Thermal Tolerances of the Spotted-Wing *Drosophila Drosophila suzukii* (Diptera: Drosophilidae)[☆]

Geraldine D. Ryan[a,d], Lisa Emiljanowicz[a,b], Francesca Wilkinson[a,c], Melanie Kornya[a], Jonathan A. Newman[a,b]

[a]*School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada*
[b]*Current address: Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada*
[c]*Current address: Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom*
[d]*Corresponding author, e-mail: geraldinedryan@gmail.com*

Abstract

The spotted-wing drosophila (*Drosophila suzukii* Matsumura) is an invasive species of Asian origin that is now widely distributed in North America and Europe. Because of the female's serrated ovipositor, eggs are laid in preharvest fruit, causing large economic losses in cultivated berries and stone fruit. Modeling *D. suzukii* population dynamics and potential distribution will require information on its thermal tolerance. Large summer populations have been found in regions with severe winter conditions, though little is known about responses to prolonged low-temperature exposure. We used controlled chambers to examine *D. suzukii* fecundity, development rate, and mortality across a range of temperatures encompassing the upper and lower thresholds (5–35 °C). Optimal temperatures (T_{opt}) were found to be 28.2 °C for the development of the egg-to-adult stage, and 22.9 °C for reproductive output. No adult eclosion occurred below 8.1 °C (T_{lower}) or above 30.9 °C (T_{upper}). We also investigated survival outcomes following prolonged (42-d) low-temperature exposure to a simulated cold winter (−5, −3, −1, 1, 3, and 5 °C). Adult survival was dependent on temperature, with a mean LT_{50} of 4.9 °C. There were no effects of sex, mating status, geographic strain, and photoperiod preexposure on overwintering survival. Thirty-eight percent of females that were mated prior, but not after, prolonged low-temperature exposure produced viable offspring, suggesting that this species may undergo sperm storage. This study provides data on the thermal tolerances of D. suzukii, which can be used for models of *D. suzukii* population dynamics, degree-day, and distribution models.

Keywords: Briére model, *Drosophila suzukii*, overwintering, spotted-wing drosophila, thermal tolerance

Introduction

Climate parameters such as humidity, temperature, and winter severity are major determinants of species distribution and abundance in poikilothermic animals. As such, patterns of distribution in insects are often related to latitude, altitude, and local topography (Hoffmann 2010). For newly introduced species, local temperatures and overwintering conditions can determine a species' ability to establish and become invasive (Bale 2002, Paradis et al. 2008). Temperature can also determine if an established insect becomes a pest, and outbreaks of insect pests have been related to changing temperature conditions (Bale et al. 2002) and overwintering success (Virtanen et al. 1996, Roland et al. 1998).

Overwintering can be defined as the way an organism endures the winter, whereby metabolic changes result in a cold-hardy state of hibernation (Leather et al. 1995). Cold tolerance is variable among species of *Drosophila*, and is often plastic between and within life stages because of acclimation and cold-hardening. Overwintering outcomes can be enhanced by acclimation occurring over periods of days to weeks, or rapid cold-hardening occurring within minutes to hours (Sinclair and Roberts 2005). Bubliy et al. (2002) examined the cold resistance (defined as survival of adults at 0 °C) of *Drosophila melanogaster* Meigen from different geographical regions and found that acclimation increased survival in all populations. However, nonacclimated flies showed higher fertility and number of progeny (Bubliy et al. 2002).

These results suggest that cold-acclimated flies are adapted to survive cold temperatures, putting fewer re-

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sources into fertility, implying fitness costs in reproduction (Bubliy et al. 2002). The spotted-wing drosophila *Drosophila suzukii* Matsumura is a fruit fly of Asian origin that has recently become invasive in North America (Hauser 2011, Walsh et al. 2011) and parts of Europe (Cini et al. 2012). In North America, the species was first detected in Santa Cruz County, CA, in 2008 on strawberries and caneberries, and in the following year, economically damaging infestations were detected in cherry orchards in Northern California (Bolda et al.2010). Because of the female's serrated ovipositor, D. suzukii can lay eggs inside of preharvest fruit, causing damage because of larval feeding in a wide variety of cultivated berry and stone-fruit hosts. In the past several years, the North American range of *D. suzukii* has expanded; it was first found in Canada (British Columbia) in 2009, and in the U.S. states of Florida, Washington, and Oregon in the same year. The first trap catches in Southern Ontario (Canada) occurred in 2010, and large infestations were found every year thereafter (Ontario Ministry of Agriculture and Food [OMAF] 2015). A recent laboratory study of the life history of *D. suzukii* found that this species has a relatively high fecundity and long life span. Emiljanowicz et al. (2014) found that *D. suzukii* had an average lifetime egg production of 636, a daily egg production of 5.7, an egg-to-adult development time of 12.8 d, and an average life span of 86 d. However, precise details of how such life history traits are altered by temperature are just beginning to be explored.

Precise information about temperature tolerances is necessary for modeling the spread and outbreak potential of *D. suzukii*. Some recent progress has been made in the area of *D. suzukii* overwintering capacity and thermal tolerance. Tochen et al. (2014) examined the effect of temperature on *D. suzukii* development rate and found that optimal development occurred at 28.1 °C. However, upper and lower thresholds were not measured directly, and the modeled upper developmental threshold of 42.1C from that study far exceeds the upper temperature limits normally experienced by other Drosophilids (e.g., Petavy et al. 2001). A study by Dalton et al. (2011) examined the overwintering capacity of flies collected from Oregon. Pupae and adults were subjected to temperature treatments ranging from 1 to 10 °C for a 6-wk period. Survival was assessed throughout the study period, and it was determined that D. suzukii survived longest at 10C, with survival decreasing below this point, whereby 100% mortality was observed by Day 17 at 1 °C. However, little is known about the overwintering capacity of *D. suzukii* in regions where average winter temperatures are below freezing, and whether different populations of *D. suzukii* are locally adapted to temperature conditions.

In this study, we examined several aspects of *D. suzukii* thermal tolerance in controlled experiments. Using a colony established from infested fruit in Southern Ontario, we examined temperature-dependent fecundity, development rate, and mortality in a range of temperatures that encompassed the upper and lower thresholds (5–35 °C). Additionally, we investigated tolerance to prolonged low temperature exposure using an overwintering assay, and examined the effects of life stage, sex, mating status, and photoperiod preexposure on overwintering survival. We also examined the effect of geographic origin by comparing the overwintering survival of an Ontario (cold winter) strain with a British Columbia (mild winter) strain.

Material and Methods

Fly Rearing and Colony Maintenance

The *D. suzukii* colonies used for this study were maintained in the same manner as described in Emiljanowicz et al. (2014). Controlled experiments on temperature-dependent development, fecundity, and mortality were carried out using a laboratory colony of flies that originated from infested fruit from a farm in Southern Ontario (mean daily winter averages $\approx$ −6 °C). For the overwintering assay, we then compared the Ontario colony with a colony that originated from D. suzukii collected from infested fruit in the lower mainland of British Columbia (mean daily winter averages $\approx$ +4 °C). These flies were maintained under lab conditions for 2 yr before our possession. We then kept these flies in a separate growth chamber set to the same settings as the chamber housing the Ontario flies. The two colonies were used to examine ecotype and genetic background effects on overwintering survival.

Temperature-Dependent Life History Measurements

Temperature-dependent development, fecundity, and survival were measured using temperature-controlled chambers. Humidity within each chamber was monitored using a digital hygrometer, and average humidity across all chambers was 47.1% RH. Each chamber was equipped with an LED light, which was set to a 16:8 (L:D) h. In an initial experiment, we examined temperature-dependent development at temperatures ranging from 5 to 35 °C in 5 °C increments. These data were used to examine the window in which the upper and lower developmental thresholds could be found.

	Comparisons			
Groups	LP *vs* SP	Male *vs* Female	Mated *vs* Virgin	ON *vs* BC
ON-M-LP	X1		X1	
ON-M-SP	X2	X1	X1	X1
ON-V-LP	X1		X2	
ON-V-SP	X2		X2	
BC-M-SP				X2
Males		X2		
Pupae				

Table 1: The first 5 groups were all females where ON—Ontario (cold winter strain), BC—British Colombia (mild winter strain), M—mated, V—virgin, LP—long photoperiod, SP—short photoperiod. The male group was exposed to a short photoperiod and was taken from a mating colony.

We then used 1 °C increments to more closely approximate these thresholds in two subsequent experiments (5-10 °C and 30-35 °C). In total, we therefore examined temperature-dependent development at: 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 31, 32, 33, 34, and 35 °C.

To obtain eggs for examining development, the lids of several centrifuge tubes (Fisherbrand 50 ml, Fisher, Ottawa, ON, Canada) were filed with 1 ml of standard lab diet and placed in a reproductive colony of *D. suzukii* for 4 h. Lids were used because the small amount of diet and dark color of the lids aided in quickly identifying and extracting eggs with forceps. Fifteen eggs were then placed in a disposable Fisherbrand 35- by 10-mm petri dish containing standard lab diet. Two dishes were placed in each temperature for a total of 30 eggs per temperature. As larval stages feeding on media cannot readily be observed with the naked eye, we measured the number of hours from egg to pupation and from egg to adult. Dishes were examined every 12 h for signs of pupation or adult eclosion. For the purposes of calculating development time, we assumed that pupation and eclosion had occurred in the middle of the 12-h window (Manly 2008). The inverse of the number of days to development was used to calculate developmental rates. Mortality rates were calculated for each dish in each temperature using the formula: (1− number emerging/initial egg number).

To conduct temperature-dependent fecundity measurements, "mating chambers" were constructed from modified centrifuge tubes (Fisherbrand 50 ml, Fisher). A hole was cut into the side of the centrifuge tube and covered with mesh for air flow. A second hole was cut in the opposite side, and a small section of clear PVC tubing was glued inside the hole where cotton could be inserted as a water source. This cotton was saturated with double-distilled water *ad libitum*. Two males and one female from a reproductive colony (515 d posteclosion) were placed in each mating chamber, and 10 mating chambers were placed into each temperature box. The mating chambers were given 4 d to acclimatize before egg counts began. The lid of each modified centrifuge tube contained 1 ml of standard lab diet and was replaced every 24 h. Following diet replacement, the number of eggs in each lid was counted every day for 10 d using a dissection microscope. Fecundity is expressed here as the number of eggs per day per female.

Overwintering Assay

To examine the effect of sustained low temperatures on D. suzukii survival and reproduction, we subjected groups of flies to a 42-d exposure to constant temperatures of −5, −3, −1, 1, 3, and 5 °C. While such constant temperatures are not representative of average fluctuating winter temperatures, we were predominantly interested in comparing the relative performance of different groups in order to make inferences about the optimal overwintering stage. Specifically, we were interested in comparing tolerance to low temperature exposure between males and females, virgins versus mated flies, short versus long photoperiod preexposure, cold winter (Ontario) versus mild winter (British Columbia) strains, and life stage. Table 1 shows the treatment groups used in the overwintering experiment and outlines the group comparisons that were made.

For each life stage outlined in Table 1, there were five petri dishes per temperature treatment, with 10 individuals per petri dish, for a total of 1,800 individuals for the whole experiment. Danby compact refrigerators (Danby Products LTD, Guelph, ON, Canada) were used as incubators to house the flies during the experiment, and HOBO data loggers (Onset Computer Corporation, MA, United States) were used to monitor temperature. Data from the data loggers were analyzed two to three times per week and adjustments were made if needed. A HUMICAP humidity indicator (Vaisala, Helsinki, Finland) was used to monitor humidity levels within the

fridges weekly. Flies were placed into a large petri dish (100 by 15mm) with a small petri dish (35 by 10mm) of banana diet that freezes at a lower temperature than standard lab diet (as described in Albers and Bradley 2006), as well as cotton saturated with double-distilled water. The petri dish was sealed with parafilm, and the lid had a hole covered with mesh for ventilation. Flies were prepared in this way before the preexposure acclimation period (see below). When the 42 d had elapsed in the temperature treatment, flies were subjected to postexposure acclimation (see below). Survival was assessed by recording the proportion of flies per petri dish that survived the exposure and acclimation periods.

Preexposure Treatments

It has been found that a combination of low temperature (15 °C) and short photoperiod (10:14 [L:D] h) will induce diapause in some Drosophila. To rear flies that were to be used in the overwintering experiment, one controlled growth chamber was set to 15 °C, 25% RH, and a photoperiod of 10:14 (L:D) h (short photoperiod conditions), and one controlled growth chamber was set to 15C, 25% RH, and a photoperiod of 15:9 (L:D) h (long photoperiod conditions). Diet dishes (as described in Emiljanowicz et al. 2014) that were left in the colony cages for 2 d were transferred to either the short or long photoperiod growth chamber and left to incubate in those conditions until adult eclosion (30 d).

Mated flies to be used in the experiment were released into a new colony cage in the respective chamber on the first day of emergence. For the following 10 d, all newly emerging flies were released into the same cage. These flies were left for 1wk to settle and mate. Diet dishes were replaced as needed. Virgin flies to be used in the experiment were separated within 12 h of emergence, and females were released into a new colony cage in the respective growth chamber. For the following 10 d, all newly emerging females were released into the same cage. These flies were left for 1wk to match the age range of the mated flies. The protocol for getting the flies into the temperature treatment was the same as for mated flies.

All flies in all groups were subjected to a step-wise acclimation process before and after the temperature treatment as follows: pretreatment, 10C with respective photoperiod for 4 d, followed by 5 °C for 4 d before being subjected to the experimental temperature treatment; post treatment, 5 °C for 4 d after 42 d in the temperature treatment, followed by 4 d at 10 °C, followed by 4 d at 15 °C, followed by 4 d at 22 °C.

Reproduction Following Overwintering

Fecundity of surviving flies was assessed for the ON-M-SP (Ontario, mated, short-photoperiod) group, as this life stage is assumed to be the overwintering stage for *D. suzukii* in the literature (Cini et al. 2012). Up to 20 surviving females from each temperature treatment group were put into a mating chamber (as described above). Half of the females were placed in a mating chamber with a male from the Ontario colony, and half of the females were put into a chamber alone. This was done to assess whether viable eggs were produced by flies that were mated only before the temperature treatment, or if a second post emergence mating was needed to produce viable eggs.

Flies were kept in mating chambers for a 6-d period in the 22 °C chamber. The diet lids of the mating chambers were replaced every second day over this period, and egg counts were performed on these diet lids. To determine viability of these eggs, the diet from the lid was transferred to a large petri dish filled with fresh diet (similar to the method described in Emiljanowicz et al. 2014 to determine sex ratio). After the 6-d period, a total of three diet lids had been transferred to the original petri dish. This procedure resulted in a total of 13 petri dishes for the females that came from the 3 °C temperature treatment (7 mated and 6 unmated after the cold exposure), and 19 petri dishes for the females that came from the 5 °C temperature treatment (9 mated and 10 unmated after the cold exposure). The petri dishes were incubated in the 22 °C chamber until all pupae had developed, and they were then counted.

Statistics

All data analysis and model fitting was performed using JMP 11.2 (SAS Institute, Cary, NC). For the overwintering assay, all survival data (proportion of adults surviving per petri dish) were analyzed using generalized linear models (GLM), using a binomial error structure and a logit link function. The temperature at which 50% of the population died (LT_{50}) and their 95% confidence intervals (CIs) were obtained from the GLM using the inverse prediction option in JMP. The LT50s were compared between groups (female vs. male, British Columbia vs. Ontario, virgin vs. mated, and long photoperiod vs. short photoperiod) by assessing CIs; group comparisons were considered significant where intervals were nonoverlapping. Post overwintering daily fecundity data were Box-Cox transformed before analysis. Both fecundity and viability of eggs of surviving females were analyzed using a two-way analysis of variance with postexposure mating status (mated

	Egg-to-pupa stage			Egg-to-adult stage			Adult females	
Temp (°C)	Mortality rate	Time to emergence (d)	SEM	Mortality rate	Time to emergence (d)	SEM	Fecundity	SEM
5	1.0	0		1.0	0		0	0
6	1.0	0		1.0	0		—	—
7	1.0	0		1.0	0		—	—
8	1.0	0		1.0	0		—	—
9	0.7	53.9	2.04	0.7	87.3	1.5	—	—
10	0.4	42.2	0.99	0.6	75.1	1.35	0.10	0.08
15	0.1	16.4	0.24	0.3	30.3	0.33	0.29	0.23
20	0.1	8.1	0.21	0.3	16.3	0.30	1.60	0.84
25	0	5.6	0.16	0.0	11.0	0.26	2.09	1.25
30	0.1	7.0	0.21	0.4	10.6	0.20	0.49	0.50
31	0.5	11.25	0.71	1.0	0		—	—
32	1.0	0		1.0	0		—	—
33	1.0	0		1.0	0		—	—
34	1.0	0		1.0	0		—	—
35	1.0	0		1.0	0		0	0

Note that fecundity was measured only in 5 °C increments (dashed line = not measured).

Table 2: Mortality rates (1−proportion emerging) and development time for the egg-to-pupa and egg-to-adult life stages, and adult female reproductive output (egg number per female per day) across all temperatures (means ± SEM)

and unmated) and temperature (3 and 5 °C) as fixed effects.

Results

Temperature-Dependent Fecundity

Egg number per reproductive female was counted daily over a period of 10 d (after an acclimation period of 4 d) and is expressed here as egg number per female per day. Table 2 shows the mean fecundity across temperatures ranging from 5 to 35 °C. We observed no egg production at 5 and 35 °C. We used a polynomial function with compact support, similar to that described by Saryazdi and Cheriet (2007), to fit to the experimental data (Fig. 1). This function is Gaussian-like, but unlike a Gaussian function, which is defined on the interval $[-\infty, +\infty]$, this function is constrained over a fixed interval $[T_{min}, T_{max}]$.

$$y = \begin{cases} \alpha \left[\frac{\gamma+1}{\pi\lambda^{2\gamma+2}} \left(\lambda^2 - \left([T-\tau]^2 + \gamma^2 \right) \right)^{\gamma} \right] & \text{if } T^2 + \gamma^2 < \lambda^2 \\ & \text{and } T_{min} < T < T_{max}; \\ 0 & \text{otherwise.} \end{cases}$$

where $\alpha = 659.06$, $\gamma = 88.53$, $\lambda = 52.32$, $\delta = 6.06$, $\tau = 22.87$, $T_{min} = 5$, and $T_{max} = 30$. The temperature at which the highest fecundity occurs (T_{opt}) was computed by calculating the real root of $\frac{\partial y}{\partial T} = 0$ for $T_{min} < T < T_{max}$:

$$\frac{\partial y}{\partial T} = -\frac{2\alpha\gamma(\gamma+1)\lambda^{-2\gamma-2}(T-\tau)\left(\delta^2 + (T-\tau)^2\right)^{\gamma-1}}{\pi} = 0$$
$$= 22.87°\text{C}$$

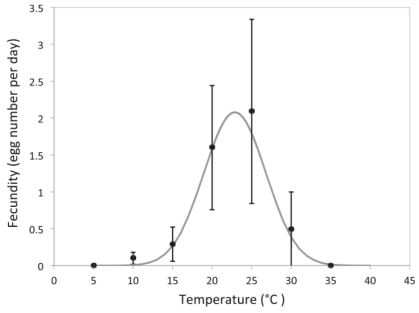

Figure 1: Temperature-dependent fecundity of *D. suzukii*. Fecundity is expressed as the number of eggs per female per day, as measured over a 10-d period following a 4-d acclimation period. Data were fitted using a polynomial function with compact support, adapted from Saryazdi and Cheriet (2007). Data points are means±SD.

Temperature-Dependent Development and Mortality

Table 2 shows the development time in days for both the egg-to-pupa and egg-to-adult life stages for all temperatures. There was no pupal emergence higher than 31C and no adult emergence higher than 30 °C. For both life stages, there was no emergence in temperatures lower than 9 °C. Corresponding mortality rates for each stage are also shown in Table 2. The development rate (the inverse of development time) was fitted to a Brie're function, a nonlinear model commonly used to describe arthropod temperature-dependent development (Brie're et al. 1999):

$$D(T) = aT(T - T_l)(T_u - T)^{\frac{1}{m}}$$

The optimal temperature is found from the positive root of

$\frac{\partial D(T)}{\partial T} = 0$, which is given by:

$$T_{opt} = \frac{\sqrt{T_l^2 (m+1)^2 - 4T_l m^2 T_u + 4m^2 T_u^2} + T_l m + T_l + 2mT_u}{4m + 2}$$

Both a and m represent empirical constants, while T represents temperature. We chose this function because it contains terms for both T_l (the lower temperature threshold; the temperature below which no development occurs) and T_u (the upper temperature threshold; the temperature above which no development occurs) and allowed us to input these empirically estimated values from our development data set. This model allowed us to estimate Topt (optimal temperature; the temperature at which the highest rate of development occurs) by calculating the local maximum of the curve. The model was fitted to both the egg-to-pupa data set and the egg-toadult data set. In running the model, T_l and T_u were allowed to vary within the window in which the thresholds were found to occur in our experiments (8.1-9.0 °C and 31.0-31.9 °C for egg-to-pupa; 8.1- 9.0 °C and 30.0-30.9 °C for egg-to-adult), and the model chose the best fit within these ranges. Table 3 shows the parameter estimates and associated SEMs for the model for both life stages. Using the Topt equation and the empirical estimates from Table 3, the optimal temperatures were found to be 26.9C for the egg-to-pupa stage and 28.2 °C for the egg-to-adult stage. Fig. 2 shows the shape of the function for the temperature-dependent development of the egg-to-pupa life stage.

Overwintering Survival

No pupae were found to survive the 6-wk exposure to any of the temperatures (between −5 and 5 °C). The proportion of adults surviving was dependent on exposure temperature ($\chi_1^2 = 527$, $P < 0.0001$; Fig. 3). The mean LT50 (the temperature at which 50% of the population dies) averaged across all data (sex, ecotype, mated status, and photoperiod exposure) was 4.88 °C (lower $CI_{95} = 4.65$; upper $CI_{95} = 5.15$). LT_{50}s were compared for all factors of interest (female vs. male, British Columbia vs. Ontario, virgin vs. mated, and long photoperiod vs. short photoperiod) and are shown in Fig. 4. The 95% confidence intervals overlapped for all groups, indicating no significant difference between the groups compared.

Postexposure Reproduction

The fecundity and offspring viability of previously mated females surviving overwintering exposures to 3 and 5 °C were examined. Surviving females were divided into two groups and were either mated or unmated postexposure. There was no effect of overwintering exposure temperature on mean daily fecundity of female survivors. However, there was an effect of mating status, whereby females that were mated again after exposure had higher daily fecundity measures than those females that were not mated again after exposure (mated: 4.4160.91 eggs/day; unmated: 0.4060.16 eggs/ day; $F_{1,27} = 24.47$, $P < 0.0001$). There was no temperature × mating status interaction for fecundity.

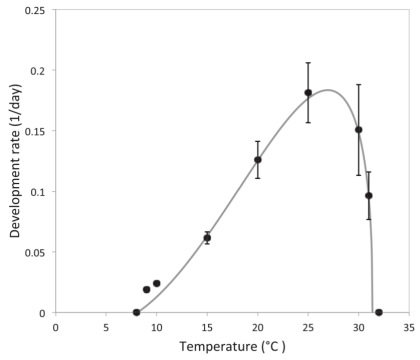

Figure 2: Temperature-dependent development rate of D. suzukii for the egg-to-pupa stage. Data were fitted with a standard model of arthropod temperature-dependent development (Brière 1999). Data points are means±SD.

Viability was measured as the proportion of eggs that developed into pupae. There were no effects of temperature, mating status, or their interaction on viability. On average, 85.6% (66.7%) of eggs laid by females following winter exposure developed into pupae.

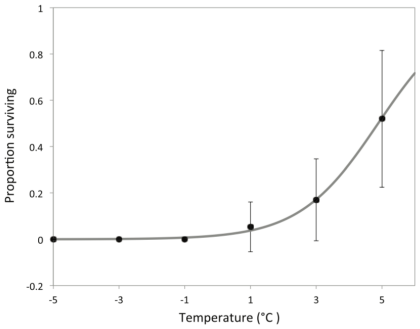

Figure 3: The dependence of adult survival on temperature for data combined across all groups. Data were fitted using a GLM with a binomial error structure and logit link function. Data points represent means±SD.

Discussion

The current study provides detailed observations of *D. suzukii* temperature- dependent survival, development, and reproductive output across experimental temperatures encompassing upper and lower temperature thresholds. Optimal temperatures were 26.9 °C for the development of the egg-to-pupa stage, and 28.2 °C for the eggto- adult stage, while optimal reproductive output (number of eggs per female per day) occurred at 22.9 °C. No adult eclosion occurred below 8.1 and above 30.9 °C, while no pupae were formed below 8.1 or above 31.6 °C. Tochen et al. (2014) recently observed a similar optimal developmental temperature of 28.1 °C in *D.*

suzukii from data also fitted with a Brie're model. However, the upper and lower developmental thresholds found in that study (42.1 and 7.2 °C, respectively) differed greatly from our observations. This is likely because thresholds were extrapolated from a narrower experimental range of temperatures in the Tochen study (10-30 °C), which may have resulted in an overestimation of the thermal tolerance of this species. However, another notable difference between the two studies was the use of synthetic medium in the present study versus host fruit (cherries and blueberries) in the Tochen study. This may account for some of the differences between the two studies, though is unlikely to account for the large deviation in the upper developmental threshold.

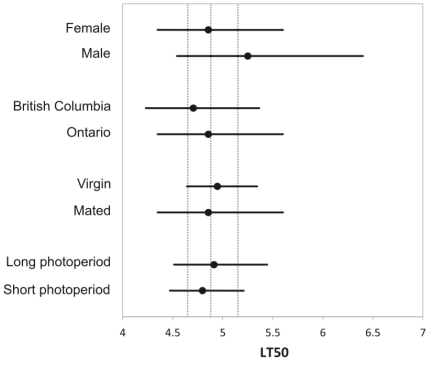

Figure 4: LT_{50}s (°C)+95% confidence limits for all group comparisons. The overlapping CIs indicate that there were no significant differences between groups. Dashed lines represent the overall LT_{50} and CI_{95} for the whole data set

The present study provides evidence to suggest that acclimated *D. suzukii* can survive 42-d exposures to 1, 3, and 5C environments, though the LT_{50} was found to be 4.88 °C across all treatments. Unsurprisingly, temperature affected survival, and higher survival was observed at higher temperatures (Fig. 3). This result is in agreement with Dalton et al. (2011). However, in their experiment, adults did not survive for ¿17 d at 1 °C, while we found that 5% of individuals survived 42 d at this temperature. No flies survived the exposure period at temperatures below freezing, suggesting that this is not a cold-tolerant species. *Drosophila suzukii* have recently been characterized as chill susceptible. Jakobs et al. (2015) found that while the supercooling point of this species was ≈ -20 °C, fly mortality tended to occur at temperatures well above the supercooling point.

While we did not find any survival below 1 °C after 42 d of exposure, it should be noted that the overwintering assay was performed at constant temperature. While the current study allowed us to compare the thermal tolerance of several groups, more information about the overwintering capacity of this species could be obtained by altering both the duration and the temperature of exposure. It has been found that duration of cold exposure can affect the survival of *Drosophila* (Marshall and Sinclair 2009). It has also been found that fluctuating temperatures can increase the survival of overwintering insects, as they are able to repair cold injuries during intermittently warmer periods (Renault et al. 2004). Fluctuating temperatures can also acclimate Drosophilids to temperature extremes. For example, Overgaard et al. (2011) found that *D. melanogaster* exposed to fluctuating temperatures during development subsequently had a higher tolerance to both heat and cold.

We observed no difference in the overwintering survival of *D. suzukii* from British Columbia versus Southern Ontario, suggesting that these populations do not represent distinct ecotypes with respect to thermal tolerance. However, one limitation of the current study may be that colonies were kept under lab conditions for multiple generations before running experiments. Studies have shown that over many generations, some *Drosophila* species may adapt to laboratory conditions (Matos et al. 2000) and values of life history traits may diverge from newly sampled wild populations. However, others have observed similar results for this species. In a study of the temperature tolerance of several Drosophilids sampled across latitudinal gradients in Japan, Kimura (2004) found that several species showed intraspecific differences in thermal tolerance depending on geographical origin. However, in the case of *D. suzukii*, Kimura found no difference in tolerance between cool-temperate and warm-temperate geographical strains.

We found that pupae did not survive a 42-d exposure to any of our experimental temperatures, suggesting that adults represent the overwintering stage. We observed no significant differences between male and female adult survival at low temperatures. This contrasts with the observations by Dalton (2011), who found that of 22 flies surviving an 84-d exposure to 10 °C, only a single individual was male. However, these results may reflect differences in the exposure period. Jakobs et al. (2015) found no difference in the supercooling point of males versus females, but found that the LT_{80} of a 1-h exposure was 7.5 °C for females and 7.2 °C for males.

Dalton et al. (2011) suggested the need for the study of female fecundity after prolonged exposure to winter conditions. We conducted such a study after exposure to a 42-d cold treatment. We compared females that were only mated before the cold exposure, and females that were mated both before and after cold exposure. We observed that some females (38%) that were mated only before cold exposure were able to lay eggs following exposure and that these eggs were viable. This observation has three possible mechanisms: 1) females were able to store sperm throughout the cold exposure and used this sperm to fertilize eggs once they were returned to favorable conditions; 2) the eggs were fertilized at time of mating, before cold exposure, but development was depressed on exposure to cold; or 3) females converted to a state of parthenogenesis with cold exposure. Sperm storage organs are common in insects, and it is known that some *D. melanogaster* can store sperm in the spermathecae, and are able to lay fertile eggs for up to 2wk after being isolated from males, until the sperm store is exhausted (Lefevre and Jonsson 1962). Collett and Jarman (2001) observed that even after

a 6-mo exposure to cold, mated Drosophila pseudoobscura females were able to use stored sperm to fertilize and lay viable eggs. It may be possible for mated *D. suzukii* to store sperm over the winter season, as they would not be exhausting this resource during this time, and use this viable sperm once conditions had become favorable to fertilize their eggs.

Low temperatures have been found to depress the pace of embryogenesis in certain insect species (Mansingh 1971, Schaefer 1977). It is possible that at the time of mating, an embryo was produced but development was arrested on entry into unfavorable conditions. Once temperatures were brought back within a favorable range, these eggs would be viable once laid. Another possibility is that of parthenogenesis. Stalker (1954) discusses the capacity for parthenogenesis in Drosophilidae. In a survey of 28 species, parthenogenesis was identified in 23, albeit at a low rate (Stalker 1954). Further, of the species that produced viable eggs, only three species were able to produce adult progeny (Stalker 1954). Although unlikely, it is possible that females that were able to produce eggs, but were not mated again after the cold exposure, actually reproduced by parthenogenesis. In this event, if males are less likely to survive the winter months, perhaps females who survive resort to parthenogenesis until a stable male population is established.

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