insect taxa (Keosentse *et al*.,

201 2021; Tarusikirwa *et al*., 2020). After 1 hour, the insects were quickly transferred to a climate

202 chamber (Memmert GmbH + Co. KG, Schwabach, Germany) set at optimal 28 °C and 65% RH

203 for recovery. The time (in minutes) it took for insects to regain consciousness i.e., self-righting

204 through e.g., movement after being chilled was described as the CCRT (Tarusikirwa *et al*., 2020

205 and citations therein). The data was recorded using a video recording camera (HD Covert

206 Network Camera, DS2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China)

207 connected to the climate chamber. The experiment was performed twice, yielding n = 20 (20

208 replications).

209

210 *Supercooling point (SCP)*

211 Supercooling points were measured using protocols outlined by Nyamukondiwa *et al*.,

212 (2013). Insects were individually placed in 2.0 ml microcentrifuge tubes; each insect was placed

213 in contact with the tip of a type-T copper-constantan thermocouple (762–1121, Cambridge, UK).

214 To ensure that the insect and the thermocouple stayed in contact, they were secured using cotton

215   wool. Thermocouples were connected to an 8-channel Picotech TC-08 (Pico Technology,

216   Cambridge, UK) interfaces and temperatures were recorded at 1 second intervals using PicoLog

217   software for windows (Pico Technology). Insects were first subjected to a temperature of 15 °C

218   for 10 minutes followed by a gradual decrease in temperature at rate of 0.25 °C/min until their

219   SCPs were recorded. SCP for each organism was determined as the lowest temperature recorded

220   before a spike in temperature due to latent heat of crystallization (Nyamukondiwa *et al*., 2013).

221   Each treatment had twenty animals/replications (n = 20), in keeping with previous similar studies

222   (see Nyamukondiwa *et al*., 2013).

223

224   Natural ambient temperature and rainfall data for the specific sampling sites for the two seasons

225   were obtained from the Department of Meteorological Services, Botswana, and were used to

226   infer environmental temperatures experienced by the insects under their natural settings and to

227   explain the effects of both long- and short-term conditions on thermal phenotypes tested.

228

229   **Data analyses**

230   Data was analyzed using Statistica 14.0.0.15 (Statsoft Inc., Tulsa, Oklahoma). The data were

231   tested for normality using Shapiro–Wilks test which indicated that data were not normally

232   distributed ($p < 0.05$); therefore, non-parametric analysis techniques were adopted. Kruskal-

233   Wallis non-parametric test was used to compare CT_{min}, CCRT and SCP of the fall armyworm

234   larvae collected in the different seasons. Kruskal-Wallis post-hoc was used to separate

235   statistically heterogeneous medians at 95% CI. Because no significant differences were observed

236   across treatments for SCPs, a frequency distribution curve was done using Statistica 14.0.0.15

237   (Statsoft Inc., Tulsa, Oklahoma). Temperature and rainfall data were plotted in OriginPro 8,

238   version 8E (Origin Lab Corporation, Massachusetts, USA).

239

240   **Results**

241   Minimum and maximum environmental temperature data collected from October 2020 to May

242   2022 within the specimen sampling areas showed that winters had expectedly lower temperatures

243   than summer seasons. Temperatures in winter (May-August) ranged from -0.5 to 32.8 °C, with

244   sub-zero temperatures being recorded thrice within the two winter seasons. However, although

245   summers (November-March) generally had high temperatures, summer 2021 was characterized

246   by significant incidences of acute low temperature episodes, including unusually low subzero

247   minimum temperatures of   -8 °C on the 16th of December 2021(**Fig. 1a**). Maximum temperature

248   recorded during the two seasons in summer from October to March was 38.7 °C and the lowest

249   minimum temperature was -8 °C (startlingly in summer) (**Fig. 1a**). Similarly, low to no rainfall

250   was recorded in both winter seasons; however, summer seasons had higher rainfall ranging from

251   0 - 98.5 mm. Apart from unusually low temperatures, summer 2021 was also characterized by

252   unusually high rainfall relative to 2020; reaching 98.5 mm in December 2021 (**Fig. 1b**).

253

254   Temporal seasonal temperature changes had significant effects on fall armyworm low

255   temperature tolerance limits measured as CT_{min} (Kruskal-Wallis test: H (4, N= 101) = 38.82554,

256   $p < 0.05$) (**Fig. 2**). The CT_{min} for all the winter seasons' larvae were generally low and not

257   statistically different from each other. Specifically, fall armyworm larvae collected in both winter

258   seasons as well as summer 2021 had the lowest CT_{min} (the highest cold tolerance) compared to

259   summer 2020 and the controls (except winter 2022 for the latter). Larval CT_{min} for summer 2020

260   was significantly higher than all other seasons ($p < 0.05$) but was statistically similar to the

261   control group (**Fig. 2**).

262

263   The sampling season had a significant effect on fall armyworm larval cold tolerance measured as

264   CCRT (Kruskal-Wallis test: H (4, N= 100) = 33.28580 7.36853, $p < 0.05$) (**Fig. 3**). The CCRT

265   for winter sampled larvae was not significantly different from the controls in both sampling

266   seasons/years. However, the summer collected larvae showed mixed results, i.e., summer 2021

267   larvae had significantly lower CCRT than control ($p > 0.05$), while summer 2020 collected

268   larvae had CCRT that showed no significant difference with the control (**Fig. 3**). In addition, the

269   CCRT for winter sampled larvae showed significant differences between the two seasons, with

270   winter 2022 sampled larvae having significantly lower CCRT (higher cold tolerance) than winter

271   2021 collected specimens ($p < 0.05$). Similarly, cold tolerance of the larvae collected in the two

272   summers significantly different from each other. Fall armyworm larvae collected in summer

273   2020 had significantly higher CCRT (lower cold tolerance) than those collected in 2021 ($p <$

274   0.05) with the latter having CCRT significantly lower than the control ($p < 0.05$). This is

275   consistent with the CT_{min} results where summer 2021 larvae had significantly lower CT_{min} than

276   both the summer 2020 and the control larvae.

277

278   Contrary to CT_{min} and CCRT results, there were no significant differences in the SCPs of fall

279   armyworm larvae across all seasons sampled and compared to the control larvae (Kruskal-Wallis

280   test: H (4, N = 100) = 7.368586, $p > 0.05$). Generally, SCPs ranged from -3.77 to -18.13 °C with

281   a median of -10.21 ± 0.296 °C for summer 2020, winter 2021, summer 2021, and winter 2022

282   and controls collectively and followed a unimodal distribution curve (**Fig. 4**)

283

Discussion

We set out to investigate whether fall armyworm critical low temperature limits to activity traits adaptively tracked the prevailing seasonal environmental temperatures through larval CT_{min}, CCRT and SCPs. Our results show that fall armyworm cold tolerance is higher in winter and lower in summer and that it likely tracks short term changes to acute environmental temperature regardless of the season, i.e., larvae could track acute dips in environmental temperatures occurring in summer, increasing their cold tolerance in summer season as short-term response. Most insects are ectothermic and as such, their body temperatures always track ambient conditions (Stevenson, 1985). Given fluctuating thermal environments in nature, the ability of organisms to quickly adapt to thermally stressful events through rapid homeostatic adjustments in response to changing environmental temperatures is key to insect survival and ecological success (reviewed in Whitman & Ananthakrishnan, 2009). Prolonged low temperature-induced seasonal acclimatization has been shown to improve thermal tolerance in insects (see Bale & Hayward, 2010; Driedzic & Gesser, 1994; Whitman 2009; Sgrò et al. 2016). Although our experiments showed mixed results, generally trends were clear that fall armyworm low temperature responses track both fine-scale ambient environmental temperature and seasonal timescales. We recorded no significant difference in the SCPs across all treatment seasons and controls, suggesting no role of supercooling ability in fall armyworm overwintering survival. However, for both traits of CT_{min} and CCRT, winter collected larvae generally had higher cold tolerance, suggesting that long term acclimatization through diurnally changing low temperatures may elicit some improved cold tolerance responses, not only during winter, but also summer (where transient bouts of acute low temperatures are experienced). We also recorded a

14

306   statistically significant difference for CT_{min} between the two summers- but not between winter -

307   seasons, suggesting that both long- and short-term acclimatization may affect cold tolerance.

308   Similar trends were observed, for example, summer 2020 collected fall armyworm larvae had

309   higher CT_{min} than those collected in summer 2021, and likewise both season treatments for

310   CCRT showing the interplay between long- and short-term acclimatization in dictating field cold

311   tolerance.

312

313   Summer collected fall armyworm larvae had significantly higher CT_{min} (lower cold tolerance)

314   except for summer 2021 treatment. However, as expected, winter collected organisms showed

315   significantly lower CT_{min} values (higher cold tolerance). Since insects do not often experience

316   sub-optimal low temperatures during summer, insects may trade-off cold tolerance and allocate

317   more resources for heat tolerance and traits that are likely more influential for reproduction and

318   survival during summer (Behrman *et al*., 2015). A study done on *Drosophila melanogaster*

319   yielded similar results with adults collected during summer having lower cold tolerance relative

320   to those collected during the fall season (Noh *et al*., 2017). Seasonal shifting of the lower thermal

321   limits has also been reported in related Dipteran communities. For example, seven mosquito

322   species including *Aedes* and *Culex* genus increased their CT_{min} with increase in temperatures

323   from spring to summer and decreased their CT_{min} when environmental temperatures decreased

324   from summer to autumn (Oliveira *et al*., 2021). Similarly, Nyamukondiwa *et al*. (2013) also

325   showed that flies shift their thermal limits diurnally with shifting temperatures. For example,

326   insects sampled early in the morning where temperatures are lower had higher cold tolerance

327   than those sampled during the afternoon where temperatures are higher suggesting the ability of

328   insects to track their ambient environments at fine scale level. Cyclic variation in seasonal

329   environmental conditions provides efficient selection pressure for certain traits over others

330   (Hoffmann & Sgrò, 2011; Chevin *et al*., 2010). Change in critical thermal limits is more evident

331   in insects from temperate and sub-tropical zones that experience large disparities in

332   environmental temperatures between seasons and thus shifting critical thermal limits is critical

333   for survival (Janzen, 1967; Oliveira *et al*., 2021). However, even for tropical insects, insects

334   significantly accumulate carbohydrate cryoprotectants as temperatures fall and day length

335   shortens with winter (Teets & Denlinger, 2013). For example, insects have higher glycerol

336   content in winter than summer (Storey, 1990) and this may help them survive stressful low

337   winter temperatures. While we did not test these biochemical mechanisms in this study, we

338   speculate, with caveats that such mechanisms may explain the higher cold tolerance of fall

339   armyworm populations collected in winter and the cold summer of 2021. We thus, recommend

340   that future research may look into some of the biochemical mechanisms associated with

341   temporary low temperature adaptation in the fall armyworm.

342

343   Larval fall armyworm collected in summer 2021 showed superior cold tolerance traits than those

344   collected in both winter treatment seasons. Acute short- to medium-term bouts of sub-zero

345   temperatures and high rainfalls experienced during this sampling season may account for this

346   higher cold tolerance anomaly.. Thus, fall armyworm potentially rapidly acclimatized to these

347   short-term changes in environment through e.g. rapid cold hardening (as in e.g. Lee, 1987;

348   Koveos, 2001; Lee *et al*., 2006) as we hypothesised. Indeed, Nyamukondiwa & Terblanche,

349   (2010) showed that both long- and short-term acclimation modulates critical thermal limits in

350   insects. This may mean that both long term changes in ambient temperature environment with

351   season and more acute temperature conditions experienced just before insect sampling may both

352 have effects on fall armyworm responses. This points to the notion that fall armyworm thermal

353 responses track the prevailing environmental temperature both in the long term (season) and

354 short-term (hours to days - immediate ambient environment). Here, our data suggests that fall

355 armyworm larvae shifted its CT_{min} phenotype in the short term (*ad hoc*), synonymous to rapid

356 hardening responses (see Sgrò *et al*., 2016; Rodrigues & Beldade, 2020). Several laboratory

357 acclimation studies performed on other Lepidopteran species such as *Chilo partellus*, *Busseola*

358 *fusca* and *Tuta absoluta* have shown that short-term exposure to sub lethal low temperatures

359 improves cold tolerance (Mutamiswa *et al*., 2018b; Tarusikirwa *et al.,* 2020). Contrary to our

360 results, Keosentse *et al*. (2021) showed 3rd instar fall armyworm larvae showed a lack of cold

361 tolerance plasticity as short-term acclimation did not influence CT_{min}. However, exact

362 experimental methodology such as length of acclimation period can affect acclimation responses

363 (Rezende *et al*., 2011; Rohr *et al*., 2018). Thus, differences in experimental protocols can

364 potentially explain the availability or lack thereof of plastic responses in insects (Sgrò *et al*.,

365 2016).

366

367 Summer 2020 collected fall armyworm larvae cold tolerance was not significantly different from

368 control insects in both CT_{min} and CCRT. Temperatures experienced during summer 2020

369 generally ranged between 26 and 30 °C which is consistent with the control rearing temperature

370 (28 °C)   Occasional bouts of temperatures above 30 °C may be buffered by insects'

371 microclimates (Pincebourde and Woods, 2020) thus explaining the lack of difference in thermal

372 responses. There are wide temperature differences in insect fine scale microclimates which may

373 slightly buffer insects from high temperatures and affect their responses in thermal limits and

374 traits (Pincebourde *et al*., 2016; Pincebourde & Wood, 2020). Classically, cold tolerance is

375 measured as the ability of insects to develop and reproduce post cold stress exposure (Overgaard

376 & MacMillan, 2017). From the weather data experienced in the natural environments (**Fig. 1a**),

377 summer 2020 lacked critical low temperatures enough to elicit acclimation responses and thus,

378 may explain the lack of significant differences with the control.

379

380 Seasonal responses to low temperature were mixed; contrary to the insignificant differences

381 observed for CT_{min} between winter seasons, winter 2022 displayed lower CCRT than winter

382 2021. The cold tolerance traits measured in this study affect different organ, physiological

383 systems and bio-chemical systems (Rodgers *et al*., 2010; Armstrong *et al*., 2012; MacMillan *et*

384 *al*., 2012; MacMillan *et al*., 2014; Andersen & Overgaard, 2019; Davies *et al.,* 2021). It is thus

385 not surprising that they gave varying results. Previous studies that worked with laboratory reared

386 and wild insect populations indicated that CCRT and CT_{min} are independent of each other and

387 thus may display divergent results across populations and genotypes (Davis *et al*., 2021; Garcia

388 *et al*., 2020). Similarly, a study on *T. absoluta* showed contrasting results for traits of cold

389 tolerance, and that short-term acclimation improved CT_{min} while compromising CCRT

390 (Tarusikirwa *et al*., 2020). This further emphasizes that although the two traits (CT_{min} and

391 CCRT) are all low temperature tolerance metrics, their underlying physiological mechanisms

392 may be decoupled.

393

394 Our results for SCPs showed no significant differences across all seasons and treatments contrary

395 to other cold tolerance metrics of CT_{min} and CCRT. SCPs are affected by many factors including

396 species, feeding status, type of diet, body water content, and availability of other ice nucleating

397 agents and related factors (Lee *et al*., 1996). For example, insects often clear their gut contents in

398   winter in preparation for lower temperatures. A related study showed that seasonal change of

399   *Cydia pomonella* SCPs were in part attributed to feeding status and diet (Khani &

400   Moharramipour, 2010; Maes *et al*., 2015). Increased gut content increases the possibility of

401   having ice nucleating agents which support the creation of ice crystals (Koch *et al*., 2004;

402   Zachariassen, 1985). Despite field and control populations in our study having originated from

403   different diets there was no significant consequence on SCPs were recorded, contrary to previous

404   studies (e.g. Spranghers *et al*., 2017). Previous studies have also shown that for fall armyworm,

405   rapid cold hardening increased glycerol content and improved SCPs. However, several other

406   factors such as the permeability of cells to cryoprotectants affect the ability of insects to rapidly

407   adjust their low temperature tolerance phenotypes (Vatanparast & Park, 2022).Several reports

408   suggest that supercooling ability may be more ecologically relevant for more temperate than

409   tropical organisms such as fall armyworm. The lowest temperature recorded during the sampling

410   period was -8 °C while SCPs ranged from -3.77 to -18.13. Inferring on SCP results only,

411   majority of fall armyworm larvae can survive winter low temperatures in Botswana, consistant

412   with results by Keosentse *et al*. (2021). Previous studies have indicated that cold tolerance of fall

413   armyworm is life stage dependent, with adults being generally having higher cold tolerance than

414   earlier developmental stages (Keosentse *et al*., 2021; Zhang *et al*., 2021). However, SCPs results

415   have generally been contrasting and often inconclusive (Keosentse *et al*., 2021; Zhang *et al*.,

416   2021). This difference may be due to clinal variation. Therefore, lack of change in the SCP

417   across seasons for a period of 2 years could mean that other intrinsic or inherent factors (likely

418   heritable), had more influence on fall armyworm SCPs. Further studies may need to test this over

419   a high number of both winter and summer seasons.

420

421 In conclusion, the results show that fall armyworm cold tolerance tracks seasonal environmental

422 temperature both in the long and short timescales. First, we show that response to seasonal

423 change in temperatures in fall armyworm larvae vary with the cold tolerance matrix being

424 measured. While CT_{min} increased with rise in temperatures from winter to summer, season had

425 an opposite effect on fall armyworm CCRT. Second, we showed that fall armyworm cold

426 tolerance tracks environmental temperature through rapid 'non-seasonal' responses to ambient

427 environment. Indeed, summer 2021 collected larvae had higher cold tolerance than summer

428 2020, and similar to the winter collected organisms owing to the unusually short-term bouts of

429 low temperatures experienced during that summer (2021). Third, for SCP, the results showed a

430 lack of seasonal plasticity in fall armyworm, suggesting that the mechanisms underlying SCPs

431 may not be ecologically 'altered' by temporal short-term or seasonal changes in environmental

432 temperature for this tropical species. Overall, these results provide evidence that fall armyworm

433 has short- to long-term responses to 'seasonally' fluctuating environmental temperatures. This

434 may be beneficial for fall armyworm survival during both short- and long-term cold snaps (in

435 whichever season they occur) which have increased in frequency due to climate change (e.g. Kug

436 *et al*., 2015) aiding both its survival and invasion success. This raises questions on its

437 overwintering strategies in warmer areas such as Botswana. Further studies on the physiological

438 basis of this environmental low temperature tracking coupled to trends on local migration

439 patterns are needed to answer these questions. Thus, both short- and long-term temperatures

440 modulate fall armyworm population dynamics and thus incorporating such data into predictive

441 models may be important in improving current invasive pest forecasts and distribution models.

442

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450   The authors declare no conflict of interest.

451

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453   The datasets during and/or analysed during the current study available from the corresponding

454   author on reasonable request.

455

459

460   **Authors & contributions**

461   Project conceptualization and management: BS HM CN.

462   Data curation: PM.

463   Formal analysis: BS HM.

464   Investigation: BS HM.

465   Visualization and validation: BS HM CN.

466   Writing – original draft: BS HM CN.

467   Writing, review and editing: BS HM CN.

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724   **Figures**

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726   **Figure 1a**

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729   **Figure 1b**

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Figure 2

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Figure 3

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736   **Figure 4**

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762   **Figure 4**: Supercooling points (SCPs) for field populations of fall armyworm 3rd instar larvae

763   collected in different seasons compared to lab reared (28±1 ℃, 65±10% RH) control population

764   (n = 20). Winter and summer test organisms were collected in the months of May and December

765   respectively in each of the years. Frequency distribution graph of supercooling points in larval

766   fall armyworm across different seasons showing a unimodal distribution curve.

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