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Cold tolerance of third-instar *Drosophila suzukii* larvae

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ABSTRACT

Drosophila suzukii is an emerging global pest of soft fruit; although it likely overwinters as an adult, larval cold tolerance is important both for determining performance during spring and autumn, and for the development of temperature-based control methods aimed at larvae. We examined the low temperature biology of third instar feeding and wandering larvae in and out of food. We induced phenotypic plasticity of thermal biology by rearing under short days and fluctuating temperatures (5.5–19 °C). Rearing under fluctuating temperatures led to much slower development (42.1 days egg-adult) compared to control conditions (constant 21.5 °C; 15.7 days), and yielded larger adults of both sexes. *D. suzukii* larvae were chill-susceptible, being killed by low temperatures not associated with freezing, and freezing survival was not improved when ice formation was inoculated externally via food or silver iodide. Feeding larvae were more cold tolerant than wandering larvae, especially after rearing under fluctuating temperatures, and rearing under fluctuating temperatures improved survival of prolonged cold (0 °C) to beyond 72 h in both larval stages. There was no evidence that acute cold tolerance could be improved by rapid cold-hardening. We conclude that *D. suzukii* has the capacity to develop at low temperatures under fluctuating temperatures, but that they have limited cold tolerance. However, phenotypic plasticity of prolonged cold tolerance must be taken into account when developing low temperature treatments for sanitation of this species.

1. Introduction

Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an emerging global pest of soft fruit (Cini et al., 2014; Lee et al., 2011; Walsh et al., 2010). *D. suzukii* lays eggs in unripe fruit. The entry wound and larval development promote fruit degradation, resulting in significant losses to blueberry, strawberry and cherry crops (Bolda et al., 2010). As with most *Drosophila* except *D. lutescens*, which may overwinter as a larva or pupa in Japan (Kimura, 1988), *D. suzukii* appears to overwinter as an adult, and there is a well-described 'winter morph' that is darker than the summer morph (Zerulla et al., 2015). This winter morph has some improved tolerances to environmental stress (Plantamp et al., 2016; Shearer et al., 2016; Toxopeus et al., 2016; Wallingford et al., 2016). However, larvae appear to be significantly less cold tolerant than adults, being killed by short exposures to sub-zero temperatures (Dalton et al., 2011) and longer exposures to temperatures near 0 °C (Kanzawa, 1939).

Insect cold tolerance strategies are usually divided into freeze tolerance (those that can withstand internal ice formation) and freeze avoidance, wherein individuals can survive cold as long as they do not freeze, but are killed when ice formation occurs (the supercooling point, SCP; Sinclair et al., 2015). The majority of insects, however, are chill-susceptible, killed by processes unrelated to ice formation at temperatures above the SCP (Sinclair et al., 2015). Strachan et al. (2011) found that larvae of 18 of 27 *Drosophila* were chill-susceptible, with another eight freeze-avoidant. Larvae of the closely-related *Chymomyza costata* and *C. amoena* are freeze tolerant when sufficiently cold-acclimated and with external ice inoculation (Koštál et al., 2011; Sinclair et al., 2009). However, no *Drosophila* larvae are currently thought to be freeze tolerant. Cold tolerance can also be phenotypically plastic. *D. melanogaster* larvae exhibit a rapid cold-hardening response (Czajka and Lee, 1990), as well as responding to longer-term acclimation (Rajamohan and Sinclair, 2009).

We observed that some late-instar *D. suzukii* larvae in field cages survived a cold snap in November 2014 that reached

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−6.9 °C and killed all the adult flies. This led us to hypothesise that acclimation or hardening may make larvae more cold-tolerant than previously reported. Moreover, because the host fruit are often exported, cold tolerance of the larvae is relevant for determining the capacity of larvae to survive chilling during processing and transport. Thus, our objective was to better characterise the cold tolerance of *D. suzukii* larvae. We measured growth and development, SCP, cold tolerance strategy and acute and chronic lethal temperatures of third-instar feeding and wandering larvae with and without an acclimation under fluctuating temperatures. For feeding larvae, we conducted experiments both within food (replicating likely field conditions) and without food (which allows us to better control the conditions and get a more precise measure of lethal limits).

2. Methods

2.1. Animal rearing and treatment groups

We established a *Drosophila suzukii* population from approximately 200 individuals collected in the Halton Hills region, Ontario, Canada (43°34′N 79°57′W). We reared flies on a banana-cornmeal-agar medium (Markow and O'Grady, 2005), at 21.5 ± 1 °C and 60 ± 5 % relative humidity under 13:11 L:D, as described elsewhere (Jakobs et al., 2015; Nyamukondiwa et al., 2011; Toxopeus et al., 2016). We used 3.7 L population cages containing approximately 300 adult flies that were two to six days post-eclosion (to reduce any parental age effect). Flies laid eggs on Petri dishes of banana food that had been dyed green with food colouring, which allowed us to separate feeding and non-feeding larvae. We removed the plates from the population cages every 24 h, and reared larvae on the Petri dishes.

To induce phenotypic plasticity in *D. suzukii* larvae, we placed the food plates with the eggs into two different rearing conditions (treatment). Eggs were placed under either control conditions (21.5 °C, 13:11 L:D) or exposed to a fluctuating thermal regime (FTR; 5.5 °C/19 °C, 11.5:12.5 L:D), simulating the average photoperiod and daily minimum and maximum temperatures from late September in London, Ontario.

We used third instar feeding and wandering larvae for experiments. We checked the food plates for larvae on a daily basis and removed larvae with a soft paintbrush. Banana food medium was carefully removed from larvae with tap water and larvae were blotted dry with a tissue. The life stage of a subset of larvae on each collection day was identified using the morphology of the mouth hooks (Fig. 1A–C) and anterior spiracles (Fig. 1D), based upon Demerec's (1965) descriptions for *D. melanogaster*. In addition, feeding third instar individuals appeared green as they still carried green food in their gut, while wandering-stage instars were transparent and lacked food in the gut (Fig. 1E).

To determine the effect of the treatments on developmental time, eggs were reared into adults under control conditions, FTR or a constant low temperature (11 °C, 10:14 LD). We removed pieces of the banana medium carrying approximately ten eggs, and transferred them into 35 mL vials containing banana medium (n = 6 vials/treatment). We collected the adults that developed from these eggs daily and stored them at −20 °C. When emergence had ended, we dried the flies over silica gel for approximately 48 h.

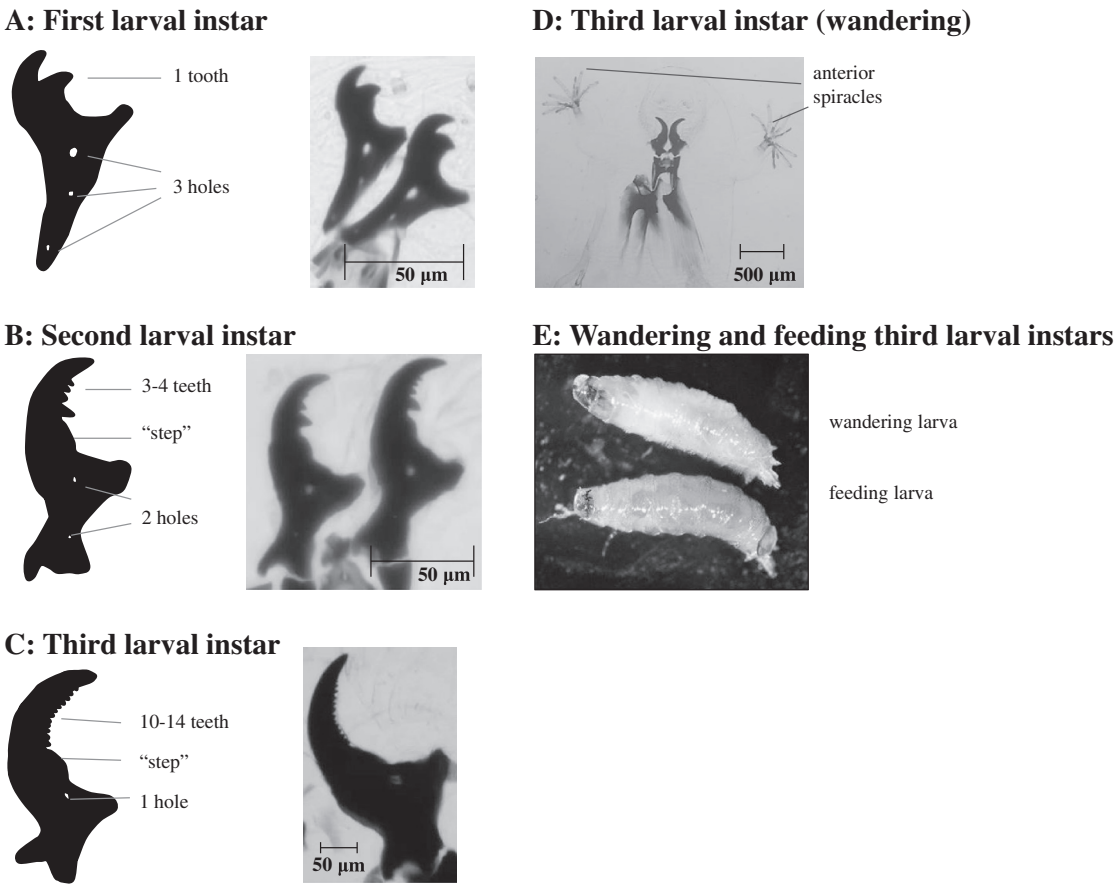

Fig. 1. Identification of larval stages of *Drosophila suzukii*. Mouthparts of first (A), second (B), and third (C) larval instars vary in size and shape (scale bar: 50 µm). Third-instar wandering larvae (D) have well-developed anterior spiracles (scale bar 500 µm), while third-instar feeding larvae do not (not shown). Dyed food (green in colour, appears dark in figure) is apparent in the gut of larvae that are feeding, whereas third-instar wandering larvae have cleared their gut and are transluscent (*E*).