Research Article

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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

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Abstract

BACKGROUND: *Drosophila suzukii* was first found in Oregon in August 2009. The threat of this pest to regional small and stone fruit production industries led to investigations on its overwintering capabilities in fruit-growing regions in the Pacific Northwest. Knowledge of its cold tolerance will help in the development of computer models to forecast seasonal population growth and decline.

RESULTS: Of 1500 adults or pupae, 22 (1.4%) individuals survived the 84 day experimental chilling period. Most (86%) of the survivors were subjected to 10 °C temperature treatments. Survival decreased significantly at lower temperature treatments. Freezing temporarily increased the mortality rate but did not significantly affect overall mortality over the trial period. Flies that emerged from pupae are estimated to survive for up to 103–105 days at 10 °C and for shorter periods at lower temperatures. Field trapping in five fruit production areas has demonstrated overwintering survival in California and Oregon, but lower survival is predicted in Eastern Washington and Michigan.

CONCLUSION: The experiments reported here indicate that long-term survival of *D. suzukii* is unlikely at temperatures below 10 °C. Field data from five climatic regions indicated extended low initial *D. suzukii* field presence in 2010 in all regions except California, where field presence was recorded earlier.

Keywords: *Drosophila suzukii*; winter survival; Pacific Northwest; berry pest insect; stone fruit; small fruit

1 INTRODUCTION

Drosophila suzukii Matsumura (Diptera: Drosophilidae) was discovered along transportation corridors in Pacific fruit production areas during 2009,[1] marking one of the first recordings of the species outside Asia. Mitsui *et al*.[2] reported high fecundity of *D. suzukii* on wild Japanese fruits that are closely related to American cultivated berry species. In California, crop damage caused by *D. suzukii* in 2008 was locally severe. Infestation and damage by *D. suzukii* was found in small and stone fruits as far north as British Columbia during 2009.[1]

Thermal tolerance is a range-limiting factor for *Drosophila* species,[3] and several mechanisms have been proposed to explain resistance to extreme hot or cold temperatures. The gradual process of acclimation may prolong survival of *Drosophila* when flies are later subjected to temperature extremes.[4–6] A similar effect of cold hardening for short time periods at near-lethal temperatures has been observed.[7] Additionally, behavioral modification may shift the timing of reproduction to occur after extreme temperature conditions have lifted.[8]

Latitudinal gradients provide range limits for *Drosophila* species.[9] In a study of Japanese drosophilids, species were divided into groups based on their geographic distributions. Generally, species sharing a northern range limit exhibited higher cold tolerance compared with species with further southern

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boundaries. A notable exception was in the case of *D. suzukii*, which had one of the broadest latitudinal ranges reported in the study. This species was less cold tolerant than most species that shared a range boundary north of Sapporo (43° 6′ N). To overcome deficiencies in cold tolerance, it is possible that *D. suzukii* may be behaviorally adapted to overwinter in man-made protected habitats.[9]

Climatic regimes in the Pacific Coast region of North America range from year-round mild temperatures in maritime areas to more severe extended winter cold periods in inland areas such as the Columbia River Gorge of Oregon and the Yakima Valley of Washington. The objective of the present study was to determine basic information on survivorship of *D. suzukii* under controlled long-term exposure to temperatures commonly experienced during the winter in North American Pacific production regions. A climatic comparison is also made between fruit-growing areas of Pacific Coast states and southwestern Michigan, where *D. suzukii* has been recently detected,[10] with emphasis on how seasonal climate differences may affect incidence of *D. suzukii* in other major fruit production regions. This study provides baseline data on minimum temperature requirements for survival as a first step in assessment of North American climates where *D. suzukii* will persist.

2 MATERIALS AND METHODS

In the present study, the survival of both early and late emerging adults was tracked by subjecting emerged adult individuals as well as late final-instar pupae to different temperature regimes within environmentally controlled temperature cabinets. The objectives were to determine the survival of *D. suzukii* adults and pupae under two general scenarios. The first scenario simulated constant mild overwintering temperatures. The second scenario represented mild temperatures with an intense cold interval designed to mimic a winter freeze event. Experiments were initiated on 14 December 2009 and continued until 8 March 2010.

Rearing containers for experimental units consisted of 163 mL plastic soufflé cups (Solo®, Urbana, IL) equipped with a moistened filter paper strip (Whatman International, Ltd, Maidstone, UK) along the interior cup wall, and a cotton square (US Cotton, LLC, Rio Rancho, NM) with a surface area of 4 cm^2. The filter paper provided texture to the vertical surface of the cup and, along with the cotton square, provided a water source for the insects. An insect diet medium was added to the cup as an artificial food source. Flies were secured in cups with tight-fitting lids pierced to provide gas exchange between the cups and the temperature cabinets.

Drosophila suzukii adults and pupae were provided by the Horticultural Crops Research Laboratory (HCRL), the United States Department of Agriculture – Agricultural Research Service, Corvallis, Oregon. Laboratory cultures of *D. suzukii* were obtained and maintained from weekly field collections made in the Willamette Valley and Columbia River Gorge of Oregon from 20 August to 31 October 2009. Individuals were reared at HCRL at 25 °C and subsequently acclimated to 10 °C in temperature cabinets (Model E-30BHO; Percival Scientific, Perry, IA) by decreasing chamber temperature by 2.5 °C every 2 days. The total period of acclimation was 12 days for all life stages. Pupae and adults of *D. suzukii* were approximately 7 and 12 days old, respectively, when trials were started, and these ages were incorporated in the statistical analysis. Acclimated individuals were divided into treatment groups of 1, 3, 5, 7 and 10 °C. Each cup initially contained either five adults or five pupae. Each temperature treatment was represented by 30 labeled cups containing adults and 30 labeled cups containing pupae.

Most cups of adults and pupae were subjected to their designated temperature treatments for a 6 week period. A subset of ten predetermined cups from each adult treatment and ten predetermined cups from each pupal treatment was placed in a dark walk-in cold room (−2 °C) for 7 days beginning on day 18 of the experiment. Forty-two days following the placement of insects at initial treatment temperatures, all units were placed at 10 °C for an additional 42 days. The total experimental period was 84 days.

Light levels and humidity were controlled within the temperature cabinets. For the first 6 week period, the photoperiod was maintained to provide 8 h of light and 16 h of darkness on a diurnal cycle. For the second 6 week period, the photoperiod was adjusted to 12 h of light and 12 h of darkness. Relative humidity was set to 80%, mimicking the 5 year mean winter humidity levels in Corvallis, Oregon, from 2004 to 2009,[11] although at 1 and 3 °C the growth chambers had difficulty maintaining high humidity levels.

Cups were observed at 21 °C for periods of no more than 5 min per observation at least twice per week for the duration of the study. Intact pupae, healthy adults and dead adults were recorded for each cup. Adults were observed for motion or feeding to determine whether they were alive or dead. Apparently dead flies were left in the cups for several observation periods to confirm that they were actually dead. Partially emerged pupae were counted as dead adults. Cups, diet, filter paper strips and cotton squares were replaced when they had become visibly contaminated with fungi and bacteria. The filter paper and cotton squares were periodically rehydrated with deionized water to prevent desiccation of *D. suzukii* adults and pupae.

Solid food diet for *D. suzukii* adults was provided by HCRL. Agar (45 g), cornmeal (125 g), sugar (200 g) and nutritional yeast (70 g) were combined with 1 L of dH_2O at room temperature. The slurry was added to 2.8 L of boiling dH_2O, and the resulting mixture was cooked at a gentle boil for 15 min under continual agitation. The mixture was cooled to 63 °C, at which point propionic acid (17.7 mL at 1 M concentration) and ethanol (33.3 mL at 95% purity) were added. The diet mixture was then poured into sterile petri dishes and vials, sealed with Parafilm (Pechiney Plastic Packaging, Inc., Chicago, IL), stored at 4 °C and used as needed. Diet was replaced in the treatment cups when it showed signs of dryness or microbial degradation. Cotton swabs were rehydrated with dH_2O as necessary to maintain sufficient moisture in the cups to prevent desiccation. Swabs were replaced upon visual evidence of microbial contamination.

One-way analysis of variance (ANOVA) was performed on data of the proportions of *D. suzukii* surviving for 84 days in order to compare differences among treatments. Using this approach, the authors compared the long-term survivability of *D. suzukii* young adults and final-instar pupae as treatments, compared freeze and no-freeze treatments and contrasted fly longevity at five temperature treatments. Factorial ANOVA of the treatments – $\text{pupae}_{\text{no freeze}}$, $\text{pupae}_{\text{freeze}}$, $\text{adults}_{\text{no freeze}}$ and $\text{adults}_{\text{freeze}}$ – was performed in order to determine differences among these treatments for the effects of temperature. Additional simple multiple regression analysis was performed in order to estimate potential survival using mean daily adult survival figures over the duration of the experiment: survival of adults with no exposure to freeze ($\text{adults}_{\text{no freeze}}$); survival of adults with exposure to freeze ($\text{adults}_{\text{freeze}}$); survival of pupae with no exposure to freeze (pupae$_{no\ freeze}$); survival of pupae with exposure to freeze (pupae$_{freeze}$). All statistical analyses were performed using Statistica 7.1 (StatSoft Inc., Tulsa, OK).

Seasonal weekly catches of *D. suzukii* in monitoring traps were recorded in five fruit-producing regions of the United States. These pooled fly counts were from: unsprayed cherry orchards in the Northern San Joaquin Valley, California (37° 44′ N, 12 traps total); Himalayan blackberry (*Rubus armeniacus*) stands in Marion County, Oregon (44° 43′ N, 50 traps total); cherry orchards and Himalayan blackberries in Wasco County, Oregon (45° 46′ N, 70 traps total); unsprayed riparian areas dominated by Himalayan blackberry in Benton County, Washington (46° 12′ N, 15 traps total); highbush blueberries in Allegan County, MI (42° 44′ N, 165 traps total). Traps were made of clear or white ca 1 L plastic cups, and each trap had 10–16 entrance holes of 4.5–9.5 mm diameter. Traps were baited with 100–150 mL of natural apple cider vinegar and 0.25–3 mL of unscented liquid soap to break water surface tension. Traps in Michigan did not contain liquid soap, but contained a small yellow sticky card. The bait was replaced weekly. All traps were hung near the fruiting zone or on a stable surface in a cool shaded area. Contents were filtered once a week into a white pan and examined with a jeweler's loupe (30×) or examined in the laboratory under a dissecting microscope for accurate fly identification. Contents from Michigan and California traps were sorted in the laboratory. All fly counts from traps were transformed to display mean weekly *D. suzukii* per trap for each of the regions. The sex ratio of captured individuals was recorded during the first 2 weeks of successful *D. suzukii* captures in order to highlight the population structure of the potential overwintering population. Mean daily temperatures from local public weather stations for 2010 are presented in conjunction with counts from monitoring traps for each region.

3 RESULTS

In all life stage and freeze exposure treatments, individual *D. suzukii* survived for the longest periods at 10 °C. Survival periods decreased at all temperatures below 10 °C. By day 17 at 1 °C, 100% mortality was observed in adults and pupae. At all life stages and temperatures except 10 °C, 99% mortality was observed° within 84 days. Five individuals survived temperature regimes

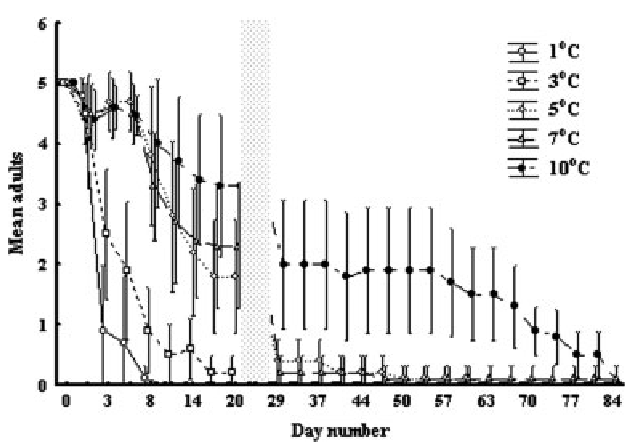

Figure 1. Mean weekly counts of *Drosophila suzukii* from monitoring traps and daily temperatures in °C during 2010 in five North American fruit production areas: (a) San Joaquin County, California; (b) Marion County, Oregon; (c) Wasco County, Oregon; (d) Benton County, Washington; (e) Allegan County, Michigan.

with a 7 day freeze period, compared with 17 surviving individuals under no-freeze conditions. One-way ANOVA comparisons of the number of young adults surviving in each treatment were performed after the 84 day experimental window had concluded. No differences in survival were found when comparing the life stages at which the experiments were initiated (young adult mean = 0.05 ± 0.02, N = 150; pupae mean = 0.09 ± 0.03, N = 150; $F_{1,298} = 1.27$; $P = 0.26$). No difference in survivorship was found when comparing the freeze (surviving adults = 0.05 ± 0.02, N = 200) and no-freeze (surviving adults = 0.09 ± 0.03, N = 100) treatments ($F_{1,298} = 0.85$; $P = 0.35$). There were more adult survivors at 10 °C (mean survivors = 0.32 ± 0.08; N = 60) compared with 3, 5 and 7 °C (mean survivors = 0.016 ± 0.016; N = 60), and no surviving adults at 1 °C ($F_{4,295} = 13.73$; $P < 0.001$). Of the surviving flies, 19 survived at 10 °C, with one fly surviving at each of the temperatures 3°, 5° and 7 °C. No pupae or adult flies survived the 1 °C temperature regimes. Compared with 21 surviving females, only one male (from the 10 °C treatment without exposure to a freeze) survived the 84 day trial period (Table 1).

3.1 10 °C adults$_{no\ freeze}$

Factorial analysis showed differences in the number of living adults alive from day 14 to day 70 when comparing 10 °C with the lower temperatures ($F_{104,2665} = 12.148$; $P < 0.001$) (Fig. 1). No significant differences were found when comparing the number of surviving adults from day 77 to day 84 in this trial. At all temperatures, adults gradually died with no freeze exposure over the 84 day period (Fig. 1). The simple regression function of adult survival on time at 10 °C is $y = 4.87 - 0.055x$ ($R^2 = 0.98$; df = 1, 25; $F = 843$; $P = 0.0001$). It is estimated that adults will survive for up to 85 days under conditions of constant 10 °C temperature. Adults at colder constant temperatures will survive for shorter periods. The majority of flies died within 8 days at 1 and 3 °C, and 20 days at 5 and 7 °C (Table 1). Mortality rates at these four temperatures decreased dramatically after 8 and 20 days.

3.2 10 °C adults$_{freeze}$

Factorial analysis displayed differences in the number of survivors from day 29 to day 70 when comparing 10 °C with the lower temperatures ($F_{104,1274} = 7.98$; $P < 0.001$) (Fig. 2). No significant differences were found for surviving adults from day 77 to day 84 in this trial. Mortality of adults held at 10 °C but including a 7 day freeze period displayed a linear relationship (Fig. 2). The simple regression function of adult survival on time at 10 °C with a freeze event is $y = 4.34 - 0.05x$ ($R^2 = 0.94$; df = 1, 25; $F = 394$; $P = 0.0001$). The estimate from this function is that adults will survive for up to 86 days at 10 °C even when subjected to a 7 day freeze period. Adults maintained at temperatures below 10 °C will survive shorter periods. The majority of flies died within 5 days at 1 and 3 °C, and within 20 days at 5 and 7 °C (Table 1). Mortality rates for all temperatures increased during the 7 day cold period, but decreased thereafter (Fig. 2).

3.3 10 °C pupae$_{no\ freeze}$

Factorial analysis indicated significant differences in the number of survivors in the pupae$_{no\ freeze}$ treatment from day 8 to day 84 when comparing 10 °C with the lower temperatures ($F_{104,2665} = 6.96$; $P < 0.001$) (Fig. 3). Pupae with no freeze exposure emerged during the first 20 days of the experiment as adult individuals (Fig. 3). Adult mortality at 10 °C after day 20 showed an approximate linear relationship. At 10 °C the simple regression function of

Table 1. Survival and sex of adult and pupal *Drosophila suzukii* with and without a week-long exposure to −2 °C, relative to the initial placement temperature in laboratory rearing chambers

Developmental stage and treatment	Temperature (°C)	Days to reach % mortality					Number of survivors at 84 days
		25%	50%	75%	99%	100%	♀:♂
Adults$_{no\ freeze}$	1	3	3	8	17	17	0
	3	3	3	8	80	–	1 : 0
	5	8	17	24	41	–	1 : 0
	7	5	14	29	60	63	0
	10	20	54	66	–	–	4 : 1
Adults$_{freeze}$	1	3	3	3	8	11	0
	3	3	3	8	29	29	0
	5	8	14	29	50	63	0
	7	8	14	29	84	–	1 : 0
	10	11	29	66	80	84	0
Pupae$_{no\ freeze}$	1	0	0	0	8	17	0
	3	0	0	0	8	8	0
	5	0	0	0	20	24	0
	7	0	0	0	34	57	0
	10	0	17	63	–	–	10 : 0
Pupae$_{freeze}$	1	0	0	0	8	8	0
	3	0	0	0	11	11	0
	5	0	0	0	29	29	0
	7	0	0	0	73	73	0
	10	0	8	44	–	–	4 : 0

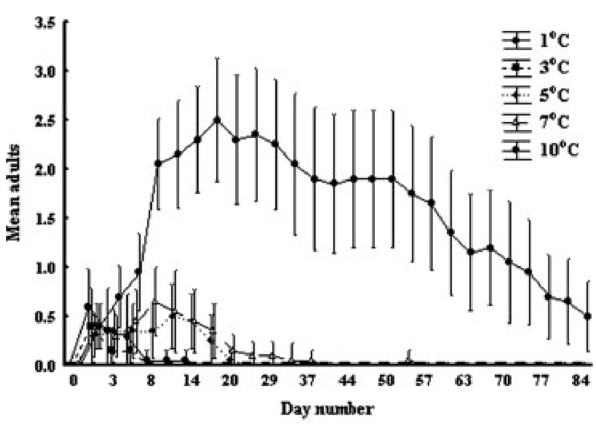

Figure 2. Mean adult *Drosophila suzukii* survival at five constant temperatures in separate growth chambers. Vertical bars indicate SEM and denote 95% confidence intervals.

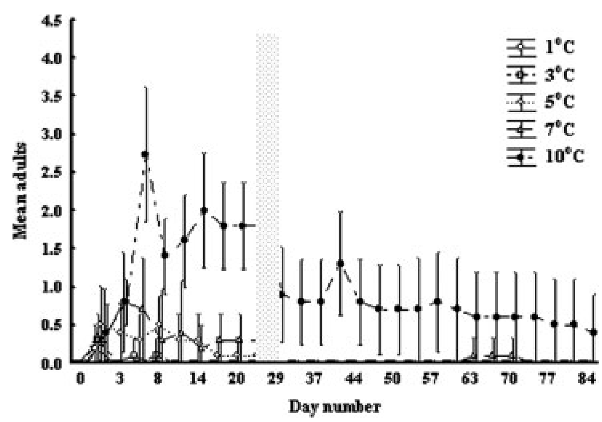

Figure 3. Mean adult *Drosophila suzukii* survival at five constant temperatures. The transparent bar indicates the period in which adults were subjected to a 7 day freeze period. Vertical bars indicate SEM and denote 95% confidence intervals.

pupae developing to adults and subsequent adult survival over time is $y = 3.11 - 0.029x$ ($R^2 = 0.94$; df = 1, 17; $F = 288$; $P = 0.0001$). It is estimated that adults emerging from pupae at 10 °C will survive for up to 105 days. Adults emerging from pupae at temperatures below 10 °C will survive for shorter periods. The majority of individuals likely died while in the pupal stage within 8 days at 1, 3, 5 and 7 °C. Mortality rates for these four temperatures remained high up to 37 days. All individuals died by day 60 at temperatures below 10 °C.

3.4 10 °C pupae$_{freeze}$

In the pupae$_{freeze}$ treatment, factorial analysis showed differences in the number of adult survivors from day 14 to day 50 when comparing 10 °C with the lower temperatures ($F_{104,1274} = 4.16$; $P < 0.001$) (Fig. 4). No significant differences were found for surviving adults from day 53 to day 84 in this trial. In the treatment where pupae were held at 10 °C and subjected to a 7 day freeze period, adults emerged during the first 18 days of the experiment preceding the onset of the freeze exposure (Fig. 4). No adult individuals emerged from pupae after exposure to freezing temperatures. Mortality of the emergent adults displayed an approximate linear relationship after day 20. The simple regression function on time of eclosed adult survival at 10 °C including freeze exposure is $y = 1.696 - 0.016x$ ($R^2 = 0.63$; df = 1, 17; $F = 484$; $P = 0.0003$). The estimate from this function is that adults will survive for up to 103 days at 10 °C, in spite of exposure to freezing conditions.

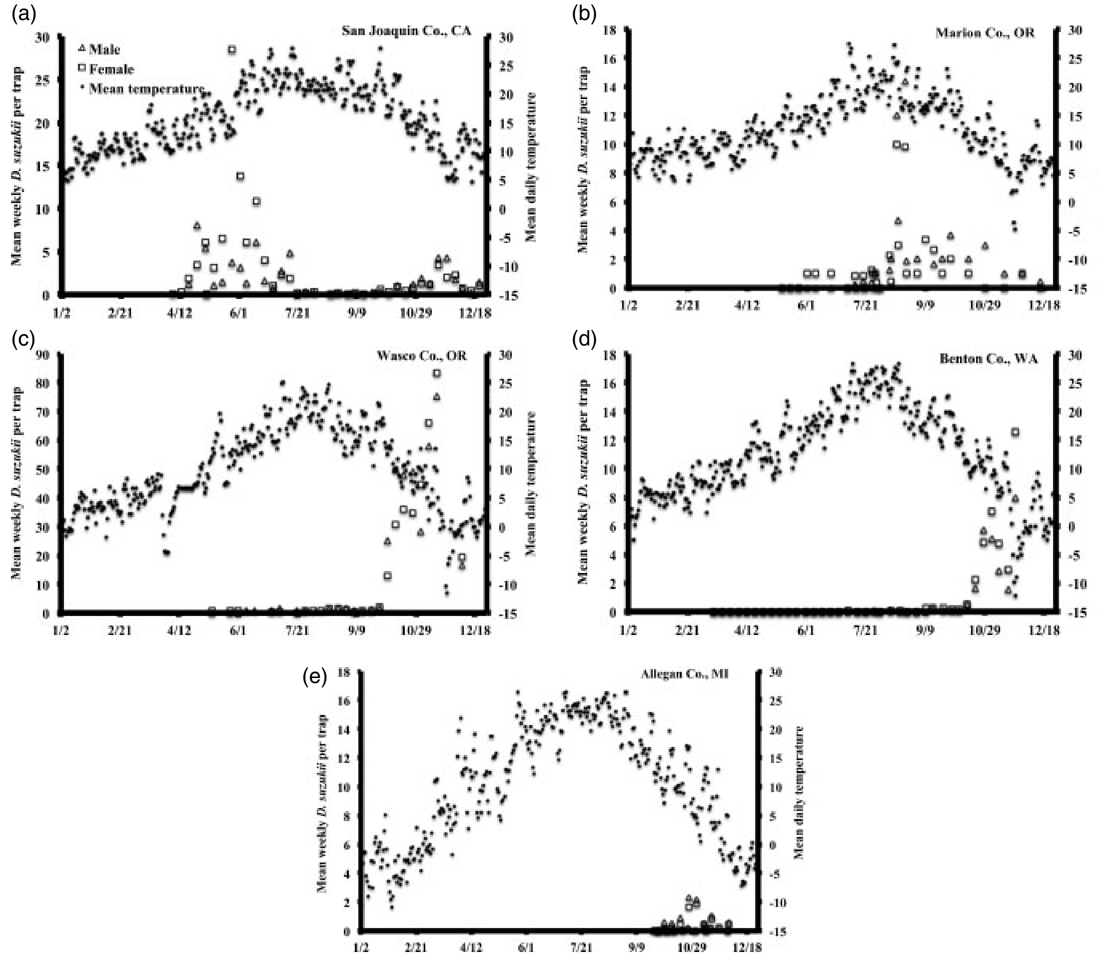

Figure 4. Mean *Drosophila suzukii* survival of adults started from the pupal stage at five constant temperatures. The number of adults initially increased as they emerged from the pupal stage, and then decreased owing to mortality. Vertical bars indicate SEM and denote 95% confidence intervals.

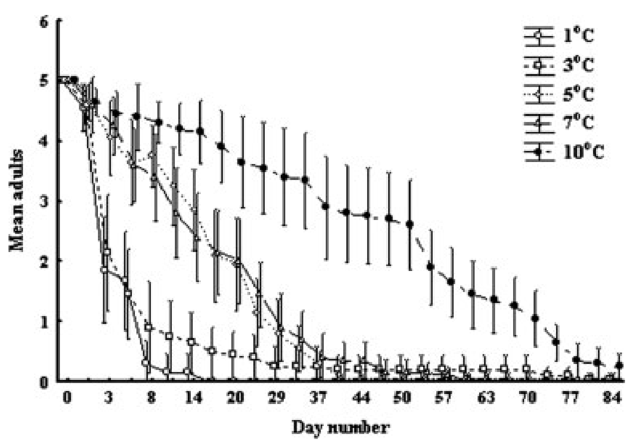

Figure 5. Mean *Drosophila suzukii* survival of adults started from the pupal stage at five constant temperatures. The number of adults initially increased as they eclosed, and then decreased owing to mortality. Adults were in addition subjected to a 7 day freeze period. This period is indicated by the transparent bar. Vertical bars indicate SEM and denote 95% confidence intervals.

3.5 Year 2010 seasonal capture in traps

In San Joaquin County, California (Fig 5a), mean temperatures preceding the first trapped adult *D. suzukii* ranged from 5 to 15 °C. From 1 January to the day when the first catches were made, there were no days with mean temperatures below freezing. The average daily mean temperature during this period was 10.6 °C. The first positive identification of *D. suzukii* in traps was made on 6 April, and numbers in traps increased to a maximum of eight males on 27 April and 28 females per trap on 26 May. During this period, the mean daily temperatures ranged from 10 to 19 °C and averaged 15.2 °C. The male : female sex ratio during the initial fly counts from 6 to 20 April was 1 : 2.1. The initial spring–summer flight dropped off to less than one fly per trap in late July. The mean daily temperatures throughout this entire initial flight period ranged from 9 to 25 °C and averaged 17.1 °C. Flight activity remained low from late July to mid-October. Mean temperatures during this low flight period ranged from 16 to 28 °C and averaged 20.4 °C. More active flight resumed on 13 October and peaked on 17 November, with an average capture of four males and three females per trap. The mean temperatures during this second peak period ranged from 9 to 23 °C and averaged 14.9 °C. After this period, *D. suzukii* numbers rapidly decreased to levels below one fly per trap on 15 December. Mean temperatures during this final period of population decrease ranged from 3 to 15 °C and averaged 9.8 °C.

In Marion County, Oregon (Fig 5b), the mean temperatures preceding the first observed capture in traps ranged from 3.9 to 18.6 °C. The first *D. suzukii* were trapped in Marion County monitoring traps from 1 to 14 June, with an observed male : female sex ratio of 0 : 1. There were no days with mean temperatures below freezing from 1 January to 1 June, and the overall mean temperature during this period was 9.9 °C. Monitoring trap fly counts increased to a maximum of 14.4 males per trap on 8 August. During this period, the mean temperatures ranged from 11.4 to 27.2 °C. After this initial population peak, *D. suzukii* numbers gradually decreased to levels below one fly per trap on 12 December 2010, with mean temperatures ranging from −4.7 to 27.2 °C during this period.

In Wasco County, Oregon (Fig 5c), the mean temperatures preceding the first observation of flies in traps ranged from −4.3 to 10.3 °C. The initial observed male : female sex ratio of flies captured in monitoring traps was 0 : 1. This period occurred from 11 to 24 May. There were 16 days from 1 January to 10 May with mean temperatures below freezing, and the mean overall temperature during this period was 4.5 °C. Fly counts in monitoring traps were very low until the end of summer. Increases in fly captures occurred from 26 September onwards and peaked at a maximum of 72 males and 83 females per trap on 17 November. During the period from 26 September to 16 November, the mean daily temperatures ranged from 1.3 to 21.1 °C. After the initial population peak, *D. suzukii* numbers decreased to levels of 16 and 19 male and female flies per trap, respectively, on 12 December, with mean temperatures ranging from −11.6 to 8.4 °C during the period from 17 November to 12 December.

In Benton County, Washington (Fig 5d), the mean temperatures from 1 January to the date of first observed *D. suzukii* in monitoring traps ranged from −2.6 to 28.3 °C. Only one day occurred with mean temperatures below freezing from 1 January to 7 July. The mean overall temperature during this period was 10.9 °C. The first capture in traps was made on 7 July 2010, and the male : female sex ratio during the period from 7 July to 24 July was 1 : 1. Numbers in the traps increased to a maximum of 12.6 males per trap on 24 November. During this period, the mean temperatures were 2.8–28.3 °C. On 24 November, temperatures reached −12.2 °C. Following the extreme temperature event, *D. suzukii* were no longer found in monitoring traps. The daily mean temperatures for this time period ranged from −12.2 to 9.3 °C.

In Allegan County, Michigan (Fig 5e), the mean temperatures preceding the first trap counts ranged from −10.8 to 26.3 °C. The male : female sex ratio during the initial trap counts from 24 September to 7 October was 1.75 : 1. From 1 January to the day when the first *D. suzukii* was trapped on 24 September, 57 days had mean temperatures below freezing, while the overall mean temperature during this period was 11.9 °C. Counts of *D. suzukii* increased to a maximum of 7.1 males and 5.85 females per trap on 26 October. During this period, the daily mean temperatures ranged from 11.4 to 27.2 °C. After this population peak, *D. suzukii* numbers gradually decreased to levels below 0.5 flies per trap on 1 December, when the mean temperature reached −2.6 °C. No flies were counted after this date, and the mean temperatures in December varied from −7.2 to 0.3 °C.

4 DISCUSSION

The present study provides evidence that acclimated adult *D. suzukii* can survive for up to 88 days at constant 10 °C, with no marked change in mortality when flies are subjected to a 7 day freeze period. Adult longevity is shown to decrease progressively at constant temperatures below 10 °C. When pupae are subjected to similar temperatures, emerged adults are predicted to live for up to 105 days at constant 10 °C and for 103 days at 10 °C including a 7 day freeze period at −2 °C. Shorter survival periods are expected at lower temperatures. Survival of pupae at all tested temperatures appears to be low.

Emerging adults may undergo reproductive diapause following eclosion at 10 °C, whereas acclimated adults may not undergo the same processes because they would have eclosed before exposure to cold temperatures. The triacylglycerol content of various *Drosophila* species has been shown to increase to a peak concentration for flies that emerge during mid-Autumn, suggesting that early or late eclosion inhibits sufficient production of TAG for long-term winter survival.[12] To the present authors' knowledge, accumulation of cryoprotectants in response to cold temperature exposure in *D. suzukii* is unknown. Understanding the mechanisms of acclimation will be helpful in determining possible climatic range limitations for *D. suzukii*.

In certain *Drosophila* species, male sterility is induced at 12 °C and below.[13] As all treatment temperatures apart from the acclimation period were below this temperature threshold for the entirety of the experiment, it is assumed that, during the present study, emerged male *D. suzukii* were rendered sterile and were unable to mate successfully with emerged females. No reproductive behavior, eggs or larvae were observed during the laboratory experiments. *Drosophila* species may show parthenogenesis,[14] but the proportion of viable eggs is unknown, and whether this is possible for *D. suzukii* is not known. In natural settings, females could mate before the arrival of cold temperatures in the fall and lay fertilized eggs the following spring.[4] The fact that mostly female individuals survived to the conclusion of the experiment may have significant implications during the following crop season in affected areas. In areas where male *D. suzukii* populations will die in the winter, pest management scouts will need to adapt early trapping efforts specifically to search for females.

This study indicates that very few individuals will survive the cool winter temperatures found in coastal production areas in the Pacific Northwest. Inland areas are subjected to more prolonged cold periods of higher intensity. For this reason, the present data suggest likely minimal survival in these areas. Mild winter temperatures and early-season captures suggest that *D. suzukii* has a higher winter survival rate in San Joaquin County, California, compared with the two areas in Oregon. The first *D. suzukii* captures in the two Oregon sites were recorded in May (Wasco County) and June (Marion County), more than 4 weeks later than those in California. In Washington the first *D. suzukii* were trapped in July. It is unclear whether these flies were survivors of eastern Washington winters. It is not understood what the significance of the sex ratio is to potential overwintering; however, more females were observed in early-season California and Oregon counts where the population most likely overwintered. In Michigan the first observations of *D. suzukii* in traps occurred in a 1 : 1 sex ratio and were made in late September, potentially indicating no survival of *D. suzukii* through the cold winter, followed by infestation by imported flies that arrived in Michigan berry-producing areas only towards the end of the 2010 growing season. However, the detection of this insect in thirteen Michigan counties during 2010 supports the earlier suggestion that winter survival in areas of extreme cold temperatures will rely on the ability of *D. suzukii* to overwinter in man-made habitats or other sheltered sites, while seasonal dispersal may allow populations to build to high numbers throughout the summer and fall.[2] Essential future studies on the overwintering capacity of *D. suzukii* include examinations of survival in protected environments with fluctuating temperatures, investigations of in-field survival in various overwintering climates, studies of female fecundity after prolonged overwintering periods and development of overwintering risk models in major US small and stone fruit production areas.

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