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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 to the fly. In a dose-dependent study, erythritol and erythrose significantly reduced fly longevity, with 100% mortality with 1, 0.5, 0.1 & 0.05 M doses after feeding for 7 days. When sucrose and erythritol 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 sucrose 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 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 microorganisms (Munro et al., 1998). The non-nutritive sugar is also 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 that the initial reduction of erythrose converts 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 learned the appetitive memory from continuous feeding (Burke and Waddell, 2011; Fujita and Tanimura, 2011). Although non-nutritious sugars contain a sweet taste these sugars were eventually excluded by the section of food from a long term memory learned by *Drosophila* fed these sugars.

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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 repeated 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 ± 5°C 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 × 95 mm height) and fed a dose of 1, 0.5, 0.1 or 0.05 M 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 0.5 M 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 + 0.5 M sucrose, water + 0.5 M erythritol, 0.5 M sucrose + 0.5 M erythritol, 0.5 M sucrose + 1 M erythritol, 1 M sucrose + 1 M erythritol, or 1 M sucrose + 2 M 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 (0.5 M/0.5 M, 0.5 M/1 M, 0.5 M/2 M, 1 M/2 M, or 1 M/2 M) 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 × 70 mm height, Fisher Scientific) (Fig. 3A). The solution (0.5 M) 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 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 on 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 (0.5 M), water + erythritol (0.5 M), or sucrose (0.5 M) + erythritol (0.5 M) 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.