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ORIGINAL PAPER

Impact of floral feeding on adult *Drosophila suzukii* survival and nutrient status

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Abstract *Drosophila suzukii*, spotted wing drosophila, is a serious pest of small fruits and cherries in many regions of the world. While host usage has been well studied at the ovipositional and larval feeding stages, little is known about the feeding ecology and nutrient requirements of adults. This study addressed the impact of feeding on the survival and nutrient reserves of adult *D. suzukii* in laboratory assays. First, access to cherry blossoms increased survival rates of both adult males and females compared to water only. This suggests that these early spring blossoms may provide a food source for *D. suzukii* in fields that may be devoid of other food sources. Second, *D. suzukii* reared on a standard laboratory diet as larvae emerged as adults with minimal glycogen and sugar levels. Adults with continued access to a carbohydrate–protein diet showed rapidly elevated carbohydrate reserves, and adults with continued access to only water showed a decline in total sugars. Third, females with access to cherry or blueberry blossoms showed elevated carbohydrate reserves when compared to those with access to water only. These results illustrate the importance of adult feeding in enhancing survival and carbohydrate reserves among *D. suzukii*.

Keywords Glycogen · Nectar · Lipid · Sugar

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Key message

- Laboratory access to cherry blossoms improves the survivorship of male and female *Drosophila suzukii*.
- Adults emerge with limited glycogen and sugar reserves and feeding on nectar/sugar sources elevates their reserves within 1 day.
- Alternate food sources are important for surviving late dormant adult *D. suzukii*.
- Such food sources should be incorporated into pest management approaches especially when sugar sources are lacking in the environment.

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), also referred to as spotted wing drosophila, is a serious economic pest of small fruits and stone fruits in the Americas, Europe, and its native range of Asia (Asplen et al. 2015; Depra et al. 2014). Unlike most other *Drosophila*, *D. suzukii* females have a specialized ovipositor that enables them to oviposit into intact fruit (Atallah et al. 2014), and adults are attracted to odors associated with fruit ripening in addition to fermentation volatiles (Keesey et al. 2015). Given the importance of *D. suzukii*, recent studies have focused on host usage, which includes oviposition by adult females and subsequent development of feeding larvae as reviewed by Hamby et al. (2016). Host usage by *D. suzukii* has been investigated among many ornamental and wild fruits (Lee et al. 2015b; Poyet et al. 2015; Kenis et al. 2016), and among fruits of varying °Brix, pH, and penetration force of skin (Burrack et al. 2013; Ioriatti et al. 2015; Kinjo et al. 2013; Lee et al. 2011,

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2015a). *D. suzukii* larvae take longer to develop and experience lower survivorship on diets containing low protein and carbohydrate levels, particularly at high larval densities (Hardin et al. 2015). While host usage has been studied during other stages, the feeding ecology of adult *D. suzukii* has been relatively unexamined. Little information is available on the role of food sources on adult *D. suzukii* during periods of low fruit availability, the optimal range of energetic requirements, and impacts of feeding on longevity, reproduction, and dispersal.

Knowledge of adult nutrient requirements and feeding ecology can improve management of *D. suzukii*. First, host usage information enables prediction of the landscape patterns of the pest. Using a mark-capture technique (Klick et al. 2014), marked *D. suzukii* adults have been observed to move from wild blackberry and cherry host borders and into the crop field (Klick et al. 2016). By identifying such adult food sources, we can further estimate the range of dispersal of *D. suzukii* as affected by improved nutrient levels obtained from these resources. Next, the adult food sources can be incorporated to enhance current control methods. Second, for instance, adding sugar to an insecticidal spray reduced *D. suzukii* larval infestation of strawberries by more than 50 % when compared to without sugar (Cowles et al. 2015). The food source likely acts as a phago-stimulant and increases the efficacy of contact-acting insecticides. Third, adult food sources may be limited in spring and fall when crop fruit is not available. In this case, food sources might be incorporated into 'attract and kill' systems to maximize efficacy. Fourth, if a food source is identified in a landscape devoid of other similar resources, then control strategies should be focused at the food source.

Current knowledge of adult feeding by *D. suzukii* includes feeding on (1) host fruits based on crop-sugar analysis (Watabe et al. 2010) and observation (Kanzawa 1939), (2) sap of wounded oak trees based on observation (Kanzawa 1939), and (3) yeasts as suggested by its presence in adult midguts and also host fruit (Hamby et al. 2012). Information on important diet components may be obtained by examining diet recipes used to rear *D. suzukii* larvae and feed adults. Current diet used for *D. suzukii* includes both carbohydrate and protein sources (Hardin et al. 2015; Dalton et al. 2011; Kinjo et al. 2014; Woltz et al. 2015).

Insight on the potential feeding requirements and physiological responses of *D. suzukii* are obtained from studies of *Drosophila melanogaster* Meigen. Yeast (protein) and sugar provide a carbon source for *D. melanogaster* egg production with roughly half of the carbon coming from each food type and with egg sugars primarily derived from adult feeding as the female ages (Min et al. 2006). A diet devoid of protein suppresses egg production in *D. melanogaster* (Lee 2015), and similar diets have been used to produce *D. suzukii* with low egg loads (A. Wallingford and G. Loeb, unpub. data). Adult feeding and optimal nutrient levels are essential for increased *D. melanogaster* longevity (Min et al. 2006). Here, sugars provide ~40 % of somatic carbon of adult *D. melanogaster*, of which 75 % were obtained from adult feeding. For *D. melanogaster*, the protein-to-carbohydrate ratio is critical (Lee 2015) as a diet too high in sugar during both the larval and adult stages can decrease cold tolerance and metabolic balance of adults (Colinet et al. 2013). Nevertheless, adult *D. melanogaster* can adjust its intake of sucrose (Edgecomb et al. 1994) and yeast (Lebreton et al. 2014) depending on nutritional status. This adjustment in behavior can increase the likelihood of maintaining optimal nutrient balance.

We initiated studies on the feeding ecology of *D. suzukii* with three objectives in mind. The first objective was to determine how access to cherry blossoms present in the early spring impacted survivorship of *D. suzukii*. The early spring period is likely devoid of food sources, and only a few wild or ornamental fruits are used by *D. suzukii* for oviposition and development during April–May (Lee et al. 2015b). The second objective was to establish baseline parameters by measuring the lipid, glycogen and sugar levels of *D. suzukii* at emergence, and with or without access to typical sugar–protein food shortly after emergence. Knowing the nutrient reserves of adult life stages at emergence and after feeding or starvation will elucidate adult feeding requirements. The third objective was to compare nutrient reserves of *D. suzukii* with or without access to cherry or blueberry blossoms.

Materials and methods

Drosophila suzukii adults were either from stock colonies established at Oregon State University in 2009 (Dalton et al. 2011) or at the USDA ARS Horticultural Crops Research Unit in 2011 (Woltz et al. 2015). Different stock colonies were used depending on the location of the trials. Measuring responses with two stock colonies provided additional support for observed trends. Stock colonies were supplemented annually with field-collected flies and maintained in large rearing containers at 22 °C, 60–65 % RH, 16:8 L:D photoperiod, and fed an artificial diet as described in Dalton et al. (2011) and Woltz et al. (2015) for the respective colonies. Flies from the Oregon State University colony were used in the survivorship and floral feeding nutrient assays. Flies from the USDA colony were used in the baseline nutrient assays. Adult *D. suzukii* used in assays were collected from emergence cages holding diet plates containing the pupal stage, this is further described for each section. While we considered

transferring pupae from diet dishes into sterile vials, we have observed very low emergence rates with this process (J.C.L. personal obs.).

Survivorship assays

Survivorship of adult *D. suzukii* was monitored in two food treatments: (1) an untreated control containing water only; and (2) blossoms from fresh unsprayed sweet cherries, *Prunus avium* var. 'Rainier' and 'Bing.' Each treatment was set up in six Bug Dorm cages (MegaView Science, Taiwan) 30.5 × 30.5 × 30.5 cm in size with mesh and plastic sides. Each cage contained 50 males and 50 females and a water supply via a wicking sponge immersed in a 100-ml container. *D. suzukii* used in these trials were collected every 24 h from emergence cages holding pupae within diet plates (Dalton et al. 2011) and hence were 0- to 24-h-old adults that might have fed on the same diet that they emerged from. Flower blossoms were collected during the naturally flowering period in Oregon and replaced weekly until the conclusion of the study. All floral treatments included a freshly picked branch of 6.5 cm length and contained at least twenty flowers. Branches were secured using parafilm within a 100-ml beaker of water. Control and cherry blossom treatments were conducted from April 14 to May 25, 2011 for 41 days. Mortality of flies was observed every 48–72 h, three times a week, and dead flies were removed and counted. The experiment with cherry blossoms terminated before all *D. suzukii* died because blossoms were no longer available.

Experimental conditions closely replicated typical spring conditions in the Mid-Willamette Valley of Oregon by placing all cages in temperature-controlled growth chambers (Percival Scientific, Perry, IA). Because of the typically earlier flowering found with cherries, flies were exposed for 14 days to cherry blossoms at diurnal fluctuating temperatures of 3–13 °C and 13:11 L:D photoperiod. The temperature was subsequently increased to 4–16 °C and 14:10 L:D for the next 14 days, and then 7–20 °C and 15:9 L:D for the remaining period. In order to standardize the different temperature regimes to physiological time, we converted temperature conditions to accumulated degree-days (Baskerville and Emin 1969; Wilson and Barnett 1983) by using a low threshold of 7.2 °C (Tochen et al. 2014) and an upper threshold of 30 °C (Asplen et al. 2015). For analysis of survivorship, Kaplan–Meier curves were generated for each treatment x sex combination: 'water ♀,' 'water ♂,' 'cherry ♀,' and 'cherry ♂.' First, a non-parametric log-rank comparison with censored observations tested all four treatments together. Second, means comparisons were done by testing each treatment pair by log-rank and an adjusted Bonferroni *P* value (α/no. pairwise comparisons = 0.05/6 = 0.00833). All statistical analyses were performed in JMP 11.0.0 (SAS Institute 2013).

Baseline nutrient assays

To establish the baseline nutrient profiles of *D. suzukii*, the nutritional state was measured at fly emergence (referred to as 'unfed 0 d'), at 1 day old when fed ('fed 1 d') or starved ('unfed 1 d'), and at 3 days old when fed ('fed 3 d'). To reduce the likelihood of adult flies feeding on the diet from the developmental dish, emergence from pupae was checked each hour. Newly emerged *D. suzukii* were transferred once they inflated their wings to avoid damaging them, and they were not observed to readily feed on food at this time (J.C.L. personal obs.). Some newly emerged flies were immediately frozen at −80 °C, and other flies were immediately placed into plastic holding cages 23 × 23 × 25 cm with a mesh sleeve held at 22 °C. Five holding cages with food and three without food were set up between February 28 and March 1, 2012, and flies from multiple cages were frozen live after 1 day and 3 days. Because unfed flies mostly died within 2 days, nutrient levels for these starved individuals were not measured at 3 days. Between 14 and 20 flies of each sex were assayed from each treatment × age combination. Unfed flies were kept in cages with a water wick, and fed flies were kept in holding cages with a water wick, 20 % sucrose-water solution and artificial diet (diet in Woltz et al. 2015). A 20 % sucrose concentration was used because it was close to the optimal sucrose concentration of 22 % for herbivores (Heyneman 1983), and within the sugar concentration range of many floral nectars (Kevan and Baker 1983).

To account for the effect of insect body size (covariate) on nutrient levels, the forewings of each fly were removed and measured for length from the humeral-costal break to the end of L3 vein. Also, any diet particle was removed from their body with tweezers. The lipid, glycogen, fructose, and total sugar content within individual *D. suzukii* were measured using a protocol adapted for parasitic wasps (Olson et al. 2000; Lee et al. 2004). Briefly, each fly was crushed with a pestle in a 1.5-ml microcentrifuge tube and 50 µl of 2 % sodium sulfate. A control that did not include a fly was run during each assay set of 20 flies. Next, 450 µl of chloroform–methanol (1:2) was added, pestle removed, and tube was centrifuged for 2 min at 13,000 rpm to form a glycogen precipitate at the bottom. The supernatant was transferred into a glass test tube, vortexed, and divided further: half for the lipid assay and half for the fructose/sugar assay.

For the lipid assay, the supernatant was boiled at 90 °C for ~8 min until a minimal amount of liquid remained, ~5 µg. Next, 40 µl of sulfuric acid was added and

heated at 90 °C for 2 min. Once cooled, 960 µl of vanillin reagent was added, vortexed, and left at room temperature for 25 min. Absorbance was read at 525 nm on a spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, Piscataway, NJ), and lipid content was estimated from the absorbance values of lipid standards made for each vanillin reagent. To calibrate the standard, 0, 1, 5, 10, 35, and 50 µg of canola oil were reacted with vanillin as described above, and the relationship between the absorbance value and lipid content was calculated by a linear equation. A similar calibration was done for glycogen, fructose, and sugar standards. For the glycogen assay, 975 µl of anthrone reagent was added, vortexed until the precipitate dissolved, and heated at 90 °C for 10 min. Once cooled, absorbance was read at 625 nm. For the fructose/sugar assay, each tube was heated at 90 °C for 2 min leaving ~25 µl of supernatant. Next, 975 µl of anthrone reagent was added, vortexed, and heated at 30 °C for 1 h. Absorbance was read at 625 nm to determine fructose levels. For the total sugar assay, the same solution used for the fructose assay was then heated at 90 °C for 7 min. Six female samples (fed 1 d and fed 3 d) used in the fructose assay could not be reused for the total sugar assay. These samples turned brown during the heating process indicating degradation of the anthrone reagent. Once cooled, absorbance was read at 625 nm. Because only half of the supernatant was used to assess lipid or sugar levels, the estimate was multiplied by two in order to represent the total level per fly.

For the baseline assay, separate analyses were done for each sex and each nutrient. $\text{Log}_{10}(x + 1)$ or square-root transformations were used to homogenize variances as needed. Four treatment groups were compared together: unfed 0 d, unfed 1 d, fed 1 d, and fed 3 d. A two-factor test by age, feeding status, and its interaction was not run because it was difficult to experimentally obtain 'fed 0 d' and 'unfed 3 d' flies for a balanced design. First, an ANCOVA tested the effect treatment on a given nutrient with wing length as a covariate. Tukey HSD compared treatments. Second, if wing lengths did not significantly affect results, a simpler ANOVA tested the effect of treatment on a nutrient followed by a Tukey HSD, and these results are reported here.

Floral feeding nutrient assays

Female *D. suzukii* were exposed to one of three treatments: (1) a 20 % sucrose-water solution (positive control), (2) water (negative control), and (3) blossoms from fresh unsprayed sweet 'Rainier' and 'Bing' cherries, or blossoms from 'Jersey' blueberries collected during the naturally flowering period in Oregon. Prior to exposure, ~2-day-old females from the colony were starved for 8 h with only water. Each treatment was set up in six cages with water similar to the survivorship assay. A separate sucrose solution was provided in a similar manner as the water. Cherry blossom, sucrose, and water treatments were set up and tested during April 15 to April 17, 2013 at 13.5:10.5 L:D and 22 °C, and blueberry blossom, sucrose, and water treatments during April 29 to May 1, 2013 at 14.2:9.8 L:D and 22 °C. Flies were collected live after 1 day or 2 days of exposure and frozen for subsequent wing length measurements and nutrient assays as described in the baseline assay. Assessments were made at 1 day or 2 days to enable feeding and metabolism, and before starved flies died. We used 18 to 20 females per assay per treatment x day combination. Lipid levels were not assessed for the cherry trial because of contamination during the biochemical assay.

For the floral feeding assay, separate analyses were done for the cherry and blueberry assays for each nutrient. The effect of food treatment, time of exposure, and food x time interactions were fixed effects in an ANCOVA with wing length as a covariate. Nutrient levels were transformed if necessary, and Tukey HSD compared treatments. If wing lengths did not affect results, two-factor ANOVAs and subsequent Tukey HSDs were tested.

Results

Survivorship assays

Survivorship of male and female adult *D. suzukii* provided with cherry blossoms was enhanced when compared to individuals provided with water only ($\chi^2 = 276.2$, $df = 3$, $P < 0.001$) (Fig. 1). Water ♀ had significantly greater survivorship than water ♂, but there were no differences between the sexes when both had access to cherry

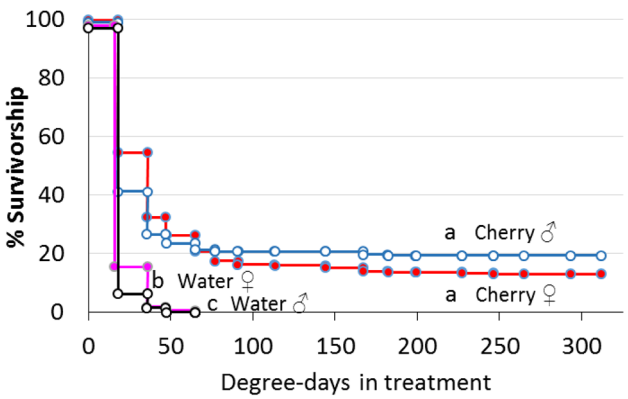

Fig. 1 Kaplan–Meier survival curves of *D. suzukii* in cages containing water only and cages containing water and cherry blossoms, and letters show log-rank comparisons between treatments

blossoms. Only 16 % of adult female and 6 % of adult male *D. suzukii* were alive after 3 days (18 DD) of exposure to water following transfer from emergence cages with rearing diet. At the same time, 55 % of females and 41 % of males exposed to cherry blossoms were alive. At the end of the experiment at 41 days (312 DD), 13 % of females and 19 % of males remained alive in the cherry treatment. While some adults given cherry blossoms died within a few days, the remaining cohort survived for a long period.

Table 1 Results of the ANOVA test of nutrient levels of *D. suzukii* in the baseline nutrient assay

Sex	Nutrient	*df*	*F*	*P*
Females	Lipid	375	9.1	<0.001
	Glycogen	375	42.1	<0.001
	Fructose	370	33.6	<0.001
	Total sugar	364	30.1	<0.001
Males	Lipid	370	29.2	<0.001
	Glycogen	370	29.8	<0.001
	Fructose	370	26.2	<0.001
	Total sugar	369	30.7	<0.001

Baseline nutrient assays

Wing lengths of *D. suzukii* were not different among females in the different food or age groups in the baseline assay (mean length 2.47 ± 0.013, ANOVA $F_{3,75} = 1.4$, $P = 0.26$) nor among males (mean length 2.34 ± 0.009, $F_{3,70} = 2.5$, $P = 0.064$). Because wing length did not significantly impact nutrient levels, and statistical outcomes on nutrient levels were similar among ANOVAs and ANCOVAs, the simpler ANOVA results are presented here.

Lipid levels significantly differed among the four treatment groups for both female and male *D. suzukii* (Table 1; Fig. 2a). For the most part, trends among females and males were similar and are discussed together unless otherwise specified. Newly emerged *D. suzukii* had significantly higher lipid levels than those fed a diet and sucrose solution, comparing unfed 0 d to fed 1 d or fed 3 d.

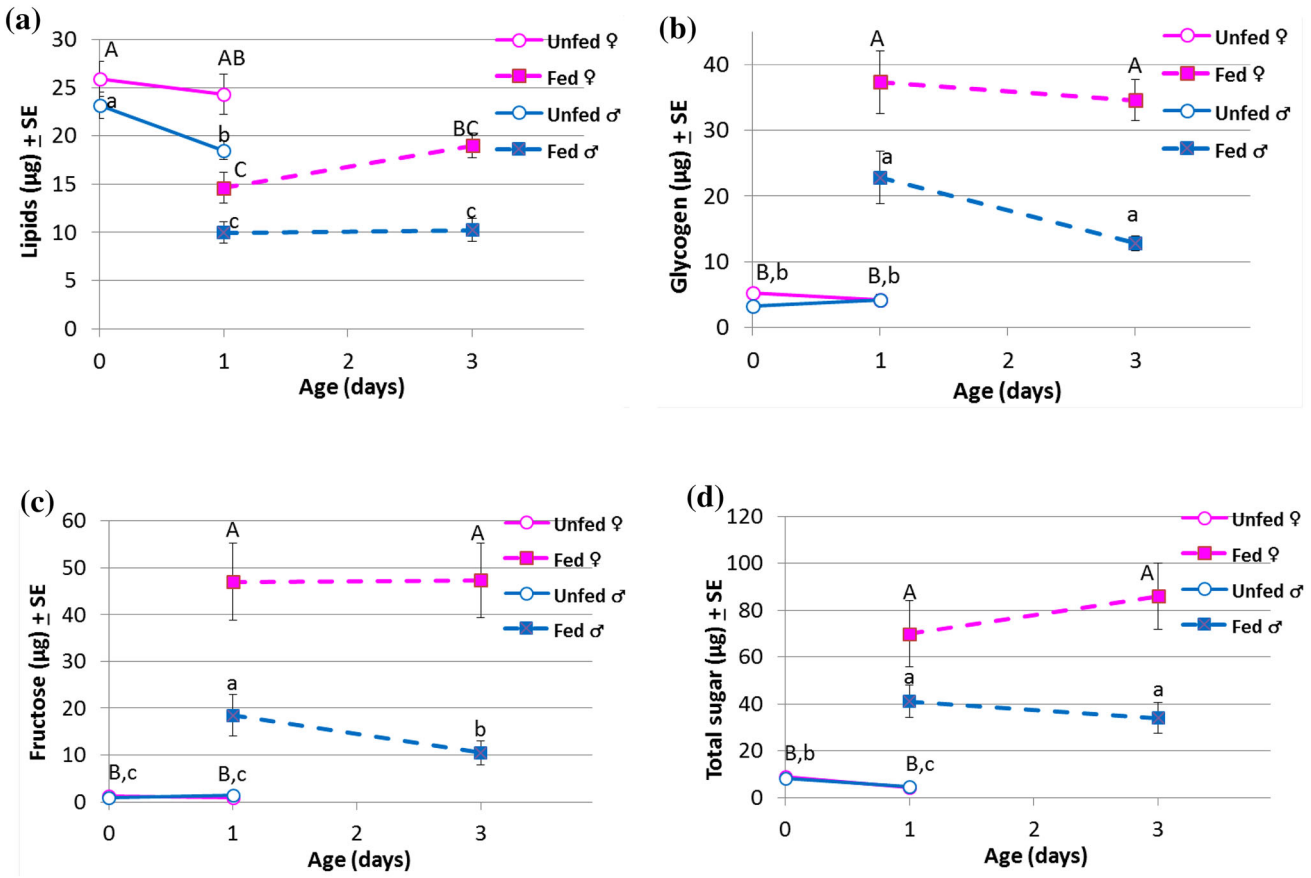

Fig. 2 Average (±SE) lipid (**a**), glycogen (**b**), fructose (**c**), and total sugar levels (**d**) per male and female *D. suzukii* with or without food at various ages. *Uppercase letters* denote significant differences by Tukey HSD among the four treatment groups of females, and *lowercase letters* denote differences among males

Table 2 Results of the ANOVA tests for nutrient levels of female *D. suzukii* in the floral feeding nutrient assay

Trial	Nutrient	Effect	*df*	*F*	*P*
Cherry blossom	Glycogen	Food	2112	56.1	<0.001
		Time	1112	6.3	0.014
		Food × time	2112	1.6	0.210
	Fructose	Food	2112	52.8	<0.001
		Time	1112	2.7	0.104
		Food × time	2112	8.3	<0.001
	Total sugar	Food	2112	28.7	<0.001
		Time	1112	6.5	0.0121
		Food × time	2112	3.4	0.0355
Blueberry blossom	Lipid	Food	2107	1.8	0.17
		Time	1107	3.1	<0.001
		Food × time	2107	4.8	0.0095
	Glycogen	Food	2107	194.5	<0.001
		Time	1107	6.5	0.012
		Food × time	2107	1.09	0.341
	Fructose	Food	2107	40.7	<0.001
		Time	1107	1.6	0.210
		Food × time	2107	2.0	0.143
	Total sugar	Food	2107	94.1	<0.001
		Time	1107	1.2	0.275
		Food × time	2107	0.93	0.399

Starved males, specifically, had significantly lower lipid levels than newly emerged males, comparing unfed 1 d to unfed 0 d. Among 1-day-old *D. suzukii*, those without food had significantly higher lipid levels than their counterparts given food, comparing unfed 1 d to fed 1 d. Glycogen levels significantly differed among the treatments (Table 1; Fig. 2b). *D. suzukii* without food had low glycogen levels (<5 μg) which was significantly lower than those given food, comparing unfed to fed of all ages. For males, glycogen levels numerically dropped as they aged, but the trend was not significant, comparing fed 3 d to fed 1 d.

Fructose is a substantial component of floral nectars (Nicolson and Thornburg 2007) and the disaccharide sucrose fed to *D. suzukii* is initially broken down into equal parts of the monosaccharides fructose and glucose. Fructose levels significantly differed among the treatments (Table 1; Fig. 2c). *D. suzukii* without food have minimal fructose levels (<1.2 μg) which is significantly lower than those given food, comparing unfed to fed of all ages. For males, fructose levels were significantly higher in 1 compared to 3-day-old fed flies. Total sugar levels were examined to provide an overall status of energetic reserves that can be quickly utilized for maintenance and mobility. Total sugars significantly differed among the treatments (Table 1; Fig. 2d). As with fructose, *D. suzukii* without food had significantly lower sugar levels than those given food. Starved males had significantly lower sugar levels than newly emerged males, comparing unfed 1 d to unfed 0 d. Total sugar levels were often twice the fructose levels among fed *D. suzukii* (Fig. 2c, d), which is consistent with the breakdown of sucrose.

Floral feeding nutrient assays

Wing lengths were not different among females in the cherry floral feeding assay (2.63 ± 0.008, $F_{5,112} = 1.9$, $P = 0.10$) nor the blueberry assay (2.32 ± 0.011, $F_{5,107} = 1.5$, $P = 0.21$). As in the earlier section, ANOVA results are presented.

Lipid levels were affected by time and food x time interactions in the blueberry assay (Table 2). Females exposed to sucrose for 2 days had lower lipid levels than those exposed only to water (Fig. 4a). Glycogen levels were affected by food in both the cherry and blueberry assays (Table 2). Females given sucrose had the highest glycogen levels, females given blossoms had an intermediate level, and those given water had the lowest (Figs. 3a, 4b). Also, there was an effect of time in the blueberry assay (Table 2), as glycogen levels slightly increased with longer exposure (Fig. 4b).

Fructose and total sugar levels showed similar trends in the cherry assay. There was an effect of food and food x time interaction on levels (Table 2). Generally, females given cherry blossoms or sucrose had higher levels than those given water (Fig. 3b, c). However, females given cherry blossoms for 2 days showed a decrease in fructose and total sugar levels compared to those given sucrose for 1 day or 2 days. In the blueberry assay, fructose and total sugar levels shared similar trends, and there was an effect of food on levels (Table 2). Females given sucrose or blueberry blossoms had higher sugar levels than those given water (Fig. 4c, d).

Discussion

As expected, *D. suzukii* exposed to cherry blossoms had higher survival than their counterparts that were exposed to water only, with 16 % overall surviving after 41 days (312 DD) and 12 % surviving after 3 days (18 DD), respectively. As found in numerous studies with other insects (rev. by Russell 2015; Wackers et al. 2007), access to flowers such as cherry blossoms would enable *D. suzukii* adults to live longer. Floral nectar is rich in three predominant sugars (sucrose, glucose, and fructose) and may contain small amounts of minor sugars, amino acids, and lipids (Nicolson and Thornburg 2007). While not studied, it is possible that blossoms can provide other nutrients via

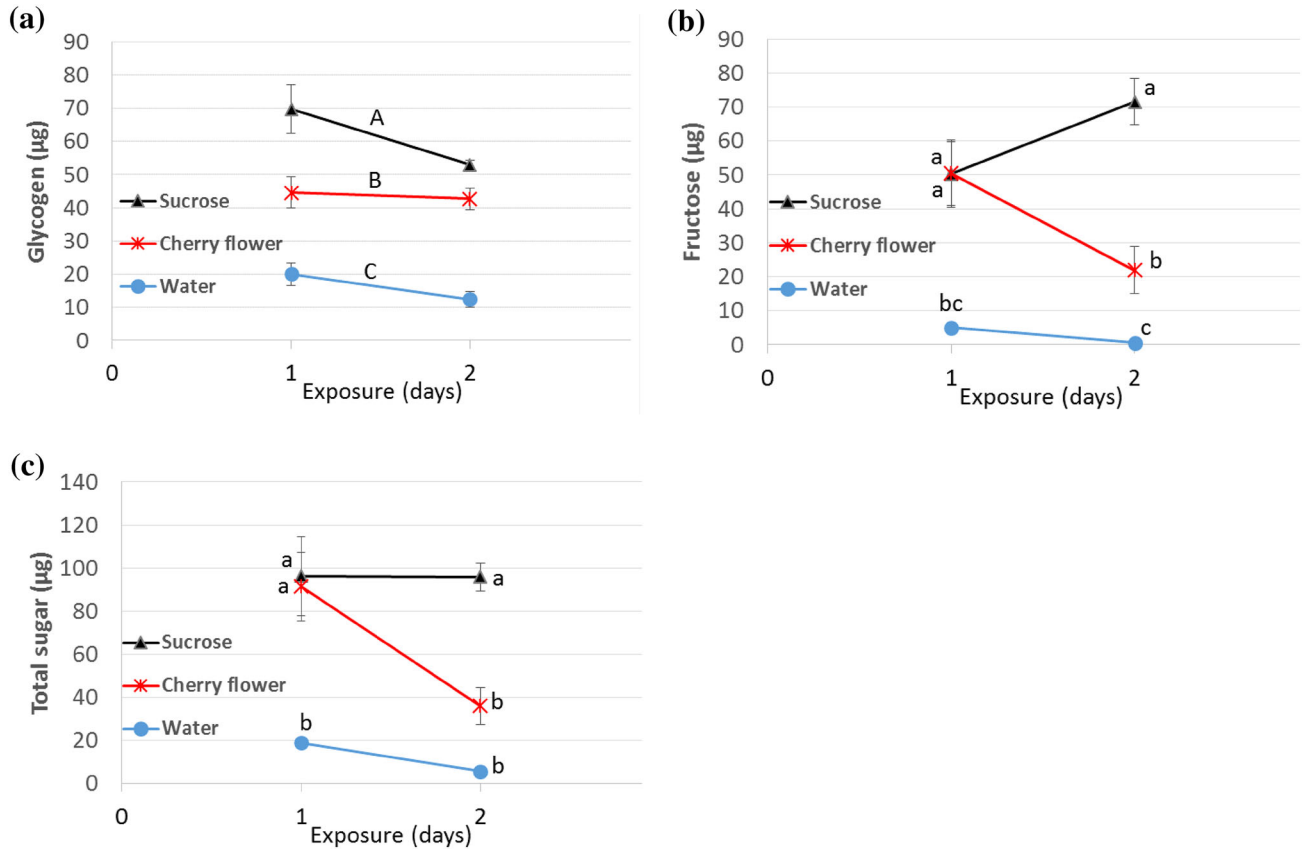

Fig. 3 Average (±SE) glycogen (**a**), fructose (**b**), and total sugar levels (**c**) per female *D. suzukii* with 1 day or 2 days of exposure to water, sucrose solution, or cherry blossoms. *Uppercase letters* denote significant differences by Tukey HSD among the three food treatment groups, *lowercase letters* denote differences among the six treatment × time combinations when food × time interactions were significant

pollen and colonized bacteria or yeasts. *D. flavohirta* Malloch adults are known to gather pollen with their proboscis (Nicolson 1994). Several yeast species are common in the midgut of *D. suzukii* (Hamby et al. 2012) and whether flowers could provide a source of yeasts is unknown. Given that floral access enhances *D. suzukii* survival in a laboratory study, *D. suzukii* in the field might be expected to feed on early spring blossoms when wounded ripe fruit or fermenting fruit are not prevalent sugar sources.

At emergence, *D. suzukii* males and females had low reserves of glycogen and sugars, and no/minimal reserve of the specific sugar fructose. Fructose is considered a gut-specific sugar obtained through feeding; this is supported by zero or low levels (<2 µg) of fructose among newly emerged or starved hymenopterans (Heimpel et al. 2004; Lee et al. 2004; Lightle et al. 2010; Olson et al. 2000; Steppuhn and Wackers 2004) and dipterans (Fadamiro et al. 2005; Moore et al. 1987). The low levels of fructose detected in *D. suzukii* may indicate trace amounts present, or result from other sugars slightly reacting with the anthrone reagent at 30 °C. Once exposed to food for 1 day, adults quickly had elevated levels of glycogen, total sugar, and fructose. Similar increases in glycogen and sugar stores occur after a carbohydrate meal in *Anopheles* mosquitoes (Briegel 1990), *Aedes aegypti* (Naksathit et al. 1999), phorid fly *Pseudacteon tricuspis* (Chen and Fadamiro 2006), and *D. melanogaster* (Colinet et al. 2013). For *An. gambiae* and *An. stephensi*, glycogen levels reached a maximum at 1–2 days and gradually declined thereafter; this suggested that glycogenesis occurs soon after adult emergence for these species. For *D. suzukii*, a longer duration of glycogen monitoring is necessary to determine if glycogenesis peaks as well. When *D. suzukii* male adults were continually starved for 1 day with water, total sugar levels significantly dropped from day 0 to day 1. These profiles of starved and fed adult *D. suzukii* could later be useful for interpreting nutrient levels of field-collected *D. suzukii*.

Drosophila suzukii larvae reared on standard diet emerged as adults with 23–26 µg of lipid reserves. Lipid levels continued to decline significantly among adult males

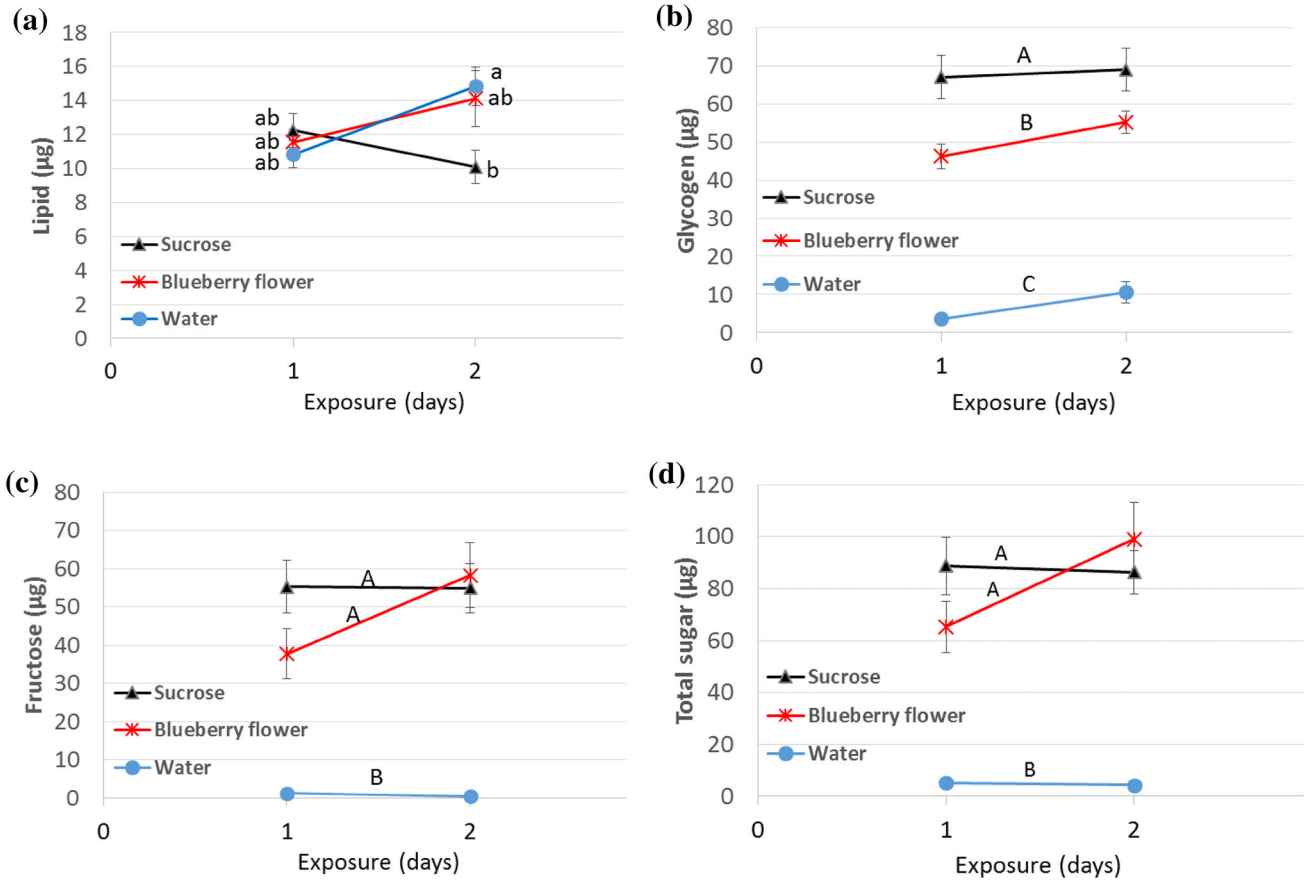

Fig. 4 Average (±SE) lipid (**a**), glycogen (**b**), fructose (**c**), and total sugar levels (**d**) per female *D. suzukii* at 1 day or 2 days of exposure to water, sucrose solution, or blueberry blossoms. *Uppercase letters* denote significant differences by Tukey HSD among the three food treatment groups, *lowercase letters* denote differences among the six treatment × time combinations when food × time interactions were significant

without access to food. There was no clear evidence of lipogenesis during the observation of 0- to 3-day-old adults; post-emergent lipid levels of 1- or 3-day-old adults did not rise above emergence levels regardless of food access. However, the potential for lipogenesis should be further examined for a longer duration. Lipogenesis has been observed in the mosquitoes *Anopheles* spp. (Briegel 1990) and *Aedes aegypti* (Naksathit et al. 1999), but not the tephritid *Ceratitis capitata* (Nestel et al. 1985). Unexpectedly, 1-day-old *D. suzukii* with access to food had lower lipid levels than when starved, and this trend is further discussed in the floral feeding experiment.

Access to blossoms of cherry and blueberry significantly increased the glycogen, sugar, and fructose levels of female *D. suzukii* compared to flies that only had access to water. This increase was moderate compared to being fed a 20 % sucrose solution, as females fed sucrose oftentimes had higher carbohydrate levels than those fed blossoms. This may suggest that females may have fed less on the blossoms than on the sucrose solution with the reasons being unknown. The flowers were accessible to *D. suzukii*. However, the nectar concentration and composition was not measured at the time of assay (Obinna et al. 2013) to discern whether different sugar concentrations or ratio of sugars may have affected intake and nutrient levels of *D. suzukii*. This should be characterized in future studies. In other studies, blueberry blossoms have had 15–45 % sugar concentration with considerable variation among soil type, age of bush, and cultivar (Starast et al. 2014) and concentrations increased from the first to fourth day of nectar secretion (Jabionski and Pliszka 1985). Interesting results were also observed in the lipid levels of female *D. suzukii*. Access to blueberry blossoms resulted in females having similar lipid levels as those with water, but access to sucrose led to a decline in lipid levels after 2 days of exposure. The unexpected outcome from sucrose feeding was also observed in the baseline study with a different stock of *D. suzukii*. To our knowledge, other studied insects have not shown lower lipid levels with access to sugar. This should be further examined with *D. suzukii* adults

reared from wild sources with the time of feeding and amount consumed closely monitored to determine the pattern and consistency of lipid decline. In contrast, sugar feeding has been associated with an enhancement of lipid levels, either through lipogenesis (Briegel 1990; Naksathit et al. 1999) or slowing the rate of lipid decline in other flies (Jacome et al. 1995; Nestel et al. 1985) and parasitic wasps (Ellers 1996; Lee et al. 2004).

In summary, this study confirmed the importance of adult feeding among *D. suzukii*. Adults emerge with limited glycogen and sugar reserves and having access to a food source, either floral blossoms or sugar solution plus yeast-based diet, led to increased carbohydrate reserves within 1 day. Access to cherry blossoms also improved survivorship. Such blossoms could be important food sources in the early spring when other fruit hosts are limited (Lee et al. 2015b). Future studies should follow up on the impacts of adult feeding and nutrient reserves on late dormant fecundity, dispersal, and foraging behavior. Additional knowledge of adult nutrient and feeding requirements can improve pest management by determining the importance of the landscape on *D. suzukii*'s survival and reproductive success. In other studies, adding adult food sources to insecticidal sprays may improve insecticidal efficacy (Cowles et al. 2015). Also, such food sources might be incorporated into 'attract & kill' systems. Identified food sources in the environment might therefore serve as targeted areas for chemical control.

Author contribution

ST, JL, and VW conceived and designed research. ST and JL conducted experiments and analyzed data. All authors contributed to writing the paper.

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