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# Comparing apples and oranges (and blueberries and grapes): fruit type affects development and cold-susceptibility of immature *Drosophila suzukii*

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## Abstract

*Drosophila suzukii* is a cosmopolitan polyphagous pest on unripe soft-skinned fruits. We sought to determine 1) temperature treatments that could be used to kill immature *D. suzukii* in fruit or packaging, and 2) whether development on different fruits led to differences in cold tolerance of immature *D. suzukii*. We reared animals from egg on a banana-based laboratory diet and diets made of apple, blueberry, cherry, grape, orange, raspberry, or strawberry homogenate in agar, and measured development time, adult body size, and cold tolerance. Diet type had complex effects on development time; in particular, flies reared on apple- or blueberry-based diets developed more slowly to a smaller adult body size than those on other diets. Cold exposure killed eggs and both first- and second-instar larvae. Survival of 24h at +4°C by feeding third-instar larvae was lowest in blueberry and cherry. Five days at +0.6°C killed all feeding third-instar larvae; this treatment is likely sufficient for targeting *D. suzukii* in fruit. Two hours at -5 or -6°C killed all wandering third-instar larvae and pupae; this exposure could be sufficient for sanitation of packaging.

## Introduction

*Drosophila suzukii* is a global economic pest of soft fruits (Asplen et al. 2015; Rota-Stabelli et al. 2013). Female *D. suzukii* lay eggs in healthy fruits and berries which are then damaged by larval feeding and associated necrosis (Rota-Stabelli et al. 2013). Both larvae and adults of *D. suzukii* are chill-susceptible, and are thus killed by low temperatures prior to any internal ice formation (Jakobs et al. 2017; Jakobs et al. 2015). Short exposures to sub-freezing temperatures (e.g. 1 h at -7.5 °C) are lethal to adults and larvae, as are prolonged exposures (i.e. one- to two-weeks) at 0 °C (Jakobs et al. 2017; Jakobs et al. 2015; Toxopeus et al. 2016). Eggs appear susceptible to cold, and are killed after 72 h at 1-2 °C (Aly et al. 2016; Kim et al. 2018). Most soft-skinned fruits, such as raspberries and strawberries, are refrigerated above 4 °C for transport, but can be stored at temperatures between 0 and 2 °C for days to weeks (Lidster et al. 1988). This post-harvest chilling may provide an opportunity to use cold exposure to control *D. suzukii* (also discussed by Aly et al. 2016; Kim et al. 2018). Such an approach is used for phytosanitation of apples that might contain *Rhagoletis pomonella* (Canadian Food Inspection Agency 2017), although the timing of this treatment (42 days at 0.6 °C or 90 days at 3.3 °C) would likely be inappropriate for the soft-skinned fruit and berries that *D. suzukii* infests. In addition, wandering larvae leave the fruit and pupate in packaging (a possible means of spread among fields; Asplen et al. 2015), thus, these life stages may be targeted by cold treatment of packaging.

Diet can modify insect thermal biology. For example, increased dietary cholesterol enhances cold tolerance of *Drosophila melanogaster*, probably by increasing cell membrane fluidity (Shreve et al. 2007), whereas increased dietary sugars create metabolic imbalances and

55 impair cold survival (Colinet et al. 2013). Insects reared on laboratory diets may have different
56 cold tolerance compared to those feeding on natural diets (Coudron et al. 2007), and the effects
57 of diet may hinge on differences in the microbial composition of the food (Colinet and Renault
58 2014). In *D. suzukii*, 72 h at 1.1 °C kills 97 % of third instar larvae and all eggs, and first and
59 second instar larvae in artificial cornmeal diet but only 61-98 % in raspberries and 76-100 % in
60 blueberries depending on the developmental stage tested (Aly et al. 2016). Although the physical
61 properties of fruits will differ, these differences among larvae reared on different fruits could be
62 because fruit-specific macro- or micro-nutrient profiles affect some aspect of metabolism, or
63 because they yield different gut microbiomes (Vacchini et al. 2017) that may affect cold
64 tolerance (Colinet and Renault 2014; Henry and Colinet 2018; Jiménez Padilla 2016). However,
65 if a *D. suzukii* larva or pupa is in a fruit shipment, we know (by definition) the fruit in which it
66 developed. Thus, any phytosanitary cold treatment can be adjusted for fruit-specific impacts on
67 cold tolerance once those have been isolated.

68 We had two objectives. First, to identify combinations of time and temperature that
69 reduce survival of immature stages of *D. suzukii*. Second, to determine whether cold tolerance of
70 these stages is affected by the composition of the larval diet. For larvae inside food (i.e. feeding
71 larvae), we chose temperatures above the freezing point of most fruits which would be suitable
72 for fruit storage and shipping. For life stages outside of food (i.e. wandering larvae and pupae),
73 we extended this to temperatures suitable for rapid treatment of pallets and storage containers.
74 We measured development time on the different diets to quantify the sublethal impacts of cold
75 exposure. To control for the physical effects of different fruits and potential microbiota, we
76 developed artificial diets based on soft-skinned fruits of economic importance (blueberries,

77 cherries, grapes, raspberries and strawberries), and compared these with the standard banana-
78 based laboratory diet we have used previously (Jakobs et al. 2017; Jakobs et al. 2015) and diets
79 based on apples and oranges, which are not within the normal host range of *D. suzukii*.

80

81 # Methods

82 *Animal rearing and collection*

83 We established a population of *D. suzukii* from flies collected in the Halton Hills region,
84 Ontario, Canada (43º00´N, 81º15´W) in 2012 (Jakobs et al. 2015). We reared the flies on generic
85 banana-based lab food (Markow and O'Grady 2005), at 25 ± 1 °C, 60 ± 5 % relative humidity,
86 and 14 h:10 h L:D cycle. We transferred 5-day old adult flies to small acrylic egg collection
87 cages (⌀ = 3.5 cm, 5.8 cm high) and maintained them on the banana medium supplemented with
88 inactive yeast for 3 d to stimulate oviposition. On the fourth day, the standard food was removed
89 and replaced with fruit-based media (100 mL deionized water, 25 g agar, 4 mL propionic acid,
90 and 500 g mashed organic frozen fruit – raspberry, blueberry, cherry, strawberry or apple, or 500
91 mL organic grape, or orange juice). The flies laid eggs overnight in the fruit-based media and we
92 transferred the eggs in the media to rearing cages (30 cm × 15 cm × 15 cm plastic boxes) until
93 the larvae reached the desired developmental stage.

94 To determine the effects of diet type on cold tolerance, we collected eggs, first- and
95 second-instar larvae, feeding third-instar larvae (the stage with the longest duration within the
96 food), wandering third-instar larvae (which have left the food), and early- and late-stage pupae.
97 We determined the age of larvae by observing the mandible structures and anterior spiracles of a

98 subset of larvae under a light microscope (Demerec 1965). Since the fruit-based food is darker
99 than standard lab food, the wandering larvae were easily identified by the lack of visible food in
100 their gut (Jakobs et al. 2017). We differentiated pharate from early pupae by the presence of eyes
101 and black wings visible through the puparium. We flooded the media plates with water – the
102 larvae crawl to the surface of the food and the pupae float. We collected the larvae and pupae
103 with a soft paintbrush, removed any food residue with tap water, and blotted them dry (cf. Jakobs
104 et al. 2017).

105

106 *Effects of low temperature exposure on D. suzukii third instar larvae and pupae.*

107 To determine the survival of third-instar feeding larvae, we placed ten larvae on top of 1
108 mL of media in a 2 mL microcentrifuge tube (five tubes/diet/temperature or time, three cohorts)
109 and allowed them 15-20 min to burrow into the food before cold exposure. We exposed the
110 remaining stages to cold in empty microcentrifuge tubes (five tubes/diet/temperature or time,
111 three cohorts), because wandering larvae and pupae are outside food. We placed the tubes into
112 aluminium blocks cooled by circulating methanol or ethylene glycol from a refrigerated bath
113 (Lauda Proline 3530, Lauda, Würzburg, Germany). We recorded the temperature via a 36-AWG
114 type-T thermocouple (Omega, Laval, Quebec, Canada) inserted into the food or touching the
115 larvae or pupae in the tubes without food. The thermocouples were connected to a computer by a
116 TC-08 interface running Picolog software (v5.24.2, Pico Technology, Cambridge, UK). After
117 exposure, all stages were transferred to 35 mL narrow *Drosophila* vials with 10 mL of the
118 appropriate food and returned to their rearing conditions. We monitored vials every 24 h until

eclosion ceased. We calculated development time as the time elapsed time from egg collection to eclosion and survival as the number of adults eclosed per vial. We determined the average fresh mass of all eclosed adults per sex for each treatment in the first cohort using a microbalance (MX5, Mettler Toledo, Columbus, OH, USA).

We exposed feeding larvae to 4 °C for one day to simulate the storage of fruits in a standard fridge or cooler for a day. We also exposed the larvae to 0.6 °C for two, four, or seven days based on Canadian government recommendations for optimal storage conditions of berries (Lidster et al. 1988; OMAFRA 2019). Additionally, we recorded larval survival after exposure to -1 °C for two or four days, which approximates the highest freezing points for strawberries and blackberries (-0.8 °C), raspberries (-1.1 °C), and blueberries (-1.3 °C) (Lidster et al. 1988; OMAFRA 2019). We exposed stages out of food (wandering larvae and pupae) to 4 °C for one or ten days, -4 °C and -5 °C for one or two hours, and -6 °C for 2 h.

We controlled for the effect of diet and handling on survival, development time, and mass by collecting feeding third instar and wandering larvae, as well as early and pharate pupae using the same technique as for the cold-treated individuals. We collected the larvae or pupae from flooded media plates with a soft brush, transferred them to fruit-media vials (five vials/cohort/stage, ten larvae or pupae per vial) and reared them under standard conditions and measured survival, development time and mass in the same manner as for the cold-exposed flies.

*Statistical analysis*

All analyses were performed in R v3.1.2 (R Development Core Team 2017) and preliminary data exploration was conducted according to Zuur et al. (2010). We used ANOVA to

compare the development time and mass of control flies, followed by Tukey's HSD *post-hoc* test. We compared survival of controls and cold-exposed flies, as well as development time following cold exposures using generalized linear models with a binomial distribution. We used analysis of deviance to determine the significance of the main effects in these models.

## Results

*Survival and development in different diets without cold exposure*

Fruit type had significant, but complex, effects on survival and development of immature *D. suzukii*. Fruit type significantly affected survival of *D. suzukii* removed from food without exposure to low temperatures (Table S1, Figure S1). Generally, development on food derived from blueberries, grapes, and raspberries decreased overall survival of *D. suzukii* compared to other fruit types (Figure S1). Diet also affected development time ($F_{7, 604} = 451.8$, $p < 0.001$; Figure 1): flies reached adulthood faster on standard (banana-based) laboratory food, but more slowly on blueberries and apples compared to other fruit types (Figure 1). Fresh mass of both males ($F_{7, 32} = 36.71$, $p < 0.001$; Figure 2A) and females (ANOVA; $F_{7, 32} = 52.72$, $p < 0.001$; Figure 2B) was dependent on food type – in general, flies reared in apple- and blueberry-based diets were smaller than the flies reared in other fruit types.

*Cold tolerance*

Cold exposure killed all eggs and first- and second-instar larvae. We exposed feeding third-instar larvae to above-zero temperatures simulating fruit storage conditions. More than half of larvae from all foods survived exposure to +4 °C for 24h; survival varied by diet (Table 1) and was

highest in larvae from the banana-based diet, and lowest in blueberry- and cherry-based diets (Figure 3A). However, when exposed to 0.6 °C, some feeding larvae from all diets survived after three days, 1-2 larvae from the banana, strawberry, orange, and cherry diets survived four days, and all larvae, irrespective of diet, were killed after five days (Figure 3B). A four-day exposure was sufficient to kill more than 90 % of feeding third-instar larvae at 0 °C, regardless of fruit (Figure S2), while 4 d at -1 °C killed all feeding third-instar larvae (see supplementary data sheet). Some feeding third-instar larvae developed dark melanised spots after exposure (Figure S3); none of the larvae that developed these dark spots successfully eclosed.

More than 50% of wandering larvae, early pupae, and pharate pupae survived a 24 h exposure to +4 °C (Figure 4), but none survived a ten-day exposure at this temperature (see supplementary data sheet. Survival did not vary among diets in wandering larvae, but was lower in blueberry-based diets than other diets (Figure 4). None of these life stages survived a five-day exposure to +0.6 °C (see supplementary data sheet).

Brief exposure to acute low temperatures caused significant mortality in post-feeding life stages but survival varied depending on diet prior to cold exposure (Table 1). We observed significant, but not complete, mortality in post-feeding life stages exposed to -4 and -5 °C for one hour (Figure S4). The effects of diet were variable among life stage and temperature, but post-feeding life stages raised on blueberry had consistently poorer survival of acute cold than other foods (Figures 5, S4). Longer exposures to subzero temperatures led to more significant mortality. Some individuals of all post-feeding life stages survived a 2 h exposure to -4 °C, and a few

pupae survived 2 h exposure to -5 °C (Figure 5); all post-feeding life stage individuals were killed by a 2 h exposure to -6 °C (see supplementary data sheet). Many third-instar wandering larvae that survived cold exposure accrued developmental abnormalities due to either incomplete pupation or malformation upon eclosion as adults (Figure S5); however, we did not quantify these effects.

*Development time following cold exposure*

Cold exposures that were less effective in reducing survival (i.e. +4 °C for 24 h, -4 °C for 1 h) did increase the development time of flies that survived long enough to eclose as adults (Figures 6 and 7; Table 2), in a diet-dependent manner. As for other treatments, rearing on apple- or blueberry-based diet had the greatest effect, slowing development more than the other foods (Figures 6 and 7). Flies reared on a banana-based diet developed fastest after a 24 h exposure to +4 °C, and if exposed to acute cold for 1 h as Wandering larvae (Figure 7), but pupae reared on apple- or cherry-based media performed best after an acute exposure of 1 h at -4 °C (Figure 7).

## Discussion

*Drosophila suzukii* is a polyphagous pest, and here we show that the cold-susceptibility of the immature stages depends on the fruit in which the animals are reared. Furthermore, flies reared on fruit-based diets often had slower development, smaller adult size, and reduced cold tolerance compared to those reared on our standard (banana-based) laboratory diet. From a control perspective, this is (partly) a positive finding: we found that performance was worse on some commercial fruits than on laboratory food, which implies that conclusions based on flies

reared on high-quality laboratory diet may be conservative. In particular, flies reared on blueberry-and cherry-based foods were particularly cold-susceptible; however, flies reared on strawberry and raspberry were comparably more cold-tolerant. By contrast, Aly et al. (2016) found that berry-reared immature stages had slightly higher cold tolerance than their counterparts reared on a cornmeal-based laboratory diet. The banana-based diet is particularly nutrient-rich, whereas cornmeal-based diets are less-so (Markow and O'Grady 2005). We expect that flies reared on cornmeal-based diets likely experience more (and different) nutrient stress compared to our fruit diets. We have observed poor performance of *D. suzukii* when reared in cornmeal-based diet (YJ-P, unpublished observations), and nutrient balance is important in overwintering of adult *D. suzukii* (Rendon et al. 2019). This among-diet variation in laboratory phenotype is increasingly acknowledged in *Drosophila* research (e.g. Ormerod et al. 2017; Rendon et al. 2019), and is important when extrapolating pest management decisions to new crops. We also included two non-host fruit diets, apples and oranges. While this is the archetypal inappropriate comparison, the effects of both of these (very different) fruits fell within the range of other fruits, suggesting that we are probably seeing close to the full range of expression of diet-related variation in phenotype in our experiments.

Our data indicate that although there is only limited mortality after a day at a typical refrigeration temperature (+4 °C), at +0.6 °C there is high mortality after three days, and complete mortality of immature life stages after four days. Thus, +0.6 °C, a temperature used for storage and transport of soft fruits (Lidster et al. 1988), appears to be an appropriate temperature to kill *D. suzukii* in fruit. While this is not useful for control (infested fruit are generally not marketable;

Rota-Stabelli et al. 2013), chilling for more than four days at +0.6 °C could be an appropriate treatment to maintain market access for shipments from known infested areas. Post-feeding life stages are more cold-tolerant, but brief (2 h) exposures led to complete mortality at -5 or -6 °C. We expect that such temperatures are readily and quickly attainable in commercial freezers, even with the buffering effect of packaging. Thus, cold treatment of packaging and pallets is a viable approach for preventing spread of *D. suzukii* among fields (Asplen et al. 2015).

We did not explore the physiological mechanisms underlying the effects of diet on *D. suzukii* performance. However, we speculate that there are likely nutritional sources of the variation we observed. The different fruit diets likely have very different nutritional properties. Bananas, oranges, and raspberries are relatively high in protein (Hulme 1972), which might enhance the melanisation response (Lee et al. 2008), and therefore repair and protection of tissues after cold exposure (see Sinclair et al. 2013 for discussion). Protein is also a source of proline and arginine, which have significant cryoprotective effects in *D. melanogaster* (Koštál et al. 2016; Koštál et al. 2012). However, high protein diets reduce lifespan and fecundity of winter morph *D. suzukii* (Rendon et al. 2019). Interactions with microbes may also mediate the effects of diet on cold tolerance. Fruit type can alter the gut microbiome (Martinez-Sañudo et al. 2018) and hence nutrient absorption and development (Bing et al. 2018). Because we used homogenised fruit and included propionic acid, it is possible that our fruit-based diets lacked beneficial fruit-specific microbes that might enhance performance in nature, or had microbe × fruit interactions that reduced performance. However, other work in our laboratory shows that flies reared on

propionic acid-containing diets (to reduce mould growth) still have a substantial gut microbiota, including yeasts (Jiménez-Padilla, Esan, Floate, and Sinclair, submitted).

We identify several possible caveats to our results. We prepared our diets using essentially homogenised fruit, which although nutritionally similar to fruit, lacks the physical structure of living fruit (Reeve 1956), or the interactions between the larva and the (living) host tissue (Corrado et al. 2012). The laboratory fruit diets probably also lack some components of the natural microbiota (discussed above). The flies that oviposited onto our fruit diets were raised on banana-based food, so there is a possibility that the larvae missed any maternal effects that would be present if their mothers were reared on the same fruit (cf. Matzkin et al. 2013). Finally, *Drosophila* larvae generally have considerable plasticity in cold tolerance (e.g. Jakobs et al. 2017; Rajamohan and Sinclair 2008; 2009), so it is quite likely that *D. suzukii* larvae reared in our fruit diets may have the capacity to improve their cold tolerance. However, we assume that larvae in commercial crops would not have been exposed to cold prior to harvest. We also reared our larvae under constant temperatures, and fluctuating temperatures can sometimes improve low temperature performance, even if they do not include significant cold spells. While it is important to consider these caveats when interpreting our results, most of the effects we describe yield effect sizes similar to those we observed, which suggests to us that the diet effects will remain a key determinant of cold tolerance in *D. suzukii* larvae.

*Conclusions*

*Drosophila suzukii* development rate, final body size, and cold tolerance are dependent on their diet. Nevertheless, immature *D. suzukii* are susceptible to cold. Feeding stages are all killed by more than four days' exposure to +0.6 °C, and post-feeding stages by a brief (c. 2 h) exposure to -5 or -6 °C. We suggest that the former would be an appropriate temperature regime for sanitising fruit from infested areas, and the latter is an achievable set of conditions for killing post-feeding stages in packaging.


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376

**Table 1.** Statistics for the main effect of diet type on survival of immature *Drosophila suzukii* exposed to a range of low temperatures for various durations. Results are from an analysis of deviance for a generalised linear model with binomial error distribution.

| Life stage | Temperature | Time (h) | df | $\chi^2$ | P |
|---|---|---|---|---|---|
| Feeding 3rd-instar | 4 ℃ | 24 | 7 | 36.8 | <0.001 |
| | 0 ℃ | 24 | 7 | 65.1 | <0.001 |
| | 0.6 ℃ | 24 | 7 | 26.4 | <0.001 |
| | -1 ℃ | 24 | 7 | 8.7 | 0.27 |
| Wandering 3rd-instar | 4 ℃ | 24 | 7 | 18.9 | <0.001 |
| | -4 ℃ | 1 | 7 | 39.4 | <0.001 |
| | | 2 | 7 | 12.4 | 0.09 |
| | -5 ℃ | 1 | 7 | 19.7 | <0.01 |
| | | 2 | 7 | 0 | 1 |
| Early pupae | 4 ℃ | 24 | 7 | 42.9 | <0.001 |
| | -4 ℃ | 1 | 7 | 198.4 | <0.001 |
| | | 2 | 7 | 82.1 | <0.001 |
| | -5 ℃ | 1 | 7 | 26.8 | <0.001 |
| | | 2 | 7 | 4.1 | 0.77 |
| Pharate pupae | 4 ℃ | 24 | 7 | 36.8 | <0.001 |
| | -4 ℃ | 1 | 7 | 135.62 | <0.001 |
| | | 2 | 7 | 54.6 | <0.001 |
| | -5 ℃ | 1 | 7 | 46.1 | <0.001 |
| | | 2 | 7 | 9.42 | 0.22 |

382 **Table 2.** Statistics for main effects of duration, temperature, and diet type on survival of
383 immature *Drosophila suzukii*. Results are from an analysis of deviance for a generalised linear
384 model with binomial error distribution.

| Life stage | Coefficient | df | $\chi^2$ | P |
|---|---|---|---|---|
| Feeding larvae | Days | 1 | 488.9 | <0.001 |
| | Fruit | 8 | 1090.7 | <0.001 |
| | Cold treatment | 5 | 431.7 | <0.001 |
| Wandering larvae | Days | 1 | 410.9 | <0.001 |
| | Fruit | 8 | 359.1 | <0.001 |
| | Cold treatment | 2 | 667.8 | <0.001 |
| Early pupae | Days | 1 | 398.7 | <0.001 |
| | Fruit | 8 | 385.7 | <0.001 |
| | Cold treatment | 2 | 463.1 | <0.001 |
| Pharate pupae | Days | 1 | 429.87 | <0.001 |
| | Fruit | 8 | 445.81 | <0.001 |
| | Cold treatment | 2 | 509.23 | <0.001 |

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## Figure Legends

**Figure 1.** Egg-adult development time of *Drosophila suzukii* reared on diets derived from different fruits. Note that the banana-based food was a standard laboratory food that included a range of additional ingredients. Boxes indicate the interquartile range, the error bars denote the minimum and maximum values, and we plot individual points that fell outside this range (n = 10 vials/diet and each vial contained 7-10 flies). Different letters signify statistically significant differences in development time among fruit types ($p < 0.05$, Tukey's *post-hoc* test; see Table S1 for statistics).

**Figure 2.** Fresh mass of (A) male and (B) female adult *Drosophila suzukii* reared on diets derived from different fruits. Note that the banana-based food was a standard laboratory food that included a range of other ingredients. Mean ± SEM shown (n = 5 vials/diet, vials contained 10-21 females and 12-29 males); different letters signify statistically significant differences in mass among fruit types ($p < 0.05$, Tukey's *post-hoc* test; see text for statistics).

**Figure 3.** Survival of exposure to +4 °C (A) and +0.6 °C (B) by feeding third-instar *Drosophila suzukii* larvae reared on diets derived from different fruits. Mean ± SEM shown (n = 15 vials/diet and each vial contained 10 flies).; different letters signify statistically significant differences in survival among fruit types ($p<0.05$, GLM with binomial error distribution test; see Table 1 for statistics). Data points on Day 3 are slightly offset to improve visibility of data.

**Figure 4.** Survival of 24 h at +4 °C by immature *Drosophila suzukii* reared on diets various fruit media. We measured survival as eclosion as adults. Mean ± SEM shown (n = 15 vials/diet and each vial contained 10 flies).; different letters signify statistically significant differences in survival among fruit types ($p<0.05$, GLM with binomial error distribution; see Table 1 for statistics).

**Figure 5 –** Survival of immature stages of *Drosophila suzukii* following acute cold exposure to -4 ℃ and -5 ℃ for 1 h. We measured survival as eclosion as adults. There was no survival of wandering larvae at -5 °C. Mean ± SEM shown (n = 15 vials/diet and each vial contained 10 flies).; different letters signify statistically significant differences in survival among fruit types ($p < 0.05$, GLM with binomial error distribution; see Table 1 for statistics).

**Figure 6.** Cumulative development of *Drosophila suzukii* third-instar larvae following cold exposure. We reared flies from egg to feeding larvae on one of eight diets derived from different fruits (apples, blueberries, cherries, grapes, oranges, raspberries, strawberries), or a banana-based control laboratory diet. We exposed feeding 3$^{rd}$-instar larvae to 4 °C for 24 hours or 0 °C for 48 hours and measured the number of days from egg to adult eclosion in surviving flies. Mean ± SEM shown (n = 5 vials/diet and each vial contained 10 flies); see Table S2 for statistics.

**Figure 7.** Cumulative development of post-feeding immature *Drosophila suzukii* following cold exposure. We reared flies from egg to wandering 3$^{rd}$ instar larvae, early pupae, and pharate pupae on one of eight diets derived from different fruits (apples, blueberries, cherries, grapes, oranges, raspberries, strawberries), or a banana-based control laboratory diet. We exposed flies to 4 °C for 24 hours 0 °C for 48 hours cold exposure and measured the number of days from egg to adult eclosion in surviving flies. Mean ± SEM shown (n = 5 vials/diet and each vial contained 10 flies); see Table S2 for statistics.