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Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii*

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Abstract:
In this study, we investigated the effects of non-nutritive sugars and sugar alcohols on the survivorship of spotted wing drosophila, *Drosophila suzukii*, and found erythritol and erythrose as potentially insecticidal effect to the fly. In a dose-dependent study, erythritol and erythrose significantly reduced fly longevity, with 100% mortality with 1, 0.5, 0.1 & 0.05M doses after feeding for 7 days. When sugar solutions were provided separately to flies for 7 days, there was no effect on survivorship regardless of erythritol concentrations. However, with a serial combination of sugar and erythritol solutions, fly survivorship was significantly decreased for the same period. Also, the higher dose of erythritol regardless of the sucrose dose combined showed in greater mortality. In a no-choice assay, *D. suzukii* ingested more erythritol than sucrose or water, indicating the fly continuously fed on erythritol for 72 h. Also under no-choice conditions, erythritol and sucrose-fed flies gained more weight than water-fed flies. However, in two-choice assays, the amount of erythritol ingested was less than sucrose or water. Total sugar and glycogen levels among erythritol and erythrose-fed flies were significantly less than mannitol, sorbitol, xylitol, and sucrose-fed flies after 48 h. This indicates that these two non-nutritive sugars can't be used a substrate for enzymes involved in sugar metabolism. Although the metabolism of erythritol and erythrose is unknown in insects, the mortality of *D. suzukii* flies ingesting these sugars might be caused by two potential physiological changes. The fly is starved by feeding of non-metabolizable erythritol and erythrose, or experiences abnormally high osmotic pressure in the hemolymph with erythritol molecules diffused from the midgut. Non-nutritive sugars might be used as an insecticide alone or combined with conventional or biological insecticides to enhance efficacy. If other sugar sources are present, a palatable sugar might be mixed with erythritol to elicit feeding.

1. Introduction

A variety of non-nutritive sweeteners including erythritol have been approved for use as a food additive and sugar alternative labeled as zero-calories in the United States (FDA, 2014). Erythritol is a four carbon-structured sugar alcohol with 75% of the sweetness of sucrose, and is produced from corn or wheat starch by enzymatic process of either yeast or fermentative micro-organisms (Munro et al., 1998). The non-nutritive sugar is also known a sweet antioxidant to help against high blood sugar (hyperglycemia) affecting diabetes (Den Hartog et al., 2010; Munro et al., 1998). Erythrose is also a tetrose carbohydrate and belongs in the aldose family with aldehyde group (Nelson and Cox, 2000). Little is known about the metabolism of erythrose. It has been suggested the initial reduction of erythrose to erythritol (Hiatt and Horecker, 1956). Erythrose was recently offered as an anti-cancer agent that inhibits tumor growth (Liu et al., 2015).

A number of sugars, sucrose, glucose and fructose, etc. obtained from soft skin fruits as food sources provide an effective energy for adult flight in *Drosophila*. The detection and selection of sugars by flies was decided with palatability and nutrient value of sugars, that flies leaned the appetitive memory to continuous feeding (Burke and Waddell, 2011; Fujita and Tanimura, 2011). Nonnutritious sugars although they contain some sweet taste were eventually excluded by the section of food from a long term memory learned by *Drosophila* fed these sugars.

Sugar acts as a phagostimulant increasing ingestion of insecticides and may play an important role by increasing insecticide effectiveness. To enhance the effectiveness of insecticides, sucrose can be added to conventional or organic insecticides targeting *Drosophila suzukii* (Matsumura) (Cowles et al., 2015). Although the non-nutritive sugars such as erythritol failed to increase the feeding activity of the ant (Vander Meer et al., 1995), recently they were shown to carry insecticidal properties against Dipteran pests (Baudier et al., 2014; O'Donnell et al., 2016; Sampson et al., 2016; Zheng et al., 2016). In the lab, *Drosophila melanogaster* (Meigen) had reduced longevity and motor coordination when fed erythritol or the artificial sweetener that contained erythritol (Baudier et al., 2014). Moreover, *D. melanogaster* actively consumed erythritol in the presence of the sucrose, and had decreased longevity. This is important because erythritol could have an insecticidal effect if the insect pest readily consumes it. Although erythritol showed some insecticidal activity on *Drosophila* (Baudier et al., 2014; Sampson et al., 2016), the corresponding physical changes following erythritol ingestion and mechanism leading to death remains unknown.

The spotted wing drosophila, *D. suzukii*, originally from Asia is now an important small fruit and cherry pest in Asia, Europe, and North America (Asplen et al., 2015). If the pest is left unmanaged, annual losses have been estimated at US$421 million for the soft fruit and cherry industry of California, Oregon, and Washington combined (Bolda et al., 2010), and 13% revenue loss in the northeastern berry production area of Italy (De Ros et al., 2015). While numerous biological, cultural, mechanical, and chemical strategies are being developed for *D. suzukii* control, insecticides are used in both conventional and organic programs. While generally effective and convenient, the application of insecticides must be repeatedly every 10-14 days based on their residual activity (Beers et al., 2011), and even more if rainfall occurs (Diepenbrock et al., 2016; Van Timmeren and Isaacs, 2013). For insecticides to be part of a more sustainable program, efforts are underway to make insecticide applications more effective and reduce overall use, such as reduced spray programs (Klick et al., 2016), and also to develop environmentally-friendly insecticides.

The first objective of our study was to determine whether erythritol, erythrose and other sugars impact on the survivorship of *D. suzukii* with a dose assay. The second objective tested erythritol feeding in various combinations with water or sucrose to determine whether mortality was caused by starvation or other physiological changes. The third objective confirmed that erythritol was ingested in capillary no-choice and choice assays. Lastly, the fourth objective measured the sugar and glycogen content of *D. suzukii* adults fed various sugars to determine whether these foods were converted for carbohydrate storage.

2. Materials and Methods

2.1. Flies, sugars and sugar alcohols

Drosophila suzukii used in these experiments were from a colony maintained at 22±5ºC under a photoperiod of L:D 16:8 h, and a relative humidity of 60±5% RH at the Horticultural Crops Research Unit, USDA ARS in Corvallis, Oregon, USA. Wildtype flies collected from infested fruits in Corvallis in October 2015 and July 2016 were used to start the colony. Standard rearing methods and diet are described by Woltz et al. (2015). Newly emerged adult males and females were collected daily and maintained in cages with water and diet until they were specific ages for experimentation. Sugars used in this study, meso-erythritol (>99%), D-erythrose (75% syrup), D-mannitol (>99%), sorbitol (>99%), sucrose (>99%), and xylitol (>99%), were purchased from Fisher Scientific (Hampton, USA).

2.2. Dose-dependent effects of sugars on fly survivorship

Ten 5-day old flies (5 males and 5 females) were introduced into a plastic vial (28 mm id x 95 mm height) and fed a dose of 1, 0.5, 0.1 or 0.05M of either sucrose, erythritol, erythrose, xylitol, mannitol, or sorbitol. Each sugar solution was soaked on a cotton stud in a 1.5 ml centrifuge tube. Survivorship of flies was checked daily for 7 days. All treatments were replicated at least three times (vials). Water and sucrose solution were used for a negative and positive controls, respectively in this and subsequent studies.

2.3. Age-dependent effects of sugars on fly survivorship

Once survivorship was confirmed to be lower on erythritol and erythrose, the age-specific susceptibility on sugars was observed to refine future assays. Ten flies (5 males and 5 females) from 1- to 7-day old were introduced into a vial as described above and given 0.5M sucrose, erythritol, erythrose, xylitol, mannitol, or sorbitol. Survivorship of flies was checked daily for 7 days.

2.4. Separate or combined sugars on survivorship

Ten 5-day old flies (5 males and 5 females) were introduced into a plastic vial and given two separate tubes (1.5 ml) containing different solutions (Fig. 2A). Various pairs were tested: water + 0.5M sucrose, water + 0.5M erythritol, 0.5M sucrose + 0.5M erythritol, 0.5M sucrose + 1M erythritol, 1M sucrose + 1M erythritol, or 1M sucrose + 2M erythritol. Survivorship of flies was checked daily for 7 days. Each pair was replicated three times.

To test combined solutions, ten 5-day old flies were introduced to feed on different ratios of a mixed sucrose and erythritol solution placed in one tube (Fig. 2C). The combination of sucrose/erythritol ratios (0.5M/0.5M, 0.5M/1M, 0.5M/2M, 1M/2M, or 1M/2M) were soaked into a cotton stud in a 1.5 ml centrifuge tube. Survivorship of flies was checked daily for 7 days. Each combination was replicated three times.

2.5. Measurement of sugar consumption and body weight gain in no-choice assay

A single fly was introduced into a glass vial (15 mm id x 45 mm length, Thermo Scientific, Rockwood, TN, USA). As a lid, a half-cut centrifuge tube with a few holes for aeration had a central capillary glass tube (70 µl, 11 mm id x 70 mm height, Fisher Scientific) (Fig. 3A). The solution (0.5M) of erythritol or sucrose, or water was filled in the capillary tube, then a mineral oil (Thermo Scientific) layer covered the treatment solution to prevent evaporation. Three identical vials without a fly were served as controls to measure actual evaporation. The amount in the capillary tube was checked daily before and after feeding. The consumed amount was calculated by subtracting the evaporated amount in the control vial from the reduced amount in the fly vial.

To measure fly body weight gain, five male and female flies were separately introduced into a glass vial with a capillary as described above. Flies were allowed to feed sucrose, erythritol, or water, and weighed before and after feeding for 48 h using a microbalance (Thermo Scientific). The average body weight from five flies in each treatment was measured, and four replications (vials) were conducted for each treatment.

2.6. Measurement of consumption amount of sugars in choice assay

Five female flies aged 5-day old were introduced into a glass vial as described above with a modified lid with 2 aeration holes and 2 glass capillary tubes (Fig. 3C). Water + sucrose (0.5M), water + erythritol (0.5M), or sucrose (0.5M) + erythritol (0.5M) solutions were filled up in the capillary tubes. The set-up with mineral oil and control vials were similar to as above as well as the weight gain calculation.

2.7. Anthrone test for carbohydrate content

Adults were fed various diets: 1) water, 2) erythritol, 3) erythrose, 4) xylitol, 5) mannitol, 6) sorbitol, and 7) sucrose. To set up assays, 5-day old flies were transferred into plastic vials (28 mm id x 95 mm height). Each vial contained 4 males or 4 females with a diet treatment for 24 h, or 5 males or 5 females held for 48 h. More flies were held for 48 h in case of mortality. In each vial, a 0.5M solution was provided in a 0.5 ml centrifuge tube plugged with a cotton wick. For all treatments, live flies were frozen at -80°C after 24 h and 48 h of exposure; dead flies in the vial were not collected. Each diet treatment and hour combination was replicated in three vials.

A standard procedure refined for parasitic wasps (Olson et al., 2000) was used to determine the amount of glycogen and sugar in each fly. This procedure has been used for *D. suzukii*, Tochen et al. (2016) describes the calibration and final determination of nutrient values. The same procedures were used except that 200 µl of the final solution was pipetted into a 96-well ELISA plate and read on an absorbance reader (ELx808, BioTek).

2.8. Statistics

For the dose study, a separate survivorship analysis was conducted at each dose. First, a Kaplan-Meier log-rank comparison with censored observations for flies alive on day 7 tested all seven treatments together for survivorship. Next, means comparisons were done by testing each treatment pair by log-rank and using an adjusted Bonferroni P-value (α/no. pairwise comparisons = 0.5/21 = 0.00238). For the separate or combined sugar survivorship assay, a similar analysis as described was used with an adjusted P-value of 0.0033 and 0.000238 for means comparisons, respectively. For the no-choice consumption and weight gain study, a standard ANOVA tested the effect of treatment; the assumptions of an ANOVA were met with untransformed data. For the choice consumption study, a separate repeated analyses was run for each paired choice, the

assumptions of a parametric model were met. The effect of diet choice, hour, and diet x hour were fixed effects, and vial was the random subject effect. Means comparisons were done by Tukey HSD or t-test. These analyses were conducted in JMP® 12.1.0 (SAS, 2015).

For the carbohydrate assay, separate analyses were conducted for males and females and for glycogen and sugar. Each nutrient level was the dependent variable with a lognormal distribution. The effect of diet treatment, hour, diet x hour were fixed effects. The vial where 4-5 flies were held together was a random effect, each fly was a replicate. Nutrient levels of flies from the other six sugar feeding treatments were compared with the water control by Dunnett's test. If the effect of treatment was significant but diet x hour was non-significant, diets were compared pooling both hours. If the diet x hour interaction was significant, a separate Dunnett's test compared treatments at 24 h and at 48 h. These analyses were conducted in Proc Glimmix in SAS 9.3 (SAS, 2010).

3. Results

3.1. Dose-dependent effects of sugars on fly survivorship

Erythritol, erythrose and water-fed flies had lower survival given 1M, 0.5M, 0.1M and 0.05M dosages compare with xylitol, mannitol and sorbitol-fed flies for 7 days (Fig. 1, Table 1). No flies survived after 6-days of feeding on erythritol, erythrose and water. The survivorship from xylitol, mannitol and sorbitol-fed flies appeared lower, but was not significantly different compared with the sucrose treatment in 1M, 0.5M and 0.1M solution. At the 0.05M concentration, mannitol, sorbitol and xylitol-fed flies had significantly lower survivorship than sucrose-fed flies by 68%, 50%, and 30%, respectively on the 7^{th} day (Fig. 1). Also, xylitol-fed flies had significantly lower survivorship than mannitol and sorbitol-fed flies.

3.2. Age-dependent effects of sugars on fly survivorship

After confirming the effects of sugar doses on fly survivorship, the 0.5M dose was selected for subsequent studies, and was introduced to 1 – 7 day old flies for 7 days (Supplementary data 1). Fly survivorship appeared to decrease with erythritol, erythrose and water from all age ranges. The most susceptible age on the sugar treatments appeared to be the 2-day old adult followed 1-day, 3-day, 4-day, 6-day, and then either 5-day or 7-day olds. Therefore, we used 5-day old flies as the most tolerant age on erythritol for following assays in this study unless stated. Survivorship from the same dose of sucrose, mannitol, sorbitol and xylitol did not appear to vary by age (data not shown).

3.3. Separate or combined sugars on fly survivorship

To determine the cause of mortality from erythritol ingestion, various concentrations of erythritol and sucrose were introduced in separate or combined tubes (Fig. 2, Table 1). When solutions were given separately, there was no effect on survivorship occurred regardless of erythritol concentrations, except for only erythritol + water (*) as a negative control (Fig. 2B). Mortality from erythritol was at 80% within 4 days (Fig. 2B).

However, with a serial combination of sugar and erythritol solutions (Fig. 2C), fly survivorship was significantly ($P < 0.0001$) decreased for 7 days (Fig. 2D). The fastest and highest mortality occurred in the 1M sucrose/2M erythritol and 0.5M sucrose/2M erythritol combinations, followed by the 1M sucrose/1M erythritol and 0.5M erythritol (= a negative control), and then the 0.5M sucrose/1M erythritol combination. But no significant mortality occurred from combinations with 0.5M sucrose/0.5M erythritol as well as 0.5M sucrose as a

positive control. The higher dose of erythritol regardless of the sucrose dose combined showed greater fly mortality (Fig. 2D).

3.4. Capillary no-choice and choice assays

To measure the amount of sugar ingested by the fly, a glass capillary tube was filled with 0.5M erythritol, sucrose or water for the fly to access in a glass container for 72 h (Fig. 3A, Table 1). The amount of erythritol ingested by the fly was significantly greater (0.12 µl per fly) than sucrose (0.01 µl per fly) and water (-0.02 µl per fly).

Choice assays with paired capillary tubes filled with water + sucrose, water + erythritol, or sucrose + erythritol were exposed to five files for 72 h (Fig. 3C, Table 1). Overall, the sucrose consumed by the flies was greater than the erythritol choice (Fig. 4D). Between a choice of sucrose or water, there was no significant difference in 24 h ($P > 0.05$), 48 h ($P > 0.05$) and 72 h ($P > 0.05$) (Fig. 4D). Between a choice of water or erythritol, water was ingested more than erythritol in 24 h ($P=0.0038$) and 72 h ($P=0.0166$), but not in 48 h ($P > 0.05$). Between sucrose and erythritol, sucrose was more ingested than erythritol in 72 h ($P=0.0287$), but not different in 24 h ($P=0.3095$) and 48 h ($P=0.6771$).

3.5. Fly weight gain from the feeding sugars

After feeding for 48 h, the average male gained 1.41 mg from sucrose which was significantly heavier than 1.2 mg from water, and similar to that of 1.41 mg from erythritol (Fig. 4). The average female gained 1.96 mg from the sucrose and 2.01 mg from erythritol, these were significantly greater than 1.72 mg gained from water (Fig. 4). Overall, both male and female flies gained more weight with sucrose or erythritol than water, and female flies gained ~40% more weight than male flies (Fig. 4).

3.6. Sugar content after fed various sugars

Both males and female had significantly elevated total sugar levels in their entire body when fed mannitol, sorbitol, xylitol, or sucrose compared with water-fed flies after 48 h. Only those fed sucrose had significantly higher sugar levels at 24 h than water controls. We confirmed that our bioassay test does not react with sugar alcohols including mannitol, sorbitol or xylitol as well as erythritol and erythrose. Flies fed erythritol and erythrose had similar sugar levels as water-fed flies (Fig. 5, Table 1). The observed difference in the sugar levels between male and female flies is expected because females are heavier than males (Fig. 4).

3.7. Glycogen content after fed various sugars

Like sugar content, both males and females had significantly elevated glycogen levels when fed sorbitol, xylitol, and sucrose compared with water-fed flies (Fig. 6, Table 1). Males had elevated glycogen when fed mannitol, whereas females did not. However, glycogen levels remained low in males and females fed erythritol and erythrose for 48 h.

3.8. Effect of time on carbohydrate metabolism

Time affected both sugar and glycogen levels in males (Table 1). Time affected glycogen levels in females, but not sugar levels. Sugar and glycogen levels were higher at 24 h than 48 h. For males and females, there was also a diet x hour interaction with resulting sugar. Only those fed sucrose had significantly higher sugar levels at 24 h than water controls (Fig. 5). By 48 h, those fed mannitol, sorbitol, xylitol, and sucrose had higher sugar levels than water. This result indicated that some time is needed before the ingested diet is metabolized into other forms of sugar.

4. Discussion

In this study, we tested various sugars including erythritol under a variety of dosages, sugar combinations, fly ages, and feeding choices to investigate its potential insecticidal activity on *D. suzukii* adults. The previous study suggested erythritol acts an insecticidal effect on *D. melanogaster* (Baudier et al., 2014). We also observed fly mortality from various doses of ingested erythritol or erythrose in *D. suzukii*. In this study, therefore we investigated whether the fly mortality is caused by starvation because these sugars could not be metabolized into nutritional carbohydrates, or by hyperosmotic imbalance in the hemolymph in *D. suzukii*. In mammals, tetra-carbon molecules are normally passed through intestinal membranes at faster rate than hexose sugars (Munro et al., 1998; O'Donnell and Kearsley, 2012). Likely in *D. suzukii*, both erythritol and erythrose could be simply absorbed and diffused through the midgut membrane. This may increase the osmotic pressure in the hemolymph before being excreted out. Further physiological studies are need to elucidate the possible mechanism.

The lowest concentration (0.05M) of xylitol and sorbitol ingested in this study led to decreased survivorship at 30% and 50% after feeding for 7 days. Death may have resulted from these sugar alcohols being slowly metabolized. Sorbitol is converted to fructose by the sorbitol dehydrogenase in mammalian tissues (El-Kabbani et al., 2004), and its metabolism is relatively slower than glucose and sucrose (O'Donnell and Kearsley, 2012). Therefore, lower doses of xylitol and sorbitol might not provide *D. suzukii* enough nutritional carbohydrates due to a slow metabolic process. A proboscis extension reflex (PER) assay to evoke the food response of *D. melanogaster* showed that flies learned sweet taste for a short period, then more likely chose sweet sugars (Burke and Waddell, 2011; Fujita and Tanimura, 2011). The survivorship on flies fed sorbitol or xylose like tasteless or less sweet sugar, was lower than sucrose-fed flies. The same 0.05M dose of mannitol did not significantly reduce longevity in *D. suzukii* compared with sucrose but appeared lower. Mannitol is metabolized to the mannose-6-phosphate from the reduction of mannose to be utilized for the glycolysis (Nelson and Cox, 2000).

Worker honey bees that ingested mannose had died, which suggests that mannose is toxic to honey bees (Sols et al., 1960). Mannose toxicity in the honey bee was due to a large accumulation of mannose-6-phosphate and decreasing ATP by a shortage of mannosephosphate isomerase, however, this was not found to occur in *D. melanogaster* (De la Fuente et al., 1986). Like *D. melanogaster*, *D. suzukii* in this study was also not susceptible to 0.1 - 1M mannitol, indicating it is readily metabolized to mannose. Recently, *D. melanogaster* that ingested 1M mannitol over 10 days had reduced the longevity of female flies more than males, but it was not clear about the sex specific toxicity (O'Donnell et al., 2016).

When given a choice, *D. suzukii* ingests more sucrose than erythritol in 72 h (Fig. 3D). When sucrose and erythritol were both available as separate solutions to *D. suzukii*, the fly survivorship was not affected regardless of the erythritol dose (Fig. 2B). This indicates that *D. suzukii* could be more preferred to sucrose than erythritol, and will feed sufficiently on sucrose to maintain itself in the presence of erythritol. This may not be surprising because the sweetness of sucrose is known to be 30% higher than erythritol (Munro et al., 1998). However, when *D. melanogaster* were given access to both 1M sucrose and 2M erythritol as separate solutions, fly longevity was reduced to 50% after two weeks compared with sucrose alone (Baudier et al., 2014). Thus, *D. melanogaster* will feed on erythritol sufficiently in the presence of sucrose, and erythritol could be a detrimental.

While erythritol may not be detrimental to *D. suzukii* if sucrose is also available separately, erythritol was confirmed to have insecticidal properties against *D. suzukii*. In our survivorship

assay that combined sucrose and erythritol together, the mortality of *D. suzukii* rapidly increased with several ratios of the two sugars combined (Fig. 2D). Actually, the most significant result obtained is when sucrose/erythritol was provided at a 1:2 or 1:4 ratio. When *D. suzukii* were given 0.5M sucrose/water or 0.5/0.5M sucrose/erythritol, they had high survivorship. In this case, the sucrose concentration is 0.5M and the same as the sucrose concentration in the 0.5/2.0M sucrose/erythritol solution which induced a high mortality.

Although there is nothing known about the absorption and biochemical metabolism of ingested erythritol, it might be conceivable that a feedback loop of food intake regulates the crop emptying and hemolymph osmolality in the fly. In Diptera, both digestion and absorption are predominantly accomplished in the midgut and the control of food intake is closely related to the rate of crop emptying and hemolymph osmotic pressure in insects (Bernays and Simpson, 1982). Diffusion of sugars from the midgut to the hemolymph elevates the osmotic pressure, which in turn decreases the rate of crop emptying in the blowflies (Thomson and Holling, 1977).

Therefore, it is possible that the non-metabolizable and non-storable erythritol in *D. suzukii* might maintain high osmotic pressure in the hemolymph that decreases the rate of crop emptying resulting in feeding inhibition followed by starvation. Another postulation is that an abnormally high concentration of sugars from erythritol and sucrose diffused to the hemolymph from the midgut should demand a large amount of water. The water dilutes to reduce the osmotic pressure, and may also be used to hydrolyze the breakdown of sucrose to glucose and fructose, or to immediately excrete excess erythritol from the body. This extreme change in osmotic pressure and over-regulated physiological change could be a critical for the fly body. Sucrose ingested with erythritol should be metabolized through a specific enzymatic process, and then utilized or stored in the glycogen form. The increasing rate of the osmolality in the hemolymph has been discussed and different depending on metabolizable or non-metabolizable carbohydrate fed in the honey bee (Roces and Blatt, 1999).

Although flies would be more preferred to sucrose than erythritol in choice conditions (Fig. 3D), the actual amount of erythritol consumed was greater than sucrose for 72 h under no-choice conditions (Fig. 3B). This could be interpreted that if flies were fully satiated after feeding sucrose, they stopped feeding, whereas the fly continuously fed on erythritol for this period. Interestingly, the actual amount of ingested water by fly was a negative value which means the evaporation of water exceeded water consumption by the fly. When *D. suzukii* feed on non-nutritive erythritol, they continue to feed because they are still hungry. A similar result has been observed in *D. melanogaster* that fed more on erythritol than sucrose (Baudier et al., 2014). Under no-choice conditions, *D. suzukii* fed the least amount on water compared with sucrose and erythritol. *D. suzukii* may have fed more on erythritol than water under no-choice because the fly responded to the sweet taste from erythritol which is 60–80% as sweet as sucrose, or table sugar (Munro et al., 1998).

In contrast to the no-choice test, in the choice test with erythritol and water, flies consumed more water than erythritol. This result implies that additional water feeding might protect the fly against the high physiological osmotic pressure caused by erythritol in the hemolymph.

Since *D. suzukii* ingested more erythritol than sucrose in no-choice tests (Fig. 3B), flies would be expected to have more weight gain from erythritol than sucrose feeding. However, weight gain was similar among sucrose and erythritol-fed flies (Fig. 4). This may have resulted if flies excreted more erythritol after feeding. In mammals, erythritol as a sweet antioxidant could be converted to erythrose in the intestinal membranes and excreted by urination because erythrose has been found in the urine of erythritol-consuming rat (Den Hartog et al., 2010). In

insects, the physiological process of absorption, metabolism and excretion from ingested erythritol would be interesting for future research.

By measuring the sugar and glycogen content of flies after feeding, we could infer whether ingested erythritol or erythrose was metabolized into certain carbohydrates. The levels of sugars and glycogen were significantly elevated in mannitol, sorbitol, and xylitol-fed flies after 24 h and/or 48 h, but did not changed among erythritol and erythrose-fed flies. The result implies that those sugar alcohols were utilized for substrates to be converted into sugar metabolisms (Nelson and Cox, 2000). Yet, erythritol and erythrose might be absorbed into midgut and were not converted or synthesized into long chain carbohydrates such as glycogen, a common storage form. Interestingly, only flies fed sucrose had significantly higher sugar levels at 24 h while those fed mannitol, sorbitol, and xylitol had higher sugar levels at 48 h. This suggests that these sugar alcohols may be slowly converted to sugars and should have more metabolic pathways than sucrose metabolism. The lack of carbohydrate metabolism with an erythritol or erythrose diet, and slower storage with sorbitol or xylitol in this assay may be related to the complete mortality with erythritol or erythrose and lowered survivorship with sorbitol or xylitol in the dose assay.

5. Conclusion

Ingested erythritol or erythrose, as a possible derivative of erythritol, reduces the survivorship of *D. suzukii*, has no carbohydrate value, and has insecticidal properties. If other sugar sources are available in the field such as from dropped fruit, then erythritol might be mixed with sucrose to elicit more feeding. This discovery emphasizes the potential of using non-nutritive sugars as an insecticide alone or combined with conventional or biological insecticides to enhance efficacy. While the present research focuses on *D. suzukii*, it can be expanded to other Dipteran pests.

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References

Asplen, M.K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K.M., . . . Desneux, N., 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. Journal of Pest Science.

Baudier, K.M., Kaschock-Marenda, S.D., Patel, N., Diangelus, K.L., O'Donnell, S., Marenda, D.R., 2014. Erythritol, a non-nutritive sugar alcohol sweetener and the main component of Truvia®, is a palatable ingested insecticide. PloS one 9, e98949.

Beers, E.H., Van Steenwyk, R.A., Shearer, P.W., Coates, W.W., Grant, J.A., 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest management science 67, 1386-1395.

Bernays, E.A., Simpson, S.J., 1982. Control of Food Intake. Advances in Insect Physiology 16, 59-118.

Bolda, M., Goodhue, R.E., Zalom, F.G., 2010. Spotted wing *Drosophila*: Potential economic impact of a newly established pest. Giannini Foundation of Agricultural Economics, 5-8.

Burke, C.J., Waddell, S., 2011. Remembering nutrient quality of sugar in *Drosophila*. Curr Biol 21, 746-750.

Cowles, R.S., Rodriguez-Saona, C., Holdcraft, R., Loeb, G.M., Elsensohn, J.E., Hesler, S.P., 2015. Sucrose improves insecticide activity against *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 108, 640-653.

De la Fuente, M., Penas, P.F., Sols, A., 1986. Mechanism of mannose toxicity. Biochem Biophys Res Commun 140, 51-55.

De Ros, G., Conci, S., Pantezzi, T., Savini, G., 2015. The economic impact of invasive pest *Drosophila suzukii* on berry production in the Province of Trento, Italy. Journal of Berry Research 5, 89-96.

Den Hartog, G.J., Boots, A.W., Adam-Perrot, A., Brouns, F., Verkooijen, I.W., Weseler, A.R., . . . Bast, A., 2010. Erythritol is a sweet antioxidant. Nutrition 26, 449-458.

Diepenbrock, L.M., Rosensteel, D.O., Hardin, J.A., Sial, A.A., Burrack, H.J., 2016. Season-long programs for control of *Drosophila suzukii* in southeastern U.S. blueberries. Crop Protection 81, 76-84.

El-Kabbani, O., Darmanin, C., Chung, R.P., 2004. Sorbitol dehydrogenase: structure, function and ligand design. Curr Med Chem 11, 465-476.

FDA, U.S., 2014. High-Intensity Sweeteners, May 19, 2014 ed.

Fujita, M., Tanimura, T., 2011. *Drosophila* evaluates and learns the nutritional value of sugars. Curr Biol 21, 751-755.

Hiatt, H.H., Horecker, B.L., 1956. D-erythrose metabolism in a strain of *Alcaligenes faecalis*. J Bacteriol 71, 649-654.

Klick, J., Yang, W.Q., Lee, J.C., Bruck, D.J., 2016. Reduced spray programs for *Drosophila suzukii* management in berry crops. International Journal of Pest Management 62, 368-377.

Liu, L., Yi, T., Zhao, X., 2015. Antitumor effect of D-erythrose in an abdominal metastatic model of colon carcinoma. Oncology Letters 769-773.

Munro, I.C., Berndt, W.O., Borzelleca, J.F., Flamm, G., Lynch, B.S., Kennepohl, E., . . . Modderman, J., 1998. Erythritol: an interpretive summary of biochemical, metabolic, toxicological and clinical data. Food Chem Toxicol 36, 1139-1174.

Nelson, D.L., Cox, M.M., 2000. Lehniger Pricinpals of Biochemistry, 3rd ed. Worth Publishers.

O'Donnell, S., Baudier, K., Marenda, D.R., 2016. Non-Nutritive Polyol Sweeteners Differ in Insecticidal Activity When Ingested by Adult *Drosophila melanogaster* (Diptera: Drosophilidae).

J Insect Sci 16.
O'Donnell, K., Kearsley, M.W., 2012. Sweeteners and Sugar Alternatives in Food Technology, 2nd ed. John Wiley & Sons, Ltd.
Olson, D.M., Fadamiro, H., Lundgren, J.G., Heimpel, G.E., 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. Physiological Entomology 25, 17-26.
Roces, F., Blatt, J., 1999. Haemolymph sugars and the control of the proventriculus in the honey bee Apis mellifera. J Insect Physiol 45, 221-229.
Sampson, B.J., Werle, C.T., Stringer, S.J., Adamczyk, J.J., 2016. Ingestible insecticides for spotted wing Drosophila control: a polyol, Erythritol, and an insect growth regulator, Lufenuron. J. Applied Entomol. 141, 8-18.
SAS, 2010. SAS 9.2. SAS Institute, Cary, North Carolina.
SAS, 2015. JMP® 12.1.0. SAS Institute, Cary, North Carolina.
Sols, A., Cadenas, E., Alvarado, F., 1960. Enzymatic basis of mannose toxicity in honey bees. Science 131, 297-298.
Thomson, A.J., Holling, C.S., 1977. A model of carborhydrate nurition in the blowfly *Phormia regina* (Diptera: Calliphoridae). Can Entomol. 109, 1181-1198.
Tochen, S., Walton, V.M., Lee, J.C., 2016. Impact of floral feeding on adult *Drosophila suzukii* survival and nutrient status. Journal of Pest Science 89, 793-802.
Van Timmeren, S., Isaacs, R., 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection 54, 126-133.
Vander Meer, R.K., Lofgren, C.S., Seawright, J.A., 1995. Specificity of the red imported fire ant (Hymenoptera: Formicidae) phagostimulant response to carbohydrates. Florida Entomologist 78, 144-154.
Woltz, J.M., Donahue, D.J., Bruck, D.J., Lee, C., 2015. Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii*. J. Applied Entomol.
Zheng, C., Zeng, L., Xu, Y., 2016. Effect of sweeteners on the survival and behaviour of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Pest management science 72, 990-996.

Figure Legends:

Figure 1. Survivorship of *D. suzukii* in different doses of sucrose, erythritol, erythrose, xylitol, mannitol and sorbitol provided as a sole food source. Different letters in figures denote significant differences by log-rank analyses (statistical analysis in Table 1).

Figure 2. Survivorship of *D. suzukii* in the separate sugar (A and B) and combined sugar (C and D) tests. Different letters denote significant differences (by log-rank analyses (statistical analysis in Table 1).

Figure 3. Sugar feeding tests in different test regimes. (A) Diagram of no-choice test. (B) Consumed amount of water, sucrose and erythritol per *D. suzukii* adult at 72 h. (C) Diagram of choice test. (D) Consumed amount per SWD fly at 24, 48, and 72 h when two solutions out of water, sucrose and erythritol were provided. Asterisks denote a higher volume consumed for the given choice when data were analyzed for each hour. Asterisk (*) in D indicates a significant difference ($P < 0.05$) by Tukey HSD between two sugars. NS: no significance (statistical analysis in Table 1).

Figure 4. Change of body weight gain of SWD feeding water, sucrose and erythritol. Fly fed 1M sucrose and 1M erythritol for 48h. Male: $F_{2,6} = 6.0$, $P = 0.037$. Female: $F_{2,7} = 21.2$, $P = 0.001$. Different letters on the bars denote significant differences by Tukey HSD.

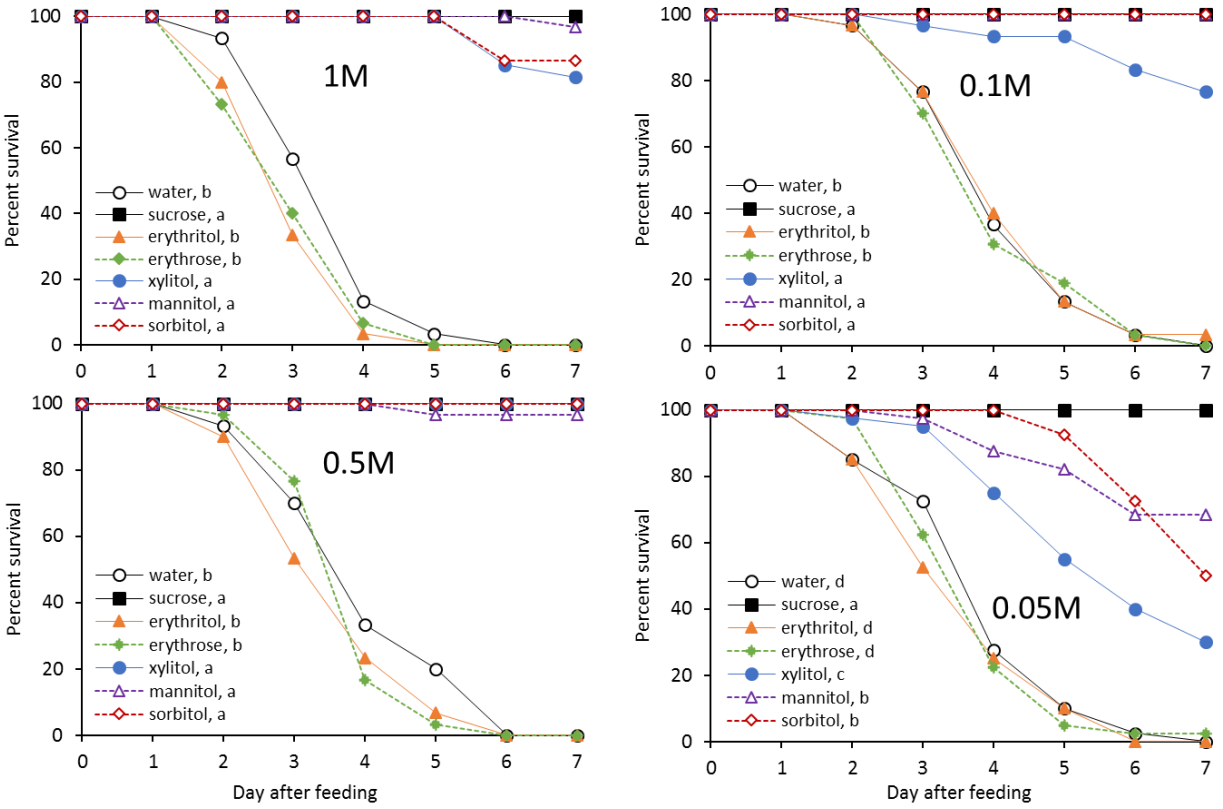
Figure 5. Average level of sugar in *D. suzukii* males and females when exposed to various sugar diets for 24 h (A) and 48 h (B). Different letters indicate significant differences analyzed by Dunnett's test (statistical analysis in Table 1).

Figure 6. Average level of glycogen in *D. suzukii* males and females when exposed to various sugar diets for 24 h (A) and 48 h (B). Different letters indicate significant differences analyzed by Dunnett's test (statistical analysis in Table 1).

Table 1. Kaplan-Meier log-rank test on survivorship between treatments, or the effects of diet treatment, time and their interaction on the volume ingested in the no-choice/choice study, and on sugar and glycogen content in the carbohydrate assay*.

Study	Depen-dent variable	Effect	df	F	P	df	F	P
Dose 1 M	Survival	Diet	6	250.7	<0.001			
Dose 0.5 M	Survival	Diet	6	251.2	<0.001			
Dose 0.1 M	Survival	Diet	6	231.4	<0.001			
Dose 0.05 M	Survival	Diet	6	230.0	<0.001			
Sep. solution	Survival	Diet	5	382.5	<0.001			
Comb. solu.	Survival	Diet	6	291.4	<0.001			
No-choice/choice study			**No-choice**			**Choice: Water or sucrose**		
	Volume ingested	Diet	2, 12	2.2	0.15	1, 4	0.36	0.58
		Hour				2, 8	1.4	0.30
		Diet x hour				2, 8	1.6	0.26
			Choice: Water or eryth.			**Choice: Sucrose or eryth.**		
	Volume ingested	Diet	1, 4	12.8	0.023	1, 4	13.2	0.022
		Hour	2, 8	13.1	0.003	2, 8	0.08	0.93
		Diet x hour	2, 8	2.6	0.13	2, 8	1.7	0.26
Carbohydrate assay			**Male**			**Female**		
	Sugar	Diet	6, 138	24.2	<.0001	6, 143	17.8	<.0001
		Hour	1, 138	14.16	0.0002	1, 143	1.43	0.233
		Diet x hour	6, 138	5.26	<.0001	6, 143	2.41	0.030
	Glyco-gen	Diet	6, 138	69.4	<.0001	6, 143	14.4	<.0001
		Hour	1, 138	174.5	<.0001	1, 143	81.6	<.0001
		Diet x hour	6, 138	9.63	<.0001	6, 143	1.88	0.088

*Measured total amounts of sugar and glycogen from 5-day old flies fed sugars in figures 1, 2, 3, 5 & 6.



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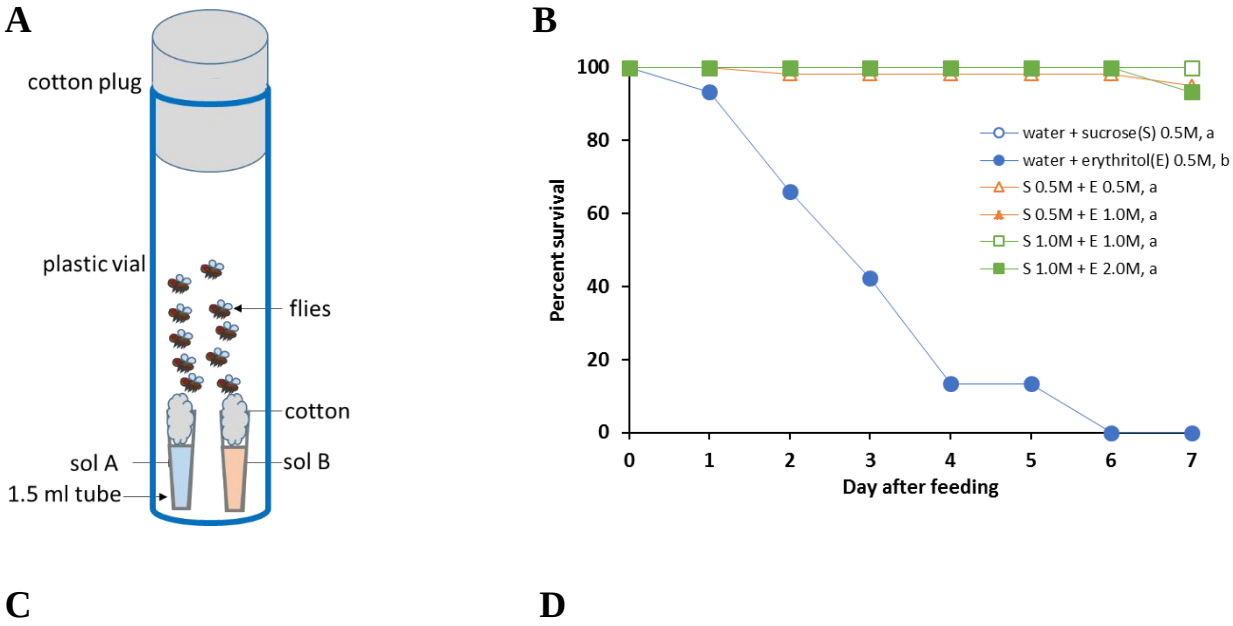

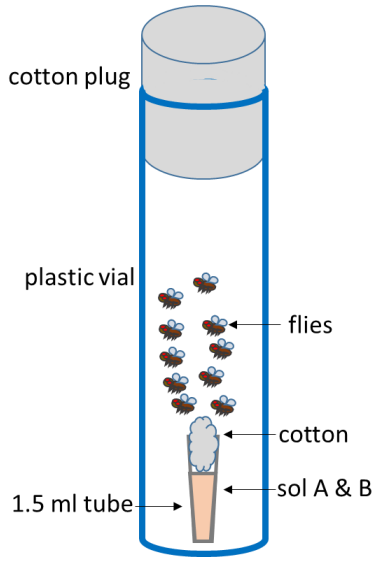

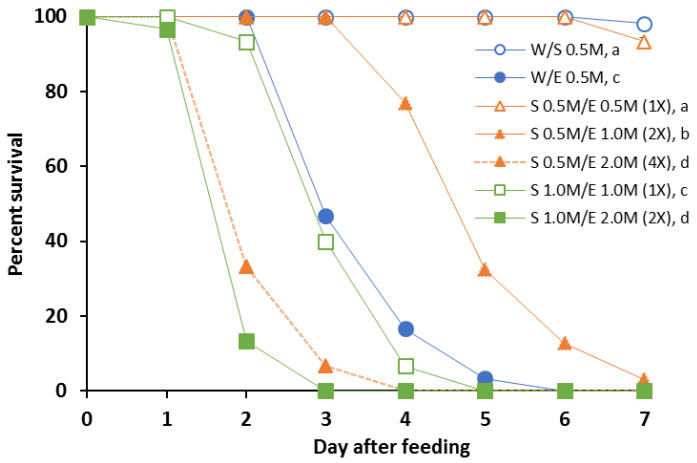

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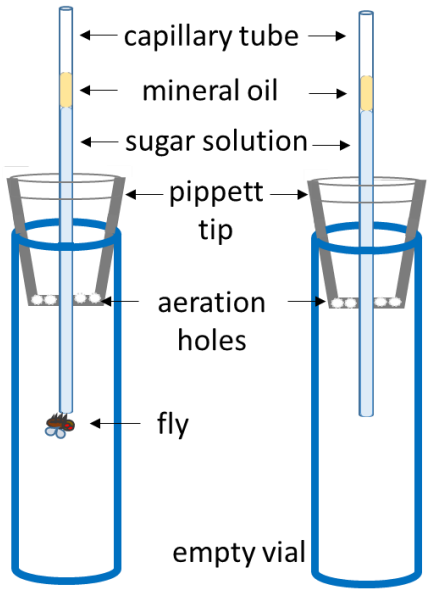

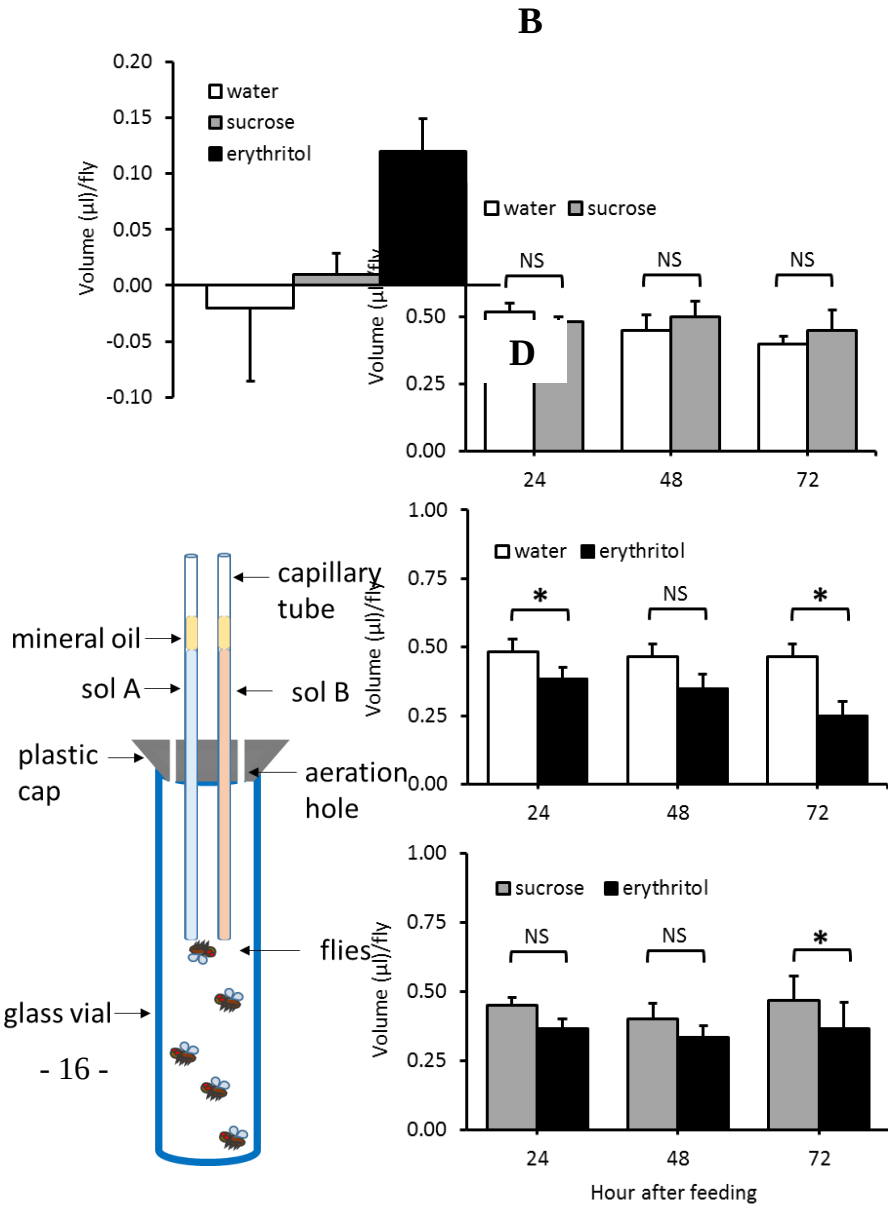

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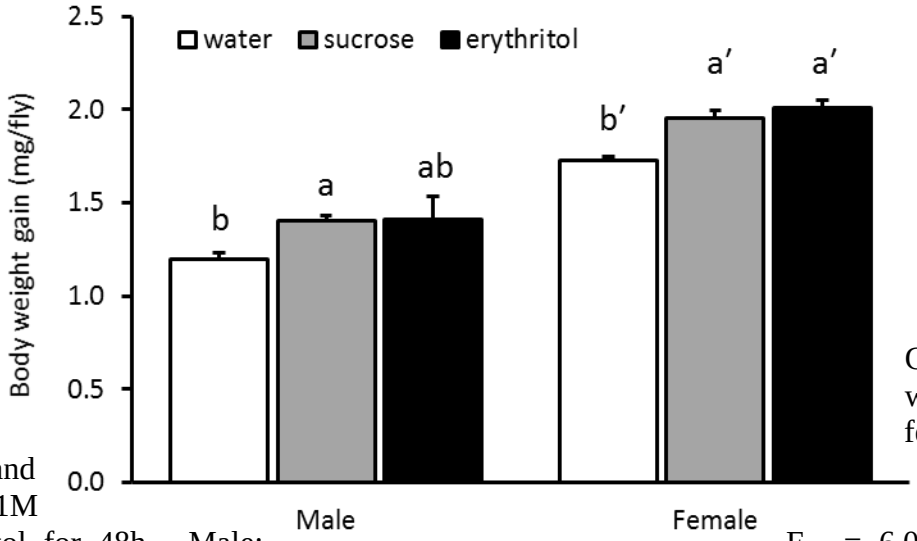

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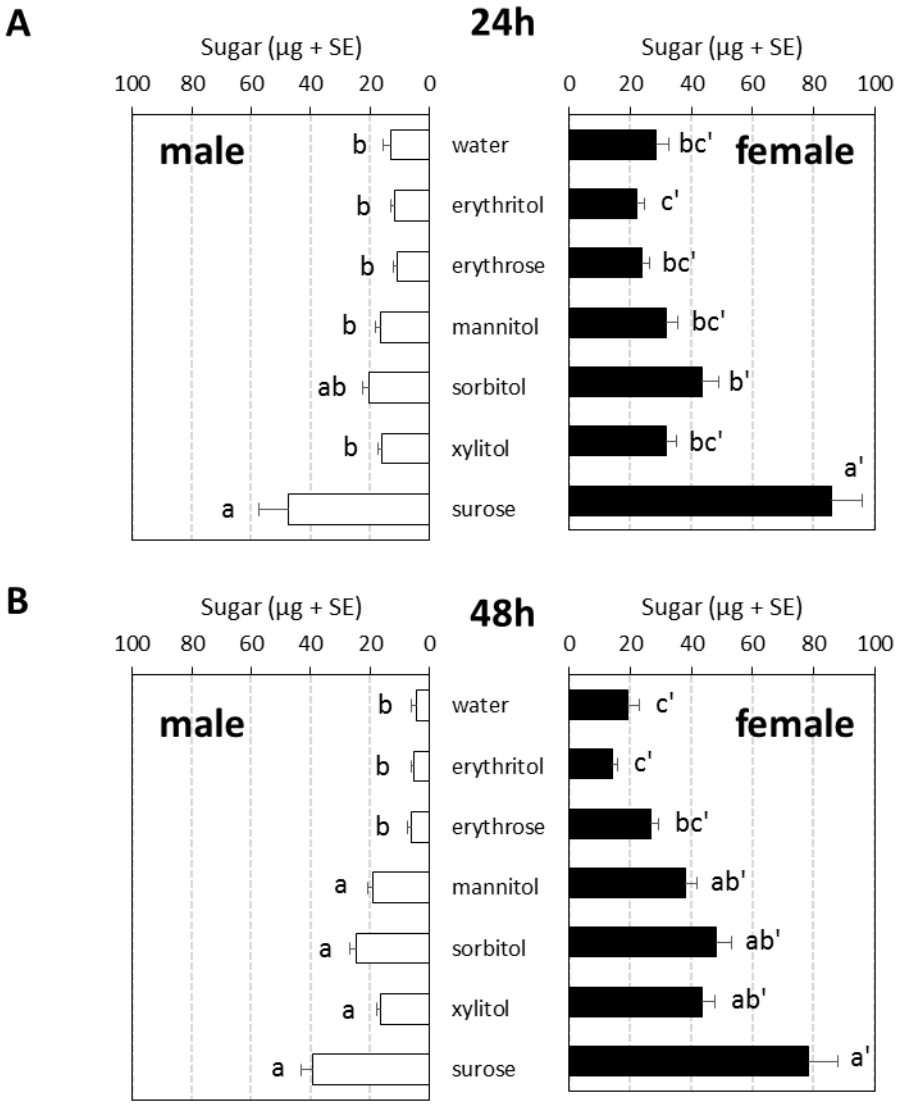

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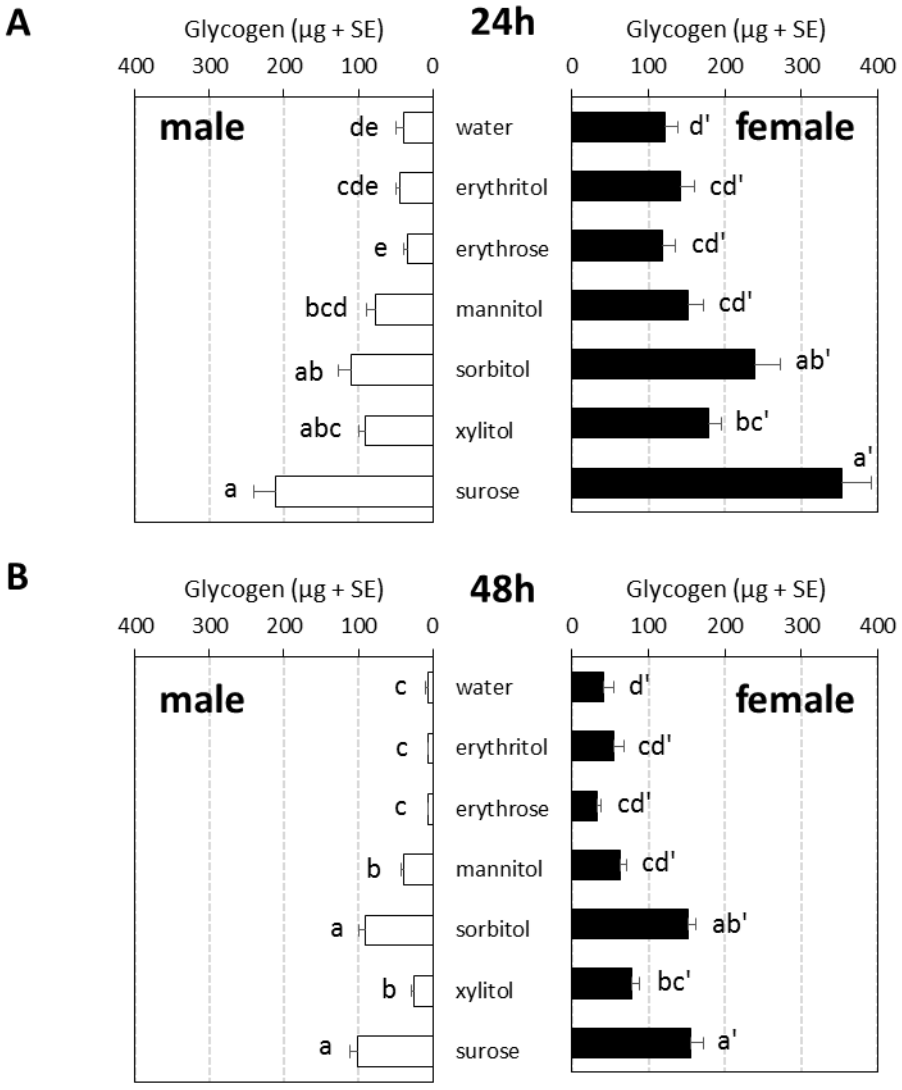

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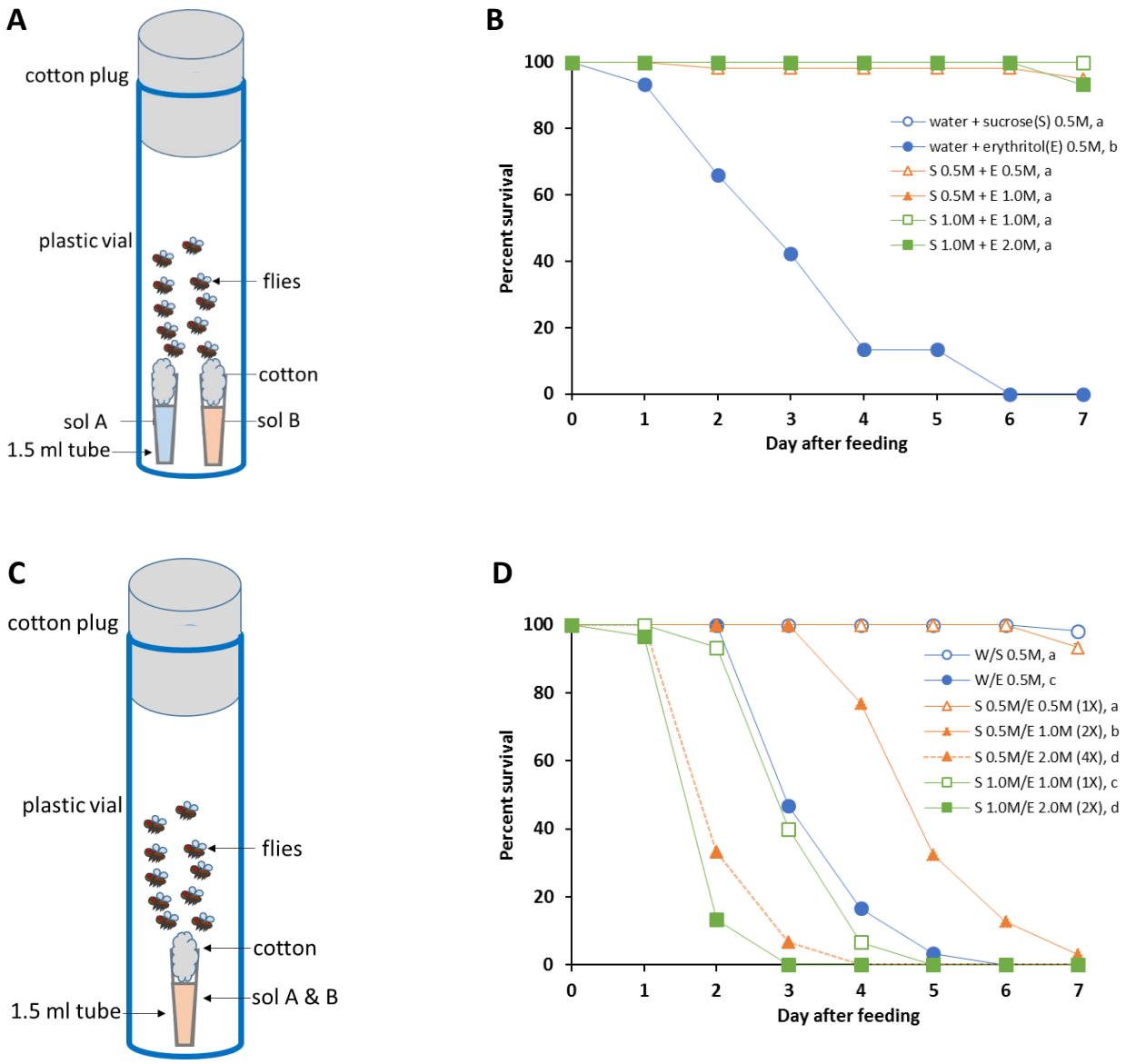

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