Cold Hardiness of Winter-Acclimated *Drosophila suzukii* (Diptera: Drosophilidae) Adults

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ABSTRACT *Drosophila suzukii* Matsumura, often called spotted wing drosophila, is an exotic vinegar fly that is native to Southeast Asia and was first detected in the continental United States in 2008. Previous modeling studies have suggested that *D. suzukii* might not survive in portions of the northern United States or southern Canada due to the effects of cold. As a result, we measured two aspects of insect cold tolerance, the supercooling point and lower lethal temperature, for *D. suzukii* summer-morph pupae and adults and winter-morph adults. Supercooling points were compared to adults of *Drosophila melanogaster* Meigen. The lower lethal temperature of *D. suzukii* winter-morph adults was significantly colder than that for *D. suzukii* summer-morph adults, while supercooling points of *D. suzukii* winter-morph adults were actually warmer than that for *D. suzukii* summer-morph adults and pupae. *D. suzukii* summer-morph adult supercooling points were not significantly different than those for *D. melanogaster* adults. These measures indicate that *D. suzukii* is a chill intolerant insect, and winter-morph adults are the most cold-tolerant life stage. These results can be used to improve predictions of where *D. suzukii* might be able to establish overwintering populations and cause extensive damage to spring fruit crops.

KEY WORDS spotted wing drosophila, *Drosophila melanogaster*, cold acclimation, cold tolerance

Nonnative insects have the potential to cause widespread damage to both natural and cultivated systems (e.g., Manchester and Bullock 2000, Pimentel et al. 2000). *Drosophila suzukii* Matsumura, frequently referred to as spotted wing drosophila, is native to Southeast Asia and was first discovered in the continental United States in 2008 (Hauser 2011). Female *D. suzukii* can oviposit into intact soft-skinned fruits including *Rubus* spp., *Prunus* spp., *Fragaria* spp., *Vaccinium* spp., and *Vitis* spp. (Lee et al. 2011, 2015; Walsh et al. 2011; Burrack et al. 2013). Larval feeding further damages the fruit and renders it unmarketable. The combined effects of lower crop yield and increased costs to control *D. suzukii* have led to substantial local reductions in revenue from berry crops (Goodhue et al. 2011).

D. suzukii is found on nearly every continent, with the notable exceptions being Oceania and Antarctica (Commonwealth of Australia 2013). This insect was discovered in Spain in 2008 and has spread rapidly across Europe (Cini et al. 2012). In 2013, *D. suzukii* was first discovered in South America in Brazil (Deprá et al. 2014). In North America, *D. suzukii* has been found in British Columbia and most US states (National Agricultural Pest Information System [NAPIS] 2014).

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Most species of *Drosophila* overwinter as adults in some sort of diapause state (e.g., reproductive or aestivation), although some may overwinter as pupae or larvae (Hoffman et al. 2003, Strachan et al. 2011). *D. suzukii* is believed to overwinter as adult winter-morphs underneath leaf litter in forested areas (Kanzawa 1936). Winter-morphs have darker coloration than summer-morphs and have an increased wing size (P. W. Shearer, personal communication). They have been found in the fall in northern climes (Kanzawa 1936), and both morphs have been successfully reared under laboratory conditions (P. W. Shearer, personal communication). Winter-morphs of *D. suzukii* are produced in the laboratory when eggs and larvae are introduced to autumn and winter conditions such as low temperatures and short photoperiod, so these morphs are thought to be cold acclimated. Large, dark morphs associated with overwintering conditions have been described in other *Drosophila* spp., including *Drosophila melanogaster* Meigen (e.g., Ayrinhac et al. 2004, Gibert et al. 2007). Overwintering females of *D. suzukii* may be in diapause (Mitsui et al. 2010, Zerulla et al. 2015) and more cold tolerant than males (Kanzawa 1936, Zerulla et al. 2015).

Cold temperature is a limiting factor in the geographic range of several *Drosophila* spp. (Kimura 1988, Andersen et al. 2015). If pupae or adults of *D. suzukii* overwinter beneath leaf litter and snow in northern climes of North America, individuals must withstand extended exposure to low temperatures (i.e., months at 0°C), with brief exposures to extreme cold temperatures. Previous studies have suggested that the lower

developmental threshold for summer-morph *D. suzukii* is 7.2°C (Tochen et al. 2014). Adult, summer-morph *D. suzukii* are unable to survive for 3 mo at 10°C (Dalton et al. 2011), but winter-morphs can survive several months at 1°C (P. W. Shearer, personal communication). This result suggests that *D. suzukii* may be as cold tolerant or more cold tolerant than unacclimated *D. melanogaster*, which can survive >45 d at 4°C (Izquierdo et al. 1991). In addition, a CLIMEX model created for *D. suzukii* suggests that much of the northern United States and southern Canada provides a suboptimal climate (Damus 2009), partially due to the effects of cold. There is a general lack of knowledge, about the relative ability of *D. suzukii* summer morphs and winter morphs to survive brief exposures to harsh cold temperatures, but Emiljanowicz (2014) and Jakobs et al. (2015) recently reported observations on the cold tolerance of summer morphs.

Insect cold tolerance frequently is quantified by using the supercooling point and the lower lethal temperature. The supercooling point is the temperature at which the insect's body fluids begin to freeze, typically below 0°C due to the colligative properties of salts, amino acids, proteins, and sugar-alcohols in the hemolymph (Denlinger and Lee 2010). Insects that succumb to chill injury before their body fluids freeze are considered "chill intolerant" (Denlinger and Lee 2010), a cold strategy common to many tropical species, including most species of *Drosophila* (Strachan et al. 2011). Jakobs et al. (2015) noted that summer morphs of *D. suzukii* are chill intolerant. Insects that survive chilling but die due to the formation of ice within their body tissues are considered "freeze intolerant," while those that are able to survive freezing are considered "freeze tolerant" (Denlinger and Lee 2010). Among temperate insects, freeze intolerance is more common than freeze tolerance (Sømme 1996). A few species of *Drosophila* larvae have been shown to survive partial freezing (Shimada and Riihimaa 1988, Strachan et al. 2011, Kostal et al. 2012).

Lower lethal temperature can be measured by briefly introducing insects to temperatures slightly above and below the supercooling point and determining mortality (e.g., Morey et al. 2012). This parameter can be used to understand mortality from brief exposures to cold temperatures (i.e., acute cold stress), although prolonged exposure to a low temperature which is not immediately lethal can also result in chill injury and death (Sømme 1996).

This study quantifies cold tolerance of adult female and male summer-morph and winter-morph *D. suzukii* by measuring the supercooling point and lower lethal temperature. The effects of morph and sex on supercooling points were compared. Supercooling points for adult summer-morph *D. suzukii* were further compared to adult *D. melanogaster* (a well-studied species known to persist in northern climates) and pupae of *D. suzukii*, all reared under the same conditions. We hypothesized that the adult female winter-morph of *D. suzukii* would be more cold tolerant than male winter-morph *D. suzukii*, adult summer-morphs and pupae of *D. suzukii* of both sexes, and that the cold hardiness of adult summer-morph *D. suzukii* would be similar to adult *D. melanogaster*.

Materials and Methods

Colony Source. *D. suzukii* were obtained from a colony at Michigan State University in East Lansing, MI, in December, 2013. This colony was established from adult flies found in a blueberry field in Allegan County, MI, in August 2013 (Steve Van Timmeren, personal communication). Adults were kept in St. Paul, MN, in narrow fly vials stoppered with foam plugs (Genesee Scientific, San Diego, CA) and where larvae were fed on a traditional agar-cornmeal-yeast diet as described in Dalton et al. (2011). A strip of filter paper was placed in each vial to reduce mortality from moisture kill. Summer-morph *D. suzukii* were reared in programmable growth chambers (Percival Scientific Inc., Perry, IA), typically at 24 ± 2°C with a photoperiod of 14:10 (L:D) h and relative humidity of ~60 ± 30%. Under these conditions, summer-morph adults lived for ~2–3 wk after eclosion. Winter-morph adult *D. suzukii* were produced by placing vials with 1-3-d-old eggs laid by summer-morph *D. suzukii* into a Percival programmable growth chamber at 10 ± 1°C with a photoperiod of 12:12 (L:D) h and relative humidity of 75 ± 20% (protocol modified from P. W. Shearer, personal communication). The colder temperatures slowed growth, and adults emerged from pupae 1 to 2 mo after being placed in the growth chamber. Winter-morph adults survived for several months. Recently eclosed adult flies (<48 h old or between 17 and 33 degree days [base temp = 7.2°C] for summer-morph flies, and <120 h old or under 14 degree days for winter-morphs flies) were used for all experiments.

Oregon-R strain of *D. melanogaster* was obtained from a laboratory colony at the University of Minnesota in June 2014. *D. melanogaster* were kept in the same type of vials and fed the same type of diet as *D. suzukii*. *D. melanogaster* were reared at room temperature (24 ± 2°C) with a photoperiod of 12:12 (L:D) h on a benchtop in St. Paul, MN, in a separate laboratory from *D. suzukii* to prevent cross-contamination, because *D. melanogaster* has previously outcompeted *D. suzukii* in mixed cultures (MKA, personal observation). *D. melanogaster* adults <48 h old were used for all experiments. *D. melanogaster* cold tolerance results were only compared with *D. suzukii* summer-morphs.

Supercooling Point Determination. Supercooling points were measured with 0.127-mm-diameter (36 AWG) type-T copper-constantan thermocouples (accuracy = ±0.17°C). Thermocouples were threaded through a milled plastic dowel fitted with an O-ring. The dowel was inserted into a microcentrifuge tube, and the O-ring sealed the tube to prevent the insect from escaping (Fig. 1). The entire apparatus was placed in the center of a 20-cm polystyrene cube plugged with a rubber stopper, and the polystyrene cube was placed in a −80°C freezer, which cooled the insect at ~1°C/min (Carrillo et al. 2004). Thermocouples were connected to a computer via an analog data acquisition unit (USB-TC, Measurement Computing, Norton,

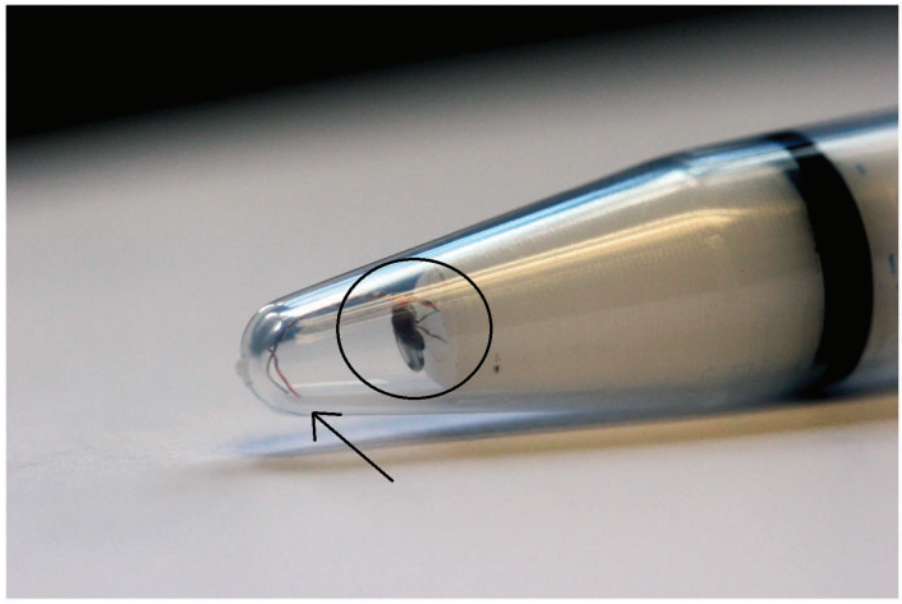

Fig. 1. *D. suzukii* male (circled) and thermocouple (arrow) in microcentrifuge tube.

MA), and temperature was recorded once per second by using TracerDAQ Pro (Measurement Computing Corporation, Norton, MA). The supercooling point was measured as the lowest temperature before the release of the latent heat of freezing, indicated by a clear spike (~0.5–1°C in our case) in temperature (Denlinger and Lee, 2010).

Supercooling point experiments followed a completely randomized design, with up to 16 individuals per batch and at least two batches per treatment. A batch consisted of a group of individuals that were placed in the −80°C freezer at the same time. Treatments consisted of species (i.e., *D. suzukii* or *D. melanogaster*), stage (i.e., pupa or adult), or morph (i.e., summer or winter) and were typically run in separate batches of different sizes due to differences in availability of individuals. The maximum number of individuals per batch was limited by the availability of thermocouples and data acquisition units. When sex was of interest, males and females were tested in the same batch. For additional details on the numbers and state of individuals tested in each batch for all treatments, see Supp. Table 1 (online only). Because a batch only included one treatment, with the exception of sex, the effects of batch and treatment are confounded factors, and potential treatment differences must be interpreted with caution. All analyses were conducted in R 3.0.0 (R Core Team, Vienna, Austria). Details of each analysis are provided below.

Adult D. suzukii. A total of 58 summer-morph and 32 winter-morph adult *D. suzukii*, approximately half of which were females, were tested. To separate *D. suzukii* from the colony, adults were anesthetized with CO_2 using a FlyPad (Genesee Scientific) and one fly was placed into each 1.5-ml microcentrifuge tube. Summer-morph adults were measured in 10 batches, while winter-morph adults were measured in three batches.

Summer-morph supercooling points were not normally distributed, so all data compared to summer-morphs were transformed by using an x^2 transformation as recommended by a Box-Cox analysis (Box and Cox 1964). A two-way ANOVA was used to test the effects of sex, morph, and their potential interaction on supercooling points of winter-morph and summer-morph *D. suzukii*. Means and confidence intervals are reported for nontransformed data.

Adult D. melanogaster. The supercooling points of 26 adult *D. melanogaster* were measured in two batches. To separate *D. melanogaster* from the colony, adults were confined on a tray in an ice bath for <5 min and transferred into individual 1.5-ml microcentrifuge tubes. Different methods for anesthetizing *D. suzukii* and *D. melanogaster* were used to reduce potential contamination of colonies. Supercooling points for *D. melanogaster* and *D. suzukii* adult summer-morphs were statistically compared by using a Welch two sample t-test, R command: t.test().

D. suzukii Pupae. Some species of *Drosophila* overwinter as pupae, so supercooling points of pupae were tested and compared to supercooling points of adults. *D. suzukii* pupae were reared at 24 ± 1°C with a photoperiod of 14:10 (L:D) h, as for summer-morphs. A total of 40 pupae, <24 h old, were transferred from the colony by using a camelhair brush and transferred to individual 1.5-ml microcentrifuge tubes. Pupae were tested in three batches. Supercooling points were determined as for adults and compared to adult *D. suzukii* summer-morphs by using a Welch two sample *t*-test.

Lower Lethal Temperature Determination. These experiments followed a randomized complete block design. Adult *D. suzukii* were cooled in

microcentrifuge tubes without food in the dark inside a Thermo Scientific refrigerated bath circulator (A40, Thermo Fisher Scientific, Newington, NH) with SIL-180 silicon oil as a coolant. Temperature of the adults was monitored using thermocouples as described for supercooling point determination. Summer-morph and winter-morph adult *D. suzukii* were cooled in separate batches at a rate of 1°C/min. For each batch, adults were cooled from 24°C to −10, −15, −20, or −25°C ± 1°C and held at the target temperature for ∼1 s. Adults that gave an exotherm were cooled to the target temperature. Control flies were held at room temperature (∼24 ± 2°C) in individual microcentrifuge tubes in the dark without food until experimental flies were finished cooling. Experimental adults were returned immediately to room temperature, and all adults were transferred to new, individual microcentrifuge 1.5-ml tubes that contained ∼0.5 ml artificial diet and a ventilation hole. Between three and six adults of each cooling treatment (including controls) were run per each batch, with eight batches for winter-morphs and 11 batches for summer-morphs. At least 20 adults of each morph were exposed to each of the temperatures (for additional details on the numbers and condition of individuals tested in each batch, see Supp. Table 2 [online only]). Mortality was measured after 24 h at room temperature to allow adults to recover, as has been used in previous studies of *Drosophila* (Czajka and Lee 1990). Survival was determined by movement and the ability of the adult to upright itself (e.g., Czajka and Lee 1990, Dalton et al., 2011). Sex was recorded at the same time as mortality by looking for spots on wings or a pointed abdomen indicating an ovipositor (Hauser 2011).

All analyses were conducted in R (R Core Team, Vienna, Austria). Abbot's correction ($P' = P - P_0 \frac{100-P}{100-P_0}$) (Healy 1952), was applied to remove mortality that could not be attributed to cold exposure, where P' is the corrected percentage of adults that died because of the effects of the cold treatment, P is the percentage of adults that died in the treatment, regardless of the cause, and P_0 is the percentage of control adults that died without experiencing cold. The percentages were then multiplied by the number of flies tested for a specific treatment prior to analysis.

The relationships between mortality and temperature for winter and summer-morphs were analyzed by using general linearized models with a logit-link in R. Survival of each individual (coded as 0 = death, 1 = survival) was the response variable and block, treatment temperature, sex, and interaction effects were explanatory variables. The LT_{50} and LT_{90} (the temperature at which 50% or 90% of the population dies after exposure for 1 s) were determined with R- function "dose.p(glm, p = 0.5 or p = 0.1)" in the MASS package. The predicted mortality curves for summer- and winter-morph *D. suzukii* were compared using a third generalized linear model with survival of each individual (coded as 0 = death, 1 = survival) as the response variable and treatment temperature as the explanatory variable. Winter morphs and summer morphs were analyzed separately.

In a final set of analyses, to determine the cold tolerance strategy, the cumulative proportion of individuals that froze by a particular temperature (i.e., a supercooling point was detected) was compared to the cumulative proportion of individuals that died at the same temperature by using logistic regression. The supercooling point data and lower lethal temperature data were combined into one dataset. The elements of the dataset were experiment (coded as SCP = supercooling point, LLtemp = lower lethal temperature), treatment temperature (i.e., exposure temperature for lower lethal temperature experiments or temperature at which the exotherm was detected for supercooling point experiments), positive outcomes (i.e., number of individuals that survived a given temperature in lower lethal temperature experiments or cumulative number of individuals that did not freeze by the given temperature in supercooling point experiments), and negative outcomes (i.e., number of individuals that did not survive a given temperature in lower lethal temperature experiments or cumulative number of individuals that froze by the given temperature in supercooling point experiments). A generalized linear model was then created with probability of observing a positive outcome as the response variable and treatment temperature, experiment, and the interaction of treatment temperature and experiment as the explanatory variables. Winter-morphs and summer-morphs were analyzed separately.

Results

Supercooling Points. Sex did not significantly affect the supercooling point of adult winter-morph or summer-morph *D. suzukii* ($F = 0.21$, df = 1, 87, $P = 0.65$; results for males and females were pooled for future analyses). Winter-morph adults had a significantly warmer supercooling point ($F = 33.8$, df = 1, 87, $P < 0.0001$) than summer-morph adults. Summer-morph adult *D. suzukii* had a mean supercooling point of −20.2°C (95% CI: −20.9, −19.6; Fig. 2), while winter-morph *D. suzukii* had a mean supercooling point of −17.5°C (95% CI: −17.7, −17.2; Fig. 2). Pupae had a mean supercooling point of −21.8°C (95% CI: −22.5, −21.0; Fig. 2). Supercooling points for pupae were significantly cooler than summer-morphs ($t = -3.6$, df = 88.3, $P < 0.001$). The mean supercooling point of *D. melanogaster* was −19.6°C (95% CI: −20.1, −19.0; Fig. 2). Summer-morph *D. suzukii* and *D. melanogaster* supercooling points were not significantly different ($t = -1.4$, df = 78.1, $P = 0.2$).

Lower Lethal Temperature. Sex did not significantly affect the lower lethal temperature of winter-morph ($F = 0.4$, df = 1, 85, $P = 0.5$) or summer-morph *D. suzukii* ($F = 0.8$, df = 1, 87, $P = 0.4$; results for males and female were pooled for future analysis). In general, for the same temperature, there was less mortality in winter-morphs than summer-morphs (Fig. 3). Logistic regression models describing mortality as a function of temperature for winter-morph and summer-morph adults had significantly different intercepts ($z = 3.4$, $P = 0.02$), though the slopes were not significantly

different ($z = 1.1$, $P = 0.280$). The intercept of 7.9 ± 1.4 ($\pm$ SE) for winter-morphs and 3.8 ± 1.13 for summer-morphs suggests that summer-morphs begin dying at a warmer temperature than winter-morphs. The predicted LT_{50} for summer-morph adults was $-10.01°C \pm 0.8$ (SE), and for winter-morph adults was $-15.3°C \pm 0.6$ (Fig. 3). The predicted LT_{90} for summer-morph adults was -15.6 ± 1.1, and for winter-morph adults was $-19.55°C \pm 0.9$.

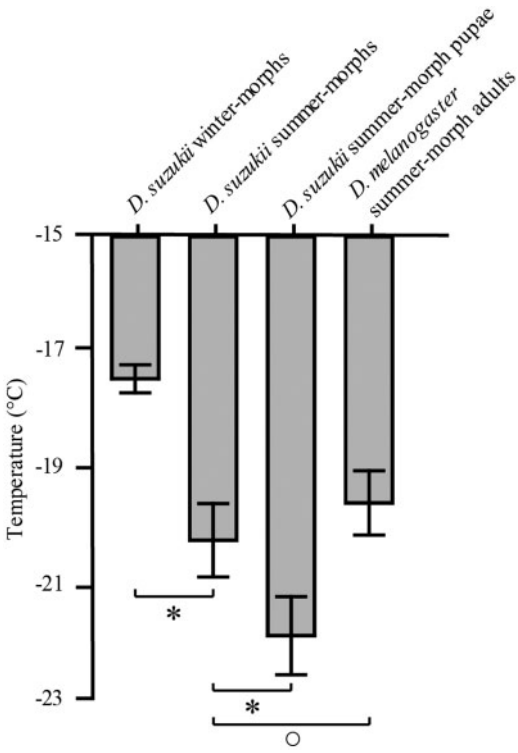

Fig. 2. Supercooling points (mean $\pm$ 95% confidence interval) of *D. suzukii* and *D. melanogaster*. Brackets indicate statistical comparisons between two species or stages. Circles indicate no statistical difference ($P > 0.05$); asterisks indicate a difference ($P < 0.05$).

Logistic regression models to determine the cold tolerance strategy of *D. suzukii* winter-morph adults compared the likelihood of freezing with the likelihood of death over a range of temperatures. The logit-link regressions had significantly different intercepts ($z = 10.5$, $P < 0.001$) and slopes ($z = 11.1$, $P < 0.001$). Winter-morphs began dying at warmer temperatures than when supercooling points were detected. The change in the proportion of individuals that froze (i.e., for which a supercooling point was detected) with a unit change in temperature was greater than the change in the proportion of individuals that died (Fig. 3). The logit-link regressions to determine the overwintering strategy of summer-morphs had significantly different intercepts ($z = -3.7$, $P < 0.001$) and slopes ($z = -2.6$, $P = 0.007$). Summer-morphs began dying at warmer temperatures than when supercooling points were detected. The change in the proportion of individuals that froze increased at a faster rate with a change in temperature than the proportion of individuals that died (Fig. 3).

Discussion

Previous research on *D. suzukii* cold tolerance has suggested that summer-morph adults should not be able to overwinter in cold climates (Kimura 2004, Dalton et al. 2011, Jakobs et al. 2015), but winter-morph adults might (P. W. Shearer, personal communication). However, while winter- morphs have been reported to survive long periods at mild temperatures (P. W. Shearer, personal communication), it is likely that they lack the ability to survive brief exposure to

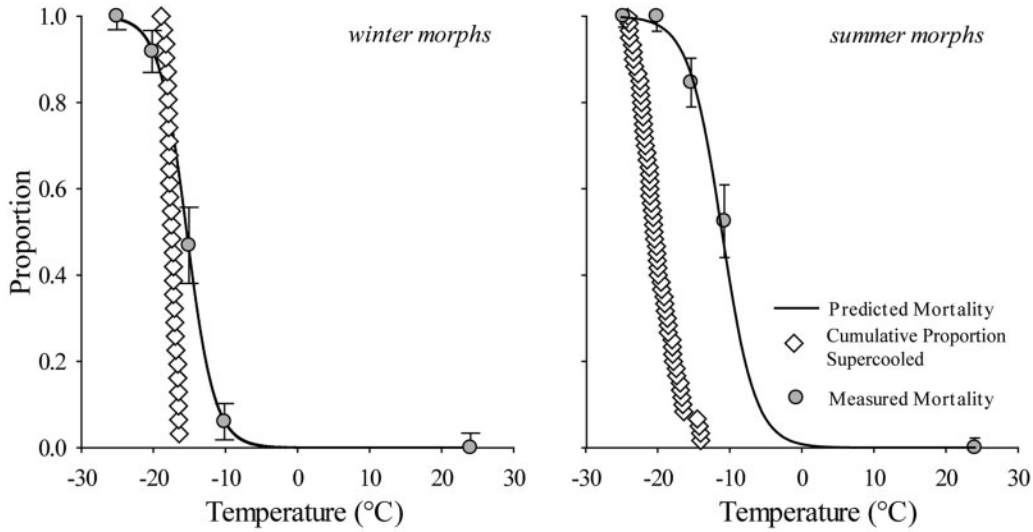

Fig. 3. Cumulative proportion of winter- and summer-morph adults of *D. suzukii* that had frozen (i.e., a supercooling point was detected) or died in response to acute exposure to cold temperatures. Mortality is mean $\pm$ SE.

extreme temperatures. This study provides a basis for which temperatures can be considered extreme for *D. suzukii* winter-morphs and summer-morphs.

Most mortality occurs before the supercooling point in both summer-morphs and winter-morphs of *D. suzukii*, although mortality in the summer-morph begins several degrees warmer than for winter-morphs. The difference between the cumulative supercooling point curves and mortality curves of *D. suzukii* suggests adults are chill intolerant, or that they die before freezing, consistent with the findings of Jakobs et al. (2015) for summer morphs. Chill intolerance is common in temperate insects, and suggests that *D. suzukii* must avoid extreme cold temperatures to overwinter. Winter-morph *D. suzukii* may have a mixture of both a chill intolerant strategy (i.e., death occurs before an individual freezes) and a freeze-intolerant strategy (i.e., death occurs once an individual freezes). The predicted mortality curve begins at temperatures before supercooling, but it follows the supercooling point curve very closely in cooler temperatures (Fig. 3). This suggests that some winter-morph *D. suzukii* may be freeze intolerant, and some may be chill intolerant. In addition, it appears that winter-morphs may not invest in types of cryoprotectants that prevent freezing, but rather invest in resources which allow them to survive chill injury.

A possible explanation for the warmer supercooling points of winter-morph adult *D. suzukii* than summer-morph adult *D. suzukii* could be an effect of suboptimal rearing conditions. O'Doherty and Bale (1985) described a warmer supercooling point of the peach-potato aphid *Myzus persicae* (Sulzer) that were reared at 5°C for 7 d than insects reared at 20°C. The authors hypothesized that this decrease in supercooling ability was due to suboptimal conditions. *D. suzukii* reared at 10°C may have an increased ability to survive over time at milder temperatures at the cost of a warmer supercooling point. Future studies are needed to investigate why cold-acclimated winter-morph *D. suzukii* adults have a warmer supercooling point despite their higher survival at cooler temperatures.

Our results suggest that the supercooling point of *D. suzukii* pupae is cooler than summer-morph *D. suzukii* adults. This result could have important ecological implications. *D. suzukii* winter-morphs may not emerge until late in the fall, and as a result, pupae must be able to survive brief exposures to cold before adults overwinter. In addition, it has been suggested that immobile stages of *Drosophila* may be more cold tolerant because they are unable to behaviorally avoid damaging temperatures (e.g., Hoffman et al. 2003). These results suggest pupae may be able to overwinter, but previous research has suggested that although pupae are able to withstand winter temperatures for a short period of time, they will emerge as adults even at low temperatures (i.e., 1°C or 3°C) and subsequently die (Dalton et al. 2011). In addition, pupae which overwinter in soil or rotting berries may be susceptible to inoculative freezing. In this study, all pupae were cooled until an exotherm was detected, and subsequent observations confirmed that these pupae were dead (A. R. Stephens, personal observation). Additional studies are needed to confirm that freezing caused mortality in this life stage, and that pupae which precede the winter morph are unable to overwinter.

Previous studies have indicated that the supercooling point of *D. melanogaster* may vary with age, but 2-d-old *D. melanogaster* should be expected to have a supercooling point between −21°C and −19°C, which is consistent with our results (Czajka and Lee, 1990). In addition, *D. melanogaster* is a chill intolerant insect. The lack of a significant difference between the supercooling point of *D. melanogaster* and *D. suzukii* summer-morphs suggests that other measures of cold tolerance, such as the lower lethal temperature of *D. suzukii* summer-morphs may be similar to *D. melanogaster*. If chill intolerant *D. melanogaster* can overwinter in northern, temperate climates as has been predicted in previous studies (e.g., Izquierdo 1991), *D. suzukii* might be able to overwinter in these climates as well. However, the ability of *D. melanogaster* to overwinter in cold climates is debated by some authors (e.g., Izquierdo 1991).

Overwintering *D. suzukii* will only survive cold in temperate climes of the northern United States and Canada if it finds a refuge to escape the cold, such as under snow or indoors. However, *D. suzukii* may not spend winter beneath snow, as previous studies have shown that *D. suzukii* is a winter-active species (Uchino 2005). The ability of winter-morphs of *D. suzukii* to survive an extended period at low temperatures has yet to be measured. Although *D. suzukii* may not be able to survive extreme cold shocks, insect survival of cold also varies based on the length of time an insect is exposed to a cold temperature (Sømme 1996). Future research on the lower lethal time, or the time it takes *D. suzukii* to die while constantly exposed to a cold stress is needed.

Our study did not find an effect of sex on supercooling point or lower lethal temperature of *D. suzukii* summer- or winter-morphs, contrary to our initial hypotheses. Likewise, Jakobs et al. (2015) did not observe an effect of sex on the supercooling point of summer-morph *D. suzukii* in the absence of acclimation, the treatment most comparable to this study, or on temperatures that caused chill coma. They also found that the temperature which caused 80% of unacclimated, summer-morph adults to die after a 1-h exposure was similar for females (-7.5 ± 0.1°C; mean ± SEM) and males (-7.2 ± 0.1°C). Females survived longer than males when exposed to a constant 0°C, but the difference was <24 h (Jakobs et al. 2014). Zerulla et al. (2015) suggested that winter-morph females are more cold hardy than males because they may enter a reproductive diapause in the fall and are more frequently found in the spring, but our results show that winter-morph females are not more likely than winter-morph males to survive brief shocks to cold.

Understanding the supercooling point and lower lethal temperatures of invasive pests can help refine risk assessments and control recommendations and may even lead to new management approaches, such as temperature-based treatment of fruit. The current

research complements existing population models for *D. suzukii* (e.g., Wiman et al. 2014; Spotted wing drosophila forecasts through Western Specialty Crops IPM-PIPE at http://uspest.org/swd/, last accessed 13 August 2015). While these models typically characterize the effects of temperatures during the growing season on population growth and structure of *D. suzukii*, our results focus on the acute effects of exposure to subzero temperatures. Pairing this information with low winter temperature records can be used to estimate overwintering survivorship of adults under the key assumptions that adults in the field have the same physiological condition as in our study and are exposed to those temperatures. Such results should be interpreted as minimum estimates of mortality (i.e., mortality should be *at least as much* as indicated by our results). Additional mortality may accrue from chronic exposures to cold or other winter-related mortality factors such as starvation or desiccation (Jakobs et al. 2015). Nevertheless, Andersen et al. (2015) found that the LT_{50} was one of the best indicators of the ability of *Drosophila* spp. to survive at different latitudes, average annual minimum temperatures, and coldest annual minimum temperatures.

Ultimately, our results indicate that both summer and winter-morph adult *D. suzukii* should not be able to successfully overwinter in cold climates without a way to escape the cold. *D. suzukii* was detected in Minnesota in 2012 and each year through 2015. In Minnesota, the first *D. suzukii* adults of 2014 were trapped from late June-early July and only appeared to be summer morphs; male and female winter morphs were captured in mid-October 2014 (E. C. Burkness, personal communication). These preliminary observations are consistent with the notion that winter morphs of *D. suzukii* are produced, but are generally unable to overwinter, in the state. The current field observations are not sufficient to reach any firm conclusions. Future research should compare winter temperatures and trap counts and examine winter indoor activity of *D. suzukii.*

Our results confirm a significant difference in cold tolerance of summer and winter-morphs of *D. suzukii*, suggesting that future ecologically relevant research on *D. suzukii* cold tolerance should focus on winter-morphs. The arrival of *D. suzukii* in poleward regions may be facilitated by other means and our results highlight the need to improve our understanding of the movement patterns and mechanisms of dispersal of *D. suzukii.* If the main form of dispersal is through fruit imports, it may be prudent to limit or monitor fruit imports from infested regions to areas where *D. suzukii* is not able to establish. Passive long-distance dispersal by wind, specifically low-level jets, might facilitate annual immigration into northern states from southern states. In addition, future research should focus on the ability of *D. suzukii* winter-morph adults to acclimate to colder temperatures. If *D. suzukii* winter-morph adults are able to acclimate to cold temperatures as summer-morph adults are (Jakobs et al. 2015), it is possible that they may be able to survive some winter cold shocks.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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References Cited

Ayrinhac, A., V. Debat, P. Gibert, A. G. Kister, H. Legout, B. Moreteau, R. Vergilino, and J. R. David. 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Funct. Ecol. 18: 700–706.

Andersen, J. L., T. Manenti, J. G. Sørensen, H. A. MacMillan, V. Loeschcke, and J. Overgaard. 2015. How to assess *Drosophila* cold tolerance: chill coma temperate and lower lethal temperature are the best predictors of cold distribution limits. Funct. Ecol. 29: 55–65.

Box, G.E.P., and D. R. Cox. 1964. An analysis of transformations. J. R. Stat. Soc. B Met. 26: 211–252.

Burrack, H. J., G. E. Fernandez, T. Spivey, and D. A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera:Drosophilidae), an invasive frugivore. Pest Manag. Sci. 69: 1173–1180.

Carrillo, M. A., N. Kaliyan, C. A. Cannon, R. V. Morey, and W. F. Wilcke. 2004. A simple method to adjust cooling rates for supercooling point determination. CryoLetters 25: 155–160.

Cini, A., C. Ioriatti, and G. Anfora. 2014. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insectol. 65: 149–160.

Czajka, M., and R. Lee. 1990. A rapid cold-hardening response protecting against cold shock injury in *Drosophila melanogaster*. J. Exp. Biol. 148: 245–254.

Dalton, D. T., V. M. Walton, P. W. Shearer, D. B. Walsh, J. Caprile, and R. Isaacs. 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manag. Sci. 67: 1368–1374.

Damus, M. 2009. Some preliminary results from CLIMEX and MAXENT distribution modelling of *Drosophila suzukii*. Version 2. CIFA Plant Health Assessment, Ottawa, Canada.

Denlinger, D. L., and R. E. Lee. 2010. Low temperature biology of insects. Cambridge University Press, Cambridge, United Kingdom.

Commonwealth of Australia 2013. Department of Agriculture, Fisheries and Forestry Biosecurity. Final pest risk analysis report for *Drosophila suzukii*. Commonwealth of Australia, Canberra, Australia.

Deprá, M., J. Poppe, H. Schmitz, D. De Toni, and V. S. Valente. 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest Sci. 87: 379–383.

Emiljanowicz, L. 2014. Life history and overwintering survival of *Drosophila suzukii* (Diptera: Drosophilidae). M.S. thesis, The University of Guelph, Guelph.

Gibert, J. M., F. Peronnet, and C. Schlotterer. 2007. Phenotypic plasticity in *Drosophila* pigmentation caused by temperature sensitivity of a chromatin regulator network. PLoS Genet. 3: 266–280.

Goodhue, R. E., M. Bolda, D. Farnsworth, J. C. Williams, and F. G. Zalom. 2011. Spotted wing drosophila infestation of California strawberries and raspberries: Economic analysis of potential revenue losses and control costs. Pest Manag. Sci. 67: 1396–1402.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag. Sci. 67: 1352–1357.

Healy, M.J.R. 1952. A table of Abbott's correction for natural mortality. Ann. Appl. Biol. 39: 2.

Hoffman, A. A., J. G. Sorenson, and V. Loeschcke. 2003. Adaption of *Drosophila* to temperature extremes: Bringing together quantitative and molecular approaches. J. Therm. Biol. 28: 175–216.

Izquierdo, J. I. 1991. How does *Drosophila melanogaster* overwinter? Entomol. Exp. Appl. 59: 51–58.

Jakobs, R., T. D. Gariepy, and B. J. Sinclair. 2015. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. J. Insect Physiol. 79: 1–9.

Kanzawa, T. 1936. Studies on *Drosophila suzukii* Mats. J. Plant Prot. 23: 66–191.

Kimura, M. T. 1988. Adaptations to temperate climates and evolution of overwintering strategies in the *Drosophila melanogaster* species group. Evolution 42: 1288–1297.

Kimura, M. T. 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia 140: 442–449.

Kostal, V., P. Simek, H. Zahradnickova, J. Cimlova, and T. Stetina. 2012. Conversion of the chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze tolerant organism. Proc. Natl. Acad. Sci. USA 109: 3270–3274.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Manag. Sci. 67: 1358–1367.

Lee, J. C., A. J. Dreves, A. M. Cave, S. Kawai, R. Isaacs, J. C. Millar, S. Van Timmeren, and D. J. Bruck. 2015. Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. 108: 117–129.

Manchester, S. J., and J. M. Bullock. 2000. The impacts of non-native species on UK biodiversity and the effectiveness of control. J. Appl. Ecol. 37: 845–864.

Mitsui, H., K. Beppu, and M. T. Kimura. 2010. Seasonal life cycles and resource uses of flower and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13: 60–67.

Morey, A. C., W. D. Hutchison, R. C. Venette, and E. C. Burkness. 2012. Cold hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. Environ. Entomol. 41: 172–179.

(NAPIS) National Agricultural Pest Information System. 2014. Purdue University. "Survey Status of Spotted Wing Drosophila - *Drosophila suzukii* (All Years)." Published: 02/24/2015. (http://pest.ceris.purdue.edu/map.php?code=IOAPAUA&year=alltime) (accessed 26 February 2015).

O'Doherty, R., and J. S. Bale. 1985. Factors affecting the cold hardiness of the peach-potato aphid *Myzus persicae*. Ann. Appl. Biol. 106: 219–228.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50: 53–65.

Shimada, K., and A. Riihimaa. 1988. Cold-acclimation, inoculative freezing and slow cooling – essential factors contributing to the freezing tolerance in diapausing larvae of *Chymomyza costata* (Diptera, Drosophilidae). CryoLetters 9: 5–10.

Sømme, L. 1996. The effect of prolonged exposures at low temperatures in insects. CryoLetters 17: 341–346.

Strachan, L. A., H. E. Tarnowski-Garner, K. E. Marshall, and B. J. Sinclair. 2011. The evolution of cold tolerance in *Drosophila* larvae. Physiol. Biochem. Zool. 84: 43–53.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. 43: 501–510.

Uchino, K. 2005. Distribution and seasonal occurrence of cherry drosophila *Drosophila suzukii* (Diptera:Drosophilidae) injurious to blueberry in Chiba Prefecture. Ann. Rep. Kanto-Tosan Plant Prot. Soc. 52: 95–97.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, S. D. O'Neal and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. Int. J. Pest Manag. 2: G1–G7.

Wiman, N. G., V. W. Walton, D. T. Dalton, G. Anfora, H. J. Burrack, J. C. Chiu, K. M. Daane, A. Grassi, B. Miller, S. Tochen, et al. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLOS One 9: e106090. (doi:10.1371/journal.pone.0106909).

Zerulla, F. N., S. Schmidt, M. Streitberger, C.P.W. Zebitz, and R. Zelger. 2015. On the overwintering ability of *Drosophila suzukii*. J. Berry Res. 5: 41–48. (doi:10.3233/JBR-150089).

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