

Effect of thermal acclimation on the tolerance of the peach fruit fly (*Bactrocera zonata*: Tephritidae) to heat and cold stress

Michael Ben-Yosef^{a,*}, Yam Altman^a, Esther Nemni-Lavi^a, Nikos T. Papadopoulos^b, David Nestel^a

^a Department of Entomology, Institute of Plant Protection, Agricultural Research Organization, Rishon Letzion, 7528809, Israel

^b Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece

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ABSTRACT

Understanding the thermal biology of insects is of increasing importance for predicting their geographic distribution, particularly in light of current and future global temperature increases. Within the limits set by genetic makeup, thermal tolerance is affected by the physiological conditioning of individuals (e.g., through acclimation). Considering this phenotypic plasticity may add to accurately estimating changes to the distribution of insects under a changing climate.

We studied the effect of thermal acclimation on cold and heat tolerance of the peach fruit fly (*Bactrocera zonata*) – an invasive, polyphagous pest that is currently expanding through Africa and the Middle East. Females and males were acclimated at 20, 25 and 30 °C for up to 19 days following adult emergence. The critical thermal minimum (CT_{min}) and maximum (CT_{max}) were subsequently recorded as well adult survival following acute exposure to chilling (0 or –3 °C for 2 h). Additionally, we determined the survival of pupae subjected for 2 h to temperatures ranging from –12 °C to 5 °C.

We demonstrate that acclimation at 30 °C resulted in significantly higher CT_{max} and CT_{min} values (higher heat resistance and lower cold resistance, respectively). Additionally, adult recovery following exposure to –3 °C was significantly reduced following acclimation at 30 °C, and this effect was significantly higher for females. Pupal mortality increased with the decrease in temperature, reaching LT₅₀ and LT₉₅ values following exposure to –0.32 °C and –6.88 °C, respectively. Finally, we found that the survival of pupae subjected to 0 and 2 °C steadily increased with pupal age.

Our findings substantiate a physiological foundation for understanding the current geographic range of *B. zonata*. We assume that acclimation at 30 °C affected the thermal tolerance of the flies partly through modulating feeding and metabolism. Tolerance to chilling during the pupal stage probably changed according to temperature-sensitive processes occurring during metamorphosis, rendering younger pupae more sensitive to chilling.

1. Introduction

Climate ultimately determines the spatial and temporal distribution limits of all organisms, and is particularly relevant in restricting the geographic distribution of temperature-sensitive, ectothermic animals such as insects (Kellermann and van Heerwaarden, 2019). Global climate change, especially global warming, increases the risk of invasion into new areas for many insect pests. Indeed, some incidences of range expansions, particularly into temperate areas, were previously linked to climate change, and generally predicted to increase due to global

heating (Robinet and Roques, 2010; Battisti and Larsson 2015; Hill et al., 2016). A common driver for some successful establishments was reduced winter mortality in newly invaded areas due to higher temperatures (Battisti and Larsson 2015). These observations correspond with the well-established latitude-dependent decline of insect lower (but not higher) thermal limits (Addo-Bediako et al., 2000), and highlight the interaction between climate and insect thermal physiology in determining their geographical redistribution under a changing climate (Kellermann and van Heerwaarden, 2019).

Predicting climate-dependent changes in range distributions of

* Corresponding author. Department of Entomology Agricultural Research Organization, Gilat Center M. P. Negev, 8531100, Israel
E-mail address: michaelb@volcani.agri.gov.il (M. Ben-Yosef).

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insects, and particularly that of pests, is of increasing concern, and requires sound biological data on the thermal tolerance of the species in question (Terblanche et al., 2015; Hill et al., 2016; Gutierrez et al., 2021). These usually include temperature thresholds for development, reproduction and mortality of different life stages under constant conditions in the laboratory (e.g. as in Gutierrez et al., 2021). Nevertheless, within the fixed limits of genetic makeup, the physiological capacity to tolerate thermal stress can be modified to support increased tolerance to temperature extremes (e.g., through thermal hardening or acclimation; Terblanche and Hoffmann, 2020). This phenotypic plasticity was comprehensively demonstrated in the lab in relation to temperature (reviewed by Sgrò et al., 2016) and associated with mechanisms such as upregulation of heat shock proteins or the capacity to maintain ion homeostasis (González-Tokman et al., 2020; Overgaard et al., 2021). Survival trials of laboratory-acclimated insects in the field indicate that acclimation contributes to thermal stress resistance *in natura* as well, and as such is also ecologically relevant (Kristensen et al., 2008; Terblanche et al., 2015). Further work on *Drosophila* flies indicates that plasticity in thermal tolerance following acclimation or thermal hardening mainly applies to cold-stress resistance (Nyamukondiwa et al., 2011; Overgaard et al., 2011; Sørensen et al., 2016; MacLean et al., 2019). Additionally, *Drosophila* species that are widespread were found to be more cold-resistant, and overall demonstrated similar or slightly higher capacity to adapt to heat and cold stress following acclimation compared to counterparts restricted to tropical habitats (Mitchell et al., 2011; Overgaard et al., 2011; Boher et al., 2016; MacLean et al., 2019). Overall, these studies point to the different ability for species to handle temperature extremes and particularly cold stress, and to its association with geographic origin and degree of spread (generally being higher for temperate compared to tropically-restricted species). Additionally, the capacity to acclimate is important for adapting to temperature stress, but its association with geographic origin seems to be weak or inconclusive (Sørensen et al., 2016; MacLean et al., 2019). Other, external factors may also affect the thermal resistance of insects. These include nutrition or microbial symbionts acting through nutrition (Andersen et al., 2010; Kostál et al., 2012; Yerushalmi et al., 2016; Raza et al., 2020; Ben-Yosef et al., 2023), which further suggests that thermal stress resistance is adaptive and responds to the environment. Accordingly, the phenotypic variation in thermal tolerance is important for assessing the thermal sensitivity of a species and to account for its geographic distribution (see Terblanche and Hoffmann, 2020). The basal and acquired components of cold tolerance may be particularly important for predicting dispersion into temperate habitats.

True fruit flies (Diptera: Tephritidae) constitute a large group of phytophagous species including some of the most devastating fruit pests worldwide (White and Elson-Harris, 1992; Papadopoulos 2014). A number of polyphagous species of the genus *Bactrocera*, native to the tropics of south-East Asia, have expanded their geographic distribution range in the past decades (Clarke et al., 2005; Gutierrez et al., 2021). Some of these, such as *B. dorsalis* and *B. zonata* have invaded and established in Africa, China, Mediterranean and Middle East countries, and pose potential risks for invading Mediterranean Europe and north America (Zingore et al., 2020; Qin et al., 2019 and references therein). Successful regulation of thermal stress, and particularly that of cold tolerance was suggested as one aspect affecting the potential geographic distribution of these flies, as well as that of other tephritids (Nyamukondiwa et al., 2010; Royer et al., 2016; Pieterse et al., 2017; Clarke et al., 2019; Moraiti et al., 2022). A growing body of evidence regarding the range expansion of *B. tryoni* - a closely related, tropical and invasive fruit fly, in temperate and sub-tropical Australia indicates that low temperatures are the major limitation to geographic distribution. Additionally, adults are the main (if not the only) developmental stage that survives through winter (reviewed by Clarke et al., 2019). However, their cold tolerance was recently found to be independent of latitude (Popa-Báez et al., 2020). Other studies on the distribution of *B. dorsalis* in China suggest an undergoing adaptation to cooler climates

corresponding with its expansion northward (Wang et al., 2014). Recently, a latitude-associated acute cold resistance was described for adult *Ceratitidis capitata* in Europe (Moraiti et al., 2022), indicating that populations differently adapt to handle cold stress as has been described for drosophilids (e.g. Hoffmann et al., 2002). These studies suggest that tropical tephritids expanding into temperate habitats are adapting to cope with cold stress. The contribution of basal thermal resistance and its adaptive aspects to dispersion, addressed in the past for some species (Nyamukondiwa et al., 2010; Pieterse et al., 2017; Weldon et al., 2018), remains important for understanding the potential distribution of other flies undergoing range expansion into temperate habitats.

Bactrocera zonata (the peach fruit fly) is currently expanding its geographic range through eastern and northern Africa and the Middle East (CABI Invasive Species Compendium, <https://www.cabi.org/isc/>). Throughout its current geographic distribution the fly has established in both temperate and hot habitats, suggesting a high climatic adaptability, and a potential for further spread. According to past and recent simulations (based on fixed thermal limits for development of all stages; Duyck et al., 2004) the potential distribution range of the fly under a scenario of global warming includes Mediterranean and subtropical regions, and most immediately, parts of Mediterranean Europe and North Africa (Ni et al., 2012; Zingore et al., 2020). The reasons contributing to its spread probably include anthropogenic and climate-related aspects, and possibly also an adaptable physiology. In this study we characterize the thermal tolerance of *B. zonata* at the adult and pupal stage, concentrating mainly on resistance to cold, which is an important climatic barrier for invading Mediterranean and temperate habitats. We examine critical thermal maxima and minima, and recovery following acute cold stress during the adult stage, hypothesizing that these measures will change correspondingly with thermal acclimation. Specifically we assumed, based on past studies (e.g. Weldon et al., 2018) that higher acclimation temperatures will promote heat tolerance but reduce resistance to cold stress. Within the frame of these experiments we additionally address the effects of sex and acclimation period on thermal tolerance. Finally, we characterize the effect of cold stress on pupal survival. Our data promote a more comprehensive understanding of the thermal sensitivity of this fly and of the associated implications for its future spread.

2. Materials and methods

Insects and acclimation treatments: *Bactrocera zonata* pupae were obtained from a laboratory colony maintained at the quarantine facility of the Plant Protection and Inspection Services laboratories in Bet-Dagan, Israel (see Gazit and Akiva, 2017). The colony's breeding population is routinely replaced with wild flies every 1–2 years (Y. Gazit, personal communication). Pupae of the F₁ generation of wild flies were obtained by propagating wild adults generated from naturally-infested guavas (*Psidium guajava*, collected in suburban Tel-Aviv) in peach fruit (*Prunus persica*) during the summer of 2020, as previously described (Gazit and Akiva, 2017). Pupae were maintained at 25 °C until adult emergence, and 2–4 day old adults were separated by sex and accommodated in three, 30 cm cubical screen cages, provided with water and a standard diet of sugar and yeast hydrolysate (3:1 ratio by weight). Each cage was subsequently maintained at one of three constant ambient temperatures: 20, 25 or 30 °C, at 50 ± 10% relative humidity and photoperiod of 16:8, hours of light:dark, respectively (henceforth acclimation treatments). In order to account for a possible effect of acclimation period on thermal tolerance, flies were acclimated for up to 19 days. Individuals from each acclimation treatment were then assayed randomly during the acclimation period for their critical thermal maxima (CT_{max}) and minima (CT_{min}), and their ability to recover from acute cold stress. For testing pupal tolerance to acute cold stress, one-day-old pupae were maintained at 25 °C for 2–9 days and directly submitted to cold tolerance assays (see below for further details).

Adult critical thermal maxima and minima: To determine the

effects of acclimation temperature and acclimation period on adult CT_{max} and CT_{min} , females and males acclimated at 20, 25 or 30 °C for 2–19 days (as described above) were weighed to the nearest 0.1 mg and confined to aerated 5 ml glass vials (one individual in each vial). Vials were subsequently incubated in a temperature-controlled water bath with continuous water circulation, where temperature was gradually increased or decreased at a rate of 0.5 °C per minute, starting from 25 °C. The maximum and minimum temperatures at which flies were stunned and dropped to the bottom of their vial (knockdown temperature, [Cowles and Bogert, 1944](#)) were determined using calibrated thermometers placed inside the vials. Critical thermal maxima were assayed using colony flies of two rearing cohorts and an additional cohort of the F_1 progeny of wild flies (overall $n = 1365$ flies). We included F_1 wild flies in these experiments with an initial intention to compare their performance with that of colony flies. However, considering that their response was similar to that of colony flies (see results), and that colony flies are routinely replaced with wild flies, we eventually regarded these as an additional cohort in the overall analysis. Critical thermal minima were determined using colony flies of three rearing cohorts (overall $n = 839$ flies). In these experiments, 5–20 individuals of each sex and acclimation temperature in each cohort (average $n = 10.17$ flies/group), were assayed randomly following different acclimation durations ($n_{(females)} = 201–274$ and $129–150$; $n_{(males)} = 173–270$ and $130–150$ individuals for each acclimation temperature, CT_{max} and CT_{min} respectively).

Adult tolerance to acute cold stress: These experiments were conducted using colony flies of three rearing cohorts (overall $n = 780$ flies). Acclimated males and females (4–15 days of acclimation) were confined to 1.5 ml test tubes (one individual in each tube) and subsequently incubated in a pre-chilled water bath containing ethylene glycol solution and set to maintain a constant temperature of 0 or -3 °C. The temperature inside the tubes was monitored by a calibrated thermometer inserted and sealed inside the exposed area of one of the tubes. Following exposure to cold stress for 2 h, vials were transferred to 30 °C for an additional 2 h, and recovery rates were subsequently recorded by counting the surviving individuals. In each cohort, 5 individuals of each sex and acclimation temperature were assayed randomly following different acclimation durations (overall $n = 5–15$ individuals in each group, $n_{(females)} = 50–65$ and $55–70$; $n_{(males)} = 65$ and 70 individuals for each acclimation temperature; 0 and -3 °C cold stress, respectively).

Pupa tolerance to acute cold stress: Additionally, we recorded the effect of acute exposure to chilling on survival during the pupal stage. Two - nine day old pupae of colony flies, maintained at 25 °C, were placed into 1.5 ml test tubes (one individual in each tube) and exposed to -12 °C, -3 °C, 0 °C or 5 °C for 2 h as described for adult flies. Subsequently, recovery at 30 °C (measured as adult emergence) was recorded. Recovery was similarly monitored in a control group of pupae maintained at 30 °C throughout development. Overall, insects from 5 rearing cohorts, randomly distributed to temperature treatment groups were used ($n = 30–90$ individuals for each temperature and age treatment groups; overall $n = 810$ individuals). Temperature was monitored as explained for adults.

2.1. Statistical data analysis

- (a) Effect of acclimation temperature on CT_{max} and CT_{min} of adults: Our intention in including a wide range of acclimation times in this study was to provide an account for the effect of acclimation period on thermal tolerance. However, examining the association with CT_{max} and CT_{min} by linear regression indicated that for most groups acclimation period had no effect on thermal tolerance. In other groups significant but inconsistent relationships were detected (tested separately for each sex, in each temperature regime and cohort, [Figs. S1 and S2](#)). Consequently, since thermal tolerance and acclimation period were inconsistently associated, we examined the effect of acclimation temperature on the CT_{max}

and CT_{min} of individual flies using a full factorial ANOVA including cohort and sex as the only additional fixed variables. This analysis thus accounts for the effect of acclimation over an extended range of acclimation periods. Additionally, we report of the results of the minimum adequate models, determined by sequentially excluding non-significant ($P \geq 0.328$) three and two-way interactions according to descending degree of interaction. Within these models, group means were separated by Tukey HSD comparisons.

- (b) Effect of acclimation temperature on adult recovery following acute cold stress: Analyses were performed separately for each exposure temperature (0 or -3 °C). Preliminary examination revealed that recovery following exposure to 0 °C remained high and did not respond to acclimation temperature and acclimation period. Consequently, the proportional recovery rates of females and males of each acclimation temperature were compared by non-parametric Kruskal-Wallis tests. Binomial data of -3 °C-exposed flies was analyzed by logistic regression where the effects of acclimation temperature, acclimation period, sex and cohort were included in a full factorial design. This model assigned highly non-significant effects to cohort and all of its involved interactions ($P \geq 0.235$), and consequently all were removed from the final analysis. Additionally, examining the recovery of flies exposed to -3 °C revealed that it was largely independent of acclimation period, but in some groups significant negative relationships were detected (tested separately for each sex, and acclimation temperature, [Fig. S3](#)). Moreover, removing acclimation period from the analysis did not affect the separation of group means by the model. Consequently, since thermal tolerance and acclimation period were inconsistently associated, we pooled the data of all acclimation periods, and the final fitted model tested the effects of sex, acclimation temperature and their interaction on adult recovery rates. Group means were separated the Tukey's-HSD comparisons.
- (c) Survival following acute cold-stress during the pupal stage: Logistic regression was performed on pooled binomial data of all five cohorts, where exposure temperature was included as the explanatory variable. This analysis thus examined the effect of temperature on a pupae having a range of ages (as naturally occurs in the field). To account for natural mortality (i.e. pupae that had died of natural causes such as insufficient nutrition) the data was corrected according to the proportion of dead pupae in our control group (unstressed pupae maintained at 30 °C; natural death rate = 12.67%, [Abbott 1925](#)). The effect of age on pupal survival was examined by logistic regression separately for each of the exposure temperatures. Recovery rates were not adjusted to exclude natural mortality.

Throughout the text means and standard errors (SE) are reported. Analyses were conducted using the statistical package JMP (SAS, Cary, NC, USA). Statistical significance was inferred using $\alpha = 0.05$.

3. Results

Acclimation temperature and adult critical thermal maxima and minima: the CT_{max} of males and females was significantly affected by acclimation temperature (ANOVA overall effects; $F_{(2, 1347)} = 9.32$, $P < 0.0001$), cohort ($F_{(2, 1347)} = 46.46$, $P < 0.0001$), and the interaction between cohort and sex ($F_{(2, 1347)} = 4.45$, $P < 0.012$; [Supplementary Table S1](#)). Mean CT_{max} values were higher for females and males acclimated at 30 °C compared to those of counterparts exposed at 20 or 25 °C (females: 42.52 ± 0.17 , 42.20 ± 0.18 , 41.94 ± 0.19 ; males: 43.09 ± 0.16 , 42.04 ± 0.17 , 42.12 ± 0.20 °C, respectively). Notably, this pattern was consistent across all cohorts as indicated by a highly non-significant interaction between cohort, acclimation temperature and sex ($F_{(4, 1347)} = 3.53$, $P = 0.841$). Sex was a marginally insignificant

predictor of CT_{max} ($F_{(1, 1347)} = 3.08$, $P = 0.079$, Fig. 1a), and did not significantly interact with acclimation temperature ($F_{(2, 1347)} = 1.11$, $P = 0.328$). However, although apparent for both sexes, the effect of acclimation temperature turned significant only for males (Tukey HSD comparisons, $P \leq 0.025$; Fig. 1a). Overall (regardless of acclimation temperature), the mean CT_{max} of males was slightly higher than that of females (42.50 ± 0.10 °C compared to females: 42.25 ± 0.10 °C). However, the overall greater male tolerance to temperature turned significant only for one of the three cohorts (colony-reared flies, t -test comparisons) resulting in the significant interaction between cohort and sex. Finally, the CT_{max} of wild and colony-reared flies was similar, and the significant effect of cohort stemmed from the overall lower CT_{max} values obtained for one cohort of colony flies. Excluding the non-significant interactions between cohort, sex and acclimation temperature, and between cohort and acclimation temperature resulted in similar effects (Table S1) without changes to the separation of group means (Fig. 1). Altogether, regardless of sex, flies acclimated at 30 °C showed a small but significant increase of 0.68 and 0.77 °C in CT_{max} , compared to counterparts acclimated at 25 and 20 °C, respectively (overall CT_{max} values: 42.8 ± 0.11 , 42.12 ± 0.12 and 42.02 ± 0.14 °C, 30, 25 and 20 °C – acclimated flies, respectively).

Adult CT_{min} was significantly affected by acclimation temperature (ANOVA overall effects; $F_{(2, 821)} = 80.33$, $P < 0.0001$), sex ($F_{(1, 821)} = 14.38$, $P = 0.0002$), cohort ($F_{(2, 821)} = 11.06$, $P < 0.0001$), and the interaction between cohort and acclimation temperature ($F_{(4, 821)} = 3.83$, $P = 0.0043$; Supplementary Table S2). Female and male CT_{min} values changed according to acclimation temperature similarly to CT_{max} values: acclimation at 30 °C significantly increased the knockdown temperature of the flies (i.e decreased their ability to tolerate chilling)

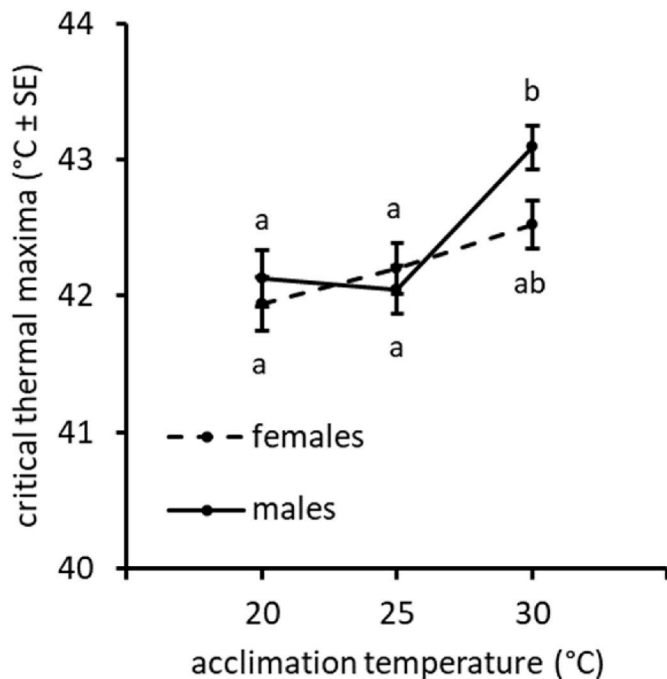


Fig. 1. Critical thermal maxima (CT_{max}) of female and male *Bactrocera zonata* acclimated at 20, 25 or 30 °C for 2–17 days. Overall, acclimation temperature significantly affected the heat tolerance of the flies, and pre-exposure at 30 °C resulted in greater CT_{max} values. This effect was significant for males and marginal for females (ANOVA followed by Tukey HSD comparisons, $P \leq 0.025$, $n = 183$ –284 flies in each group). Acclimation period had a negligible effect on the temperature tolerance of the flies regardless of cohort or acclimation temperature. Nevertheless, for a few groups significant or marginally significant correlations were detected (linear regression analysis; Supplementary Fig. S1). Means separated by different letters are significantly different (letters indicated below and above the SE bars correspond to females and males, respectively).

compared to 25 or 20 °C - acclimated counterparts (females: 6.21 ± 0.12 , 4.79 ± 0.11 , 5.03 ± 0.13 °C; males: 6.60 ± 0.11 , 5.22 ± 0.11 , 5.36 ± 0.13 ; respectively). This effect was consistent in all cohorts (non-significant interaction between cohort, acclimation temperature and sex; $F_{(4, 821)} = 0.78$, $P = 0.536$) and applied to both sexes (Tukey-HSD comparisons, $P \leq 0.037$; Fig. 2a). The significant effect assigned to sex resulted from the overall lower CT_{min} of females (5.38 ± 0.07 °C) compared to males (5.76 ± 0.07 °C). Nevertheless, the model did not differentiate between the mean CT_{min} of females and males acclimated at the same temperature (Fig. 2a). Additionally, the overall mean CT_{min} of the flies was significantly higher for one of the three cohorts assayed, accounting for the significant effect assigned to cohort. Finally, the change in CT_{min} induced by acclimation temperature, although consistent in all cohorts, was not equal in magnitude in all cohorts, resulting in the significant interaction between cohort and acclimation temperature. Excluding the non-significant interactions between cohort, sex and acclimation temperature, and between cohort and sex resulted in similar effects (Table S1) without changes to the separation of group means (Fig. 2a). Ultimately, acclimation at 30 °C resulted in a significant increase in the knockdown temperature of the flies when exposed to cold stress. This translated to a reduced tolerance to chilling, averagely differing by 1.39 and 1.21 °C from that of flies acclimated at 25 or 20 °C, respectively (overall CT_{min} values: 6.40 ± 0.08 , 5.01 ± 0.08 , 5.19 ± 0.09 °C; 30, 25 and 20 °C – acclimated flies, respectively).

Acclimation temperature and adult recovery from acute cold-stress: Survival following exposure to acute cold stress depended foremost on treatment severity. While little mortality occurred following chilling at 0 °C for 2 h, regardless of acclimation temperature, period or sex (0.91–1.00 recovery rate in all groups; Kruskal-Wallis test: $\chi^2_{(5)} = 6.79$, $P = 0.236$, results not shown), the ability of males and females to survive chilling at –3 °C was essentially compromised, and significantly affected by acclimation temperature (logistic regression analysis; overall effect: $\chi^2_{(2)} = 9.78$, $P = 0.0075$). Sex was also a significant predictor of response to acute cold stress ($\chi^2_{(1)} = 25.53$, $P < 0.0001$) as well as the interaction between sex and acclimation temperature ($\chi^2_{(2)} = 11.22$, $P = 0.0036$; Supplementary Table 3). Flies acclimated at 30 °C recovered at significantly lower proportions compared to counterparts pre-conditioned at 20 °C (females: 0.38 ± 0.094 and 0.74 ± 0.098 , males: 0.78 ± 0.045 and 0.97 ± 0.022 , respectively; logistic regression followed by post-hoc Tukey HSD comparisons, $P \leq 0.037$; Fig. 2b). Survival rates of females and males maintained at 25 °C (0.86 ± 0.04 and 0.86 ± 0.02 , respectively) were also high compared to 30°C-acclimated flies. Nevertheless, in agreement with the significant interaction between sex and acclimation temperature, the effect was significant only for females (Tukey HSD comparisons, $P \leq 0.0001$; Fig. 2b). Males were overall significantly more tolerant to acute chilling at –3 °C than females (overall recovery: 0.87 ± 0.02 and 0.66 ± 0.06 , respectively).

Survival following acute cold-stress during the pupal stage: Pupal survival rates were significantly affected by chilling temperature (logistic regression analysis: $\chi^2_{(1)} = 86.53$, $P < 0.0001$). While low natural mortality occurred at 30 °C (87.33% recovery), survival ratios (not adjusted for natural mortality) steadily decreased with chilling temperature to 80.83, 60.83, 50 and 20% at 5, 2, 0 and –3 °C (respectively). The adjusted survival ratios (corresponding to mortality specifically due to cold stress) similarly and significantly decreased with chilling temperature to 92.55, 69.65, 57.25 and 22.9% at 5, 2, 0 and –3 °C (respectively, Fig. 3). Pupae exposed to –12 °C for 2 h were unable to recover and had all died during the experiments. Accordingly, the predicted LT_{50} at 2 h was –0.32 °C (0.21 and –0.92 °C, upper and lower 95% CI, respectively) and LT_{95} at 2 h was –6.88 °C (–5.5 and –8.94 °C, upper and lower 95% CI, respectively).

Similar analysis examining the effect of age on survival following exposure to each of the temperature treatments (excluding the group exposed to –12 °C, where no survivals were recorded) indicated that recovery was significantly and positively affected by age for pupae exposed to 0 and 2 °C (logistic regression analysis: $\chi^2_{(1)} \geq 8.66$, $P \leq$

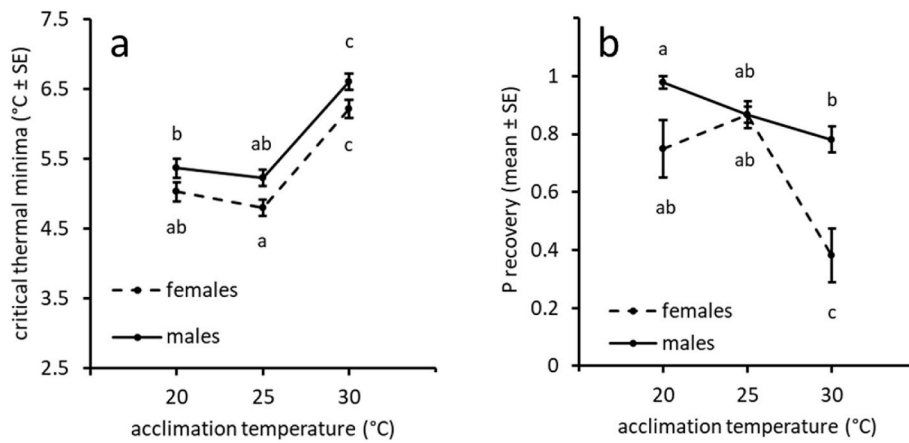


Fig. 2. (a) Critical thermal minima (CT_{min}) of *Bactrocera zonata* females and males acclimated at 20, 25 or 30 °C for 6–19 days. Acclimation at 30 °C significantly elevated the CT_{min} values of males and females (ANOVA followed by Tukey HSD comparisons, $P \leq 0.037$, $n = 129$ –150 flies in each group). Acclimation period had a negligible effect on CT_{min} regardless of cohort or acclimation temperature, except for a few groups (linear regression analysis; [Supplementary Fig. S2](#)). (b) Recovery rates of males and females following acute cold shock (−3 °C for 2 h) as affected by acclimation temperature (20, 25 or 30 °C for 4–15 days). Acclimation at 30 °C significantly reduced the ability of males and females to tolerate acute cold stress, and females were significantly more sensitive than males (logistic regression followed by Tukey-HSD comparisons, $P \leq 0.037$, $n = 55$ –70 flies in each group). Recovery rate following exposure to 0 °C for 2 h varied between 91 and 100% regardless of acclimation temperature, acclimation period or sex (results not shown). Each data point represents the

mean recovery over 9 time points during the acclimation period. Means separated by different letters are significantly different (letters indicated below and above the SE bars correspond to females and males, respectively).

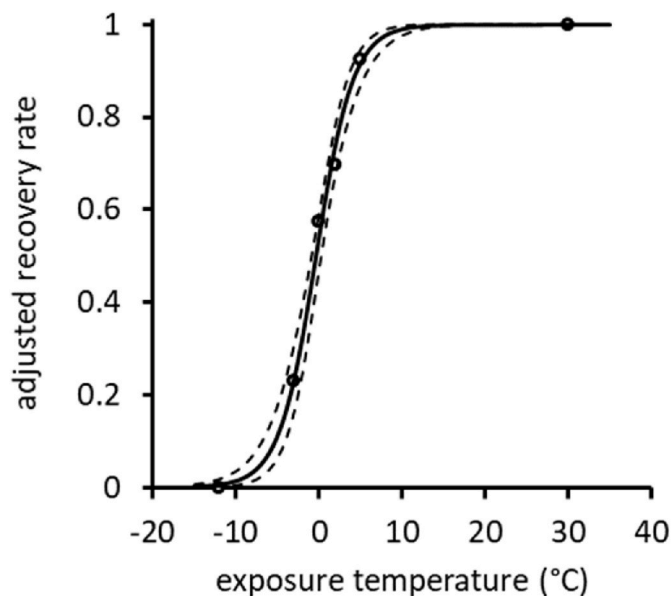


Fig. 3. Logistic regression analysis depicting the change in recovery rates of 2–9 day old pupae following a 2-h exposure to −12, −3, 0, 2, 5 and 30 °C. Recovery from treatment and adult ecdysis proceeded at 30 °C. Each data point represent the proportion of survival out of 60–300 individuals (total $n = 810$ pupae). Recovery rates were adjusted according to the natural death rate of unstressed pupae maintained at 30 °C (12.67%). Regression line depicts predicted survival ratios. Dashed lines depict the upper and lower 95% confidence intervals. LT_{50} and LT_{95} (at 2 h) = −0.32 °C and −6.88 °C respectively.

0.0032). The recovery of pupae exposed to lower (−3 °C) or higher (5 and 30 °C) temperatures remained unaffected by age ([Supplementary Fig. S4](#)).

4. Discussion

Successful range expansions by insects ultimately result from the combined effects of biotic, abiotic and anthropogenic factors (e.g. [Trombik et al., 2022](#)). The physiological ability to survive acute or prolonged periods of extreme temperature is one attribute contributing to the invasive success of some species over others ([Kelley, 2014](#); [Nyamukondiwa et al., 2022](#)). Insects of tropical origin, such as *B. zonata*,

which are adapted to warm and relatively stable climates are expected to successfully withstand high temperatures and to a lesser extent cold stress – an obstacle for colonizing temperate habitats having high temperature shifts ([Addo-Bediako et al., 2000](#)). Accordingly, the ongoing expansion of the geographic distribution of *B. zonata* (and that of other tephritids of tropical origin) into sub-tropical and temperate habitats raises questions regarding its thermal resilience, particularly regarding cold tolerance, and how this may affect its potential distribution. Herein we provide a first account for the effect of acclimation on the ability of *B. zonata* to cope with temperature extremes. Our study, together with former work defining tolerance limits to other abiotic stressors (e.g. [Duyck et al., 2004, 2006](#); [Ben-Yosef et al., 2021, 2023](#)), provide a framework to understand the climatic limits of the fly's expanding geographic range.

Acclimation temperature and adult critical thermal maxima and minima: our results point that, depending on acclimation temperature, the upper and lower thermal endpoints of *B. zonata* vary between 41.94–42.52 °C and 4.79–6.21 °C (females), and 42.04–43.09 °C, and 5.22–6.6 °C (males; CT_{max} and CT_{min} , respectively). These CT values set a range for the ability of *B. zonata* to survive extreme heat or cold events (e.g., during heat waves or winter period) and may contribute to accurately modeling its distribution. Additionally we found that acclimation at 30 °C significantly affected the thermal tolerance of *B. zonata*, and resulted in higher CT_{max} and CT_{min} values, and a decreased capacity to recover from acute cold stress ([Figs. 1 and 2](#)). According to our assays, the upper and lower thermal endpoints were elevated by 0.32–0.58 and 0.97–1.05 °C (CT_{max}), and by 1.18–1.42 and 1.24–1.38 °C (CT_{min} ; females and males, respectively) following acclimation at 30 °C compared to values of the other treatment groups. Comparable effects were previously recorded for other fruit flies such as *B. dorsalis* ([Motswagole et al., 2019](#)), *C. capitata* and *C. rosa* ([Nyamukondiwa and Terblanche 2010](#); [Weldon et al., 2018](#)) subjected to similar experimental procedures, pointing to a common effect of acclimation on the thermal endpoints of these flies as well as other insects (reviewed by [Weaving et al., 2022](#)). Nevertheless, directly comparing between the CT values of these studies and our results should take into account the rate at which temperature changed during the assay. The temperature ramping rate in our study (0.5 °C/min) is relatively high, and may have affected the CT values we recorded, as have been previously shown for other fruit flies and *Drosophila* (e.g. [Motswagole et al., 2019](#); [Salachan et al., 2019](#)). This however, seems to apply mainly to CT_{max} ([Motswagole et al., 2019](#); [Chown et al., 2009](#)). Nevertheless, the variable rates used in this and other studies are all probably higher than temperature shifts occurring

in the field, and their relevance to actual microclimatic conditions experienced by the flies remains unclear (e.g. Sinclair, 2001; see also Terblanche et al., 2011). Thus, determining the precise CT values of *B. zonata* will require further consideration of the variable climate in the field. In any case, our main aim in this study was to examine the effect of acclimation, which is likely to remain valid within the temperature ramping rates commonly applied when experimentally testing thermal tolerances.

Similarly to other fruit flies and *Drosophila* (Nyamukondiwa et al., 2011; Overgaard et al., 2011; Nyamukondiwa and Terblanche 2010), we found that acclimation induced larger changes to CT_{min} compared to CT_{max}. This pattern corresponds with the closer position of the upper, and farther position of the lower lethal temperature limits relative to the thermal optimum for insects in general, allowing for greater variation in CT_{min} values following acclimation (see Terblanche et al., 2015). Nevertheless, the implications of this phenotypic plasticity and thermal thresholds we recorded to the potential distribution of *B. zonata* are difficult to estimate from our study alone. Comparable studies on other fruit flies show that induction of cold tolerance by cold hardening contribute to the greater thermal resilience and invasiveness of *C. capitata* over *C. rosa* (Nyamukondiwa et al., 2010). Contrarily, other studies point that the highly invasive *B. dorsalis* responds to cold or heat hardening to a lesser extent than *C. capitata* and *C. rosa* (Pieterse et al., 2017), and while not thermally challenged under a tropical climate (e.g. Botswana, Motswagole et al., 2019) it may be inhibited where larger temperature shifts occur (e.g. in the Western Cape, Pieterse et al., 2017). Comprehensive analyses of niche repartitioning and altitudinal redistribution of tephritid species following invasions of *B. zonata* and *B. dorsalis* to La Reunion, and *B. dorsalis* to the Comoros Islands and Hawaii seem to support this possibility. These studies suggest that invasive *Bactrocera* species are successfully displacing *Ceratitis* species in warm, tropical lowlands, but lose their competitive advantage at cooler, higher altitudes (Vargas et al., 1995; Hassani et al., 2016, 2022, reviewed by Duyck et al., 2004b). Similarly, the distribution of *B. tryoni* in Australia suggests that low temperature during winter is a main constraint for colonizing temperate habitats (Clarke et al., 2019). Overall, it seems that tropical *Bactrocera* species, much like tropical drosophilids (e.g., MacLean et al., 2019) are less adapted to deal with cold climates compared to subtropical or temperate family members. Based on *Drosophila* studies, basal cold tolerance probably contributes an important part to this phenotype (MacLean et al., 2019). This probably also applies for tephritid fruit flies; however, the contribution of plasticity in cold tolerance remains to be determined.

Regardless of the relatively low plasticity expected for heat tolerance (van Heerwaarden et al., 2016; Nyamukondiwa et al., 2011), some studies found changes in heat tolerance to be greater in invasive insects and other ectotherms compared to non-invasive counterparts (Kelley 2014; Boher et al., 2016; Nyamukondiwa et al., 2022). Similarly, *B. dorsalis* showed a higher increase in heat tolerance following hardening during the larval stage compared to *B. correcta*, supporting the relative wider geographic distribution of *B. dorsalis* in China (Hu et al., 2014). These reports seem to contrast comparisons between tropical and temperate drosophilids pointing to similar capacities to acclimate to heat, which do not systematically associate with their distribution (MacLean et al., 2019; Sørensen et al., 2016; Overgaard et al., 2011), or only weakly associate with spread (Mitchell et al., 2011). Possibly, the weak association between phenotypic plasticity in heat tolerance and geographic distribution apply less to invasive insects regardless of their geographic origin, due to their greater adaptability in general (see Nyamukondiwa et al., 2022). The physiological mechanisms supporting such traits in fruit flies may be related to expression of heat shock proteins (e.g. as in Hu et al., 2014). Additionally, the timing of acclimation may be important. In our experiments we measured the heat tolerance of *B. zonata* following acclimation during the adult stage. Nevertheless, acclimation during the larval or pupal stages may result in similar or perhaps larger effects (Hu et al., 2014). Establishing these possibilities

requires further work.

Additionally, we could not assign a consistent effect to the duration of acclimation on the CT_{max} and CT_{min} values in our experiments (Figs. S1 and S2), suggesting that changes to thermal tolerance take effect following exposure for relatively short time periods. We assume that our acclimation regimes asserted an effect relatively fast similarly to the rapid effects of heat and cold hardening which were previously demonstrated for other tephritids (*B. dorsalis*, Motswagole et al., 2019; *C. capitata*, Nyamukondiwa and Terblanche 2010). Over long acclimation periods thermal tolerance could eventually be reduced due to the effects of aging (Nyamukondiwa and Terblanche 2009). We also observed a small and inconsistent negative effect of acclimation period on thermal tolerance in some treatment groups (Figs. S1 and S2), that may have resulted from aging during the experiments. Nevertheless, *Bactrocera* flies, including the recently-established strain that we used in our study (generated from wild flies every 1–2 years), usually have a relatively long lifespan. We thus assume that an age-related effect on thermal tolerance of *B. zonata* will be consistently apparent after longer acclimation periods.

Finally, we found that cohort and some of its interactions significantly affected the CT values recorded. Notably, the effect of acclimation temperature remained consistent for both sexes and in all cohorts throughout the experiments, indicating that flies were similarly affected by acclimation temperature in all cohorts. We suspect that the variance introduced to thermal tolerance by rearing cohort is related to larval nutrition. Larval diet, and in some cases specific nutrients were previously shown to affect the thermal tolerance of *Drosophila* and lepidopteran larvae (Košťál et al., 2012; Morey et al., 2016; Mutamiswa et al., 2020). Larval diet was additionally shown to affect adult thermal tolerance in *Drosophila* (e.g. Andersen et al., 2010), and we recently found a similar effect in *B. zonata* (Ben-Yosef et al., 2023). In line with these studies, we assume that inconsistencies in larval nutrition between rearing cohorts, originating from variation in larval density or possibly in diet composition, may have affected the thermal tolerance of adult flies, resulting in the effects of cohort in our experiments.

Acclimation temperature and adult recovery from acute cold-stress: we found that *B. zonata* adults are essentially resistant to chilling at 0 °C for 2 h, as over 90% of the flies recovered, regardless of acclimation temperature. Contrarily, acute chilling to –3 °C for 2 h resulted in significant mortality, which depended on acclimation temperature and sex. Similar to the effect on CT_{min} and CT_{max} values, acclimation at 30 °C was responsible for the main effect of temperature on acute cold tolerance. While acclimation at 20 and 25 °C resulted in similar and relatively high survival, flies acclimated at 30 °C scored significantly lower survival values. These results resemble those of other studies which examined the acute cold tolerance of fruit flies, where lower hardening temperatures prior to exposure were associated with higher survival rates under cold stress (Nyamukondiwa and Terblanche, 2009; Pieterse et al., 2017). Similarly to these studies, our results highlight the importance of preconditioning temperature when determining the acute cold tolerance of the flies. Additionally, we demonstrate that males were significantly more resistant to acute chilling compared to females, which was particularly apparent when flies were acclimated at 30 °C (Fig. 2b).

Currently, we cannot assign definitive mechanisms to the effects of acclimation temperature and sex on the cold tolerance of the flies in our experiments. Greater resistance to cold stress is generally associated with an increased capacity to regulate ion homeostasis under cold conditions and avoid loss of neuromuscular function (chill coma) and chill injury during prolonged exposure (reviewed by Overgaard and MacMillan 2017). Acclimation temperature-governed changes to membrane permeability through membrane lipid composition or transport proteins, may have affected the capacity of the flies to maintain ion homeostasis under cold conditions in our study, as have been documented for other insects (Overgaard et al., 2021). Additionally, feeding state was found to affect thermal stress resistance in fruit flies: fed flies were more resistant to cold and heat stress (Nyamukondiwa and

Terblanche, 2009) whereas starvation resulted in reduction of heat knockdown time in female but not male flies (Mitchell et al., 2017). We assume that acclimation temperature affected the general activity of the flies in our experiments, including metabolism, accumulation of weight, and sexual maturation. Female weight significantly increased during acclimation and was higher when females were maintained at 30 °C. Male weight changed similarly but the effect of temperature was nonetheless insignificant (Supplementary Fig. 5). Overall these results suggest that flies, and particularly females, were metabolically more active and fed more frequently when maintained at 25 °C and 30 °C compared to counterparts maintained at 20 °C. Nevertheless, an explanation based on feeding state do not seem to correspond with the reduced cold tolerance and the sex-related differences in acute cold resistance we observed in flies acclimated at 30 °C.

Other studies note similar sex-related differences in acute chill tolerance of *C. capitata* (Moraiti et al., 2022) and other flies (e.g. Coleman et al., 2015), where a dietary protein-driven onset of oogenesis was found to reduce acute cold tolerance in females (Coleman et al., 2015). Additionally, an interplay between starvation resistance and cold resistance which is specific to females was previously recorded for *Drosophila* (Hoffmann et al., 2005), pointing to a sex-specific association between metabolism and cold tolerance. Notably, the sex-related differences we observed in recovery from acute cold stress were not apparent when testing for thermal minima: males and females scored similar CT_{min} values, regardless of acclimation temperature, as have been demonstrated for other flies as well (Nyamukondiwa and Terblanche, 2009). These results probably point that the temperature leading to chill coma is similar for both sexes but females accumulate chill injury faster than males. Thus, an integrative explanation to these results may be that sex-specific metabolism related to reproduction which is governed by acclimation temperature have resulted in the increased susceptibility of females to acute cold stress in our study.

Survival following acute cold-stress during the pupal stage: Pupal survival significantly depended on chilling temperature. Our calculated LT values indicate that soil temperatures reaching −6.88 °C for 2 h will lead to 95% mortality of pupae. Comparably, the mortality of 3-day-old, *B. dorsalis* pupae was considerably lower when subjected to a similar temperature and time period (Wang et al., 2014), suggesting that *B. zonata* is more sensitive to cold stress during the pupal stage compared to *B. dorsalis*. A recent review by Clarke et al. (2019) point that tropical and subtropical tephritids, unlike many temperate species, do not usually overwinter as pupae. Additionally, as far as known today, the expansion of *B. tryoni* into temperate Australia is facilitated mainly, if not exclusively by adult rather than pupal overwintering since pupae do not survive during winter (Clarke et al., 2019 and references therein). Nevertheless, assessment of the cold tolerance of *B. dorsalis* at different developmental stages and from different regions of China (Wang et al., 2014) concluded that pupae are the most cold tolerant. Additionally, this study suggests that overwintering of *B. dorsalis* in temperate areas of China is mainly achieved by pupae whereas southern populations survive mainly as adults. Evidence also point that larval cold hardiness correlate with the latitude of collection sites suggesting that *B. dorsalis* is undergoing adaptation to cold conditions with its expansion northward in China (Wang et al., 2014). Thus, concluding about the capacity of *B. zonata* to survive cold stress as pupae from that of other tropical *Bactrocera* is difficult since tolerance values at the moment seem to be species-specific. The implications of these results together with our own data to invasion risk assessments are still not clear.

Finally, we found that tolerance to cold stress significantly depended on pupal age. Results show that mortality at temperatures <5 °C predominantly occurred in young (ca. 2–4 day old) pupae, whereas older (6–9 day old) pupae were more cold-hardy. We assume that this pattern is related to the temperature-sensitivity of specific developmental processes undergoing during metamorphosis, rendering younger pupae to be more sensitive to chilling, as have been previously described for other insects (Neuenschwander et al., 1981; Wang et al., 2016; Banahene

et al., 2018).

Bactrocera zonata is currently spreading in North Africa and the Eastern Mediterranean, and in risk of invading northern temperate habitats (Ni et al., 2012). The fly is currently classified as an A1 quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO), and introduce dire economic risks into newly invaded areas. The climatic limits of its expansion still need to be studied in order to produce more accurate risk maps and prepare preventive actions to reduce risk of introduction. Considering the basal temperature tolerance of this fly and its phenotypic plasticity in thermal stress resistance, together with the changing global climate may contribute to accurately predicting the potential distribution range of this pest.

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Author's contribution

DN conceived and planned the study. YA and EN-L executed the experiments. MBY contributed to calibrating the experiments, analyzed the data and wrote the manuscript. DN and NP commented on, and finalized the manuscript. All authors approved the submitted version of the paper.

Data accessibility

The raw data required to reproduce the above findings are available to download at the Zenodo data repository under the Digital Object Identifier (DOI): 10.5281/zenodo.8195037 (<https://doi.org/10.5281/zenodo.8195037>).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2023.103677>.

References

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18 (2), 265–267.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proc. Roy. Soc. Lond. B Biol. Sci.* 267 (1445), 739–745.
- Andersen, L.H., Kristensen, T.N., Loeschcke, V., Toft, S., Mayntz, D., 2010. Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *J. Insect Physiol.* 56 (4), 336–340.
- Banahene, N., Salem, S.K., Faske, T.M., Byrne, H.M., Glackin, M., Agosta, S.J., et al., 2018. Thermal sensitivity of gypsy moth (Lepidoptera: erebidae) during larval and pupal development. *Environ. Entomol.* 47 (6), 1623–1631.
- Battisti, A., Larsson, S., 2015. Climate change and insect pest distribution range. *Climate Change and Insect Pests*. CAB International, Wallingford, pp. 1–15.
- Ben-Yosef, M., Altman, Y., Nemni-Lavi, E., Papadopoulos, N.T., Nestel, D., 2023. Larval nutritional-stress and tolerance to extreme temperatures in the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae). *Fly* 17 (1), 2157161.

- Ben-Yosef, M., Verykoui, E., Altman, Y., Nemni-Lavi, E., Papadopoulos, N.T., Nestel, D., 2021. Effects of thermal acclimation on the tolerance of *Bactrocera zonata* (Diptera: Tephritidae) to hydric stress. *Front. Physiol.* 12.
- Boher, F., Trefault, N., Estay, S.A., Bozinovic, F., 2016. Ectotherms in variable thermal landscapes: a physiological evaluation of the invasive potential of fruit flies species. *Front. Physiol.* 7, 302.
- Chown, S.L., Jumbam, K.R., Sørensen, J.G., Terblanche, J.S., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* 23 (1), 133–140.
- Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K., Yeates, D.K., 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annu. Rev. Entomol.* 50, 293–319.
- Clarke, A.R., Merkel, K., Hulthen, A.D., Schwarzmueller, F., 2019. *Bactrocera tryoni* (Froggatt)(Diptera: Tephritidae) overwintering: an overview. *Aust. Entomol.* 58 (1), 3–8.
- Coleman, P.C., Bale, J.S., Hayward, S.A., 2015. Meat feeding restricts rapid cold hardening response and increases thermal activity thresholds of adult blow flies, *Calliphora vicina* (Diptera: calliphoridae). *PLoS One* 10 (7), e0131301.
- Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. AMNH* 83 article 5.
- Duyck, P.F., David, P., Quilici, S., 2004b. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecol. Entomol.* 29 (5), 511–520.
- Duyck, P.F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Réunion. *J. Anim. Ecol.* 75 (2), 518–526.
- Duyck, P.F., Sterlin, J.F., Quilici, S., 2004. Survival and development of different life stages of *Bactrocera zonata* (Diptera: Tephritidae) reared at five constant temperatures compared to other fruit fly species. *Bull. Entomol. Res.* 94 (1), 89–93.
- Gazit, Y., Akiva, R., 2017. Toxicity of Malathion and Spinosad to *Bactrocera zonata* and *Ceratitis capitata* (Diptera: Tephritidae). *Fla. Entomol.* 385–389.
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R.A., Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev.* 95 (3), 802–821.
- Gutierrez, A.P., Ponti, L., Neteler, M., Suckling, D.M., Cure, J.R., 2021. Invasive potential of tropical fruit flies in temperate regions under climate change. *Commun. Biol.* 4 (1), 1–14.
- Hassani, L.M., Delatte, H., Ravaomanarivo, L.H.R., Nouhou, S., Duyck, P.F., 2022. Niche partitioning via host plants and altitude among fruit flies following the invasion of *Bactrocera dorsalis*. *Agric. For. Entomol.* 24 (4), 575–585.
- Hassani, L.M., Raveloson-Ravaomanarivo, L.H., Delatte, H., Chiroleu, F., Allibert, A., Nouhou, S., et al., 2016. Invasion by *Bactrocera dorsalis* and niche partitioning among tephritid species in Comoros. *Bull. Entomol. Res.* 106 (6), 749–758.
- Hill, M.P., Bertelsmeier, C., Clusella-Trullas, S., Garnas, J., Robertson, M.P., Terblanche, J.S., 2016. Predicted decrease in global climate suitability masks regional complexity of invasive fruit fly species response to climate change. *Biol. Invasions* 18 (4), 1105–1119.
- Hoffmann, A.A., Anderson, A., Hallas, R., 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.* 5 (5), 614–618.
- Hoffmann, A.A., Hallas, R., Anderson, A.R., Telonis-Scott, M., 2005. Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *J. Evol. Biol.* 18 (4), 804–810.
- Hu, J.T., Chen, B., Li, Z.H., 2014. Thermal plasticity is related to the hardening response of heat shock protein expression in two *Bactrocera* fruit flies. *J. Insect Physiol.* 67, 105–113.
- Kellermann, V., van Heerwaarden, B., 2019. Terrestrial insects and climate change: adaptive responses in key traits. *Physiol. Entomol.* 44 (2), 99–115.
- Kelley, A.L., 2014. The role thermal physiology plays in species invasion. *Conserv. Physiol.* 2 (1).
- Košťál, V., Šimek, P., Zahradníčková, H., Cimlová, J., Štětina, T., 2012. Conversion of the chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze tolerant organism. *Proc. Natl. Acad. Sci. USA* 109 (9), 3270–3274.
- Kristensen, T.N., Hoffmann, A.A., Overgaard, J., Sørensen, J.G., Hallas, R., Loeschke, V., 2008. Costs and benefits of cold acclimation in field-released *Drosophila*. *Proc. Natl. Acad. Sci. USA* 105 (1), 216–221.
- MacLean, H.J., Sørensen, J.G., Kristensen, T.N., Loeschke, V., Beedholm, K., Kellermann, V., Overgaard, J., 2019. Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. *Philosoph. Transac. Royal Soc. B* 374 (1778), 20180548.
- Mitchell, K.A., Boardman, L., Clusella-Trullas, S., Terblanche, J.S., 2017. Effects of nutrient and water restriction on thermal tolerance: a test of mechanisms and hypotheses. *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 212, 15–23.
- Mitchell, K.A., Sgrò, C.M., Hoffmann, A.A., 2011. Phenotypic plasticity in upper thermal limits is weakly related to *Drosophila* species distributions. *Funct. Ecol.* 25 (3), 661–670.
- Moraiti, C.A., Verykoui, E., Papadopoulos, N.T., 2022. Chill coma recovery of *Ceratitis capitata* adults across the Northern Hemisphere. *Sci. Rep.* 12 (1), 1–12.
- Morey, A.C., Venette, R.C., Nystrom Santacruz, E.C., Mosca, L.A., Hutchison, W.D., 2016. Host-mediated shift in the cold tolerance of an invasive insect. *Ecol. Evol.* 6 (22), 8267–8275.
- Motswagole, R., Gotcha, N., Nyamukondiwa, C., 2019. Thermal biology and seasonal population abundance of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae): implications on pest management. *Int. J. Insect Sci.* 11, 1179543319863417.
- Mutamiswa, R., Machezano, H., Nyamukondiwa, C., Chidawanyika, F., 2020. Host plant-related responses on the thermal fitness of *Chilo partellus* (Swinhoe) (Lepidoptera: crambidae). *Arthropod-Plant Interactions* 14, 463–471.
- Neuenschwander, P., Michelakis, S., Bigler, F., 1981. Abiotic factors affecting mortality of *Dacus oleae* larvae and pupae in the soil. *Entomol. Exp. Appl.* 30 (1), 1–9.
- Ni, W.L., Li, Z.H., Chen, H.J., Wan, F.H., Qu, W.W., Zhang, Z., Kriticos, D.J., 2012. Including climate change in pest risk assessment: the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae). *Bull. Entomol. Res.* 102 (2), 173–183.
- Nyamukondiwa, C., Terblanche, J.S., 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *J. Therm. Biol.* 34 (8), 406–414.
- Nyamukondiwa, C., Terblanche, J.S., 2010. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short-term responses to temperature. *Physiol. Entomol.* 35 (3), 255–264.
- Nyamukondiwa, C., Kleynhans, E., Terblanche, J.S., 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecol. Entomol.* 35 (5), 565–575.
- Nyamukondiwa, C., Machezano, H., Chidawanyika, F., Mutamiswa, R., Ma, G., Ma, C.S., 2022. Geographic dispersion of invasive crop pests: the role of basal, plastic climate stress tolerance and other complementary traits in the tropics. *Curr. Opin. Insect Sci.* 100878.
- Nyamukondiwa, C., Terblanche, J.S., Marshall, K.E., Sinclair, B.J., 2011. Basal cold but not heat tolerance constrains plasticity among *Drosophila* species (Diptera: Drosophilidae). *J. Evol. Biol.* 24 (9), 1927–1938.
- Overgaard, J., MacMillan, H.A., 2017. The integrative physiology of insect chill tolerance. *Annu. Rev. Physiol.* 79, 187–208.
- Overgaard, J., Gerber, L., Andersen, M.K., 2021. Osmoregulatory capacity at low temperature is critical for insect cold tolerance. *Curr. Opin. Insect Sci.* 47, 38–45.
- Overgaard, J., Kristensen, T.N., Mitchell, K.A., Hoffmann, A.A., 2011. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? *Am. Nat.* 178 (S1), S80–S96.
- Papadopoulos, N.T., 2014. Fruit fly invasion: Historical, biological, economic aspects and management. (Trapping and the detection, control, and regulation of Tephritid fruit flies. Springer), pp. 219–252.
- Pieterse, W., Terblanche, J.S., Addison, P., 2017. Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)? *J. Insect Physiol.* 98, 1–6.
- Popa-Báez, Á.D., Lee, S.F., Yeap, H.L., Prasad, S.S., Schiffer, M., Mourant, R.G., et al., 2020. Climate stress resistance in male Queensland fruit fly varies among populations of diverse geographic origins and changes during domestication. *BMC Genet.* 21 (2), 1–19.
- Qin, Y., Wang, C., Zhao, Z., Pan, X., Li, Z., 2019. Climate change impacts on the global potential geographical distribution of the agricultural invasive pest, *Bactrocera dorsalis* (Hendel)(Diptera: Tephritidae). *Climatic Change* 155 (2), 145–156.
- Raza, M.F., Wang, Y., Cai, Z., Bai, S., Yao, Z., Awan, U.A., et al., 2020. Gut microbiota promotes host resistance to low-temperature stress by stimulating its arginine and proline metabolism pathway in adult *Bactrocera dorsalis*. *PLoS Pathog.* 16 (4), e1008441.
- Robinet, C., Roques, A., 2010. Direct impacts of recent climate warming on insect populations. *Integr. Zool.* 5 (2), 132–142.
- Royer, J.E., Wright, C.L., Hancock, D.L., 2016. *Bactrocera frauenfeldi* (Diptera: Tephritidae), an invasive fruit fly in Australia that may have reached the extent of its spread due to environmental variables. *Aust. Entomol.* 55 (1), 100–111.
- Salachan, P.V., Burgaud, H., Sørensen, J.G., 2019. Testing the thermal limits: non-linear reaction norms drive disparate thermal acclimation responses in *Drosophila melanogaster*. *J. Insect Physiol.* 118, 103946.
- Sgrò, C.M., Terblanche, J.S., Hoffmann, A.A., 2016. What can plasticity contribute to insect responses to climate change. *Annu. Rev. Entomol.* 61 (1), 433–451.
- Sinclair, B.J., 2001. Field ecology of freeze tolerance: interannual variation in cooling rates, freeze-thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinque maculata*. *Oikos* 93 (2), 286–293.
- Sørensen, J.G., Kristensen, T.N., Overgaard, J., 2016. Evolutionary and ecological patterns of thermal acclimation capacity in *Drosophila*: is it important for keeping up with climate change? *Curr. Opin. Insect Sci.* 17, 98–104.
- Terblanche, J.S., Hoffmann, A.A., 2020. Validating measurements of acclimation for climate change adaptation. *Curr. Opin. Insect Sci.* 41, 7–16.
- Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.* 214 (22), 3713–3725.
- Terblanche, J.S., Karsten, M., Mitchell, K.A., Barton, M.G., Gibert, P., 2015. Physiological variation of insects in agricultural landscapes: potential impacts of climate change. *Clim. Change Insect Pests* 8, 92.
- Trombik, J., Ward, S.F., Norrbom, A.L., Liebhold, A.M., 2022. Global drivers of historical true fruit fly (Diptera: Tephritidae) invasions. *J. Pest. Sci.* 1–13.
- Van Heerwaarden, B., Kellermann, V., Sgrò, C.M., 2016. Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol.* 30 (12), 1947–1956.
- Vargas, R.I., Walsh, W.A., Nishida, T., 1995. Colonization of newly planted coffee fields: dominance of Mediterranean fruit fly over oriental fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 88 (3), 620–627.
- Wang, J., Zeng, L., Han, Z., 2014. An assessment of cold hardiness and biochemical adaptations for cold tolerance among different geographic populations of the *Bactrocera dorsalis* (Diptera: Tephritidae) in China. *J. Insect Sci.* 14 (1).
- Wang, Q., Xu, X., Zhu, X., Chen, L., Zhou, S., Huang, Z.Y., Zhou, B., 2016. Low-temperature stress during capped brood stage increases pupal mortality, misorientation and adult mortality in honey bees. *PLoS One* 11 (5), e0154547.

- Weldon, C.W., Nyamukondiwa, C., Karsten, M., Chown, S.L., Terblanche, J.S., 2018. Geographic variation and plasticity in climate stress resistance among southern African populations of *Ceratitidis capitata* (Wiedemann)(Diptera: Tephritidae). *Sci. Rep.* 8 (1), 1–13.
- Weaving, H., Terblanche, J.S., Pottier, P., English, S., 2022. Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nat. Commun.* 13 (1), 5292.
- White, I.M., Elson-Harris, M.M., 1992. Fruit flies of economic significance: Their identification and bionomics. CAB international.
- Yerushalmi, G.Y., Misyura, L., Donini, A., MacMillan, H.A., 2016. Chronic dietary salt stress mitigates hyperkalemia and facilitates chill coma recovery in *Drosophila melanogaster*. *J. Insect Physiol.* 95, 89–97.
- Zingore, K.M., Sithole, G., Abdel-Rahman, E.M., Mohamed, S.A., Ekesi, S., Tanga, C.M., Mahmoud, M.E., 2020. Global risk of invasion by *Bactrocera zonata*: implications on horticultural crop production under changing climatic conditions. *PLoS One* 15 (12), e0243047.