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Impact of short- and long-term heat stress on reproductive potential of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae)

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ABSTRACT

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive vinegar fly of Asian origin now distributed throughout North America. Due to the unique morphology of females, this fly has become one of the most serious pests of thin-skinned fruits including blueberry, blackberry, cherry, raspberry, and strawberry. Prophylactic insecticide applications are commonly used to control this fly. A more sustainable approach to managing this invasive pest may not be possible without a clear understanding of the biology of this species under extreme environmental conditions. Specifically, high temperature is known to interfere with development and reproduction of drosophilids; however, the impact of high temperature on *D. suzukii* needs to be further investigated. The objective of the present study was to investigate the impact of exposure to constant and relatively short-term heat stress on reproductive success of *D. suzukii*, and potential for recovery. Results show that the development and reproduction of *D. suzukii* were negatively affected by constant and relatively short-term heat stress. Under constant heat stress, oviposition rate and adult lifespan decreased as temperature increased from 24 °C to 33 °C and reproduction was completely absent at 33 °C. Under relatively short-term heat stress, oviposition, pupation, and adult eclosion were significantly decreased as temperature increased from 28 °C to 34 °C. The short-term heat stress greatly reduced the fertility of both male and female *D. suzukii* which was recovered eight days after treatment. This study provides basic information on thermal biology of *D. suzukii* to help us better understand the trends commonly observed in *D. suzukii* trap captures in regions with hot summer conditions, and the results can be used in population models to predict its population dynamics in regions where high temperatures prevail during the field season.

1. Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is one of the most serious pests of thin-skinned fruits including blueberry, blackberry, cherry, raspberry, and strawberry (Beers et al., 2011; Goodhue et al., 2011; Klick et al., 2016; Lee et al., 2011). Since its first detection in the mainland United States in California in 2008, *D. suzukii* has expanded its range worldwide including Europe (Grassi et al., 2011), North America (Burrack et al., 2012), and South America (Deprá et al., 2014). Female *D. suzukii* possess a serrated ovipositor that enables oviposition in fruit that is ripe or ripening and undamaged, as opposed to other drosophilids that oviposit into overripe or previously damaged fruit (Lee et al., 2011; Beers et al., 2011; Kim et al., 2015). Larvae consume the flesh of the fruit while completing development, causing it to become soft and rot rapidly. Larval feeding inside the fruit, makes the fruit unmarketable for fresh consumption. This results in reduced crop yields and significant financial losses that have been estimated at $718 million annually in the United States (Beers et al., 2011; Goodhue et al., 2011). Additionally, annual costs to control *D. suzukii* are estimated at $1161 and $2933 per hectare for conventional and organic growers respectively (Farnsworth et al., 2017).

Currently prophylactic applications of broad-spectrum insecticides are used as the primary means to control *D. suzukii* (Beers et al., 2011; Van Timmeren and Isaacs, 2013; Asplen et al., 2015). Development of more reliable management strategies and population models depend on accurate biological information with respect to the impact of climatic conditions including temperature and relative humidity on development and reproduction of this pest (Araripe et al., 2004; Tochen et al., 2016). Temperature is one of the most impactful abiotic factors that influences the activity, development, and reproduction of insects (Denlinger and Yocum, 1998). Exposure to extreme temperature is known to affect survival rate, knock down time or recovery, and reproductive success of drosophilids (Araripe et al., 2004; David et al., 2005; Fasolo and Krebs, 2004; Hoffmann et al., 2003; Imasheva et al.,

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1997; Marshall and Sinclair, 2010; Ryan et al., 2016). *D. melanogaster* and other insect taxa have displayed male sterility when exposed to short-term (heat shock) and long-term (acclimation/hardening) heat stress (Araripe et al., 2004; Chakir et al., 2002; Civetta and Gaudreau, 2015; David et al., 2005; Jorgensen et al., 2006). Although temperature stresses usually have a faster and greater impact on males, the impact of stress on females cannot be ignored (Araripe et al., 2004; David et al., 2005). Heat shock can also cause injury to the female reproductive system, specifically the oocytes and ovarian development, which could lead to the abolishment of or decreased egg production (Fasolo and Krebs, 2004; Thanh Manh et al., 2013).

Our understanding of the effects of high temperature on fitness and reproduction of *D. suzukii* is still in its early stages. The optimal development of *D. suzukii* occurs at 24–26 °C while 30 °C was reported to be the upper developmental threshold, with reproductive activity completely inhibited at 31.5 °C for *D. suzukii* under simulated field conditions (Kinjo et al., 2014; Tochen et al., 2014; Asplen et al., 2015). In the southeastern United States, *D. suzukii* routinely experience high temperatures (32–38 °C) during the hottest parts of the day that far exceed the upper temperature threshold of 30 °C reported for *D. suzukii* and other drosophilids (Petavy et al., 2001; Araripe et al., 2004; Pedersen et al., 2011), yet growers report fruit infestations. The degree-day accumulation models also do not accurately predict *D. suzukii* populations during the hot summer months because the trap captures drop significantly in regions with hot summer conditions (Wiman et al., 2014; Grant, 2016). The factors limiting *D. suzukii* population growth, the physiological mechanisms and behavioral adaptations enabling a fraction of the population to survive during hot summer conditions are largely unknown (Overgaard and Sorensen, 2008).

The objective of the present study was to determine the impact of constant and short-term heat stress on reproductive success of *D. suzukii*, and the potential for recovery. Observations were recorded on several parameters of survivorship, development and reproduction including oviposition, pupation, adult eclosion and recovery from temporary sub-fertility or sterility induced as a result of exposure to heat stress. Our findings will establish a foundation for understanding the thermal biology of *D. suzukii* to help us better understand its population dynamics in regions where physiologically unfavorable high temperatures prevail in the field.

2. Materials and methods

2.1. Insect rearing and treatment groups

Drosophila suzukii used in this study were taken from a laboratory colony established using wild specimens captured in Clarke County, GA, during the summer of 2013. The colony was initiated by creating single female isogenic lines which were maintained in the laboratory to allow interbreeding for several generations. The fly colony was reared on standard fly diet as described in Jaramillo et al. (2015). Tegosept and propionic acid were used as additives to slow the growth of fungus/mold in the rearing bottles (Jaramillo et al., 2015). Fly cultures were maintained in 177-ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) containing approximately 50 ml of fly diet and plugged with bonded dense-weave cellulose acetate plugs. Incubators (Model I36VLCB, Percival Scientific, Perry, IA) set to 24 °C and 70% relative humidity, and a photoperiod of 14 h:10 h (L:D) were utilized for colony maintenance.

2.2. Experiment 1: Impact of long-term exposure to heat stress on fecundity, development, and lifespan

Male and female *D. suzukii* used for this experiment were 3–5 day old virgin flies, which were isolated as pupae to prevent mating prior to the experiment. The 3–5 day old age was selected because female *Drosophila* have a spike in fecundity starting on day 4 after eclosion with a slow linear decrease until the end of the fertile period (Revadi et al., 2015; Chakir et al., 2002). Virgin flies (1 male and 1 female) were then paired in 1.5 oz plastic cups (DART/SOLO Corporation, Mason, MI) containing 3.0 ml of rearing diet as a food source and oviposition substrate. Each pair was kept at ambient temperature of 24 °C for 24 h to allow for mating to occur. The mating pairs were then placed inside the incubators set at each of the four constant temperature treatments including 24, 27, 30, and 33 °C for the duration of the life of the female fly. The 24 °C treatment also served as a control for this experiment because the colony was maintained at this temperature. All pairs were held under their respective experimental treatment conditions for the duration of the study. Incubators used in this study were maintained at 70% relative humidity with 14 h:10 h light:dark photoperiods (Tochen et al., 2016; Wiman et al., 2014). A total of 20 replicates was set up at each of the four temperature treatments. Pairs of flies were transferred every 24 h to new plastic cups containing fresh media to achieve a measure of daily oviposition as well as to avoid crowding among the progeny (Jorgensen et al., 2006). Starting seven days after the first transfer, each cup containing eggs was observed daily for the formation of pupae and the total number of pupae was recorded.

In addition to taking egg and pupal counts, adult emergence was recorded in each replicate. Adult counts began when the first adult was observed and then continued on a daily basis. Containers were kept for one week after the last adult enclosed to ensure all adults had been counted. Following eclosion, all adults were removed from experimental conditions to prevent further mating. Data for adult and pupal counts were presented in percent pupation ([number of pupae/number of eggs] × 100) and percent adult eclosion ([number of adults/number of eggs] × 100). The time between pupation and adult emergence was recorded daily for each replicate. This provided a measure of average pupal development time. Each replicate was then followed until both male and female flies had died. This resulted in a measure of adult lifespan in days. The lifespan of the offspring was not measured.

2.3. Experiment 2: Effect of short-term exposure to heat stress on reproduction and potential for recovery

In a follow-up experiment, male and female *D. suzukii* were individually exposed to short-term heat stress individually. Five- to seven-day-old virgin flies were held in groups inside 6 oz square bottom polypropylene bottles (Genessee scientific, San Diego, CA) including a damp cotton ball and covered with mesh fabric. Temperature treatments used in this study were 28, 30, 32, and 34 °C. The treated flies were exposed to the respective temperatures for 24 or 72 h. These exposure times were selected based on a pilot study where < 24 h exposure didn't cause any observable sublethal effects and > 72 h exposure resulted in high mortality. At the end of the exposure time, male and female *D. suzukii* were paired such that only male or only female or both sexes were exposed to temperature treatments. A set of 20 replicates was set up for each combination at each exposure period. The virgin flies were paired in 1.5-oz plastic cups (DART/SOLO Corporation, Mason, MI) containing 3.0 ml of rearing diet as a food source and oviposition substrate. The pairs of flies were placed at laboratory conditions of 24 °C temperature and 70% relative humidity to allow them to recover. The pairs were then followed for 8 days to observe any change in fecundity and fertility over time and to determine whether recovery was possible. Each pair was transferred daily to new cups allowing close inspection for the presence of eggs in the medium. Data were collected at 2 and 8 days after heat treatment. The recovery time depends on exposure temperature and at temperatures above 30 °C recovery periods of 7–9 days have been reported in other species (Denlinger and Yocum, 1998). Because this study was the first to investigate recovery of *D. suzukii* from heat stress, the two and eight days after treatment were chosen to provide a baseline for more targeted studies in the future.

Data were collected for egg, pupal, and adult counts. Egg count data