AN ABSTRACT OF THE THESIS OF

Briana Price for the degree of Master of Science in Horticulture presented on December 1, 2021.

Title: Non-nutritive Sugars as a Management Tactic for Spotted-Wing Drosophila (*Drosophila suzukii*) and Non-Target Effects

Abstract approved:

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With confirmation of non-nutritive sugar, erythritol, having insecticidal properties to Dipterans, this thesis research focuses on use of erythritol as a human-safe management tool for small berry and cherry pest, *Drosophila suzukii*, commonly referred to as spotted-wing drosophila. *Drosophila suzukii* is a destructive invasive fruit fly from Asia with an advantageous ovipositor that allows females to lay eggs into ripening fruit. Current pest management involves revolving insecticide application of various classes of insecticides despite the well-known detrimental impacts to the environment, human health, and beneficial insects. This thesis covers three main topics concerning use of erythritol when combined with sucrose and another sweet non-nutritive sugar, sucralose. Erythritol has insecticidal properties to *D. suzukii* if they consume a lethal dose, so our formulation uses 1.5 molar concentration and is combined with something sweet to entice flies to feed a large amount. Testing sucralose is of particular interest because an erythritol+sucralose combination would be completely non-nutritional to spotted-wing drosophila, which consequently has potential to quicken mortality if flies are unable to use sucralose for energy.

First, laboratory tests were performed directly comparing efficacy between erythritol+sucrose and erythritol+sucralose formulations on *D. suzukii* feeding preference, survival, and oviposition behavior. We found that erythritol combined with sucralose enhanced feeding, quickened mortality, and reduced oviposition rates, in comparison to erythritol+sucrose

combination; thus, we suggest that sucralose could be a suitable, non-nutritional phagostimulant and replace sucrose for our purposes. Second, we explored how *D. suzukii* metabolizes sucralose as it is a chlorinated form of sucrose. We performed various physiological experiments to investigate whether sucralose can be converted to a usable carbohydrate in the fly body or if it is truly non-nutritive. Much like erythritol, sucralose consumption leads to starvation, heightened pressure inside the body and desiccation that negatively effects fly survival. Through anthrone and vanillin tests, we found that *D. suzukii* cannot convert sucralose to any usable bodily carbohydrates or storage in lipids. Third, we conducted multi-year field trials to test performance of our two erythritol formulations on blueberry cultivars and examined non-target impacts to honeybee brood (*Apis mellifera*) and western yellow jacket (*Vespula pensylvanica*).

During both years of field trials, leaf damage was observed which could indicate that our sprays are slightly phytotoxic to the various blueberry cultivars, although there was no detectable negative impact of our sprays on blueberry fruit quality (firmness, size, penetration force to pierce fruit epidermis and °Brix). Additionally, during field trials, yellow jacket visitation was plentiful to both erythritol+sucrose and erythritol+sucralose sprayed bushes. Yellow jackets are considered nuisance pests, thus we tested toxicity of these treatments by feeding these compounds to adults in a feeding assay. Our results showed little to no toxicity of erythritol or sucralose to yellow jackets. Honeybee visitation was scarce and similar among all treatments which indicates that they are not lured towards the sweet sprays. Although there was minimal visitation, the impacts of our erythritol treatments to honeybee brood was still of interest and this was tested by directly dripping treatment solutions into larval brood cells and monitoring mortality until completion of adult development. Our results showed that there is no detectable difference in toxicity of both erythritol and sucralose to honeybee brood in comparison to control (distilled water). Our formulations, erythritol+sucrose and erythritol+sucralose, kill and reduce oviposition of spotted-wing drosophila efficiently in lab settings, but were inconclusive in field settings because of low wild *D. suzukii* infestation rates and abnormally hot weather. This work provides vital non-target information as both of our formulations cause negligible non-target damage to blueberry fruit, yellow jackets, and honeybees.

Non-nutritive Sugars as a Management Tactic for Spotted-Wing Drosophila (*Drosophila suzukii*) and Non-Target Effects

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Briana Price, Author

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CONTRIBUTION OF AUTHORS

Briana Price designed and conducted experiments and helped analyze data. Jana Lee analyzed data, edited many draft revisions, designed spotted-wing drosophila field performance, mortality, and oviposition experiment methods, and provided guidance with experimental design for yellow jacket mortality experiments. Man-Yeon Choi edited draft revisions and provided guidance with experimental design for spotted-wing drosophila hemolymph and frass gas chromatography-mass spectrometry and feeding preference experiments. Ramesh Sagili and Carolyn Breece helped develop methodology to assess honeybee brood mortality and provided honeybee hives for the experiments.

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CHAPTER 1

General Introduction

Briana Price

1.1 INVASIVE DIPTERAN PESTS IN AGRICULTURE

The Food and Agriculture Organization (FAO) of the United Nations estimates that nearly 40% of global crops are damaged by insect pests and plant diseases each year, costing the global economy around US$220 billion from plant disease damage and US$70 billion from insect pests (*FAO*, 2015). There are two families of flies that are commonly referred to as fruit flies. The first, belong to the Tephritidae family; a large portion of this group contains species that are extremely destructive to a wide variety of commercially grown fruits, vegetables and ornamental plants (*USDA APHIS | Fruit Flies*, 2020). The other family is Drosophilidae; only a few species in this family are harmful to commercially-grown fruit such as *Zaprinonus indianus* Gupta (Steck & Steck, 2005), *Zaprionus tuberculatus* Malloch (Raspi et al. 2014) and the focal pest of this thesis work: *Drosophila suzukii* Matsumura (Bolda et al. 2010).

1.2 NATURAL HISTORY AND ECOLOGY OF SPOTTED-WING DROSOPHILA

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), also known as spotted-wing drosophila (SWD), is a polyphagous vinegar fly that favors small fruits such as berries and cherries. *D. suzukii* is native to Eastern Asia and was first observed in Japan in 1916, where it was observed to inflict serious damage to the cherry industry (Kanzawa, 1939; Walsh et al. 2011). In the last decade, SWD has rapidly expanded its range via fruit importation (Cini et al. 2012) to North America, South America and Europe (Asplen et al. 2015, Deprá et al. 2014) and has become an extreme threat to the small berry industry in the United States; the estimated economic impact in California, Oregon and Washington reaches US $511 million annually if untreated (Bolda et al. 2010, Goodhue et al. 2011). This species has been documented in Hawaii since 1980 without reports of any damage (Kaneshiro 1983, Nishida 1997, Beardsley et al. 1999, O'Grady et al. 2002) and first observed in the North American continent on commercial caneberry and strawberry fields in Watsonville, California in 2008 (Bolda et al. 2010). During the time *D. suzukii* was first observed in North America, the only other drosophilid pest in the Western U.S. was observed in 2006 – *Zaprionus indianus* – a pest to figs (Van der Linde et al. 2009). At first observation in North America, *D. suzukii* was mistakenly identified as *D. biarmipes* (Hauser, 2011) but detriment to ripening crops was quickly recognized. *D. suzukii* is unique as it lays eggs in developing or ripening fruit, unlike most drosophilids which lay eggs in rotten or fallen fruit. Since its arrival into North America, extensive research has been conducted

to understand its biology, physiology and most effective control methods because of the damaging impact on the small-fruit industry (Hamby et al. 2016). The common name, spotted-wing drosophila, refers to the single black spot present on both apical wings of mature males (Hauser, 2011). SWD is successful in invading a wide range of host fruits because of its morphologically advantageous female anatomy, the serrated ovipositor.

The serrated ovipositor allows easy puncture into ripe tough-skinned fruits (Atallah et al. 2014). In contrast, other *Drosophila* spp. with smaller, dull ovipositors restrict them to decaying or overripe fruits as hosts (Kenis et al. 2016). According to Atallah et al. (2014), *D. suzukii'*s closest relatives are *Drosophila subpulchrella, Drosophila biarmipes and Drosophila mimetica*. A figure from their paper (right) shows their model of the ovipositor evolution *D. suzukii* has established: enlarged, pigmented bristles, a sharper ovipositor tip, increased length to width ratio and ability to puncture tougher fruit skin such as grapes. Although *D. subpulchrella* has similar enlarged ovipositor bristles they are less successful in laying eggs through tough fruit skin and *D. suzukii* has a longer ovipositor (Atallah et al. 2014). Green et al. (2019) suggests the longer length of *D. suzukii* ovipositor in comparison to *D. melanogaster* is due to a combination of expanded apical cells and improved anisotropic rearrangements of the cells in the tissue.

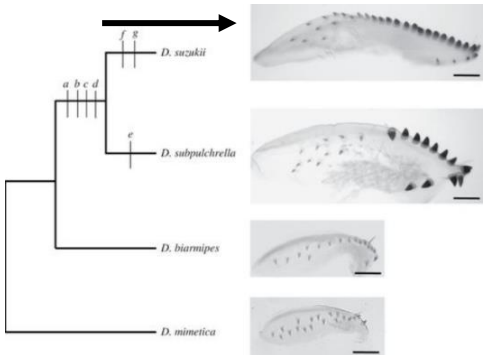

Figure 6 from Atallah et al. (2014). A model of ovipositor evolution. The phylogeny is based on previous work (Prud'homme et al. 2006, Barmina and Kopp 2007) and only the topology is shown. Lettering is used to indicate the timing of specific evolutionary changes. (*a*) Increase in ovipositor area. (*b*) Evolution of modified (enlarged, pigmented) ovipositor bristles. (*c*) Evolution of a sharper ovipositor tip. (*d*) Evolution of the ability to puncture the skin of raspberries and cherries. (*e*) Evolution of a distal bulb. (*f*) Evolution of a streamlined ovipositor (increased length to width ratio). (*g*) Evolution of the ability to puncture the skin of grapes. Scale bars, 50 µm.

After the ovipositor punctures through ripening berry skin, an egg is deposited and develops in 1-3 days. After the egg matures, larvae develop through three instars, usually taking 3-13 days (Kanzawa 1939, rev by. Cini et al. 2012), using the internal fruit pulp as nutrition (Deprá et al. 2014). The pupation period can last from 4-42 days depending on temperature and humidity (Tochen et al. 2014). While SWD does pupate within the fruit, it oftentimes exits fruit as wandering larvae, and pupates in the soil (Woltz & Lee, 2017). Adults live, on average, 20-56 days allowing for prolific reproduction (Tochen et al.

2014). Overwintering adults are known to survive more than 200 days (Poyet et al. 2015). It is estimated that over a few months, a single female can produce three-thousand offspring (Sampson et al. 2019). This pest is difficult to contain as an estimated ~13 generations can prosper with successful migrations to multiple hosts (Tochen et al. 2014). SWD uses visual, mechanical and odor cues to detect a suitable host (Cloonan et al. 2018). Cranberries do not attract *D. suzukii* and have been observed as unsuitable for oviposition given its thick fruit skin, though in a laboratory study SWD successfully laid and developed in wounded cranberries (Steffan et al. 2013). Since this pest has no natural enemies in the North America and has a specialized ovipositor and olfactory system, it has a unique biological niche and ability to use multiple host plant genera.

Along with utilizing a wide variety of small berries as hosts, SWD is known to use non-crop wild and ornamental plants as means of shelter or food source. *D. suzukii* has the ability to utilize plants from more than 19 host plant families, such as the genera *Cornus, Prunus, Rubus* and *Sambucus* (Kenis et al. 2016). Tochen et al. (2016) conducted an experiment with cherry blossoms to investigate feeding ecology with surrounding floral resources. Their results showed SWD live longer when given access to cherry blossom, indicating that the nectar (containing sucrose, fructose, and glucose) could be a useful nutrition source on and off-season (Lee et al. 2015). Surrounding wild vegetation in a crop system may harbor overwintering and early-season populations of SWD, so research into managing those areas is also being examined (Lee et al. 2015).

To better understand SWD nutritional physiology, carbohydrate assays can measure energetic reserves in insects manipulated under different conditions and give insight on behavior such as reproduction and flight (reviewed by Lee 2019). Carbohydrates are required for SWD adult survival and egg production, while protein is required for maturation (Plantamp et al. 2017). According to Plantamp et al. (2017) SWD is more likely to oviposit in healthy, ripe fruit but prefer feed on damaged fruit where carbohydrate content may be higher. With a heavily fruit-based diet, protein intake is limited; Bing et al. (2018) suggests that microbes eaten while consuming fruit play a key role in providing enough protein required for healthy egg and larval development. Adults of *D. suzukii* actively select habitat that will provide suitable space for mating and feeding; fruit volatiles are involved in this discovery (Cloonan et al. 2018). Like other vinegar flies, SWD is also actively attracted to fermentation odors. A female will locate a

site containing fermenting fruit knowing she will find suitable mates, then after mating, locates fruit suitable for oviposition (Karageorgi et al. 2017). Using this knowledge about life cycle, temperature tolerance, and host plants *D. suzukii* utilizes, as well as how *D. suzukii* scouts for its hosts, integrated pest management tactics can be combined with other current managements to limit destruction to a crop system.

1.3 CURRENT MANAGEMENT TACTICS FOR SPOTTED-WING DROSOPHILA CONTROL

One year from the initial detection of SWD, targeted control programs were implemented in the Pacific Northwest (Farnsworth et al. 2017). Due to its specialized ovipositor, they thrive in ripe fruit, so rotational insecticide application is commonly applied several times from fruit ripening to harvest. Strategic preparation is required when planning for application including, timing and sequence of numerous insecticides. Commonly used, effective conventional insecticide types to control SWD include: synthetic pyrethroids, organophosphates, spinosyns, and a few carbamates and neonicotinoids (Haviland & Beers, 2012).

Field application of conventional insecticides leaves residual that last 5-14 days and follow-up applications are dependent on pest pressure. Rotation of spray type and reapplication of sprays are usually required to keep SWD population numbers minimal (Bruck et al. 2011) and prevent development of insecticide resistance (Garcia, 2020). Residual of insecticides vary depending on equipment type, precipitation, water volume, canopy density, application type etc. (Haviland & Beers, 2012; Van Timmeren & Isaacs, 2013). Thousands of organic berry farms exist in the United States and many are located along the West Coast. In organic crop production, spinosad is the only available insecticide to control SWD that is certified by the Organic Materials Review Institute. Though there are regulations that require rotation to pyrethrin sprays to prevent resistance evolution; pyrethrin is not effective in killing SWD and rotations result in greater levels of infestation in organic production. There is evidence that SWD is building resistance against spinosad (Gress & Zalom, 2019). While continuous chemical control is effective in controlling SWD, it is difficult to simultaneously protect beneficial insects and not exceed maximum residue limit regulations, a measurement of pesticide residue present on food that is considered safe for humans to consume (Haviland & Beers, 2012).

There are various non-insecticidal, integrated-pest managements that effectively aid in managing SWD populations such as **biocontrol** (rev. by Lee et al. 2019), **RNAi-based control methods** (Abrieux and Chiu 2016; Murphy et al. 2016a; Murphy et al. 2016b; Taning et al. 2016; Ahn et al. 2019), **cultural control** (Rendon et al. 2020; Schöneberg et al. 2021)**, semiochemical control** (Hampton et al. 2014; Cloonan et al. 2018; El-Sayed et al. 2009; Githiomi et al. 2019), and **non-nutritive sugars** (Choi et al. 2017, 2019; Goffin et al. 2017; Sampson et al. 2017, 2019; Tang et al. 2017).

Biocontrol is defined as controlling a pest with a natural enemy (Roderick et al. 2012). Two resident pupal parasitoids are known to effectively control spotted-wing drosophila populations in the Pacific Northwest: *Pachycrepoideus vindemiae* Rondani (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) (Miller et al. 2015, Wang et al. 2018, rev. by Lee et al. 2019). Both species can locate SWD pupae in soil and fruit, though natural parasitism is generally below 10% as they are generalists and will parasitize other dipterans (Supp appendix [online only] rev by. Lee et al. 2019). *Trichopria drosophilae* is a pupal idiobiont endoparasitoid, laying eggs inside host tissue (Chabert et al. 2012), while *Pachycrepoideus vindemiae* is an ectoparasitic idiobiont, laying eggs between pupae and pupal casing (X. Wang & Messing, 2004). *Pachycrepoideus vindemiae* concurrently sustains itself by host-feeding on the pupae of SWD as well as parasitism with no effect on parasitoid offspring (Bezerra Da Silva, Price, & Walton, 2019). Both species are considered efficient biological control parasitoids; *P. vindemiae* has parasitism capacity of 400-600 pupae in a lifetime (Bezerra Da Silva, Price, Soohoo-Hui, et al. 2019) and has wider temperature tolerance than *Trichopria drosophilae* (X.-G. Wang et al. 2018) whereas *T. drosophilae* has shown higher parasitism rates when in presence of competitors (X.-G. Wang et al. 2016). Another biological control option includes using fungi. Although field trials of fungal sprays were deemed less effective, fungal spores could be used as a ‘lure-and-infect’ strategy to trap and inoculate *D. suzukii* (Yousef et al. 2018, rev. by Lee et al. 2019).

RNA interference (RNAi) control uses double stranded RNA (dsRNA) to initiate sequence specific gene silencing. Introduced dsRNA debases complementary messenger RNA (mRNA). Without critical mRNA coding the targeted genes, the organism will die (Murphy, West, et al. 2016). Control of many Lepidopteran, Coleopteran, Hemipteran, and Dipteran pests (Mamta and Rajam 2017) have been considered successful through various dsRNA delivery

methods such as use of transgenic plants (Baum et al. 2007; Mao et al. 2007), bacteria-mediated feeding (Ahn et al. 2019; Zhu et al. 2011), oral feeding and microinjections (Taning et al. 2016). Taning et al. (2016) was first to investigate efficacy of RNAi on *Drosophila suzukii*; results indicated that RNAi was functional in SWD and inducible through both microinjection and orally ingesting dsRNA. To demonstrate efficacy of orally delivering dsRNA, Murphy et al. (2016) demonstrated that SWD ingestion of genetically modified yeast (Abrieux & Chiu, 2016) is a successful delivery method; locomotor activity and egg laying decreased. They suggest that the primary cause of decreased fitness was certainly due to RNAi successfully silencing the target-genes as the other experimental Dipterans such as *D. melanogaster* and *D. simulans* showed no effect on their fitness.

Since *D. suzukii* occupy wild vegetation surrounding crop systems, habitat alteration such as removal of a host plant or fruit could help manage the pest within the surrounding landscape but there is little information available on the efficacy of this in reducing infestation (Lee et al. 2015). *D. suzukii* has the ability to pupate below the soil surface and cultural control methods such as mulching and using sawdust or weed mats below crop bushes can inhibit larvae from burrowing under the surface to pupate (Rendon et al. 2020). Other cultural control methods utilized include exclusion netting, pruning, sanitation or picking up fallen berries and creating an unfavorable microclimate inside a host bush can help reduce *D. suzukii* infestation (Schöneberg et al. 2021)

Semiochemical control uses volatiles to alter SWD behavior; earlier mentioned, adults of *D. suzukii* actively select habitat that will provide suitable space for mating and feeding through fruit volatiles such as fermentation odors. Using knowledge of chemical ecology and behavior of SWD, attractants and deterrents can be utilized to control invasion of crop systems. Mass trapping, push-pull and attract-and-kill technology has been developed using volatile lures. Mass trapping has been deemed ineffective because only a small portion of the flies may drown in the trap while the bigger portion still succeeds in infesting surrounding fruit (Hampton et al. 2014). Another less-effective tool incorporating odors is the push-pull system which involves using both attractants and deterrents; aversive odors being placed within the crop system and attractive odors outside of crop system (Wallingford et al. 2018). Attract-and-kill method is most useful in reducing the risk of a spill-over effect (El-Sayed et al. 2009; Githiomi et al. 2019). A successful

strategy uses a combination of attract-and-kill approach with targeted insecticide application (Cloonan et al. 2018).

To expand selections of integrated management tactics effective in controlling SWD, research into efficacy of non-nutritive sugars as human-safe insecticides have been conducted (Choi et al. 2017, 2019; Goffin et al. 2017; Sampson et al. 2017, 2019; Tang et al. 2017) and are explored further in this thesis.

1.4 A NEW, PROSPECTIVE CONTROL TACTIC: NON-NUTRITIVE SUGARS, ERYTHRITOL AND SUCRALOSE

Erythritol (also known as meso-erythritol) is a tetrose sugar alcohol that is naturally produced in a wide variety of plants such as mushrooms, grapes and watermelon (Shindou et al. 1989; Corti, 1999) and fermented foods (Bernt et al. 1996). Currently, a cost-effective method to manufacture erythritol on an industrial scale uses biotechnological processes such as fungal or lactic acid bacterial fermentation. Large scale production of erythritol commonly uses glucose from hydrolyzed corn or wheat as the principal substrate and then fermenting-microorganisms are separated through ion exchange chromatography (Rzechonek et al. 2018). Sucralose is a substituted disaccharide synthesized by chlorination of sucrose, changing its molecular structure. Three hydroxyl groups of sucrose are replaced with chlorine ions, making sucralose. It is considered a high-intensity non-nutritive sugar as it is 600× sweeter than sucrose (Binns 2003; Qiu et al. 2007).

Erythritol and sucralose are safe for human consumption at high levels. Due to the chemical composition of erythritol, it does not stimulate changes in blood insulin levels and it is easily absorbed into the small intestine instead of being metabolized (Munro et al. 1998). Sucralose is also poorly absorbed in humans and does not accumulate in body tissues (Binns, 2003). Both erythritol and sucralose have become increasingly popular to use as a food additive and zero-calorie sugar substitute, especially for those with diabetes and obesity (Grotz & Munro, 2009; Moon et al. 2010). In recent years, erythritol has been observed as insecticidal to *Drosophila melanogaster* (Baudier et al. 2014; O'Donnell et al. 2018), *Bactocera* flies (Zheng et al. 2016), house flies (Burgess and King 2017; Fisher et al. 2017), mosquitoes (Gilkey et al. 2018), termites (Caponera et al. 2019), psyllids (Wentz et al. 2020), spider mites (Schmidt-Jeffris

et al. 2021) and *D. suzukii* (Choi et al. 2017; Goffin et al. 2017; Sampson et al; 2017, 2019; Tang et al. 2017)

The earlier literature that pioneered exploration of non-nutritive sugars and insects was conducted by Baudier et al. (2014). They investigated effect name-brands Truvia, Equal, Splenda, Sweet'N Low and PureVia versus nutritive sugar, sucrose, on the survival of common fruit fly, *Drosophila melanogaster.* Their results showed high mortality in flies which fed on Truvia treatments - Truvia is a stevia-based sweetener with erythritol being main ingredient; this suggested that erythritol was highly insecticidal to *Drosophila* spp. when ingested (Baudier et al. 2014). All other non-nutritive sugars had little to no influence on mortality, including Splenda® which main ingredient is sucralose (Grotz & Munro, 2009).

Various sugars (i.e sucrose, glucose and fructose) in soft-skinned fruits are the main energy source for *Drosophila* spp. and nutritional value of sugars can be detected and learned through adaptive memory (Burke & Waddell, 2011). A sugar resource is recognized by gustatory receptors (Amrein & Thorne, 2005) and is sought after in a crop system; if sugar is added to other insecticides it can have a phagostimulant effect, or stimulates increased insect feeding (Allan 2011; Tochen et al. 2014; Cowles et al. 2015; Roubos et al. 2019). Erythritol by itself has been demonstrated as a phagostimulant candidate to replace sucrose in the entomopathogenic fungus for the house fly (Burgess et al. 2018), and in insecticides used in *D. suzukii* management (Gullickson et al. 2019). However, the sweetness of erythritol is 30% lower than sucrose that would be less attractive to flies. In a choice study, *Drosophila melanogaster* readily consumed erythritol in presence of sucrose and died. This gives promise to erythritol as an effective insecticide, even more so if combined with sucrose (Baudier et al. 2014).

To investigate if erythritol acts as an effective insecticide to SWD, various experiments have tested responses in mortality, fecundity, and physiological processes. Choi et al. (2017) examined feeding preference between erythritol and sucrose in a choice study, sucrose was preferred. However, in a no-choice assay, the volume of erythritol consumed was higher than sucrose, suggesting that the fly may have not been satiated after feeding (Choi et al. 2017). After testing a sucrose-erythritol combination, within which flies had adequate nutrition to maintain themselves, they still died. This indicated an insecticidal property in erythritol beyond just starving.

Tang et al. (2017) investigated physiological effects from consumption of erythritol and measured sugar levels present in hemolymph and excrement (frass) in erythritol versus sucrose-fed flies. Excretion rids of un-used waste because erythritol cannot be metabolized. Results showed that erythritol-fed flies excreted much more often than sucrose-fed flies. The amount of erythritol present in frass of flies was less than the amount present in the hemolymph. This indicates that the excretion process may have been hindered because of hyperosmotic pressure in the fly body (Tang et al. 2017). The digestive processes in *Drosophila* spp. is completed in the midgut and related to the rate of the crop emptying (Lemaitre & Miguel-Aliaga, 2013), it is possible that non-metabolizable erythritol slows the crop emptying, permitting faster diffusion into the hemolymph instead of processing through to the hindgut for excretion (Choi et al. 2017). Simple tetra-carbon molecules, like erythritol, are known to diffuse though mammalian intestinal membranes faster than hexose sugars, like sucrose (Mitchell, 2008; Munro et al. 1998). Erythritol cannot be metabolized into a usable energy or storage sugar, which ultimately creates hyperosmolarity in the fly body that interferes with normal muscle contraction needed for oviposition and excretion and flies will die after a few days. In summary, erythritol can kill *Drosophila suzukii* by hyperosmotic pressure generated in the fly body and by starving if no sucrose is present (Choi et al. 2017).

Five-day old flies are considered the most tolerant age of erythritol and were therefore used in Choi et al. (2017) assays exploring survivorship when fed various combinations of sucrose and erythritol. The fastest and highest mortality happened when fed 1M erythritol: 0.5M sucrose and 2M erythritol: 0.5M sucrose, resulting in 100% mortality in three to four days (Choi et al. 2017). Fecundity of erythritol-fed flies also decreased; this could have been confounded by these same females having a decreased lifespan (Tang et al. 2017). Since fewer females were alive in treatments with erythritol, fewer live females would be able to oviposit during the 7-d experiment. More studies were continued to determine if there were behavioral changes in oviposition rate while females remained alive, or physiological changes in their egg load.

Choi et al. (2019) tested flies with availability to feed on erythritol alone and erythritol-sucrose combination with normal blueberries for 2-d. Oviposition rates were reduced comparing to sucrose-fed (control), suggesting that fewer eggs were produced in their ovaries. Interestingly, dissection of the ovaries gave insight to the decreased egg laying; erythritol-sucrose-fed flies laid fewer eggs but had, but had more eggs still in their ovaries – this indicates that feeding on

erythritol somehow inhibits the oviposition mechanism or coated berries could deter oviposition (Choi et al. 2019). With success in killing and deterring oviposition, further examination in Chapter 2 of this thesis tests whether erythritol solutions are an ovipositional deterrent effect when flies were given blue berries dipped in those solutions.

Although there is coveted success with killing SWD with erythritol+sucrose formulas, using sucrose as a phagostimulant risks fueling *D. suzukii* with nutritional carbohydrates if flies do not ingest a lethal amount, or may encourage microbial development on the host fruits. Thus, the main theme of this thesis work is investigating the effectiveness of a non-nutritive phagostimulant to replace sucrose. Since sucralose, the main ingredient in Splenda®, is ~600x sweeter than sucrose, and safe for human consumption (Binns 2003), it has potential as a zero-calorie phagostimulant. To our knowledge, there is no current research on effects of sucralose on *Drosophila suzukii* behavior or physiology.

This thesis aims to bridge this unknown gap and develop a prospective completely non-nutritive, human-safe, insecticide option. During Chapter 2, we investigate the effect of non-nutritive sugar, sucralose, on *Drosophila suzukii* mortality and oviposition behavior as well as finding if sucralose is phagostimulative, or enticing, to flies during choice and no-choice capillary feeding assays. During Chapter 3, we examine physiological changes within *Drosophila suzukii* after consuming erythritol, sucrose and sucralose combinations. This includes finding out if sucralose is metabolized by *D. suzukii* by measuring sugar levels in hemolymph and frass of flies fed various combinations much like (Tang et al. 2017) and utilizing the anthrone and vanillin tests to quantify lipid, glycogen, and sugar reserves within flies ed various treatments. The nutritional chemistry behind anthrone and vanillin assays (Olson et al. 2000) involve an insect being crushed into a solution and centrifuged until a glycogen precipitate forms. Lipids react with a vanillin reagent and turn pink while sugars and glycogen react with anthrone and turn greenish-blue. After the reactions take place, densities are translated on an absorbance reader (rev. by Lee 2019). This tool has proven useful in many carbohydrate assays to explore SWD nutritional physiology: nutrient gain through floral feeding (Tochen et al. 2016), interaction between diet and flight (Wong, Cave, et al. 2018), nutrient acquisition from host berries with oviposition punctures (Wong, Wallingford, et al. 2018), nutrient reserves and metabolism between summer and winter morphs (Rendon et al. 2019; Wong, Wallingford, et al.

2018) and glycogen, as a long-chain sugar, in overwintering SWD (Toxopeus et al. 2016). In Chapter 4, we focus on field efficacy of our formulations and non-target effects, see below.

1.5 FIELD EFFICACY AND NON-TARGET EFFECTS OF NON-NUTRITIVE SUGARS

For these erythritol formulations to become available as a viable option to growers, it is helpful to understand how it performs in the field as well as non-target effects. Erythritol formulations are expected to perform efficiently in a field setting as aerosol sprays on small fruits but need investigation in multi-year field trials because of the presence of wild competing sugar sources. Since erythritol works through direct consumption, a concentrated dose, 1.5M erythritol combined with 0.5M sucrose (E+S) is tested in this thesis work during field trials (2020-2021), as well as testing our new completely non-nutritive formulation, 1.5M erythritol: 0.1M sucralose (E+Sul). In chapter 4, multi-year field efficacy trials test our focal erythritol formulations on blueberries around Corvallis, Oregon as well as examine non-target effects. Erythritol has been seen as supplemental to the development of radish, garlic and mushrooms (Kuroda et al. 2008) but phytotoxic to corn and tomato (Scanga et al. 2018). During the first year of field trials, slight foliage damage was seen on Elliot blueberry cultivar in 2020 that could be assumed phytotoxic; there was presence of speckled discoloration on leaf surfaces under sugar droplets so during 2021 trials, fruit quality was inspected.

Understanding the effect of erythritol on non-dipteran insects such as bees is imperative; bees are vital in pollinating flowering plants and trees, especially crop plants that humans rely on for food (Ollerton et al. 2011). While bees primarily visit crops while in bloom, some do visit post-harvest to use fallen fruits as food source when nectar sources are scarce (Shackleton et al. 2016). Large numbers of managed honeybee (*Apis mellifera*) (Hymenoptera: Apidae), hives are trucked to various crop systems across the U.S. to pollinate (Morse and Calderone 2000). Bees most often visit crops while they are in bloom, whereas our erythritol sprays would be applied post-bloom, minimizing exposure to pollinators while maximizing exposure to SWD.

The Oregon State University Honey Bee Lab tested the effect of erythritol on adult honeybees at a high-exposure rate in a laboratory cage-incubation study in 2018. The bees were fed and observed for seven days – after the experimental period, survivorship did not decrease in erythritol-fed bees compared to those fed only sucrose (Choi et al. 2019). Honeybee physiology is different than that of dipterans; nectar sources are stored in the honey stomach instead of

instantly diffused into hemolymph or insect blood. A main reason why erythritol is toxic to spotted-wing drosophila is because erythritol directly diffuses into hemolymph and flies do not have enzymes to metabolize it, thus high osmotic pressure is created inside the fly body (Choi et al. 2019). After storage in the honey stomach, bees bring back provisions and perform trophallaxis to nectar receiver workers who then either store as honey or feed developing brood (Wright et al. 2018). Although results showed no toxicity to adult honeybees, toxicity to developing brood is unknown. In chapter 4, we investigate non-target effects on *Apis mellifera* (honeybees) through berry-visiting frequency surveys, foliage visitation to sprayed areas in contrast to distance (m) from hives, and direct erythritol toxicity to developing honeybee brood. Yellow jacket foragers frequent fruit fields and are a nuisance pest during harvesting season because they are attracted to fallen fruit odors. *Vespula* spp. are strongly attracted to fruit odor cues from viable carbohydrate sources (Jarau & Hrncir, 2009). Given they are a nuisance pest and another non-target organism that we observed visiting erythritol sprayed bushes, we also explore the effect of erythritol on yellow jacket mortality during choice and no choice feeding assays in Chapter 4.

CHAPTER 2

Erythritol combined with non-nutritive sucralose increases feeding by *Drosophila suzukii,* quickens mortality and reduces oviposition

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Abstract:

Drosophila suzukii, spotted-wing drosophila, is a fly pest of small fruits and cherries often managed by chemical insecticides, and finding a convenient, human-safe alternative is a challenge. Erythritol, a non-caloric sugar, is safe for humans and toxic to *D. suzukii* by causing an osmotic imbalance. Combining erythritol with sucrose as a phagostimulant can enhance fly mortality. However, sucrose is sticky when applied on plants and provides flies with nutritional carbohydrate if a non-lethal dose is ingested. Therefore, our objective was to find a sucrose alternative, a non-caloric sugar that also has phagostimulative properties, where the formulation will enhance mortality, and reduce daily oviposition rates before females die. Through capillary no-choice and choice experiments, flies fed on 0.1M sucralose (Sul) readily, and flies consumed more 1.5M erythritol: 0.1M sucralose (E+Sul) formulation than erythritol alone. In eleven different mortality assays, E+Sul induced mortality as did the 1.5M erythritol: 0.5M sucrose (E+S) formulation, while E+Sul was more successful than E+S when *D. suzukii* could also drink water to offset water loss. Sucralose (0.1M) itself had modest insecticidal properties, but when combined with erythritol it increased the insecticidal effect. Both E+Sul and E+S formulations reduced oviposition when it was directly fed to *D. suzukii* in a cotton wick or applied on blueberry fruit. The latter suggests that the sugary coating on fruits has a deterrent effect. Given the potential of E+Sul, additional studies on the effect of sucralose on fly osmolar regulation, sugar metabolism, and field efficacy trials are underway to provide growers an efficacious tool.

Highlights:

- Sucralose combined with erythritol is phagostimulative to *D. suzukii*
- Erythritol and sucralose coated blueberries deter oviposition
- Erythritol:sucralose formulation induces fast mortality

Keywords: *Drosophila suzukii*, phagostimulant, erythritol, sucralose, pest management

1. Introduction

Erythritol, or meso-erythritol, is a sugar alcohol used in sugar substitutes such as Truvia®. Furthermore, it can be a human-safe insecticide as it is lethal to insects such as *Drosophila melanogaster* (Baudier et al. 2014, O'Donnell et al. 2018), *Bactocera* flies (Zheng et al. 2016), house flies (Burgess and King 2017, Fisher et al. 2017), mosquitoes (Gilkey et al. 2018), termites

(Caponera et al. 2019), psyllids (Wentz et al. 2020), and spider mites (Schmidt-Jeffris et al. 2021). The first studies with *Drosophila suzukii*, spotted-wing drosophila (SWD), confirmed toxicity of erythritol (Choi et al. 2017, Goffin et al. 2017, Sampson et al. 2017). Further research supported that consumption increased osmotic pressure in *D. suzukii* , in turn, decreasing motor skills such as muscle contraction needed for oviposition and excretion, and leading to death after 2-3 days (Choi et al. 2019, Tang et al. 2017). *Drosophila suzukii* is a serious pest of berry and cherry crops, with estimated crop losses of US$718 million annually if left unmanaged (Bolda et al. 2010). Finding effective management options that are non-toxic to humans that growers readily adopt is challenging. Therefore, our goal is to improve the effectiveness of erythritol formulations that can later be sprayed on field crops (Sampson et al. 2019).

First, erythritol formulations may be improved with a phagostimulant. Previously, combining erythritol with sucrose was more effective in inducing mortality than erythritol alone (Choi et al. 2017). In the same study, *D. suzukii* preferred sucrose over erythritol in choice studies over the course of 72 hrs. This could be due to sucrose being sweeter or its nutrient value which flies can learn through appetitive memory with continuous feeding (Burke and Waddell 2011, Fujii et al. 2015). Similarly, sucrose added as a phagostimulant to insecticides (Cowles et al. 2015, Roubos et al. 2019) has increased mortality by *D. suzukii* . However, using sucrose as a phagostimulant risks fueling *D. suzukii* with nutritional carbohydrates if flies do not ingest a lethal amount, or may encourage microbial development on the host fruits. Thus, we aimed to find a non-nutritive phagostimulant to replace sucrose. Since sucralose, the main ingredient in Splenda®, is ~600x sweeter than sucrose, and safe for human consumption (Binns 2003), it has potential as a zero-calorie phagostimulant. Our first objective examines the preferred concentration of sucralose, and tests phagostimulation and preferences between various sugar combinations in no-choice and choice consumption tests.

Previous studies revealed that the 2M erythritol: 0.5M sucrose formulation effectively killed *D. suzukii* (Choi et al. 2017) but the solution was observed to be too viscous. To develop a practical field formulation, a less sticky 1.5M erythritol: 0.5M sucrose formulation is considered here since lower erythritol concentrations were similarly effective (Tang et al. 2017). Also, survivorship of *D. suzukii* after consumption of sucralose alone or in formulation is unknown. Thus, our second objective compares *D. suzukii* survivorship on 1.5M erythritol: 0.5M sucrose and various sucralose formulations in eleven mortality assays.

Erythritols' lethality to *D. suzukii* is due to it being non-metabolizable and causing hyperosmolarity (Tang et al. 2017). Hyperosmolarity in an insect body can foreseeably interfere with physiological processes such as muscle contraction needed for digestion, excretion for unused waste or egg laying (Choi et al. 2017). In an attempt to restore homeostasis, insects may regurgitate or excrete excessively which results in water loss. Continuous water loss without replenishment can cause desiccation or "shriveled" bodies as seen in erythritol-fed *D. suzukii* (Sampson et al. 2017). In general, insects that drink water in a field setting could reduce the potency of erythritol by decreasing the hyperosmotic pressure, in turn, encouraging more excretion to rid of unused waste and bringing homeostasis back faster (Lee et al. 2021). Thus, our second objective not only assesses survivorship on various erythritol formulations but also survivorship on formulations in the presence/absence of water.

The serrated ovipositor of female *D. suzukii* allows them to attack intact fruit, thereby, disrupting oviposition is an important target. Erythritol consumption decreases *D. suzukii* female fecundity by shortening lifespan (Goffin et al. 2017, Sampson et al. 2017, Tang et al. 2017), and altering oviposition behavior (Choi et al. 2019). The latter study examined oviposition of *D. suzukii* before they died from erythritol. Previously, oviposition rates were reduced with erythritol- or erythritol:sucrose- feeding compared to a sucrose control, which we suspected was due to fewer eggs being produced among flies given some type of erythritol. Interestingly, dissection of the ovaries revealed that erythritol:sucrose-fed flies laid fewer eggs but had more eggs in their ovaries – this indicates that physiological imbalance by hyperosmotic pressure could interfere with muscle contractions of the ovipositor needed for egg laying. Since the impacts of sucralose feeding on oviposition are also unknown, our third objective tests how sucralose formulations affect oviposition rates. Though not tested in Choi et al. (2019), which compared how variously fed flies oviposited on normal berries, spraying fruit causes a sticky surface which may deter oviposition. Our third objective also tests whether oviposition differs from direct feeding or coating a fruit with formulations.

In Objective 1, we hypothesize that sucralose is phagostimulative, and needs only a low concentration due to its high sweetness. In Objective 2, we propose that sucralose combined with erythritol can be just-as or more effective in killing *D. suzukii* than when erythritol is combined with sucrose, including in the presence of water. In Objective 3, we propose that feeding on

erythritol:sucralose formulations will lower oviposition rates of females before they die, and that the sugar coating can be deterrent. These formulations could be a practical option for growers.

2. Materials and Methods

2.1 Flies and sugar solutions

Drosophila suzukii were reared at 22°C, 16L:8D and 60% RH on a cornmeal diet (Woltz et al. 2015). Experiments were conducted with the F_1-F_3 generation of flies from wild adults and were maintained at 22°C, 16L:8D and 60% RH as well. Each year, wild adults were collected from infested caneberries from Corvallis, Oregon throughout June to October to rear F_1 progeny on diet for experiments. As the parental generation died, F_1 flies were set up in parental cages to produce F_2 progeny, and later F_2 parents were set up to produce F_3. Emerged adult flies were maintained in cages with water and diet until 5-days old for experimentation since they were the most tolerant age (Choi et al. 2017).

Sugars used in this study were meso-erythritol (> 99%), sucralose (> 99 %), and sucrose (> 99%) (Fischer Scientific, Hampton, NH). All sugar solutions were dissolved in purified water. Sugar solution wicks or water control wicks consisted of soaked cotton in a 1.5 mL centrifuge tube.

2.2 Sucralose concentration for phagostimulation in capillary feeding assay

To find a suitable sucralose concentration for a phagostimulant effect, the capillary feeding assay was modified from Choi et al. (2017). Five female flies (5-6 days old) were aspirated into each glass vial (25 mm id x 60 mm length, Thermo Scientific, Rockwood, TN) with one glass capillary tube sitting in an inverted pipette tip, glued to a severed centrifuge tube lid (Fisherbrand Microhematocrit Capillary tubes, 70 µl, 1.1 mm id × 75 mm height, Fisher Scientific) (Fig. 3A). Flies were given sucralose concentrations: 0.001M, 0.01M, 0.05M, 0.1M and 0.5M or water, with a layer of mineral oil (Thermo Scientific) above the solution to prevent evaporation. Three identical vials filled with the corresponding solution with mineral oil layer, without flies, served as controls to measure evaporation for each treatment. Vials were placed in a secondary plastic container with water-soaked sponges to slow evaporation. The amount in the capillary tube was measured using a digital caliper after 48 h by marking the capillary tube with a sharpie ($mark_{start}$ and $mark_{48hr}$). This was replicated 15 times per treatment over several trial dates from November 25 to December 31 2020. The amount consumed was calculated using formula adapted from (Diegelmann et al. 2017); [food uptake (µL) = (measured distance (mm)/0.9 mm) – average

evaporation]. A generalized linear mixed model (GLMM) tested whether consumption varied by treatment as a fixed effect, and trial as a random effect using a gamma distribution. Statistics were analyzed in SAS 9.4 Proc Glimmix (SAS Institute 2016).

2.3 Capillary CAFÉ choice-test

To compare consumption when a choice of solutions is offered, the CAFÉ system was used with two capillary tubes. Five female flies (5-days old) were aspirated into glass vials as described above, per treatment. A severed centrifuge lid was modified to accommodate two capillaries with aeration holes. Various molarities of sucrose and erythritol reflect concentrations used in dose-dependent effects in Choi et al. (2017, 2019). Treatment solutions available for flies to feed on were combinations: 1) water vs. 0.5M sucrose, 2) water vs. 1.5M erythritol, 3) water vs. 0.1M sucralose, 4) 1.5M erythritol vs. 0.5M sucrose, 5) 1.5M erythritol vs. 0.1M sucralose, 6) 0.5M sucrose vs. 0.1M sucralose, 7) 1.5M erythritol: 0.1M sucralose vs. 1.5M erythritol, or 8) 1.5M erythritol: 0.05M sucralose vs. 1.5 M erythritol. Choice 7 and 8 tested the phagostimulative effect of sucralose compared to erythritol alone. All trial vials were set up as described above over several dates from February 17 to March 10, 2020. The amount in the capillary tube was checked after 24 h. Choice tests 1-7 and choice test 8 were replicated 17 times and 13 times, respectively with five flies in each replication. Due to erythritol inducing quick mortality, the number of dead flies was accounted for and results reported are “consumption per fly”. Consumption amounts as calculated above were compared in all eight choice tests with paired t-tests, with consumption from both capillary tubes within a vial as the pair. Lastly, a GLMM compared the consumption of erythritol:sucralose formulations in choice tests 7 and 8, with sucralose concentration of 0.05 or 0.1 M as the fixed effect, and trial as the random effect. The dependent variable was the consumption of the formulation divided by total consumption in the vial [erythritol:sucralose / erythritol:sucralose + erythritol] using a binomial distribution.

2.4 Mortality

Ten different mortality assays investigated the insecticidal effect from consumption of erythritol, sucrose and sucralose formulations. Ten adults, 5 male and 5 females (5-days old), were placed per container, and mortality was assessed daily for 7 days. The arena type, number of replicates, and sugar concentrations and ratios of formulations used in each assay are summarized in Table 1.

Mortality 1 and 2 assessed whether different concentrations of sucralose induce death when in combination with erythritol compared to a positive sucrose control. Also, erythritol:sucralose formulations were compared to erythritol alone to test for added potency and phagostimulation. While flies feeding on only non-nutritive sucralose are expected to die of starvation, Mortality 3 tested whether sucralose itself has insecticidal properties by comparing sucralosE+Sucrose formulations to sucrose alone. Mortality assays 1-3 were in conducted in a flat-bottom vial (28 mm id x 95 mm height, Genesee Scientific, San Diego, CA), with a solution-soaked cotton wick at the bottom of the vial.

Mortality 4-6 tested the efficacy of two less viscious formulations than the previously used 2M erythritol: 0.5M sucrose (Choi et. al 2019) in different sized arenas; a flat-bottom vial where fly mobility is limited (Mortality 4), a 946 mL (32 oz.) cup (Mortality 5), and a plastic cage (L23x W23 x H26 cm) where flies could fly (Mortality 6). In the cup, the solution-soaked cotton wick was hung from the lid with a wire. In the cage, the solution wick was placed sideways on the bottom. Wicks were replaced on day 4 to ensure the sugar was not crystalized in the cup and cage arenas.

Mortality 7 and 8 tested whether formulations still induce mortality when flies have access to water, with assays done in a 532 mL (18 oz.) cup. Each cup had one water wick and one treatment wick suspended from the top with wire. Mortality 9 compared mortality from consumption of erythritol formulations with and without water access, with assays done in a 946 mL cup. Wicks were replaced on day 4. Mortality 10 examined mortality from formulations when there is simultaneous access to 0.5M sucrose. Each cup had one 0.5M sucrose wick and one treatment wick suspended from the lid. Mortality 11 compared E+Sul and E+S formulations, sucrose or water to confirm that formulations induce death quicker than by starvation and support that formulations are phagostimulative. This has been confirmed with prior erythritol:sucrose formulations (Choi et al. 2017), but not for erythritol:sucralose.

In all mortality assays, the cumulative proportion of flies dead per day was compared in a GLMM, with treatment, day and their interaction as fixed effects, and treatment*replicate (i.e., specific vial) and date initiated as random effects. Because non-independent observations were made daily from each treatment*replicate, an autoregressive correlation structure was used for repeated measures. An appropriate error distribution was selected (i.e., binomial), and a Tukey

HSD separated means. For visualization, survivorship curves (the inverse of mortality) are shown, such that it is clear that no flies have survived when curves go to zero.

2.5 Oviposition

Oviposition 1 was a no-choice study testing whether treating berries with erythritol or sucralose reduced oviposition. Berries were dipped in: 1) water, 2) 0.1M sucralose, 3) 2.0M erythritol: 0.5M sucrose (older formulation in Choi et al. 2019, Tang et al. 2017), 4) 1.5M erythritol: 0.1M sucralose (E+Sul), or 5) water and presented alongside a vial of E+Sul in a 1.5 ml centrifuge tube plugged with cotton. In each replicate, 10 berries were dipped, air dried for 30 minutes, and placed in a L23 x W23 x H25 cm plastic cage with a water wick in a 59 mL (2 oz.) cup with a soaked sponge. Each cage had 5 female and 4 male *D. suzukii* that were 5-12 days old to provide reliable oviposition. After 24 h, berries were removed, and eggs laid on the berries were counted under a microscope. Then berries were reared for 2 weeks to monitor development. Treatments were replicated 15 times. The number of eggs laid, and proportion of eggs developing to adults were tested separately as dependent variables. In a GLMM, treatment was a fixed effect, and trial date as a random effect using a Poisson or binomial distribution. A Tukey HSD separated means.

Oviposition 2 was a choice study testing whether females preferentially laid eggs on berries that were untreated or treated with erythritol formulations. In each cage, 5 water-dipped berries were placed ~10 cm from 5 berries dipped in: 1) 1.5M erythritol: 0.5M sucrose (E+S), or 2) E+Sul. The experiment was set up as in Oviposition 1 using the same cages, water wicks and fly description. After 24 h, eggs on berries were counted, and berries were reared to monitor adult emergence. Treatments were replicated 15-16 times over. The number of eggs laid was compared in a paired t-test, choices from the water- or treated berry in the same cage were a pair. The proportion of eggs surviving to adulthood was compared in a GLMM with treatment as a fixed effect, and date as a random effect with a binomial distribution.

Oviposition 3 was a no-choice study testing whether females fed sugar solutions laid eggs differently when berries were untreated or treated with the same sugar solution. In each cage, 5 females and 4 males as described earlier were provided: 1) E+S vial & water-dipped berries, 2) E+S vial & E+S berries, 3) E+Sul vial & water berries, 4) E+Sul vial & E+Sul berries, 5) sucrose vial & water berries, or 6) sucrose vial & sucrose berries. Five berries and a water wick were placed in each cage. After 48 h, berries were removed for egg counting. All treatments were

replicated 15 times. The number of eggs laid was compared in a GLMM with vial, berry treatment (water vs. sugar) and the interaction as fixed effects, and date as a random effect with a Poisson distribution. Since differences between sugar- and water-dipped berries were most interesting among flies given the same food; treatments were compared by LSMeans sliced by vial treatments.

3. Results

3.1 Sucralose concentration for phagostimulation in capillary feeding assay

The no-choice capillary feeding results showed that consumption varied among treatments (GLMM $F_{5,33}$=5.60, P=0.0008). The quantities of 0.05M, 0.1M and 0.5M sucralose concentration consumed were similar to the quantity of water consumed which shows that there is no aversion to sucralose (Fig 1b).

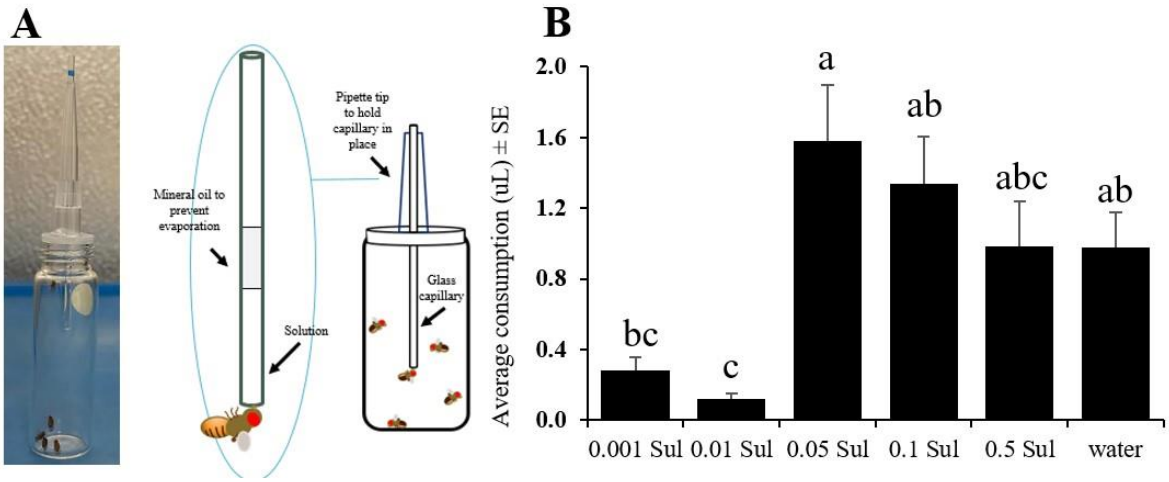

Figure 1. Testing the feeding preference of *D. suzukii* for sucralose in a CAFÉ system (A), and average amount consumed per vial in 48 h when various molar concentrations of sucralose (Sul) or water were provided (B); different letters denote statistical significance (*P <0.05) by Tukey HSD.

3.2 Capillary feeding choice-test

Between the choice of sucrose or water in Choice 1, flies preferred to feed on sucrose (paired t-test *P <0.0001). In Choice 2 and 3, there was no significant preference between water or erythritol (P=0.259), and water or sucralose (P=0.149), again confirming no aversion. When given the choice between sucrose and non-nutritive sugars, flies preferred sucrose over both erythritol (P* <0.0001) in Choice 4, and sucralose (*P <0.0001) in Choice 6. There was no preference when given choice between erythritol and sucralose (P=0.354) in Choice 5. Choice 7 and 8 tested erythritol alone versus two formulations of erythritol: sucralose to confirm that

sucralose was phagostimulative. When given choice between 1.5M erythritol alone and a erythritol:sucralose formulation, flies preferred to feed on the 1.5M erythritol: 0.1M sucralose solution (P=0.030) and 1.5M erythritol: 0.05M sucralose (P=0.012) (Fig. 2). Further analysis showed that there was no difference between 0.1M and 0.05M sucralose concentrations, in terms of the proportion consumed relative to total consumption in the vial (GLMM $F_{1,23}$=1.8, P=0.191).

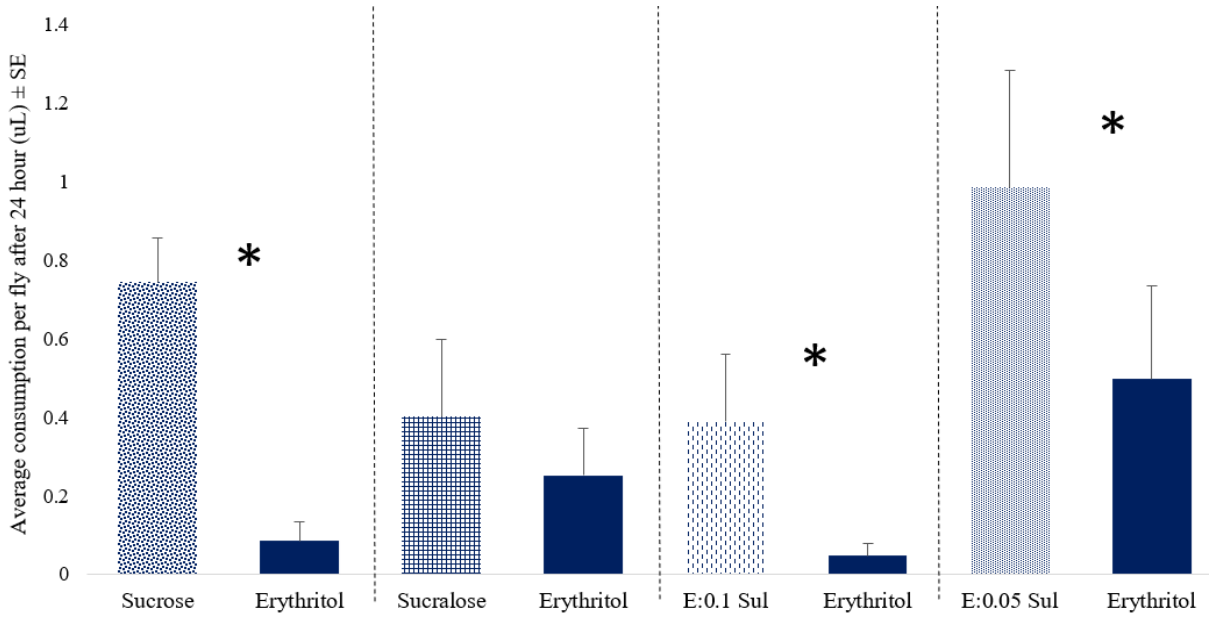

Figure 2. Average consumption per *D. suzukii* fly after 24 h when given a choice between two sugar solutions; E:0.1Sul = 1.5M erythritol: 0.1M sucralose, E:0.05Sul = 1.5M erythritol: 0.05M sucralose. Asterisks denote significant difference by paired t-test (*P <0.05).

3.3 Mortality

Statistics for all mortality assays are in Table 1, and graphs not shown in the manuscript are in Supplementary data. Two formulations repeatedly tested are abbreviated in the results as E+S (1.5M erythritol: 0.5M sucrose), and E+Sul (1.5M erythritol: 0.1M sucralose).

Mortality 1 assay showed that *D. suzukii* died within 4 days after consuming any formulation containing erythritol:sucralose, whereas *D. suzukii* lived to 5 days after consuming 0.5M erythritol alone. The formulations of 2M E: 0.1M Sul and 1.5M E: 0.1M Sul induced the fastest mortality, dying on average, within 36 h (Fig. 3a). Since the 0.1M sucralose concentration was comparable to the 0.05M in causing phagostimulation (Fig. 2), and the 0.1M sucralose in the erythritol formulation elicited quick mortality, 0.1M sucralose was further tested in the formulation ‘E+Sul’. Mortality 2 assay continued testing various sucralose concentrations with erythritol. Formulations with 1.5M erythritol fared similarly as with 2M erythritol when

sucralose was present at 0.1M or 0.01M concentrations (Fig. S1). Because 2M erythritol is stickier than 1.5M erythritol, subsequent assays used 1.5M erythritol, or E+S. In Mortality 3, *D. suzukii* died quicker when fed sucralose combined with 0.5 M sucrose compared to 0.5 M sucrose alone, with 69% compared to 83% survival after 7 days (Fig. S2). This shows that sucralose is modestly, though not highly toxic, to *D. suzukii*. A sucralose: sucrose formulation was no longer explored for inducing mortality.

Mortality 4-6 assessed *D. suzukii* survival with E+S, E+Sul, 0.5M erythritol or 0.5M sucrose in various sized arenas (Fig. 3b-d). In Mortality 4, where flies were in close proximity in a vial, E+Sul-fed flies died the quickest, followed by erythritol-fed and then E+S-fed flies, all dying within 2-4 days in comparison to the positive control sucrose. In Mortality 5 where flies were in 532 mL cups, with space to expel energy, E+S-fed and E+Sul-fed flies died quickest, within 2 days. In Mortality 6, flies in a cage had space to fly; E+S-fed and E+Sul-fed flies died quickest, within 24 h. These data show that with increased physical activity, death after consumption of erythritol and sucralose formulations is expedited.

Mortality 7 and 8 tested mortality when a separate water source is present with the solutions, and Mortality 9 compared mortality with or without a water source in the cup arena. With a water source present, Mortality 7 showed death within 3-4 days with erythritol alone and E+Sul formulation (Fig. S3a). Notably, E+S-fed flies survival decreased by only 33% within 7 days compared to sucrose controls. Mortality 9 showed that survivorship of *D. suzukii* significantly increased with water present than absent (Fig. 3e). About 34% more flies survived in E+Sul, and 79% more flies survived in E+S treatments with water by Day 2 than without water. Together, these results confirm that in the presence of water, E+S solutions are not as effective in killing *D. suzukii* than the zero-calorie E+Sul formulation. In Mortality 8, sucralose, water or erythritol alone, all killed *D. suzukii* within 4 days when in the presence of water (Fig. S3b). This is expected as these three solutions do not provide flies with nutrition. In Mortality 10, flies survived near 100% when given E+S, E+Sul or erythritol alone with a separate sucrose solution present. This suggests that *D. suzukii* may readily feed on sucrose if available. In Mortality 11, flies died quickest on E+S and E+Sul formulations with all dead by 4 days, and died slower on water over 7 days, and all survived on sucrose (Fig. 3f). This confirms that formulations induce faster death than starvation alone, and supports that formulations are phagostimulative.

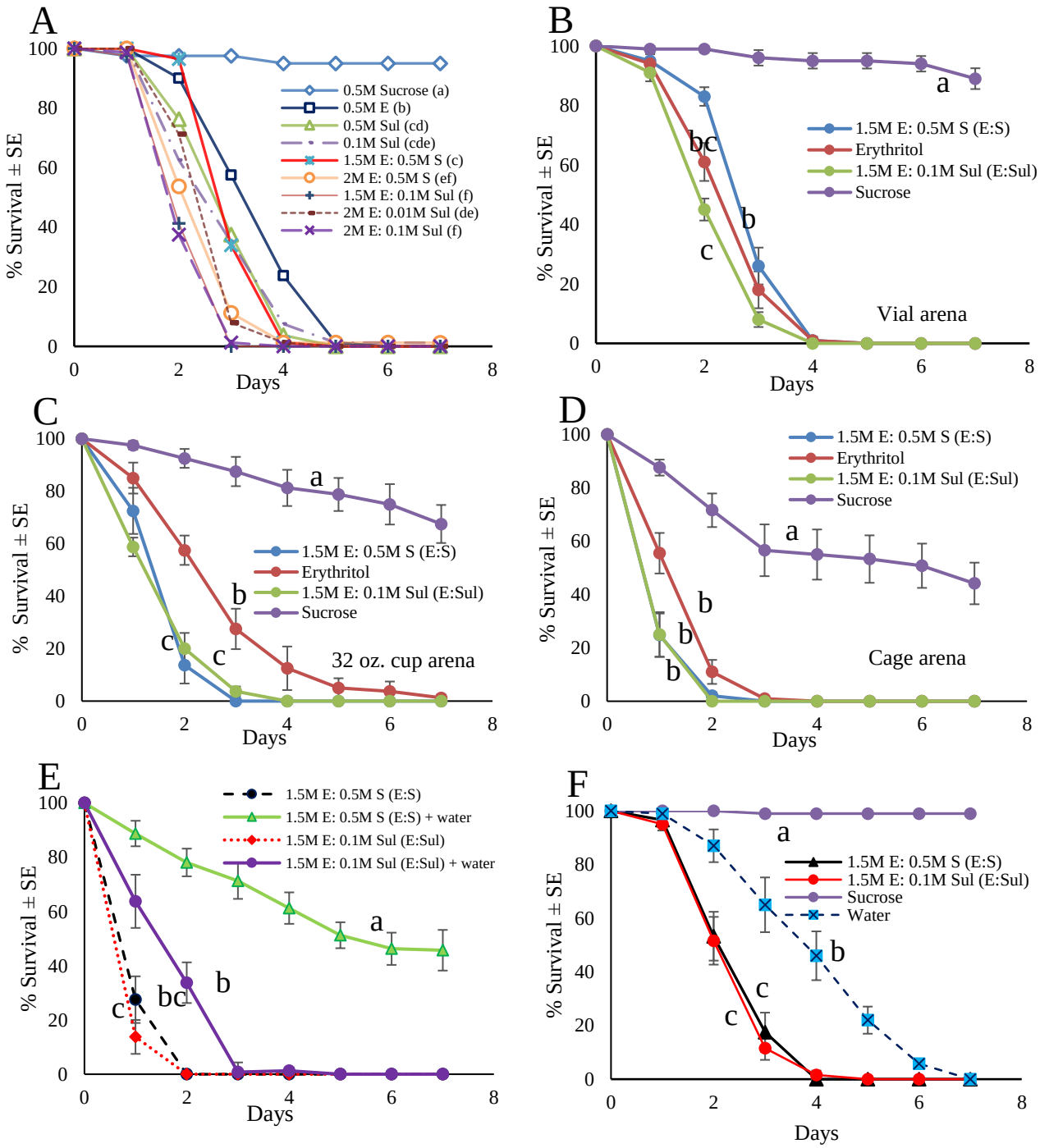

Figure 3. Survivorship of *D. suzukii* after consumption of various ratios of erythritol: sucralose in comparison to control (0.5M sucrose) in Mortality 1 experiment (A) (sucrose=S, erythritol=E, sucralose=Sul); the same four treatments compared in a vial in Mortality 4 (B), 32 oz. cup in Mortality 5 (C), and cage in Mortality 6 (D); comparisons between formulations in the presence or absence of a separate water source in a cage (E); comparisons between formulations, water or sucrose in vial, Mortality 11 (F). Different letters denote (*P <0.05) differences by Tukey HSD.

Table 1. Summary of experimental trials, replicates, treatments, and statistics from *D. suzukii* mortality assays from order of high to low mortality, or low to high survival (E = erythritol, S = sucrose, Sul = sucralose).

Exper.	Arena, Reps	Treatments (molar conc.)	Tukey[1]	Effects	F	(NDF, DDF)	P
Mort. 1	Vial	2 E: 0.1 Sul	a	Treatment	365.3	8, 59	< 0.001
	8 reps	1.5 E: 0.1 Sul (E+Sul)	a	Day	1380	6, 381	< 0.001
		2 E: 0.5 S	ab	Treat*day	34	48, 381	< 0.001
		2 E: 0.01 Sul	bc				
		0.1 Sul	bcd				
		0.5 Sul	cd				
		1.5 E: 0.5 Sul	d				
		0.5 Erythritol	e	Fig. 3 shows treatments in reverse from highest			
		0.5 Sucrose	f	to lowest survival			
Mort. 2	Vial	2 E: 0.01M Sul	a	Treatment	314.2	9, 30	< 0.001
	4 reps	2 Erythritol	ab	Day	480.3	6, 180	< 0.001
		2 E: 0.002 Sul	abc	Treat*day	10.4	54, 180	< 0.001
		2 E: 0.1 Sul	abc				
		1.5 E: 0.1 Sul (E+Sul)	abc				
		1.5 E: 0.01 Sul	abc				
		1.5 Erythritol	bdc				
		1.5 E: 0.002 Sul	cd				
		0.5 Erythritol	d				
		0.5 Sucrose	e				
Mort. 3	Vial	0.1 Sucralose	a	Treatment	145.0	4, 40	< 0.001
	9 reps	0.5 Erythritol	a	Day	91.4	6, 240	< 0.001
		0.1 S: 0.1 Sul	b	Treat*day	15.1	24, 240	< 0.001
		0.5 S: 0.1 Sul	bc				
		0.5 Sucrose	c				
Mort. 4	Vial	1.5 E: 0.1 Sul (E+Sul)	a	Treatment	460.5	3, 28	< 0.001
	8 reps	0.5 Erythritol	ab	Day	366.8	6, 168	< 0.001
		1.5 E: 0.5 S (E+S)	b	Treat*day	40.2	18, 168	< 0.001
		0.5 Sucrose	c				
Mort. 5	946 mL cup	1.5 E: 0.5 S (E+S)	a	Treatment	145.2	3, 28	< 0.001
	8 reps	1.5 E: 0.1 Sul (E+Sul)	a	Day	97.3	6, 168	< 0.001
		0.5 Erythritol	b	Treat*day	8.88	18, 168	< 0.001
		0.5 Sucrose	c				
Mort. 6	Cage	1.5 E: 0.5 S (E+S)	a	Treatment	47.5	3, 38	< 0.001
	12 reps	1.5 E: 0.1 Sul (E+Sul)	a	Day	45.4	6, 223	< 0.001
		0.5 Erythritol	a	Treat*day	2.74	18,223	0.0003
		0.5 Sucrose	b				
Mort. 7	532 mL cup	1.5 E: 0.1 Sul (E+Sul) + water	a	Treatment	918.1	3, 32	< 0.001
	10 reps	0.5 Erythritol + water	b	Day	413.8	6, 192	< 0.001
		1.5 E: 0.5 S (E+S) + water	c	Treat*day	108.3	18, 192	< 0.001
		0.5 Sucrose + water	c				
Mort. 8	532 mL cup	0.1 Sucralose + water	a	Treatment	1836.8	3	< 0.001
	7 reps	0.5 Erythritol + water	a	Day	664.8	6	< 0.001
		Water + water	a	Treat*day	74.5	18	< 0.001
		0.5 Sucrose + water	b				
Mort. 9	946 mL cup	1.5 E: 0.1 Sul (E+Sul)	a	Treatment	66.8	1, 28	< 0.001
	8 reps	1.5 E: 0.5 S (E+S)	ab	Moisture	138.7	1, 28	< 0.001
		1.5 E: 0.1 Sul (E+Sul) + water	b	Treat*moist	56.9	1, 28	< 0.001
		1.5 E: 0.5 S (E+S) + water	c	Day	7.92	6, 162	< 0.001
				Treat*day	1.72	6, 162	0.1184
				Moist*day	7.66	6, 162	< 0.001
				Tr*mo*day	6.0	6, 162	< 0.001
Mort. 10	532 mL cup	0.5 S + 0.5 S	ns	Treatment	0.15	3, 26	0.9266
	8 reps	1.5 E: 0.5 S (E+S) + 0.5 S		Day	2.17	6, 162	0.0482
		0.5 E + 0.5 S		Treat*day	1.13	18, 162	0.3315
		1.5 E: 0.1 Sul (E+Sul) + 0.5S					
Mort. 11	Vial	1.5 E: 0.5 S (E+S)	a	Treatment	314.9	3, 44	< 0.001
	12 reps	1.5 E: 0.1 Sul (E+Sul)	a	Day	165	6, 264	< 0.001
		Water	b	Treat*day	24.2	18, 264	< 0.001
		0.5 S	c				

[1] Letter indicates highest rate of mortality for treatments denoted by 'a' and decreasing rates.

3.4 Oviposition

In Oviposition 1 under no-choice, there was a significant effect of treatment in 24 h ($F_{4,62}$=38.6, *P <0.001), and the fewest eggs laid with berries dipped in 2M E: 0.5M S, a 74% reduction compared to the water-dipped berries (Fig. 4a). A 26% and 39% reduction in eggs laid was also found with untreated berries presented next to a vial of E+Sul and berries dipped in E+Sul, respectively.

Berries dipped in sucralose alone did not show a reduction. This supports that direct feeding on the E+Sul solution or treating berries in the solution lowers oviposition in 24 h. The proportion of *D. suzukii* eggs in these berries surviving to adulthood was also impacted by treatment ($F_{4,59}$ = 4.01, P=0.006), with highest percent survival in berries dipped in E+S or E+Sul versus water (65%, 59% and 45% mean survival, respectively). This result is probably due to the lower densities of eggs laid in treated berries, and hence higher survivorship. In any case, these results show that treating berries with erythritol formulations will not further lower development rates inside the berry. The main impact of the formulations is by preventing oviposition.

In Oviposition 2 under choice conditions, *D. suzukii* laid 70% and 44% fewer eggs on berries treated with E+S or E+Sul than treated with water in 24 h (paired t-test, P=0.0085, P=0.014, respectively, Fig. 4b). This shows a preference to lay eggs on non-sticky berries. The proportion of eggs surviving to adulthood was not different between water versus E+S-dipped berries ($F_{1,12.3}$ = 1.01, P=0.32) and marginally higher in E+Sul-dipped berries than water-dipped berries (70% and 38% mean survival, respectively, $F_{1,15}$=3.8, P=0.071). Like Oviposition study 1, this difference may reflect the lower density of *D. suzukii* eggs laid in treated berries, and higher survivorship. Again, there is no evidence that treating berries with erythritol will inhibit larval development.

In Oviposition 3 under no-choice, the solution given to *D. suzukii* in a vial, whether the berry was dipped in solution or water, and their interaction all affected the number of eggs laid in 48 h (Vial solution $F_{2,76}$ =8.4, *P <0.001, Berry dipping $F_{1,76}$ = 54.5, *P <0.001, Vial*berry $F_{2,76}$ = 4.5, *P=0.014). The sliced LSMeans comparisons were significant, where sugar- versus water-dipped berries were compared separately for each sugar vial treatment. Notably, flies that fed on E+S in vials laid 55% fewer eggs on E+S-dipped than water berries, flies fed sucrose laid 41.3% fewer eggs on sucrose-dipped than water berries, and flies fed E+Sul laid 23.4% fewer eggs laid

on E+Sul-dipped than water berries (Fig. S4). Dipping berries in some type of sugary solution, whether detrimental or not, deterred oviposition.

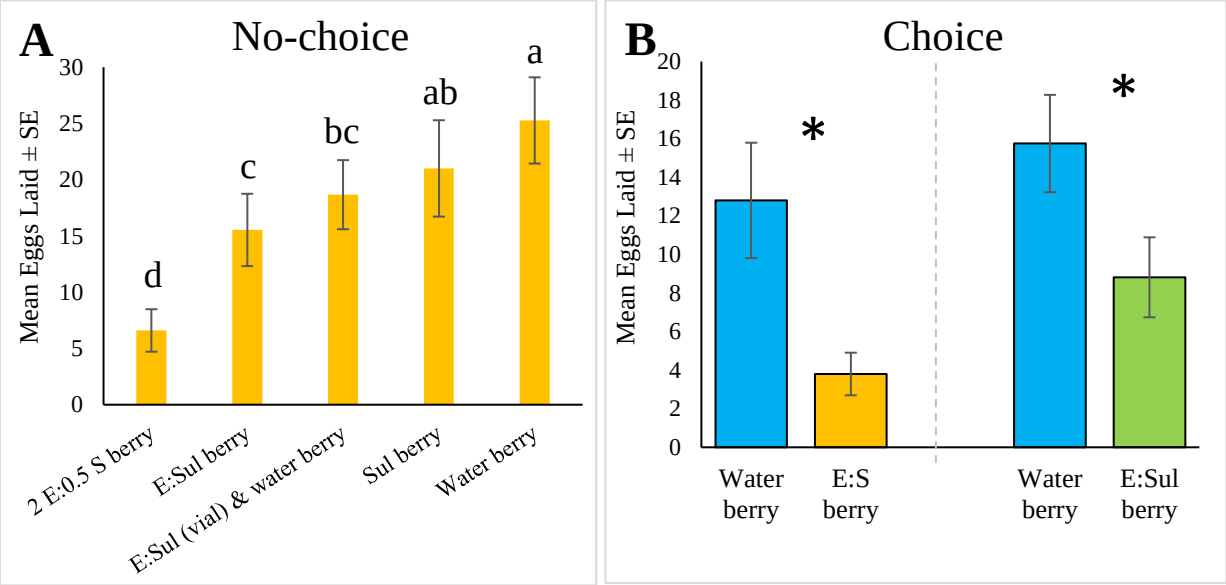

Figure 4. Mean number of eggs laid by *D. suzukii* females in variously treated blueberries (A), in treated versus water-dipped blueberries in choice cages (B); E+Sul = 1.5M Erythritol: 0.1M Sucralose, E+S = 1.5M Erythritol: 0.5M Sucrose. Different letters denote differences by Tukey HSD, and asterisks denote significant difference by paired t-test (*P <0.05).

4. Discussion

This study aimed to find a non-nutritive phagostimulant to replace sucrose in a previously researched erythritol:sucrose formulation using sucralose. By confirming enhanced feeding rates, quickened mortality, and reduced oviposition rates, sucralose is a suitable sucrose alternative. In capillary feeding assays, *D. suzukii* consumed 0.05M sucralose alone the most, followed by 0.1M over very light concentrations of 0.001 and 0.01M sucralose. Consumption of all concentrations of sucralose were comparable to water which means that sucralose is not as enticing as sucrose is, but flies were not deterred from feeding on it. In the capillary CAFÉ choice tests *D. suzukii* preferred sucrose over both erythritol and sucralose which was anticipated because *Drosophila* can sense differences in nutritive value (Fujii et al. 2015). Moreover, in choice tests between erythritol alone vs. erythritol:sucralose formulations, flies preferred to feed on formulations containing either 0.1M or 0.05M sucralose. While sucralose is not enticing alone, it elicits more feeding when combined with erythritol. Our mortality assays revealed that erythritol formulations with 0.1M sucralose were quickly detrimental in nine studies that included the E+Sul formulation. Four studies showed that flies fed E+Sul died quicker than erythritol alone,

and lastly, flies fed E+Sul died quicker than by starvation on water. These feeding and mortality results support the hypothesis of sucralose being phagostimulative, and a 0.1M sucralose concentration as appropriate.

To compare formulations, E+S (1.5M erythritol: 0.5M sucrose), E+Sul (1.5M erythritol: 0.1M sucralose), erythritol (1.5M) alone, and sucrose (0.5M) alone, were given to *D. suzukii* in different-sized arenas. E+Sul consistently induced total mortality within 2-4 days. Flies survived longer with E+S solutions in a vial-sized arena, though when placed into an area where they could move more or fly, there was no differences detected in mortality rate between E+S and E+Sul fed flies. Flies that were in a cage arena had lower survival rates in all treatments in comparison to smaller arenas like a plastic vial or 946 mL cup. A cage setting could allow more energy expenditure as experienced in field settings; all treatments containing erythritol (E+S, E+Sul and erythritol alone) induced mortality within 48 h. A previous trial questioned whether erythritol would still be detrimental while flies had access to wounded fruits. Choi et al. (2019) showed that erythritol:sucrose-fed flies still died quicker compared to sucrose-fed flies; it can be assumed that E+Sul will still be detrimental to *D. suzukii* survival in a natural setting where competing sugar sources are available. Field trials with other erythritol formulations likewise demonstrate efficacy in field settings (Sampson et al. 2017, 2019).

Efforts to understand the physiological processes of what makes erythritol toxic to insects suggests that consumption causes hyperosmolarity of the insect hemolymph. Excretion is the only means to rid of non-nutritive sugars when erythritol cannot be metabolized. (Tang et al. 2017) showed that erythritol-fed flies excreted more often than sucrose-fed flies. The amount of erythritol present in frass of flies was lower than in the hemolymph; indicating metabolic process may have been hindered because of high osmotic pressure. The digestive processes in *Drosophila spp.* is completed in the midgut and related to the rate of the crop emptying (Lemaitre and Miguel-Aliaga 2013). It is possible that non-metabolizable erythritol slows crop emptying, permitting faster diffusion into the hemolymph instead of processing through to the hindgut for excretion (Bernays and Simpson 1982). Choi et al. (2017) suggested that more water is required to dilute and decrease osmotic pressure, encouraging more excretion to rid of unused carbohydrate molecules. Our mortality assays also investigated whether E+S or E+Sul formulations are still effective when water is present. Several experiments showed that, while still effective, erythritol formulations kill *D. suzukii* slower when water is available. Notably,

when in the presence of a water source, the E+S formulation is not as effective in killing *D. suzukii* as the zero-calorie E+Sul formulation. The CAFÉ choice tests also shed light on the role of sucralose. When given a choice between non-nutritive sugars or water, there was no significant difference between consumption of water vs. erythritol, or water vs. sucralose. Like erythritol, sucralose is not consumed more than water, or feeding on it may induce water feeding to bring homeostasis. It is possible that sucralose also causes some physiological imbalance due to it being a non-caloric sugar that flies are unable to metabolize. More information on physiological processes involved in sucralose metabolism, with and without erythritol, are being investigated.

Our three oviposition experiments support that erythritol formulations given to flies in some form will reduce oviposition. First, when flies were fed E+Sul in solution and given normal berries, they laid fewer eggs. This confirms Choi et al. (2019) where lowered oviposition occurred on normal berries among flies fed erythritol or erythritol:sucrose. Interestingly, ovarial dissection revealed that flies fed erythritol-sucrose had more eggs in their ovaries – the fact that they had an ample egg supply indicates that feeding on erythritol somehow inhibits the oviposition process. Next, our second and third oviposition studies suggest that coating berries with erythritol creates a deterrence effect. In choice tests, *D. suzukii* preferred to lay on untreated berries than berries dipped in E+S or E+Sul. When flies were consistently fed E+S in a vial, a 55% reduction in oviposition occurred when flies were given E+S-dipped berries than normal (water) berries in no-choice tests. Previously, Goffin et al. (2017) tested egg laying ability on erythritol-infused agar media and found that erythritol is repellent, even in no-choice conditions. Lastly, our results suggest that surface-treating berries with erythritol did not reduce survival of *D. suzukii* developing inside. Previously, erythritol was found to have larvicidal properties on *D. suzukii* (Goffin et al. 2017, Sampson et al. 2019) and *D. melanogaster* (O'Donnell et al. 2018). In those studies, erythritol was mixed into media and was toxic to developing *D. suzukii* . The lack of impact in our studies may show that erythritol applied on the blueberry skin may not leach into the fruit to substantially affect developing larvae.

Based on eleven mortality assays and three oviposition tests, **e**rythritol clearly has insecticidal and reproductively suppressive effects on *D. suzukii* adults when applied with sucrose or sucralose. Our feeding preference and mortality assays support sucralose as a phagostimulant that will increase and hasten *D. suzukii* mortality. To further develop these

formulations for practical use, current research is investigating field efficacy in cherry and blueberry fields, impacts on plant health and impacts on non-target insects since sugar sprays are used to attract beneficials (Wade et al. 2008). Erythritol is not notably harmful to predatory mites (Schmidt-Jeffris et al. 2021) and adult honeybees (Choi et al. 2019) in laboratory assays. Erythritol has shown phytotoxicity in corn and tomato (Scanga et al. 2018), and further studies are needed to clarify whether erythritol negatively impacts host plants of *D. suzukii* .

Acknowledgements

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References

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

Bernays, E. A., and S. J. Simpson. **1982**. Control of food intake, pp. 59–118. *In* Berridge, M.J., Treherne, J.E., Wigglesworth, V.B. (eds.), Advances in Insect Physiology. Academic Press.

Binns, N. M. 2003. Sucralose - all sweetness and light. Nutr Bulletin. 28: 53–58.

Bolda, M. P., R. E. Goodhue, and F. G. Zalom. **2010.** Spotted wing drosophila: potential economic impact of a newly established pest. 4.

Burgess, E. R., and B. H. King. **2017**. Insecticidal potential of two sugar alcohols to *Musca domestica* (Diptera: Muscidae). Journal of Economic Entomology. 110: 2252–2258.

Burke, C. J., and S. Waddell. **2011**. Remembering nutrient quality of sugar in *Drosophila*. Current Biology. 21: 746–750.

Caponera, V., M. Barrett, D. R. Marenda, and S. O'donnell. **2019**. Erythritol ingestion causes concentration-dependent mortality in eastern subterranean termites (Blattodea: Rhinotermitidae). Journal of Economic Entomology. toz260.

Choi, M.-Y., H. Lucas, R. Sagili, D. H. Cha, and J. C. Lee. **2019**. Effect of erythritol on *Drosophila suzukii* (Diptera: Drosophilidae) in the presence of naturally-occurring sugar sources, and on the survival of *Apis mellifera* (Hymenoptera: Apidae). J Econ Entomol. 112: 981–985.

Choi, M.-Y., S. B. Tang, S.-J. Ahn, K. G. Amarasekare, P. Shearer, and J. C. Lee. **2017**. Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii.* Journal of Insect Physiology. 99: 86–94.

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

Diegelmann, S., A. Jansen, S. Jois, K. Kastenholz, L. Velo Escarcena, N. Strudthoff, and H. Scholz. **2017**. The CApillary FEeder assay measures food intake in *Drosophila melanogaster*. J Vis Exp.

Fisher, M. L., F. E. Fowler, S. S. Denning, and D. W. Watson. **2017**. Survival of the house fly (Diptera: Muscidae) on Truvia and other sweeteners. Journal of Medical Entomology. 54: 999–1005.

Fujii, S., A. Yavuz, J. Slone, C. Jagge, X. Song, and H. Amrein. **2015**. *Drosophila* sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. Current Biology. 25: 621–627.

Gilkey, P. L., D. T. Bolshakov, J. G. Kowala, L. A. Taylor, S. O'Donnell, D. R. Marenda, and L. K. Sirot. **2018**. Lethal effects of erythritol on the mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae). J Appl Entomol. 142: 873–881.

Goffin, J., N. Gallace, N. Berkvens, H. Casteels, M. De Ro, D. Bylemans, and T. Beliën. **2017**. Toxicity of erythritol, a sugar alcohol and food additive, to *Drosophila suzukii* (Matsumara). Acta Hortic. 843–848.

Lee, S.-H., D.-H. Choe, and C.-Y. Lee. **2021**. The Impact of artificial sweeteners on insects. Journal of Economic Entomology. 114: 1–13.

Lemaitre, B., and I. Miguel-Aliaga. **2013**. The digestive tract of *Drosophila melanogaster*. Annu. Rev. Genet. 47: 377–404.

O'Donnell, S., K. Baudier, K. Fiocca, and D. R. Marenda. **2018b**. Erythritol ingestion impairs adult reproduction and causes larval mortality in *Drosophila melanogaster* fruit flies (Diptera: Drosophilidae). J Appl Entomol. 142: 37–42.

Roubos, C. R., B. K. Gautam, P. D. Fanning, S. Van Timmeren, J. Spies, O. E. Liburd, R. Isaacs, S. Curry, B. A. Little, and A. A. Sial. **2019**. Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). J Appl Entomol. 143: 609–625.

Sampson, B. J., M. W. Easson, S. J. Stringer, C. T. Werle, D. Magee, and J. J. Adamczyk. **2019**. Laboratory and field assessments of erythritol derivatives on the survival, reproductive rate, and control of *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology. 112: 173–180.

Sampson, B. J., C. T. Werle, S. J. Stringer, and J. J. Adamczyk. **2017**. Ingestible insecticides for spotted wing drosophila control: a polyol, erythritol, and an insect growth regulator, lufenuron. J. Appl. Entomol. 141: 8–18.

Scanga, S. E., B. Hasanspahič, E. Zvorničanin, J. S. Koženjić, A. K. Rahme, and J. H. Shinn-Thomas. **2018**. Erythritol, at insecticidal doses, has harmful effects on two common agricultural crop plants. PLOS ONE. 13: e0192749.

Schmidt-Jeffris, R. A., E. H. Beers, P. Smytheman, and L. Rehfield-Ray. 2021. Erythritol, an artificial sweetener, is acaricidal against pest mites and minimally harmful to a predatory mite. Journal of Economic Entomology 114: 1701-1708.

Tang, S. B., J. C. Lee, J. K. Jung, and M.-Y. Choi. **2017b**. Effect of erythritol formulation on the mortality, fecundity and physiological excretion in *Drosophila suzukii.* Journal of Insect Physiology. 101: 178–184.

Wade, M. R., M. P. Zalucki, S. D. Wratten, and K. A. Robinson. **2008**. Conservation biological control of arthropods using artificial food sprays: Current status and future challenges. Biological Control. 45: 185–199.

Wentz, K., W. R. Cooper, D. R. Horton, R. Kao, and L. B. Nottingham. **2020**. The artificial sweetener, erythritol, has insecticidal properties against Pear Psylla (Hemiptera: Psyllidae). Journal of Economic Entomology. 113: 2293–2299.

Woltz, J. M., K. M. Donahue, D. J. Bruck, and J. C. Lee. **2015**. Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii.* J. Appl. Entomol. 139: 759–770.

Zheng, C., L. Zeng, and Y. Xu. **2016**. Effect of sweeteners on the survival and behaviour of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Effect of sweeteners on *Bactrocera dorsalis*. Pest. Manag. Sci. 72: 990–996.

Supplementary Data

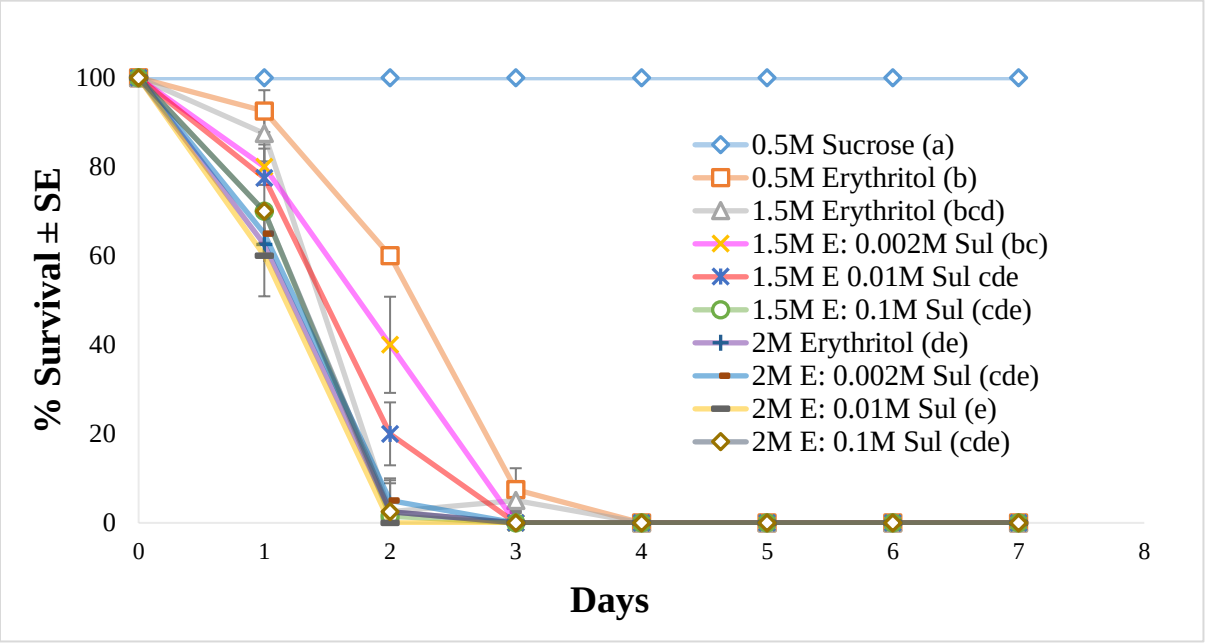

Figure S1. Testing impact of various ratios of erythritol with lower concentrations of sucralose formulations on survival of *D. suzukii* , (sucrose=S, erythritol=E, sucralose=Sul) (Mortality 2 experiment); different letters denote (*P <0.05) differences by Tukey HSD.

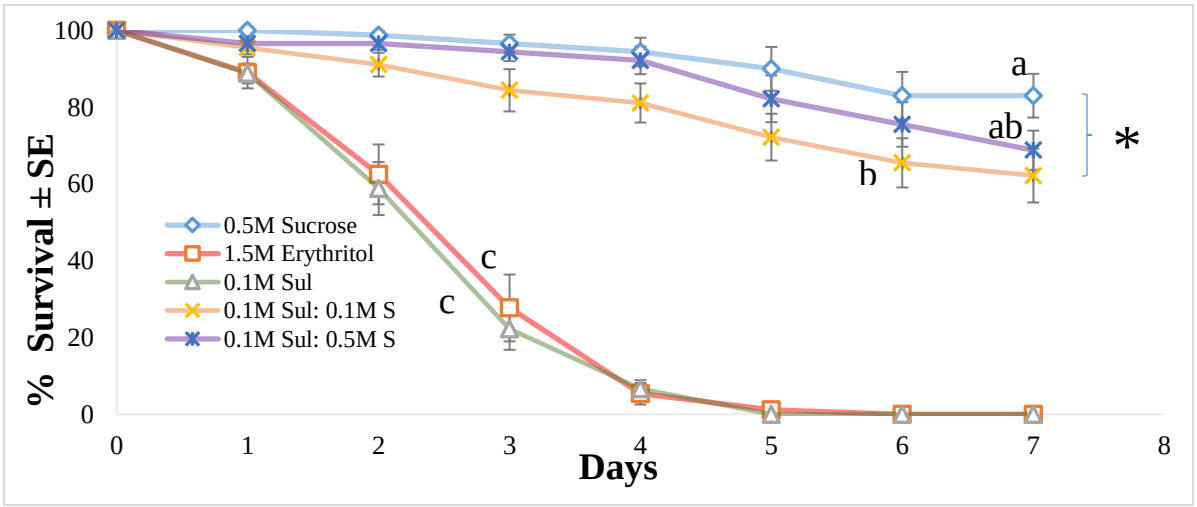

Figure S2. Testing impact of various ratios of sucralose (Sul): sucrose (S) formulations on survival of *D. suzukii* (Mortality 3 experiment); different letters denote (*P <0.05) differences by Tukey HSD. Asterisk shows difference on day 7; treatment*day interactions were significant.

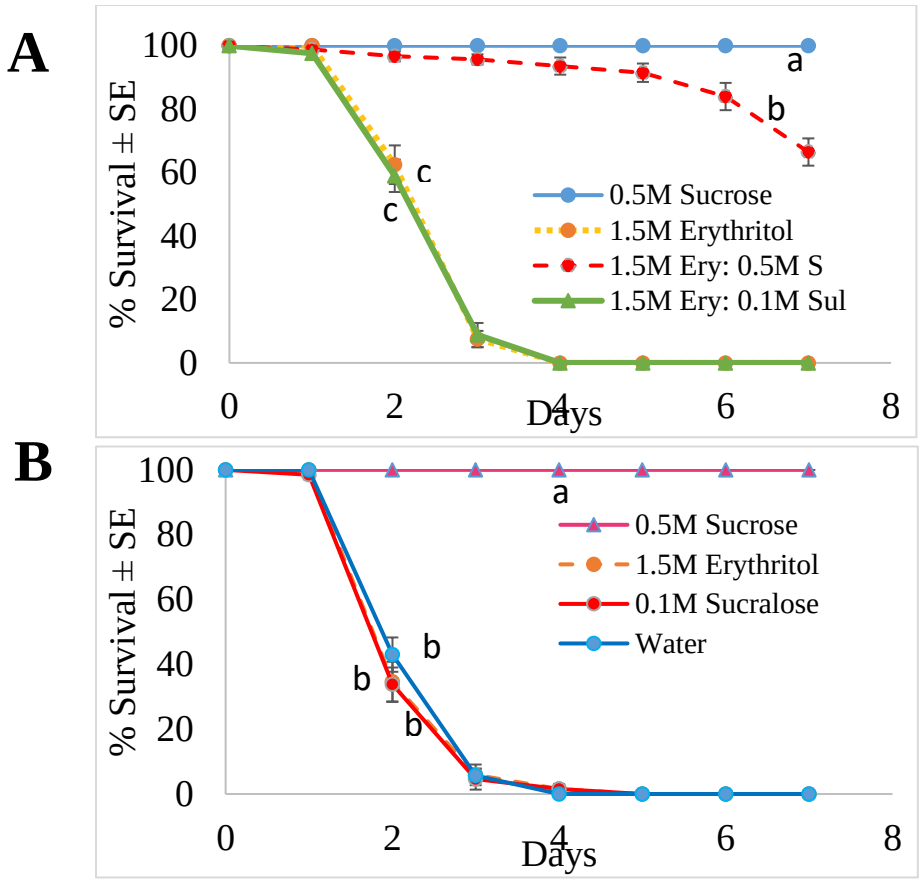

Figure S3. Survivorship of *D. suzukii* with access to water in 18 oz. cup arena, accompanied by various formulations (Mortality 7 experiment (A)) and individual sugars or water (Mortality 8 (B)). Sucrose=S, erythritol=Ery, sucralose=Sul; different letters denote differences by Tukey HSD.

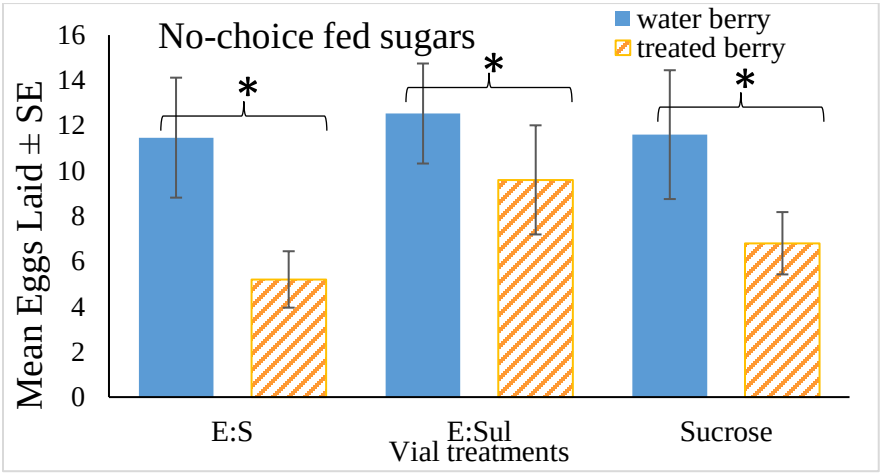

Figure S4. Mean number of eggs laid in variously treated blueberries, in water-dipped vs. treatment-dipped blueberries in no-choice experiment (Oviposition 3); asterisks denote significant difference by paired t-test (*P <0.001).

CHAPTER 3

Effects of non-nutritional sugars on lipid and carbohydrate content, physiological uptake and excretion in *Drosophila suzukii*

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Abstract: The non-nutritive sugar, erythritol, has the potential to be a human-safe management tool for the small fruits and cherry pest, *Drosophila suzukii*, or spotted-wing drosophila. Feeding on erythritol decreases fly survival and oviposition by starving and creating an osmotic imbalance in the body. Recently, we demonstrated that erythritol combined with another non-nutritive sugar, sucralose, was fed upon more than erythritol alone and hastens *D. suzukii* mortality. This suggests that sucralose is a suitable non-nutritive phagostimulant alternative to sucrose. Although promising, the nutritional and physiological impacts of sucralose on *D. suzukii* are unknown. In this study, we investigated whether sucralose is metabolized or excreted by *D. suzukii* when fed various erythritol, sucrose, and sucralose formulations. We found that sucralose cannot be metabolized or converted into any nutritional substitutes or storage carbohydrates in *D. suzukii*. Instead, sucralose molecules were largely accumulated in the hemolymph and slowly excreted from the body, creating a significant osmotic imbalance in *D. suzukii*. To excrete unused sugars, flies will use their own body fluids to restore homeostasis, resulting in losing a substantial amount of body weight and becoming desiccated in the process. In summary, ingesting sucralose leads to starvation and hyperosmotic pressure in the body, causing a decrease in fitness. With confirmation of sucralose being non-metabolizable and phagostimulative to *D. suzukii*, the erythritol+sucralose formulation is a promising insecticide for growers to use.

Keywords: Non-nutritive sugars, sucralose, erythritol, spotted-wing drosophila, insecticide

Introduction

Zero-calorie artificial sweeteners such as erythritol, the main ingredient in Truvia®, and sucralose, the main ingredient in Splenda®, are used as sugar substitutes that are safe for humans to consume (Tetzloff et al. 1996, Storey et al. 2007). Recently, erythritol has been demonstrated to have insecticidal properties against various insect species: *Drosophila melanogaster* (Baudier et al. 2014, O'Donnell et al. 2016, 2018), *Bactocera dorsalis* (Zheng et al. 2016), *D. suzukii* (Choi et al. 2017, Goffin et al. 2017, Sampson et al. 2017, 2019, Tang et al. 2017), *Musca domestica* (Burgess & King, 2017; Fisher et al. 2017), *Stomoxys calcitrans* (Burgess & Geden, 2019), *Aedes aegypti* (Gilkey et al. 2018), *Cacopsylla pyricola* (Lee et al. 2021; Wentz et al. 2020), and *Tetranychus urticae* (Schmidt-Jeffris et al. 2021).

Sucrose is a commonly used as a phagostimulant or substance to enhance consumption. When combined, sucrose increases the efficacy of insecticides or toxic baits to fly pests because flies are enticed to feed more frequently (Allan, 2011; Cowles et al. 2015; Roubos et al. 2019; Tochen et al. 2014). However, adding sucrose fuels the insects with a nutritional carbohydrate or may encourage microbial development on the sprayed fruits. Erythritol has been demonstrated as a phagostimulant candidate to replace sucrose in the entomopathogenic fungus for the house fly (Burgess, Johnson, & Geden, 2018), and in insecticides used in *D. suzukii* management (Gullickson et al. 2019). However, the sweetness of erythritol is 30% less than sucrose (Perko and DeCock 2008). It is consumed less according to Choi et al. (2017), where flies preferred sucrose more than erythritol in choice-conditions and erythritol might not be perceived as sweet to *D. suzukii* like observed in the fire ant (Vander Meer et al. 1995).

To find an alternative to sucrose, we focus on sucralose, which is ~600x sweeter than sucrose (Binns, 2003). In 16 previous feeding preference, survivorship, and oviposition trials, we confirmed sucralose to have insecticidal and phagostimulative properties (Price et al. 2021). Those trials showed that *D. suzukii* fed up to 40% more on erythritol+sucralose formulations than on erythritol alone. Also, the 1.5M erythritol+0.1M sucralose was most efficient in killing flies within one to two days without water access and within three days when flies had simultaneous access to water. Presence of water aids homeostasis to the fly body (Lee et al. 2021). Without water, flies have difficulty restoring homeostasis and regurgitate or excrete excessively to expel the non-metabolized sugars; this behavior causes water loss. Continuous water loss without replenishment can desiccate and "shrivel" the bodies as seen in erythritol-fed

D. suzukii (Sampson et al. 2017). Consumption of non-metabolizable erythritol is toxic to *D. suzukii* because it starves and creates hyperosmotic pressure in the fly body, in turn, interfering with the ability to contract muscles needed for oviposition and excretion (Choi et al. 2017; Tang et al. 2017).

Drosophila suzukii, is a damaging pest to berries and cherries; potentially costing an annual $511 million if left unmanaged in the Pacific Northwest, United States (Bolda et al. 2010). Major control methods for this pest currently rely on chemical insecticides, which can be detrimental to human and environmental health (Haviland and Beers 2012, Walsh et al 2011, Ansari et al. 2014). Finding alternative management options to replace these insecticides and efficiently control *D. suzukii* populations is a challenge. Erythritol combined with sucralose is a promising management option since our past laboratory feeding trials indicate that the erythritol+sucralose combination and sucralose alone have insecticidal and phagostimulative effects on *D. suzukii* (Price et al. 2021).

In this study, we investigated the nutritional and physiological impacts of sucralose on *Drosophila suzukii.* First, we tested if sucralose is metabolized or digested in *D. suzukii* by quantifying sucralose levels in the hemolymph and frass of flies fed various sugar formulations. Secondly, we directly examined fly weight gain, consumption volume and amount of frass droplets excreted when fed those various formulations. Thirdly, we determined whether sucralose can be converted to a long chain carbohydrate such as glycogen or affected nutrient reserves in *D. suzukii* though the anthrone and vanillin assays.

Materials, Methods, and Statistics

Flies, sugars, and sugar alcohols

Drosophila suzukii were maintained with the standard rearing methods and diet described by Woltz et al. (2015), at an average 23 ± 5 ℃ under a photoperiod of L:D 16:8 h, and a relative humidity of 50 ± 5% at the Horticultural Crops Research Unit, USDA-ARS in Corvallis, Oregon, USA. All experiments were also conducted at the conditions above. Tested flies were the F_1 and F_2 generation from wild type parents. Wild type parents originated from infested berries, not previously treated with insecticides, in Corvallis, Oregon collected in July-September 2020. Only females were utilized in this study because they are the target sex, although digestive metabolism

between *D. suzukii* sexes are similar (Tochen et al. 2016). Emerged adult flies were maintained in cages with water and diet until 5-days old for experimentation. This age is the most tolerant in prior erythritol studies (Choi et al. 2017). Sugars and polyols used in this study were D-Glucose (>99%), D-mannitol (>99%), D-trehalose (>99%), meso-erythritol (>99%), sucralose (>99 %), and sucrose (>99%), purchased from Sigma-Aldrich (St. Louis, USA). All compounds were dissolved in distilled water.

Preparation of hemolymph and frass from flies

Flies were starved for 24 h prior to experiments. Ten flies were introduced into each plastic vial (3 cm diam. × 10 cm long) to feed on four equimolar treatment solutions: 0.5M sucrose, 0.5M erythritol+0.5M sucrose (E+S), 0.5M sucrose+0.5M sucralose (S+Sul), and 0.5M erythritol+0.5M sucralose (E+Sul) solutions for 24 h. Each test was replicated four times per treatment.

Methods for collecting and quantifying frass of flies were modified from Tang et al. (2017). To collect frass from flies, each plastic vial was lined with the glossy side of transparent laminate film (4.5 cm × 8, Swingline EZuse thermal laminate size 3 mil, Lincolnshire, Illinois). While flies were able to feed on solutions from a cotton wick soaking in a 1.5 ml conical tube, the flies deposited their frass on the glossy lining of the wall. After 24 h, the lining was removed from the vial using forceps. Twenty frass spots of relatively similar size and twenty empty spots, as a control, were marked on the opposite side of the film under light and magnification. Then, 2 µl of purified water was dropped on each frass or empty dot, gently washed by pipetting, and finally transferred into 20 µl purified water. This step was repeated to collect 20 fecal dots. The samples were stored in −20 °C until the derivatization process prior to gas chromatography-mass spectrometry (GC-MS) analysis.

Collecting hemolymph from the flies fed sugars was modified from the method described by MacMillan and Hughson (2014). To collect hemolymph, each vial containing ten fed-flies were immobilized on ice. Each fly was removed from the vial using forceps and placed head-first into a modified pipette tip. The constructed apparatus (Fig. 1A) allowed air pressure to lodge the fly body into the end of the pipette tip with the head protruding from the tip. Each antenna was amputated using forceps, after which a hemolymph droplet immediately formed (Fig. 1B, C). Hemolymph droplets were then collected into a microhematocrit capillary tube (70 µl, 1.1 mm id

× 75 mm height, Fisher Scientific). After hemolymph was collected from all flies within a vial, it was transferred from the glass capillary tube into a conical tube with 2 µl pure water and stored at −20 °C until the GS-MS analysis below.

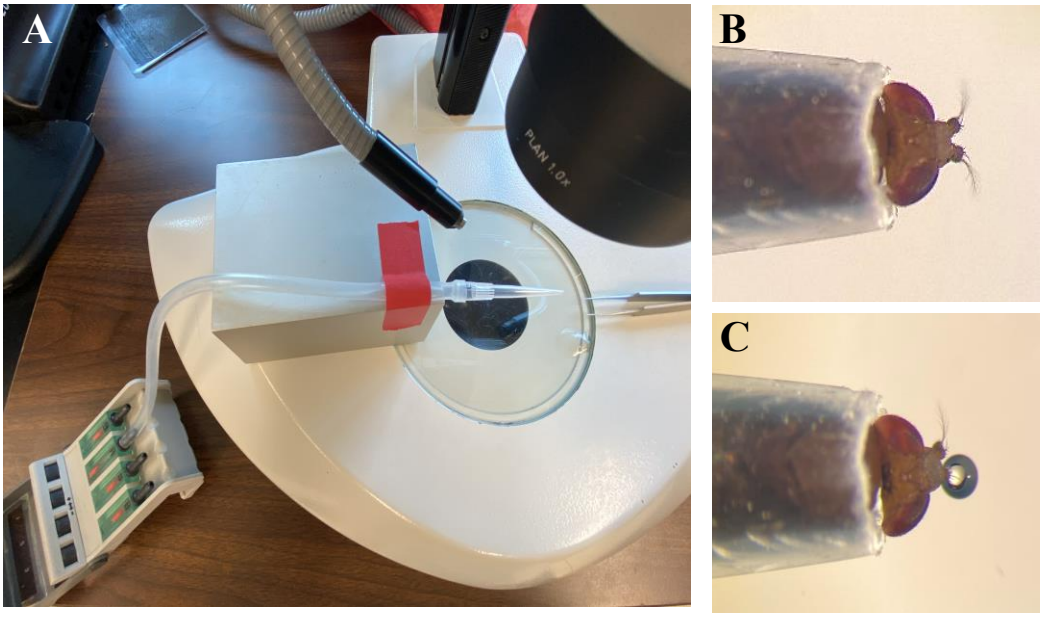

Figure 1. Photo of hemolymph extraction experimental set up and procedure (A), *Drosophila suzukii* lodged into pipette tip with air pressure (B), and hemolymph droplet formed after amputation of antenna (C).

Analysis of sugars in hemolymph and frass using GC-MS

Derivatization of the sugars collected from hemolymph and frass was conducted according to Wahjudi et al. (2010). Mannitol (1 µg) was added in each sample as an internal standard (IS) (Fig S1). First, 100 µl of methoxylamine hydrochloride (0.18 M in pyridine) was added to each sample and heated at 70 °C for 1 h. Then, 100 µl acetic anhydride was added and heated under 45 °C for 1 h. The end products were left to air dry in a fume hood. Samples were dissolved with 50 µl ethyl acetate prior to GC–MS and GC-FID analysis.

All sugar and polyol derivatives made by standard sugars were determined by their molecular structures and retention times first by a GC (7890B, Agilent Technologies, Santa Clara, CA) equipped with a MS (5977B, Agilent Technologies) and a HP-5MS column (30 m× 0.25 mmID, 0.25 µm film thickness; Agilent). Helium was the carrier gas. The oven temperature was programmed at 80 °C for 1 min, increased to 300 °C at 10 °C/min, and held for 10 min. Injector temperature was 250 °C. Then, sugars collected from the hemolymph and frass samples

were processed for sugar derivatization, and identified by the GC-FID equipped with a HP-5MS column (30 m × 0.25 mm ID, 0.25 μm film thickness; Agilent) using the same retention times determined by the GC-MS with carrier gas helium. The oven temperature was programed as above. Each sugar from the sample was quantified by comparing its area with the area of the IS. Thus, the concentrations are not absolute but, instead, relative to the IS. Sugar content in frass had many undetected sugars so no analysis was done. For flies fed sucrose, the resulting hemolymph levels of sucrose, glucose, or trehalose (effect) were compared using a normal distribution. Since multiple measurements come from each fly, each fly was a random effect. Similar analyses were done for flies fed E+S, S+Sul, and E+Sul with resulting hemolymph sugars Statistics here and below were analyzed in SAS 9.4 Proc Glimmix (SAS Institute 2016).

Measurement of body weight gain, sugar consumption and frass excretion in no-choice capillary feeding assay

To measure body weight gain, ten flies were starved 24 h prior, then aspirated into each plastic vial (3 cm × 10 cm L) and weighed. Flies were able to feed on equimolar treatment solutions described above: 1) sucrose, 2) E+S, 3) S+Sul and 4) E+Sul solutions from a cotton wick for 24 h then transferred to a clean vial and weighed again. Transferring flies to the clean vial ensured that any frass excreted was not included in final weight. This was repeated five times over two trial dates. Weight gain of flies within each vial was measured using an analytical scale (OHAUS®, Parsippany, New Jersey) and averaged per fly.

To measure sugar consumption and frass excretion, a no-choice capillary feeding assay modified from Choi et al. (2017) was conducted. Flies were starved 24 h prior to experimentation. Five flies were introduced into each plastic vial (3 cm x 7 cm L) with a plastic lid punctured to hold one glass capillary tube and one aeration hole. Each vial was lined with the glossy side of transparent laminate film as described above. Flies were given solutions listed above for 24 h. A layer of mineral oil (Thermo Scientific) was inserted to each capillary above the solution to prevent evaporation. The identical vials filled with the corresponding solution with mineral oil layer, without flies, served as controls to measure evaporation for each treatment. Vials were placed in a secondary plastic container with water-soaked sponges to slow evaporation. The amount in the capillary tube was measured after 24 h with a digital caliper. The reported amount consumed was calculated using formula modified from (Diegelmann et al. 2017); [food uptake (μl) = (measured distance (mm)/0.9 mm) – average evaporation]. The

laminate film was removed with forceps and frass dots were counted. This method was replicated ten times per treatment in several trials.

Separate generalized linear mixed models (GLMM) tested whether body weight gain, the number of frass droplets or consumption varied. Treatment was a fixed effect, and the trial was a random effect. A lognormal, normal, or Poisson distribution was used in models depending on fit.

Analysis of lipids, sugars and glycogen using anthrone and vanillin assays

Individual flies were fed various diets though capillary tubes: 1) water, 2) 0.5M sucrose, 3) 1.5M erythritol+ 0.5M sucrose (E+S), 4) 0.5M sucrose+ 0.1M sucralose (S+Sul), or 5) 0.1M sucralose for 24 h. Afterwards, each fly was transferred into 1.5 ml microcentrifuge tubes and stored at −80 °C.

These formulations differed from the concentrations in our other experiments. A higher-dose (1.5M) erythritol and lower-dose sucralose (0.1M) was tested here since these concentrations induced the highest mortality in Price et al. (2021) and are being used in field trials. A comparison of flies fed sucrose-only to E+S or S+Sul reveals whether simultaneous consumption of erythritol or sucralose might impede sucrose metabolism. A comparison of flies fed only sucralose to only water or sucrose reveals whether flies convert sucralose to usable carbohydrates. Previously, a comparison of flies fed only erythritol to only sucrose/water has revealed that erythritol is not converted to usable carbohydrates (Choi et al. 2017).

A standard procedure from Olson et al. (2000) was adapted for *D. suzukii* (Wong et al. 2018) to determine the amount of glycogen, lipids, and sugars in the fly bodies. In each tube, 50 µl of 2% sodium sulfate was added before homogenizing fly body with a plastic pestle. Leaving the pestle in the tube, 450 µl of chloroform-methanol (1:2) was added to rinse the pestle off. After removing pestle, tubes were vortexed and centrifuged at 13,000 rpm for 3 min. After centrifuging, 220 µl of the supernatant was transferred to a glass test tube (12 mm diam. × 75 mm length, Fisherbrand, Waltham, Massachusetts) for the sugar assays and 220 µl was transferred to a similar glass tube for the lipid assay. The remaining precipitate in the microcentrifuge tube was used for the glycogen assay. All glass tubes were heated at 90 °C until all solution was evaporated from the lipid tubes and approximately 25 µl of solution remained in the sugar tubes.

Lipids. Sulfuric acid (40 µl) was added to each tube containing the lipid solution and heated for 2 min at 90 °C. The tubes were cooled on ice and 480 µl of a vanillin-phosphoric acid reagent as prepared in van Handel (1985b), was added and vortexed. The solutions were left to react for 20 min at room temperature, vortexed again before 200 µl of contents were pipetted into a 96-well plate.

Glycogen. Anthrone-sulfuric acid reagent (975 µl) as prepared in van Handel (1985a), was added to the microcentrifuge tubes containing a precipitate and heated at 90 °C for 10 min. The tubes were cooled on ice, vortexed and 200 µl of solution were pipetted into a 96-well plate.

Total sugars. Anthrone-sulfuric acid reagent (975 µl) was added to each tube, vortexed and heated 90°C for 8 min. Then, tubes were cooled on ice, vortexed and 200 µl were pipetted into a 96-well plate.

For quantifying lipids, absorbance at 490 nm was read and for quantifying glycogen and total sugars, absorbance at 630 nm was read using an ELISA plate absorbance reader (ELx808, BioTek, Winooski, VT).

Standard curves. Known amounts of sucrose, glycogen, and lipid solutions (1 mg/ml) were prepared as in van Handel (1985a, 1985b). Canola oil in chloroform was used to generate the lipid standard. Pure grade glycogen (Fisher Scientific, Waltham, MA) dissolved in water and pure grade sucrose (Fisher Scientific) dissolved in 25% ethanol: 75% distilled were used for the glycogen and sucrose standards. All standard stock solutions were added in amounts of 1, 5, 10, 20, 30, 40 and 50 µl, and reacted with anthrone reagent (for glycogen and sugars) and vanillin reagent (for lipids) as described earlier. Three replicates per amount and the anthrone- or vanillin-only as a control were pipetted into a 96-well plate. Absorbance was read at 490 nm for lipids and at 630 nm for sugars and glycogen. Linear regression equations were based on the amount of lipid/glycogen/sugar on the x-axis, and absorbance readings on the y-axis. Absorbance readings from each fly were then divided by the slope to estimate nutrient content. Lipid and sugar content was multiplied by two because the supernatant was divided in half for these reactions. Separate GLMMs tested whether glycogen, lipid and sugar concentrations varied. The effect of treatment was a fixed effect, trial as a random effect with a lognormal distribution.

Results

Sugar contents in hemolymph and frass from flies

Sugar amounts in hemolymph and frass from flies fed equimolar solutions were determined (Fig. 2). Sugars were derivatized prior to GC-MS analysis and their retention times were obtained. Mannitol was used as the internal standard (Fig. S1). Trehalose and glucose were present in all hemolymph samples, as they are major blood sugars in insects but are not excreted in the frass (Fig. 2). Sucrose-fed flies were used as a positive control. In the hemolymph of erythritol and sucrose (E+S)-fed flies, sucrose, trehalose and glucose were found in much smaller amounts than erythritol (Fig. 2A). E+S-fed flies had 7x more erythritol in frass than in hemolymph. Similarly, in hemolymph of sucrose and sucralose (S+Sul)-fed flies, a large amount of sucralose (4x-15x) was found compared to the other three sugars (Fig. 2A). The flies had almost 4-times more sucralose in frass than hemolymph (Fig. 2B). When flies fed on the two non-nutritional sugars erythritol and sucralose (E+Sul), erythritol was 20x higher than sucralose in hemolymph (Fig. 2A). In the frass, however, sucralose was 1.7x higher than erythritol with no statistical difference (Fig. 2B). These results indicate that more sucralose could be excreted as waste in frass with a smaller amount remaining in the hemolymph.

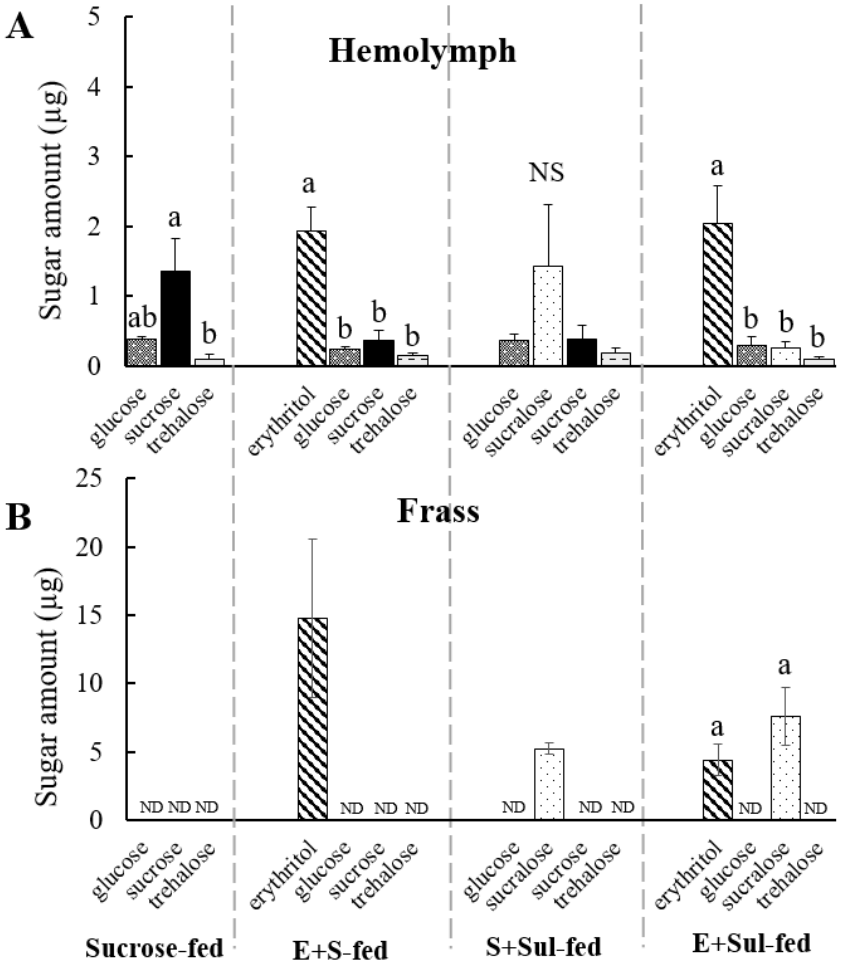

Figure 2. Average amount of sugars determined in hemolymph (A) and frass (B) of fed-flies ± standard error (SE). All sugar solutions were same molar concentration, 0.5M. E= erythritol, S= sucrose, Sul= sucralose. ND: not detected. NS: not significant. Different letters denote significant differences ($P < 0.05$) by Tukey honestly significant difference (HSD).

Body weight gain, sugar consumption in no-choice CAFÉ and frass excretion

When flies were given no-choice to feed on equimolar solutions, control (sucrose-fed) flies gained, on average, 0.75 mg within 24 h (Fig 3A). E+S-fed flies weighed 47% less than control, and S+Sul-fed flies weighed 92% less than control (Fig 3A). Flies fed the completely non-nutritive option E+Sul weighed 134% less than control flies ($F_{3, 15}$=13.08, *P < 0.001) (Fig. 3A). The abdomen size of flies-fed E+Sul was considerably shrunken compared to the control (Fig. 3B). These results could be from starvation and desiccation in the flies.

Sucrose-fed flies, for the control, consumed the most (avg. 7.67 ul) in comparison to other treatments, then S+Sul, E+S, and non-nutritive option, E+Sul, was consumed the least ($F_{3, 35}$=3.7, *P = 0.021) (Fig. 3C). Although flies fed on E+Sul the least, they excreted more than all

other treatments (avg. 54.5 frass droplets) (Fig. 3C). The remaining three treatments excreted similar amounts of frass droplets ($F_{3, 35}$=9.6, *P > 0.001). This indicates that when flies have sucrose as a source of nutrition carbohydrate in the presence of a non-nutritive sugar, they will utilize sucrose as expected and excrete relatively normal amounts.

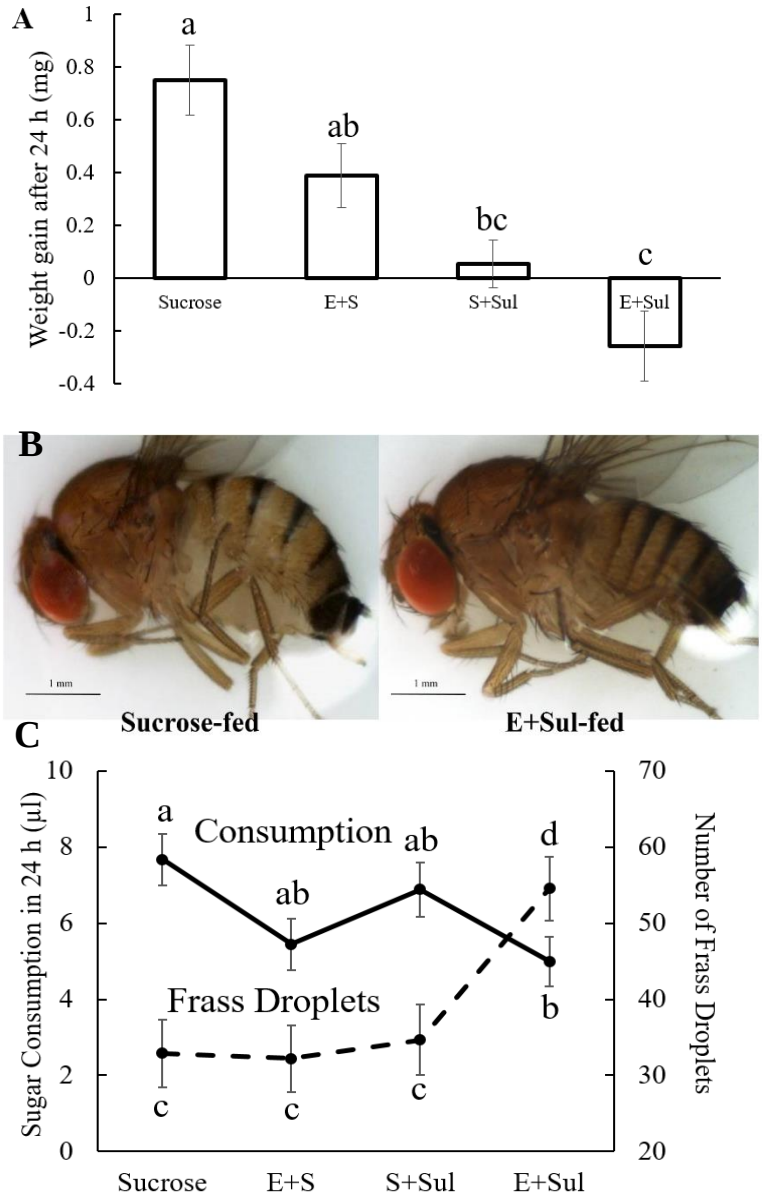

Figure 3. *Drosophila suzukii* body weight gain per fly (µl) after 24 h feeding on various sugars ± mean standard error (MSE) (A). Images of difference in abdomen size between sucrose-fed flies (left) and erythritol+sucralose-fed flies (right) from body weight gain experiment (B). Average consumption of sugars (C, left solid) and average number of frass droplets excreted (C, right dotted) after 24 h no-choice capillary CAFÉ feeding assay, ± MSE. (Sucrose=0.5M, E+S= 0.5M erythritol+0.5M sucrose, S+Sul= 0.5M sucrose+0.5M sucralose, and E+Sul= 0.5M erythritol+0.5M sucralose) Different letters denote significant differences (P<0.05) by Tukey HSD.

Amounts of lipid, sugars and glycogen in flies

Lipid, sugar, and glycogen amounts were measured in flies fed various solutions (Fig. 4). There was no detectable difference in lipid content between treatments ($F_{4, 94}$=1.91, P = 0.11) (Fig. 4A). The result indicates that none of these sugars are converted to the lipid or affect lipid metabolism in the body within 24 h. Treatment solutions affected total sugars ($F_{4, 94}$=4.55, *P < 0.01) and glycogen levels in the fly body ($F_{4, 94}$=4.79, *P < 0.01). As expected, flies fed 1.5M erythritol + 0.5 M sucrose (E+S) or 0.5M sucrose + 0.1M sucralose (S+Sul) had higher sugar levels than those fed only 0.1 M sucralose or water (Fig. 4B). Also, flies fed E+S or E+Sul had body sugar (Fig. 4B) and glycogen levels (Fig. 4C) no different than flies fed 0.5M sucrose. This suggests that when flies are fed non-nutritive sugars combined with sucrose, total body sugars increase, and that non-nutritive sugars do not noticeably affect sucrose metabolism. Next, flies fed sucralose alone had similar body sugar and glycogen levels as flies fed water, but lower than flies fed sucrose (positive control) (Fig. 4B,C). Both results indicate that *D. suzukii* adults cannot metabolize sucralose and convert it to sugar or glycogen stores.

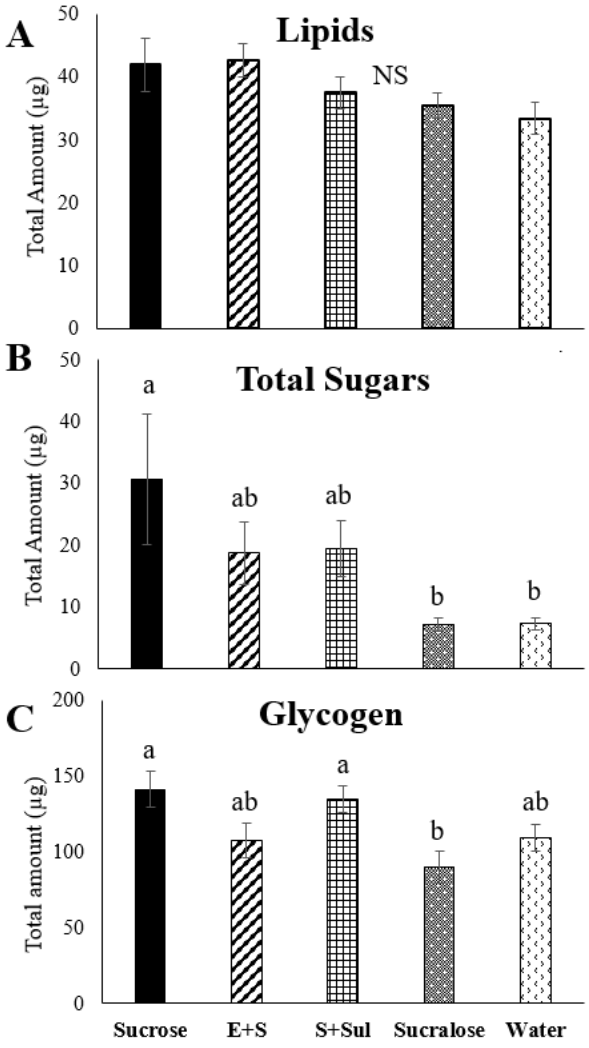

Figure 4. Amount (ug) of lipids (a), total sugars (b) and glycogen (c) in *Drosophila suzukii* fed various solutions using anthrone and vanillin methodology ± SE. Sucrose=0.5M, E+S= 1.5M erythritol+0.5M sucrose, S+Sul= 0.5M sucrose+0.1M sucralose, Sucralose=0.1M. Different letters denote significant differences ($P < 0.05$) by Tukey HSD.

Discussion

Our previously tested 1.5M erythritol+0.5M sucrose and 1.5M erythritol+0.1M sucralose formulations were found to have insecticidal effects to *Drosophila suzukii* (Price et al. 2021). The erythritol+sucrose combination (E+S) provides flies with a nutritional carbohydrate that flies can break down into glucose and fructose during sucrose metabolism (Lehninger, Nelson, & Cox, 2000). Since sucralose is non-nutritive and sweeter than sucrose, we tested and confirmed that 0.1M sucralose mixed with 1.5M erythritol was the most detrimental to *D. suzukii* survival compared to other formulations and deterred oviposition (Price et al. 2021). In this study, we

investigated how *D. suzukii* metabolizes sucralose (C_{12}, MW 397) because it is structurally similar to sucrose (C_{12}, MW 342). We performed various physiological experiments to investigate whether sucralose can be converted to a usable carbohydrate in the fly body. This was first done by analyzing the sugars present in the hemolymph and frass of flies fed various equimolar solutions via GC-MS. As expected, glucose and trehalose, were always detected in the hemolymph since they are insect blood sugars, but not excreted in the frass.

Simple tetra-carbon molecules, like erythritol, are known to diffuse though mammalian intestinal membranes faster than hexose sugars, like sucrose and sucralose (Mitchell, 2008; Munro et al. 1998). Erythritol likely diffuses though *D. suzukii* 's midgut quickly since it cannot be metabolized, which ultimately creates hyperosmolarity in the fly body. Hemolymph and frass analyses from sucralose (Sul)-fed flies showed that, much like erythritol, sucralose is excreted in large amounts with a smaller proportion remaining in the hemolymph. When flies were fed equimolar sucrose+sucralose (S+Sul) in this study, the accumulation of sucralose in the hemolymph was comparable to erythritol accumulation among erythritol+sucrose (E+S)-fed flies from Tang et al. (2017). However, erythritol was excreted in large amounts, up to 3x more than the amount of sucralose detected in frass. This suggests that the excretion of sucralose is relatively slow possibly due to it being a larger molecule (3x) than erythritol (Fig. 2).

Interestingly, when flies were fed both non-nutritive sugars in the E+Sul treatment, erythritol accumulated in the hemolymph similarly to E+S-fed flies. However, E+Sul flies excreted one-third the erythritol in their frass (4.4 µg) compared to E+S-fed flies (14.8 µg). Sucralose amounts (7.6 vs 5.2 µg) excreted from E+Sul and S+Sul flies were not different (Fig. 2). Flies fed E+Sul had less sucralose in the hemolymph than erythritol. An explanation for this could be due to sucralose being a larger molecule than erythritol and structurally different. Sucralose is a substituted disaccharide manufactured from sucrose though chlorination, changing its molecular structure, three hydroxyl groups of sucrose are replaced with a chloride side chain (Qiu et al. 2007). Another possibility for lower sucralose in the hemolymph than erythritol is that the sucralose molecule is structurally similar to sucrose, sucrase enzymes may attempt to bind to the sucralose in cells and slow down its transport back into hemolymph. More research on this enzyme activity and sugar biochemical metabolism is needed to confirm this.

While E+Sul-fed flies excreted one-third the erythritol (4.4 µg) as E+S-fed flies (14.8 µg), the total amount of the non-nutritive sugars (12.0 µg) including sucralose in their frass was similar between E+Sul and E+S flies (Fig. 2). By excreting non-metabolized sugars, flies can reduce osmotic imbalance in the hemolymph. During osmotic imbalance, flies need significant water intake to restore homeostasis in the body. Flies fed E+Sul have two non-nutritive sugars of different chemical compositions that need to be excreted; the erythritol excretion process could have been inhibited due to a large amount of sucralose present in the body.

Our second set of experiments show that ingestion of high concentrations of non-nutritive sugar requires a significant amount of water intake to restore homeostasis and excrete unused waste. The no-choice feeding and body weight gain assay revealed that feeding on E+Sul is the most detrimental, flies lost 0.26 mg in body weight and appeared extremely desiccated after 24 h (Fig. 3A, B). Next, the no-choice consumption and frass droplet assay revealed that flies feed the least on E+Sul but excrete the most than flies fed other solutions with nutritive sucrose. The Malpighian tubules is a primary excretory organ that regulates water balance and ion exchange in insects (Beyenbach, 2003; Dow et al. 1995). Malpighian tubules can secrete body water and electrolytes in response to large amounts of food intake (Maddrell, 1991). Desiccation is likely due to flies using more their own fluids to attempt restoration of physiological homeostasis. Multiple neuropeptide hormones such as corticotrophin releasing factor (CRF)-like diuretic hormone 44; DH44 (Zandawala et al. 2018) and calcitonin-like diuretic hormone 31; DH31 (Coast et al. 2001; Johnson et al. 2005) are known to regulate fluid secretion, water regulation and ion balance in flies. More research on gene expressions of these neuropeptides should be explored in flies fed non-nutritive sugars.

Lastly, Choi et al. (2017) used anthrone assays to show that when flies feed on erythritol alone, they do not convert it to a useable carbohydrate, but rather have similar glycogen and sugar reserves as water-fed flies. By examining glycogen, lipid and sugar levels in flies fed sucralose in this study, we confirmed that *D. suzukii* do not metabolize sucralose to any substantial extent; sucralose-fed flies had similar glycogen reserves and sugar levels as water-fed flies. Whether or not erythritol interferes with sucrose metabolism was previously unknown. Both E+S-fed and S+Sul-fed flies had similar glycogen and sugar reserves as sucrose-fed flies, but more than water or sucralose-only fed flies. These results also suggest that both non-nutritive

sugars, erythritol and sucralose, may not interfere with sucrose molecules converting into other metabolic or storage carbohydrates such as glycogen.

In conclusion, the non-nutritive sugar, sucralose, cannot be metabolized to any nutritional substitute or storage carbohydrate in *D. suzukii* , is slowly excreted slowly from the fly body, and accumulated in hemolymph. Sucralose consumption leads to starvation and hyperosmotic pressure that negatively affects fly survival. With confirmation of sucralose being non-metabolizable and our previous research showing that it is phagostimulative to *D. suzukii* , there is potential for the erythritol+sucralose combination to be a human-safe insecticide for growers to use. Further research on field efficacy of the formulation is underway.

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References

Allan, S. A. (2011). Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, *36*(1), 59–67. https://doi.org/10.1111/j.1948-7134.2011.00141.x

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*(6), e98949. https://doi.org/10.1371/journal.pone.0098949.

Beyenbach, K. W. (2003). Transport mechanisms of diuresis in Malpighian tubules of insects. *Journal of Experimental Biology*, *206*(21), 3845–3856. https://doi.org/10.1242/jeb.00639.

Binns, N. M. (2003). Sucralose—All sweetness and light. *Nutrition Bulletin*, *28*(1), 53–58. https://doi.org/10.1046/j.1467-3010.2003.00307.x

Bolda, M. P., Goodhue, R. E., & Zalom, F. G. (2014). Spotted wing drosophila: Potential economic impact of a newly established pest (p., 4) University of California: Giannini Foundation of Agricultural Economics.

Burgess, E. R., & Geden, C. J. (2019). Larvicidal potential of the polyol sweeteners erythritol and xylitol in two filth fly species. *Journal of Vector Ecology*, *44*(1), 11–17. https://doi.org/10.1111/jvec.12324.

Burgess, E. R., Johnson, D. M., & Geden, C. J. (2018). Mortality of the house fly (Diptera: Muscidae) after exposure to combinations of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) with the polyol sweeteners erythritol and xylitol. *Journal of Medical Entomology*, *55*(5), 1237–1244. https://doi.org/10.1093/jme/tjy083.

Burgess, E. R., & King, B. H. (2017). Insecticidal potential of two sugar alcohols to *Musca domestica* (Diptera: Muscidae). *Journal of Economic Entomology*, *110*(5), 2252–2258. https://doi.org/10.1093/jee/tox190.

Choi, M.-Y., Tang, S. B., Ahn, S.-J., Amarasekare, K. G., Shearer, P., & Lee, J. C. (2017a). Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii. Journal of Insect Physiology*, *99*, 86–94. https://doi.org/10.1016/j.jinsphys.2017.04.001.

Choi, M.-Y., Tang, S. B., Ahn, S.-J., Amarasekare, K. G., Shearer, P., & Lee, J. C. (2017b). Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii. Journal of Insect Physiology*, *99*, 86–94. https://doi.org/10.1016/j.jinsphys.2017.04.001.

Coast, G. M., Webster, S. G., Schegg, K. M., Tobe, S. S., & Schooley, D. A. (2001). The *Drosophila melanogaster* homologue of an insect calcitonin-like diuretic peptide stimulates V-ATPase activity in fruit fly Malpighian tubules. *The Journal of Experimental Biology*, *204*(Pt 10), 1795–1804.

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*(2), 640–653. https://doi.org/10.1093/jee/tou100.

Diegelmann, S., Jansen, A., Jois, S., Kastenholz, K., Velo Escarcena, L., Strudthoff, N., & Scholz, H. (2017). The CApillary FEeder assay measures food intake in *Drosophila melanogaster*. *Journal of Visualized Experiments : JoVE*, *121*. https://doi.org/10.3791/55024.

Dow, J., Maddrell, S. H. P., Görtz, A., Skaer, N., Brogan, S., & Kaiser, K. (1995). The Malpighian tubules of *Drosophila melanogaster*: A novel phenotype for studies of fluid secretion and its control. *The Journal of Experimental Biology*, *197*, 421–428.

Fisher, M. L., Fowler, F. E., Denning, S. S., & Watson, D. W. (2017). Survival of the house fly (Diptera: Muscidae) on Truvia® and other sweeteners. *Journal of Medical Entomology*, *54*(4), 999–1005. https://doi.org/10.1093/jme/tjw241.

Gilkey, P. L., Bolshakov, D. T., Kowala, J. G., Taylor, L. A., O'Donnell, S., Marenda, D. R., & Sirot, L. K. (2018). Lethal effects of erythritol on the mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae). *Journal of Applied Entomology*, *142*(9), 873–881. https://doi.org/10.1111/jen.12538.

Goffin, J., Gallace, N., Berkvens, N., Casteels, H., De Ro, M., Bylemans, D., & Beliën, T. (2017). Toxicity of erythritol, a sugar alcohol and food additive, to *Drosophila suzukii* (Matsumara). *Acta Horticulturae*, *1156*, 843–848. https://doi.org/10.17660/ActaHortic.2017.1156.124.

Gullickson, M. G., Rogers, M. A., Burkness, E. C., & Hutchison, W. D. (2019). Efficacy of organic and conventional insecticides for *Drosophila suzukii* when combined with erythritol, a non-nutritive feeding stimulant. *Crop Protection*, *125*, 104878. https://doi.org/10.1016/j.cropro.2019.104878.

Johnson, E. C., Shafer, O. T., Trigg, J. S., Park, J., Schooley, D. A., Dow, J. A., & Taghert, P. H. (2005). A novel diuretic hormone receptor in Drosophila: Evidence for conservation of CGRP signaling. *The Journal of Experimental Biology*, *208*(Pt 7), 1239–1246. https://doi.org/10.1242/jeb.01529.

Lee, S.-H., Choe, D.-H., & Lee, C.-Y. (2021). The impact of artificial sweeteners on insects. *Journal of Economic Entomology*, *114*(1), 1–13. https://doi.org/10.1093/jee/toaa244.

Lehninger A. L., Nelson D. L., & Cox M. M. (2000). Lehninger principles of biochemistry. New York: Worth Publishers.

MacMillan H. A., B. N. Hughson. (2014). A high-throughput method of hemolymph extraction from adult *Drosophila* without anesthesia. Journal of Insect Physiology, 63: 27-31. https://doi.org/10.1016/j.jinsphys.2014.02.005.

Maddrell, S. H. P. (1991). The fastest fluid-secreting cell known: The upper Malpighian tubule of *Rhodnius*. *BioEssays*, *13*(7), 357–362. https://doi.org/10.1002/bies.950130710.

Mitchell, H. (2008). *Sweeteners and Sugar Alternatives in Food Technology*. John Wiley & Sons.

Munro, I. C., Bernt, W. O., Borzelleca, J. F., Flamm, G., Lynch, B. S., Kennepohl, E., Bär, E. A., & Modderman, J. (1998). Erythritol: an interpretive summary of biochemical, metabolic, toxicological and clinical data. *Food and Chemical Toxicology*, *36*(12), 1139–1174. https://doi.org/10.1016/S0278-6915(98)00091-x.

O'Donnell, S., Baudier, K., Fiocca, K., & Marenda, D. R. (2018). Erythritol ingestion impairs adult reproduction and causes larval mortality in *Drosophila melanogaster* fruit flies (Diptera: Drosophilidae). *Journal of Applied Entomology*, *142*(1–2), 37–42. https://doi.org/10.1111/jen.12409.

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). *Journal of Insect Science*, *16*(47), Article 47. https://doi.org/10.1093/jisesa/iew031.

Olson, D. a. W. N. M., Fadamiro, H., Lundgren, J. O. nathan G., & Heimpel, G. E. (2000). Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology*, *25*(1), 17–26. https://doi.org/10.1046/j.1365-3032.2000.00155.

Price, Briana E., Jana C. Lee, and Man-Yeon Choi. (2021). Erythritol combined with non-nutritive sucralose increases feeding by *Drosophila suzukii*, quickens mortality and reduces oviposition. *Crop Protection*, 105812. https://doi.org/10.1016/j.cropro.2021.105812.

Qiu, W., Wang, Z., Nie, W., Guo, Y., & Huang, L. (2007). GC–MS Determination of sucralose in Splenda®. *Chromatographia*, *66*(11), 935–939. https://doi.org/10.1365/s10337-007-0422-4.

Roubos, C. R., Gautam, B. K., Fanning, P. D., Van Timmeren, S., Spies, J., Liburd, O. E., Isaacs, R., Curry, S., Little, B. A., & Sial, A. A. (2019). Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). *Journal of Applied Entomology*, *143*(6), 609–625. https://doi.org/10.1111/jen.12620.

Sampson, B. J., Easson, M. W., Stringer, S. J., Werle, C. T., Magee, D., & Adamczyk, J. J. (2019). Laboratory and field assessments of erythritol derivatives on the survival, reproductive rate, and control of *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, *112*(1), 173–180. http://dx.doi.org.ezproxy.proxy.library.oregonstate.edu/10.1093/jee/toy274

Sampson, B. J., Werle, C. T., Stringer, S. J., & Adamczyk, J. J. (2017a). Ingestible insecticides for spotted wing drosophila control: A polyol, Erythritol, and an insect growth regulator, Lufenuron. *Journal of Applied Entomology*, *141*(1–2), 8–18. https://doi.org/10.1111/jen.12350.

Schmidt-Jeffris, R. A., Beers, E. H., Smytheman, P., & Rehfield-Ray, L. (2021). Erythritol, an artificial sweetener, is acaricidal against pest mites and minimally harmful to a predatory mite. *Journal of Economic Entomology*, *114*(4), 1701–1708. https://doi.org/10.1093/jee/toab101.

Storey, D., Lee, A., Bornet, F., & Brouns, F. (2007). Gastrointestinal tolerance of erythritol and xylitol ingested in a liquid. *European Journal of Clinical Nutrition*, *61*(3), 349–354. https://doi.org/10.1038/sj.ejcn.1602532.

Tang, S. B., Lee, J. C., Jung, J. K., & Choi, M.-Y. (2017). Effect of erythritol formulation on the mortality, fecundity and physiological excretion in *Drosophila suzukii. Journal of Insect Physiology*, *101*, 178–184. https://doi.org/10.1016/j.jinsphys.2017.07.015.

Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, *43*(2), 501–510. https://doi.org/10.1603/EN13200.

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*(3), 793–802. https://doi.org/10.1007/s10340-016-0762-7

Tetzloff, W., F. Dauchy, S. Medimagh, D. Carr, & A. Bär. (1996). Tolerance to subchronic, high-dose ingestion of erythritol in human volunteers. *Regulatory Toxicology and Pharmacology*. 24(2), S286-S295. https://doi.org/10.1006/rtph.1996.0110.

van Handel, E. (1985a). Rapid determination of glycogen and sugars in mosquitos. Journal of the American Mosquito Control Association, 1, 299-301.

van Handel, E. (1985b). Rapid determination of total lipids in mosquitos. Journal of the American Mosquito Control Association, 1, 299-301.

Wahjudi, P. N., Patterson, M. E., Lim, S., Yee, J. K., Mao, C. S., & Lee, W.-N. P. (2010). Measurement of glucose and fructose in clinical samples using gas chromatography/mass spectrometry. *Clinical Biochemistry*, *43*(1–2), 198–207. https://doi.org/10.1016/j.clinbiochem.2009.08.028.

Wentz, K., Cooper, W. R., Horton, D. R., Kao, R., & Nottingham, L. B. (2020). The artificial sweetener, erythritol, has insecticidal properties against pear psylla (Hemiptera: Psyllidae). *Journal of Economic Entomology*, *113*(5), 2293–2299. https://doi.org/10.1093/jee/toaa124.

Woltz, J. M., Donahue, K. M., Bruck, D. J., & Lee, J. C. (2015). Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii. Journal of Applied Entomology*, *139*(10), 759–770. https://doi.org/10.1111/jen.12200.

Zandawala, M., Marley, R., Davies, S. A., & Nässel, D. R. (2018). Characterization of a set of abdominal neuroendocrine cells that regulate stress physiology using colocalized diuretic peptides in Drosophila. *Cellular and Molecular Life Sciences: CMLS*, *75*(6), 1099–1115. https://doi.org/10.1007/s00018-017-2682-y.

Zheng, C., Zeng, L., & Xu, Y. (2016). Effect of sweeteners on the survival and behaviour of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Effect of sweeteners on *Bactrocera dorsalis*. *Pest Management Science*, *72*(5), 990–996. https://doi.org/10.1002/ps.4079.

Supplementary Data

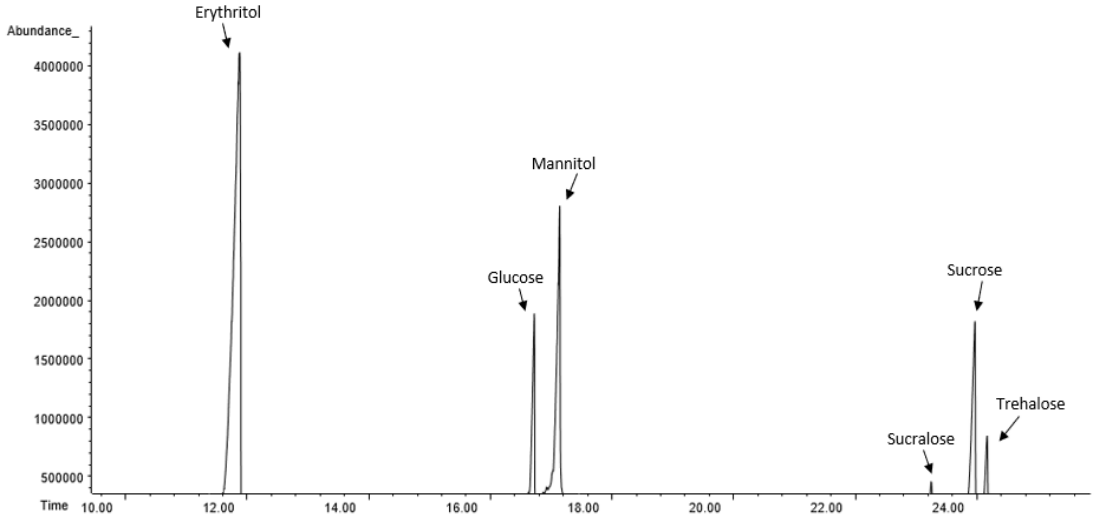

Figure S1. GC-MS gram of sugar derivatives in sugar standards. Mannitol used as internal standard. Sugars were derivatized, as described in methods, for GC-MS analysis (See detail in the Method and Materials).

CHAPTER 4

Field performance of erythritol for *Drosophila suzukii* control: effects on target and non-target species

Briana E. Price, Man-Yeon Choi, Carolyn Breece, Ramesh Sagili, Jana C. Lee

Abstract

Drosophila suzukii, commonly known as spotted-wing drosophila (SWD), is a major pest of small fruits and cherries and managed by extensive application of conventional chemical insecticides (Haviland & Beers, 2012). Here we investigate erythritol, a human-safe alternative insecticide, with two sweetening additives: sucrose and sucralose. Erythritol is a non-nutritive sugar that is insecticidal to SWD. In our previous work, we found that a 1.5M dose of erythritol increases mortality in flies when used in combination with 0.5M sucrose or 0.1M sucralose. For erythritol to be developed as an option for growers, several aspects are investigated here: non-target effects on honeybees (*Apis mellifera*), yellow jackets (*Vespula pensylvanica*), and the blueberry crop itself during multiyear field trials. We monitored blueberry bushes sprayed with experimental solutions for non-target insect visitation frequency and found that honeybees do not visit sprayed vegetation any more than unsprayed vegetation, although yellow jackets visited both areas sprayed with erythritol formulations significantly more than unsprayed. Further, we directly exposed honeybee brood to our erythritol treatments and found no negative impacts to brood development. Due to abundant visitation of yellow jackets, we tested erythritol toxicity by providing our formulations to adult yellow jackets to feed on for 7 days. There was no detectable toxicity from erythritol and sucralose to yellow jackets. Sugar sprays were applied once berries started to ripen and become vulnerable to *D. suzukii* infestation. During field trials, there was minimal spotted-wing drosophila infestation, making results for efficacy of erythritol sprays inconclusive; hence more field trials should be conducted. However, there were valuable findings pertaining to how sprays affected blueberry leaves; both solutions caused reddish-brown spotting on leaves that are likely caused by the sugar droplet residue. Although there was leaf damage, fruit quality assessments showed that our sprays did not negatively impact the firmness, size, or penetrative force required to pierce the epidermis and °Brix of the fruit. Overall, these results show that our two erythritol formulations cause undetectable non-target damage to honeybees, yellow jackets, and blueberry fruit.

Introduction

Spotted-wing drosophila (SWD), *Drosophila suzukii*, is a destructive invasive pest from Asia that attacks a wide range of ripening small fruits and cherry crops, with estimated crop losses of US$718 million annually if left unmanaged (Bolda et al. 2010). Primary fruit injury is due to larval feeding, though damage from oviposition can also lead to mold, pathogen infection, and attracts secondary pests such as *Drosophila melanogaster* (Walsh et al. 2011, Rombaut et al. 2017). Current management of spotted-wing drosophila populations extensively consists of rotational application of conventional chemical insecticides including synthetic pyrethroids, organophosphates, spinosyns, and a few carbamates and neonicotinoids despite detriment to the environment, human health, and non-target organisms (Haviland & Beers, 2012). The current decline in biodiversity of many insect taxa and insectivorous birds is attributed to agricultural practices and use of organophosphates, pyrethroids and neonicotinoids (Hallmann et al. 2014; Sánchez-Bayo & Wyckhuys, 2019).

The prospective environmentally friendly and human-safe insecticide erythritol is a non-nutritional sugar alcohol. Erythritol is safe for human consumption (Munro et al. 1998), insecticidal to spotted-wing drosophila (Choi et al. 2017, 2019; Goffin et al. 2017; Sampson et al. 2017, 2019; Tang et al. 2017), and has the potential to be utilized as an aerosol spray on small fruits. Sucrose acts as a phagostimulant, or an enticing additive, when added to insecticides, and has shown potential in increasing mortality in *D. suzukii* (Cowles et al. 2015, Roubos et al. 2019). Using sucrose as a phagostimulant risks fueling *D. suzukii* with nutritional carbohydrate if flies do not ingest a lethal amount (Tang et al. 2017) or may encourage microbial development on the host fruits as seen on plants coated with honey dew (Nelson, 2008). As an alternative to sucrose, sucralose is a potential candidate as a non-nutritive phagostimulant since it is nearly 600x sweeter than sucrose. Sucralose, the main ingredient in Splenda®, is safe for human consumption (Binns, 2003) and can be combined with erythritol in miniscule amounts while achieving the sweetness comparable to sucrose. Sucralose has been observed to be phagostimulative in laboratory conditions (Price et al. 2021). Since erythritol works most efficiently by direct consumption (Tang et al. 2017), Price et al. (2021) investigated mortality when using sucralose as a phagostimulative additive to erythritol. Both 1.5M erythritol: 0.5M sucrose and 1.5M erythritol: 0.1M sucralose efficiently killed spotted-wing drosophila in under

seven days, though the formulation with sucralose kills faster with no water access (~24-36 hours) compared to when access to water was present (~72 hours) because erythritols lethality is related to *D. suzukii* hyperosmolarity in the fly body and sucralose being non-nutritional; flies die faster with erythritol and sucralose combination because they are starving and the body is not maintaining homeostasis. Here we further investigate the target and non-target effects of these two formulations.

While erythritol has detrimental effects on a variety of pests including other flies such as: *Drosophila melanogaster* (Baudier et al. 2014, O'Donnell et al. 2016, 2018), *Bactocera dorsalis* (Zheng et al. 2016), *Musca domestica* (Burgess & King, 2017; Fisher et al. 2017), and *Stomoxys calcitrans* (Burgess & Geden, 2019), as well as the mosquito *Aedes aegypti* (Gilkey et al. 2018), and the pear psyllid, *Cacopsylla pyricola* (Lee et al. 2021; Wentz et al. 2020), impacts on non-target arthropods pose a concern. For erythritol to become available as a viable option for spotted-wing drosophila management, several aspects need consideration, such as the impact on non-target organisms and efficacy of the sugar in a field setting where *D. suzukii* are naturally found. Erythritol sprayed on plants would undoubtedly be encountered by non-target, potentially beneficial insects. For instance, sucrose sprays are often used to supplement and attract beneficial predators and parasitoids (Wade et al. 2008). To date, laboratory studies have found that erythritol is minimally harmful to a non-target predatory mite (Schmidt-Jeffris et al. 2021) and honeybee adults (Choi et al. 2019).

Bees are vital in pollinating flowering plants and trees, especially crop plants that humans rely on for food (Ollerton et al. 2011). While bees primarily visit crops while in bloom, some do visit post-harvest to use fallen fruits as food source when nectar sources are scarce (Shackleton et al. 2016). Erythritol sprays would be applied post-bloom, thus minimizing risk to pollinators while maximizing exposure to spotted-wing drosophila. Erythritol is water-soluble (Fry, 2012) and can easily wash off by rainfall, overhead irrigation, or by hose at the end of harvest. Erythritol residue may remain present on plant matter after harvest, making toxicity to non-target organisms a concern. Thus, our first objective was to observe visitation by non-target insects to post-bloom erythritol-sprayed blueberry during target field trials, as well as unsprayed blueberry and wild Himalayan blackberry bushes in different proximity of honeybee hives. We

hypothesized that visitation rates by honeybees would be similar between both groups since bees primarily forage for pollen and nectar.

As mentioned before, erythritol had no significant impacts on adult honeybees in a laboratory cage-incubation study (Choi et al. 2019). Bees were fed and observed for seven days, and survivorship among erythritol-fed bees was like those fed only sucrose. Honeybee physiology is different than that of dipterans; nectar is stored in the honey stomach instead of instant ingestion and absorption into hemolymph or insect blood. A main reason why erythritol is toxic to spotted-wing drosophila is because erythritol immediately diffuses into hemolymph, causing high osmotic pressure inside the fly body (Choi et al. 2019). After storage in the honeybee crop (honey stomach), bees bring back provisions and perform trophallaxis to nectar receiving workers, then can store the provisions as honey (Wright et al. 2018) and nurse bees can use these resources to produce brood food to developing honeybee brood. Although honeybees feed developing larvae nutrients produced in mandibular and hypopharyngeal glands (He et al. 2016), potential mandibular contamination from erythritol residues may effect developing larvae. This brings up concern of erythritol toxicity to honeybee brood; our second objective will assess how a high-dose exposure impacts the developing brood from larval stage to pupation and adult emergence. We hypothesize that high-dose (worst-case scenario) exposure of our field formulations containing erythritol to honeybee larvae will cause higher mortality when compared to controls.

In preliminary field trial observations, yellow jackets were observed more frequently in erythritol-sprayed bushes than any other insect. Yellow jacket foragers (*Vespula* spp.) are strongly attracted to fruit odor cues from viable carbohydrate sources (Jarau & Hrncir, 2009), making them a nuisance pest during harvest season. Given that they are a nuisance pest and non-target organism, our third objective evaluates yellow jacket survival on erythritol formulations in no-choice and choice laboratory assays.

While erythritol is successful in killing spotted-wing drosophila in laboratory and greenhouse trials, field trials are needed because other factors may reduce the effectiveness of erythritol in the field. Such factors include competing sugar sources; *D. suzukii* may favor other berries such as raspberry or blackberry over our experimental blueberry crop (Bellamy et al. 2013), erythritol sprays could wash off with rain or irrigation, and since it is sprayed, inner

canopy of bushes may not be completely covered and could harbor unaffected *D. suzukii*. Sampson et al. (2019) field tested various erythritol derivatives without a sweet additive on blueberries and found a 64-82% reduction in *D. suzukii* infestation. Given the success of our erythritol+sucrose and erythritol+sucralose formulas in past laboratory trials (Price et al. 2021), our fourth objective was to further test these two formulations in the field. During 2020-2021 field seasons, blueberries were sprayed with either formulation and compared to an unsprayed control. We hypothesized that fruit infestation, adult *D. suzukii* collected in baited traps, and oviposition rates would be lower in erythritol-sprayed plots when compared to unsprayed blueberry plots.

Erythritol has been observed as supplemental to the development of radish, garlic and mushrooms by promoting root growth (Kuroda et al. 2008) but seen to cause dose-dependent toxicity to other agricultural plants such as corn and tomato seed germination (Scanga et al. 2018). Honeydew, or sugary residues left from sap-sucking insects such as aphids, is known to cause sooty mold on leaves which can inhibit photosynthesis (Nelson, 2008). Whether our erythritol sprays affect host plants or fruit quality must be taken into consideration if used as a management tool for *D. suzukii* . Thus, in our fourth objective, we also monitored bushes for presence of leaf damage and mold. During the first year, discoloration throughout blueberry leaves was observed in erythritol treatments, we hypothesize that the erythritol sprays could be detrimental to fruit quality such as firmness of fruit epidermis, size and sugar content (Duarte et al. 2009, Lee et al. 2016). Firmness is especially important because it can affect storability and approval by consumers (Ehlenfeldt & Martin, 2002). Thus, post-harvest fruit quality assessments were performed during blueberry field trials in the second year.

2. Materials, Methods and Statistics

2.1 *Sugars and polyols*

Sugars and polyols used in this study were erythritol (Baolingbao Biology Co. Dezhou, China), sucralose (Bulk Supplements, NV, USA), and sucrose (Kroger, OH, USA). Solutions were dissolved in distilled water. Formulations used in this study are 1.5M erythritol: 0.5M sucrose (E+S) and 1.5M erythritol: 0.1M sucralose (E+Sul).

2.2 *Non-target species visitation during field trials of erythritol for spotted-wing drosophila control*

In the 2020-2021 outdoor field trials, the frequency of non-target insect visitation was observed for 2 min per plot weekly (Section 2.5). This duration was chosen as Couvillon et al. (2015) assessed bee visitation for one minute per plant from initial spotting. During both years, we focused on honeybee and yellow jacket visitation, but also noted other non-target insect visitations and reported them by order. In 2021, an additional trial examined whether honeybees preferentially visit erythritol-sprayed over unsprayed vegetation (non-flowering) at varying distances from hives. Three sets of honeybee hives managed by Oregon State University were located near sprayed areas. In August, a 4m swath of Himalayan blackberry or 'Reka' blueberries were sprayed E+S, E+Sul, or nothing as a control. Among the five blocks, sprayed areas were ~8 m, 10 m, 30 m, 70 m, and 300 m away from honeybee hives. Treatments in each block were spaced ~20 m apart. An initial observation was made immediately after spraying, four days after spraying, then weekly for three weeks on sunny days, with a total of five observations over time. To increase the chances of counting different bees, two 3-min observations were made per replicate at least 30 min apart on a given day for a total of 6 min per day.

The number of bees or yellow jacket observed during outdoor 2020-2021 trials were tested as a dependent variable in generalized linear mixed models (GLMM) with treatment, week and treatment*week interactions as fixed effects, block as a random effect, and treatment*block as a random subject effect for repeated measures. A Poisson distribution was used. For the 2021 additional trial, the first analysis compared the number of bees as the dependent variable. Since the distance of sprayed bushes varied from beehives, distance was categorized into two groups: <30 m and >30 m. The GLMM included treatment, distance group, their interaction, and time as fixed effects, and farm location, block, and plant type (blueberry or wild blackberry) as random effects. A Poisson distribution was used. Treatments were compared by Tukey HSD within each distance group. The second analysis compared the number of yellow jackets as the dependent variable in a GLMM with treatment, time, and their interaction as fixed effects, and farm, block

and plant type as random effects using a Poisson distribution. Analyses were done in Proc Glimmix using SAS 9.4 (SAS Institute 2016).

2.3 *Honeybee brood survival when exposed to high-dose erythritol*

To evaluate whether high exposure of erythritol solutions reduced honeybee brood survival, solutions were directly added into brood cells. Eight-frame Langstroth hives of varying population density with sister queens were chosen. In each hive, frames were examined for appropriate age larvae; young (1-2 d) and old (4-5 d) and marked. Transparent acetate sheets have been used to track *Apis mellifera* brood of known ages in other experiments (Human et al. 2013), and thus were adapted for our study. Three clusters of 28 larvae were marked by drawing circles around brood cells on acetate sheets (30.4 x 45.7 cm, Oasis Supply, Atlanta, GA), then all circles were punched into 0.64 cm holes using a drip irrigation hole puncher (DIG, CA, USA). By using a novel approach of pipetting through the acetate sheet holes, each larva was exposed to 2 µl of experimental E+S solution, E+Sul solution, or distilled water for the control (Fig. 1). By aligning the acetate sheets over each hive frame, survival was monitored daily for the first 3 d, then weekly for 3 wk to monitor effects until completion of adult development (from larvae to pupation until adult emergence). This was repeated twice for young larvae and twice for older larvae in each hive and repeated in three sister hives with varying population.

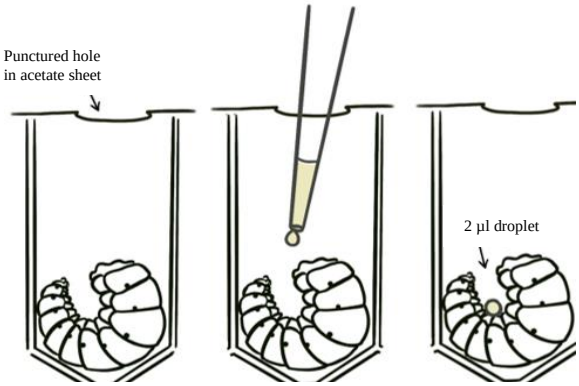

Figure 1. Diagram of high-dose exposure methods for erythritol relative to honeybee brood toxicity; novel method of pipette dripping 2 µl of solution on to larvae through acetate sheet. *Illustrated by Briana Price.*

Survivorship between days 1-21 was compared between each treatment and larval age combination by Kaplan-Meier estimates and log-rank test in Proc Lifetest in SAS 9.4 (SAS Institute 2016). Remaining survivors at 21 d were censored data. A non-parametric approach was used because survivorship lines crossed. Multiple comparisons were done using an adjusted Sidak P-value. Since treatments did not vary within each age group, a simplified log-rank test pooled young and old bees together to compare the three treatments. An end assessment compared the proportion of successfully capped cells or emerged adults as a dependent variable

in GLMMs with age, treatment, age*treatment as fixed effects, and hive as a random effect using a binomial distribution in Proc Glimmix.

2.4 *Adult western yellow jacket survival after erythritol consumption*

Wild adult western yellow jackets (*Vespula pensylvanica*) were be captured at Lewis Brown Farm, in Corvallis, Oregon using no-kill RESCUE® traps (Spokane, WA, USA) with a heptyl butyrate lure. Traps were placed near a nest, so captured wasps were most likely from the same nest but not the same age. Yellow jackets were trapped, transported back to lab in a cooler, and then temporarily immobilized at 4ºC. Once immobilized, two to four individuals were transferred via soft forceps into 32 oz. (946.35 ml) plastic cup arenas with aerated lids. Under no-choice conditions, wasps were given: 1) E+S solution, 2) E+Sul solution, or 3) 0.5M sucrose solution soaked in a sponge in a 1 oz. cup (30 ml), with water available in a second sponge-cup. Under choice conditions, yellow jackets were given a choice to feed on E+S and sucrose, or E+Sul and sucrose, with water available in a third sponge-cup. Yellow jackets were maintained at room temperature and survival was monitored for seven days. Both no-choice and choice assays were conducted simultaneously. After deaths, all individuals were pinned and confirmed as *V. pensylvanica* using morphological identification (Akre et al. 1980).

Each choice and no choice assay were repeated with 28 individuals per treatment across several weeks in July-August 2021. Survival from days 1-7 was compared between treatments by Kaplan-Meier estimates and log-rank analysis in Proc Lifetest, and multiple comparisons used an adjusted Sidak P-value. Remaining survivors at 7 d were censored data.

2.5 *Performance of erythritol for spotted-wing drosophila control in field setting – larval infestation, adult trapping, and oviposition behavior*

During 2020-2021, field trials in various blueberry cultivars were conducted. The ‘outdoor 2020’ trial was conducted in ‘Elliot’ blueberry variety from a 40 x 64 m field. The ‘outdoor 2021’ trial was conducted in a 40 x 53 m field of ‘Bluecrop’ variety, and in an experimental Oregon State University ORUS-235-3 clone planted in a 5 m row. Another ‘hoop house 2021’ trial was conducted among two rows of field-grown Elliot recently enclosed in a 6.5 x 80 m mesh hoop house. Plot size, separation, replication, dates, and assessments are summarized in Table 1. Bushes were sprayed with either E+S or E+Sul solutions using a

pressurized CO_2 sprayer at 38 PSI (R & D Sprayers, Opelousas, Louisiana), or nothing as a control.

Cultivars utilized in 2021 were treated differently from one another due to the following constraints: Bluecrop was managed with rotational spray applications of Malathion and Mustang Max, so fruit collected for measuring larval infestation occurred only at the beginning and end of trials. Only one replicate was conducted in OSU ORUS-235-3 clones and a second reapplication of erythritol sprays occurred during week two since these blueberries were over-head irrigated. The hoop house trial had several hundred laboratory-reared flies released weekly followed by weekly fruit collection to measure larval infestation. Since those blueberry bushes were in an enclosed setting, we did not trap adult flies nor count non-target insect visitation (Obj. 3).

Table 1. Experimental details of field trials.

Trial	Plot per replicate	Design	Dates	Assessments
Outdoor 2020	2 bushes	Completely Randomized Design 5 replicates per treatment in Elliot, plots 15+ m apart	July 14 to August 25	**Weekly:** larval infestation[1], adult trapping[2], oviposition[3], non-target visitation[4], mold[5] **End:** Leaf damage, mold[6]
Outdoor 2021	3 bushes	Randomized Complete Block Design (RCBD) 4 blocks in Bluecrop, 7+ m apart 1 block in clones, 0.5 m apart	June 23 to July 19	**Weekly:** larval infestation, adult trapping, oviposition, non-target visitation, leaf damage **End:** mold, fruit quality[7]
Hoop House 2021	3 bushes	RCBD 5 blocks in Elliot, 6+ m apart	June 30 to August 11	**Weekly:** larval infestation **End:** Leaf damage, mold, fruit quality

[1]Collect fruit, weigh fruit, let sit in screen-top container for two weeks, then count adult *D. suzukii* emergences.
[2]Bait traps with apple cider vinegar:white wine (1:1) with a drop of unscented soap and hang in the inner canopy, replace bait weekly, count *D. suzukii* adults.
[3]Place mesh bag over unripe berries week 0. Once ripened, add laboratory reared flies to clusters for 24 h, then count eggs laid.
[4]Watch for honeybee and yellow jacket visitation for two minutes at each plot, weekly.
[5]Visibly scan berries for signs of powdery mildew or *Botyrtis* mold as described in Polashock et al. (2017).
[6]See section: *Plant health and fruit quality assessment during field trials of erythritol for spotted-wing drosophila control* for methodology per year and crop cultivar.
[7] Measure the firmness of flesh, fruit size, penetration force of the fruit epidermis, and °Brix as described in *Plant health and fruit quality assessment during field trials of erythritol for spotted-wing drosophila control* section.

In all trials, larval infestation was measured by collecting ~300 g of ripe blue fruit per plot and rearing them in mesh containers at room temperature in the laboratory. Adult emergence was then recorded two weeks post collection. In outdoor trials, activity of spotted-wing drosophila adults was monitored using baited traps. A 32 oz. (946.35 ml) plastic cup with twenty holes drilled, 4 mm in diameter, and five pieces of 5 x 8 cm red duct tape containing about 200 ml of apple cider vinegar:white wine (1:1) with a drop of unscented soap was hung in the inner canopy and replaced weekly. Trapped *D. suzukii* adults were identified to species morphologically and subsequently sexed using a stereoscope (Leica Microsystems, Wetzlar, Germany).

Larval infestation remained low in all three trials; emerging adults from collected fruit were summed per treatment and no statistical analyses were done. The number of adult *D. suzukii* in baited traps was compared as the dependent variable in a GLMM with treatment, week and their interaction as fixed effects, and plot as a random subject effect for a repeated measures. Block was a random effect if relevant for the trial, and cultivar was a random effect since the outdoor 2021 trial consisted of Bluecrop and clone varieties. A Poisson distribution was used, and Tukey HSD for multiple comparisons.

To test if formulations deter oviposition, laboratory-reared *D. suzukii* were exposed to ripe berries in the outdoor 2020 and 2021 trials. Initially, clusters of un-ripened green berries were bagged using drawstring mesh bags (30 x 18 cm) to prevent wild *D. suzukii* infestation. Once ripened, ten female flies were placed on clusters of 3-15 berries in mesh bags, with a cotton water wick, overnight. The following day, clusters of berries were collected, and the number of eggs laid in the berries were counted under a stereoscope. For both years, four mesh bag replicates were set up per plot over several weeks. The number of eggs laid (dependent variable) was compared in a generalized linear model (GLM) with treatment, week, and their interaction as fixed effects with a Poisson or negative binomial distribution.

2.6 *Plant health and fruit quality assessment during field trials of erythritol for spotted-wing drosophila control*

Leaf damage and mold. We observed reddish-grey spots congruent with the sugar droplets and inferred this as a possible sign of phytotoxicity, as it differs from normal disease, insect or sun

damage (Polaschock et. al. 2017) (Figure 2). In 2020, each plot was assessed at the end of the trial for phytotoxic damage. For each plot, the total number of branches and branches displaying phytotoxic damage were recorded. Also in 2020, blueberry plants were scanned weekly for visible signs of *Botyrtis*, powdery mildew, or other mold as described in Polashock et al. (2017). As a side project, phytotoxic damage was observed and documented via photolog on separately sprayed branches of plants in non-trial plots for several weeks (Appendix A). The purpose of this was to see how long it took for bushes to show symptoms of phytotoxicity after the initial spray.

In the outdoor 2021 trial, we quantified the incidence of leaf damage per week. On Bluecrop blueberries, fifteen branches from each plot were randomly chosen, flagged and total number of leaves on each branch were recorded. All branches were monitored weekly for four weeks. Each week, the number of leaves showing signs of phytotoxicity were counted. In the 2021 hoop house trial, an end assessment of phytotoxic damage was made. Ten branches were randomly chosen, the total number of leaves on each branch and number of leaves with phytotoxic damage were recorded. Presence of mold was also assessed at the end of the outdoor and hoop house trials. Berries throughout the bush were randomly checked for mold until 100-200 total berries were scanned.

For end assessments on phytotoxic damage in the outdoor 2020 and hoop house 2021 trials, the number of damaged leaves out of total leaves (dependent variable) was compared in GLMMs with treatment as a fixed effect. Block was a random effect if relevant, and treatment*replicate was a random effect to recognize each branch/observation within an experimental unit as subsamples. For the outdoor 2021 trial, weekly phytotoxicity was compared with damaged leaves out of total leaves (dependent variable) in a GLMM with treatment, week, treatment*week interactions, and canopy position as fixed effects. Treatment*replicate*branch was a random subject effect in a repeated measures, and block was random. A binomial, lognormal or normal distribution was used depending on fit in these GLMM. Very few or no mold was observed in trials, and total counts are reported instead of statistical analyses.

Fruit quality assessments. During 2021 trials, fruit from outdoor Bluecrop and clones, and Elliot in a hoop house were randomly collected at harvest to measure the firmness of flesh, fruit size, penetration force of the fruit epidermis, and total soluble solids (TSS), also known as °Brix. These are suitable procedures for measuring blueberry quality and correlate with *D. suzukii*

susceptibility (Lee et al. 2016). Collected fruit were stored at 4°C, and all measurements were taken within 24 h of collection. Fifty berries were randomly chosen for measuring fruit firmness and diameter with Fruit Firm 1000 (CVM, Inc., Pleasanton, CA). Flesh firmness is reported as g/mm, the pressure needed to depress the fruit per 1 mm. Separately, fifty berries per plot were used to test penetration force of the fruit skin and °Brix. First, penetration force was measured with a penetrometer consisting of a gram-force gauge (Wagner Instruments, Greenwich, CT) and a No. 3 insect pin (Elephant Brand, Austria) attached with foam and protruding 3 mm from the end. The ball of the pin was removed, and the blunt end was used to poke fruit. Readings from the penetrometer are reported in centiNewtons (cN) (Burrack et al. 2012, Lee et al. 2016). Three readings were taken per fruit on the girth and averaged. The TSS were measured in five groups of ten macerated berries, using a digital refractometer (Hanna Instruments Inc., Woonsocket, RI), with three subsamples taken per maceration and averaged.

The flesh firmness, diameter, penetration force of epidermis, or TSS of fruits (dependent variable) were compared in GLMMs with treatment as a fixed effect, with block and treatment*block as random effects; block*treatment was used to recognize subsamples within an experimental unit. Cultivar was a random effect in the outdoor 2021 trial, and the person taking the measurement was a random effect for comparing penetration force. A lognormal or normal distribution was used depending on fit for these GLMMs.

3. Results

3.1 *Non-target visitation during field trials of erythritol for spotted-wing drosophila control*

In the outdoor 2020 trial, there was no difference in the frequency of honeybee visits among treatments ($F_{2, 15}$=0.97, P=0.40), although there was a significant difference in yellow jacket visitation ($F_{2, 15}$=28.23, P<0.001). Nearly 200 yellow jackets visited E+S sprayed bushes, while 52 visited unsprayed control and 41 visited E+Sul sprayed bushes. In 2021, there was minimal honeybee visitation amongst all treatments and no analysis. Much like 2020, yellow jacket visitation differed among treatments ($F_{2, 12}$=4.8, P=0.029) with E+Sul having the most yellow jacket visitors and control the least, although the overall number of visitations was much less during 2021. Total honeybee and yellow jacket visitation are reported in Table 2. While observing honeybee and yellow jacket visitation both years, other non-target insects belonging to

Diptera, Hymenoptera, and Coleoptera were also observed. No analysis was done for these because visitation was similar among all treatments; total visitation is also reported in Table 2.

In 2021, our additional trial examining whether honeybees preferentially visited erythritol-sprayed vegetation (non-flowering) with varying distances to established hives indicated that honeybee visitation did not differ among treatments (Fig. 2a, $F_{2, 9}$=1.42, P=0.29). Bushes near hives were visited marginally more frequently than bushes 30+ m away from hives (Fig 2a, $F_{1, 56}$=3.81, P=0.056), and no treatment*distance interaction occurred ($F_{2,56}$=0.11, P=0.89). Yellow jacket visitation was impacted by treatment ($F_{2,12}$=5.4, P=0.021), and higher in E+S than control bushes (Fig. 2b). With this additional trial and consideration of 200+ yellow jackets visiting in 2020, there seems to be an overall higher attraction to E+S by yellow jackets than E+Sul.

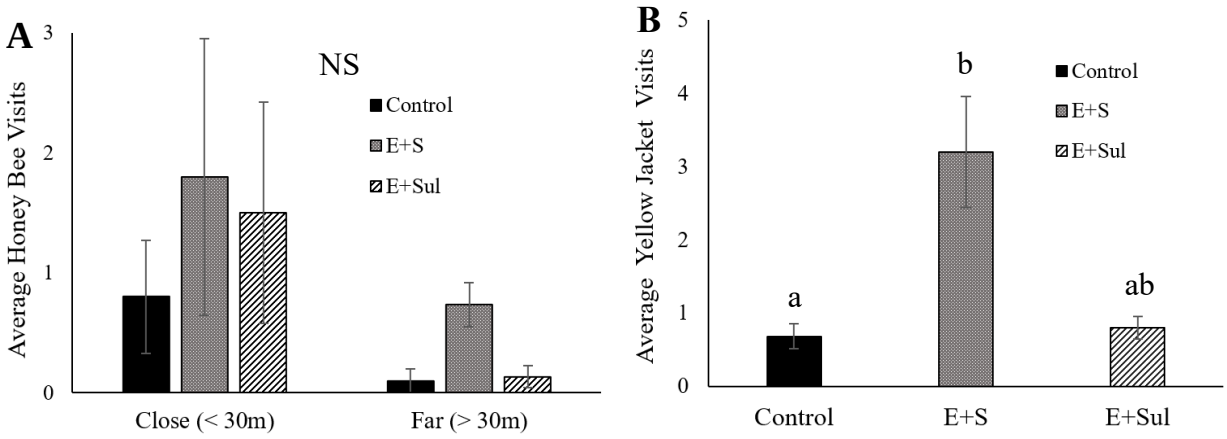

Figure 2. Average number of honeybees (a) or yellow jackets (b) ± SE visiting blackberry and blueberry bushes sprayed with erythritol formulations and unsprayed control over four weeks. Bushes are various distances (see methods) from established honeybee hives and grouped together <30 m and >30 m for statistical analysis. E+S= (1.5M Erythritol: 0.5M Sucrose) and E+Sul (1.5M Erythritol: 0.1M Sucralose). NS: no significance.

Table 2. Total count of non-target insect visitations by visual observation during field trials

Trial	Treatment	Yellow Jackets	Honeybees	Other Diptera[1]	Other Hymenoptera[2]	Other Coleoptera[3]
Outdoor 2020	Control	52	7	29	20	2
	E+S	204	12	28	22	2
	E+Sul	41	6	20	21	0
Outdoor 2021	Control	2	0	23	0	0
	E+S	6	1	31	6	0
	E+Sul	11	2	20	1	0

[1]Syrphidae and Muscidae, not identified to species
[2]Apidae (*Bombus* sp.), Vespidae, and other small bees not identified to species
[3]Chrysomelidae*: Diabrotica undecimpunctata*, spotted-cucumber beetle, Coccinellidae: (lady birds, not identified to species)

3.2 *Honeybee brood survival relative to high-dose erythritol exposure*

Survivorship differed between the six treatment*age combinations (χ^2=118.1, df=5, P<0.001). Older larvae had higher survival rates than young larvae. Survivorship within an age group was similar between treatments, multiple comparisons revealed the following trend: E+Sul*old = Control*old = E+S*old > Control*young = E+S*young E+Sul*young. When pooling young and old larvae together, survival on the three treatments was similar (Fig. 3, χ^2=2.8, df=2, P=0.241). During the final assessment, the proportion of successfully capped cells and emerged bees were quantified. Among all treatments, 55-60% young larvae and 85-100% old larvae pupated or capped their cells (Treatment: $F_{2, 22}$=1.08, P=0.83, Age: $F_{2, 22}$=22.19, P<0.001, Age*Treatment: $F_{2, 22}$=0.51, P=0.61). Additionally, 55-60% young larvae and 80-100% old larvae emerged as adults (Treatment: $F_{2, 22}$=0.2, P=0.82, Age: $F_{1, 22}$=18.9, P=<0.001, Age*Treatment: $F_{2, 22}$=0.31, P=0.74).

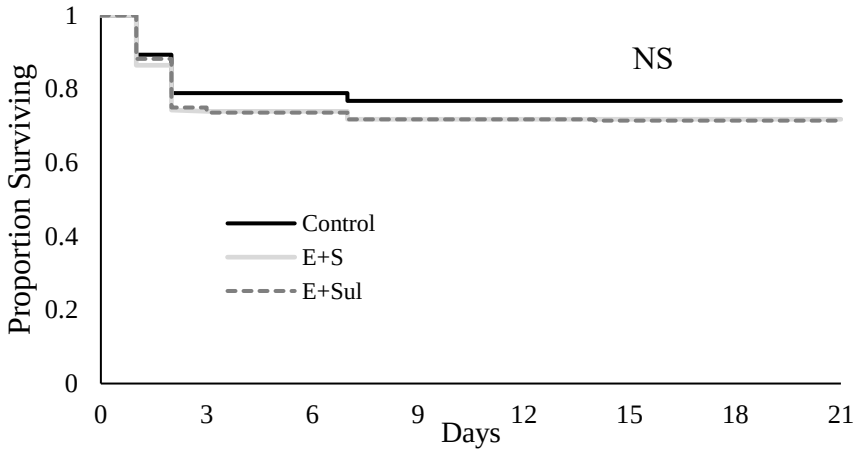

Figure 3. Kaplan-Meier survival curves of developing honeybee brood after larvae were exposed to control (distilled water), E+S (1.5M Erythritol: 0.5M Sucrose) and E+Sul (1.5M Erythritol: 0.1M Sucralose) to capped cell pupation, to emergence after 21 days. NS: no significance.

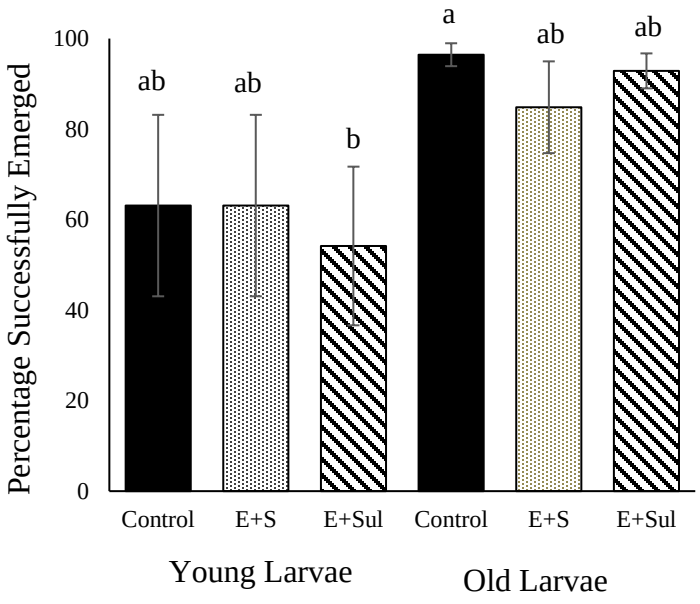

Figure 4. Percent of honeybee brood that successfully emerged ± SE after exposure to control (distilled water), E+S E+S= (1.5M Erythritol: 0.5M Sucrose) and E+Sul (1.5M Erythritol: 0.1M Sucralose). Young larvae were 1-4 days old and old larvae 5-8 days old at the time of exposure. Different letters denote statistical significance (*P <0.05) by Tukey HSD.

3.3 *Adult yellow jacket survival after erythritol consumption*

Yellow jacket adult survival rates were different among treatments (χ^2 =22.93, df=4, P<0.001). Overall, survivorship was not strong among yellow jackets fed sucrose. Individuals survived similarly to the positive control sucrose when fed E+S and E+Sul formulas with supplemental sucrose available. When fed E+Sul alone, 80% yellow jackets died in 24 h, but when E+Sul & sucrose was available, 80% mortality was seen by 4 d (Fig. 5). Among the two no-choice treatments, E+S and E+Sul, mortality was slower in E+S-fed yellow jackets, with 35% survival by day 3.

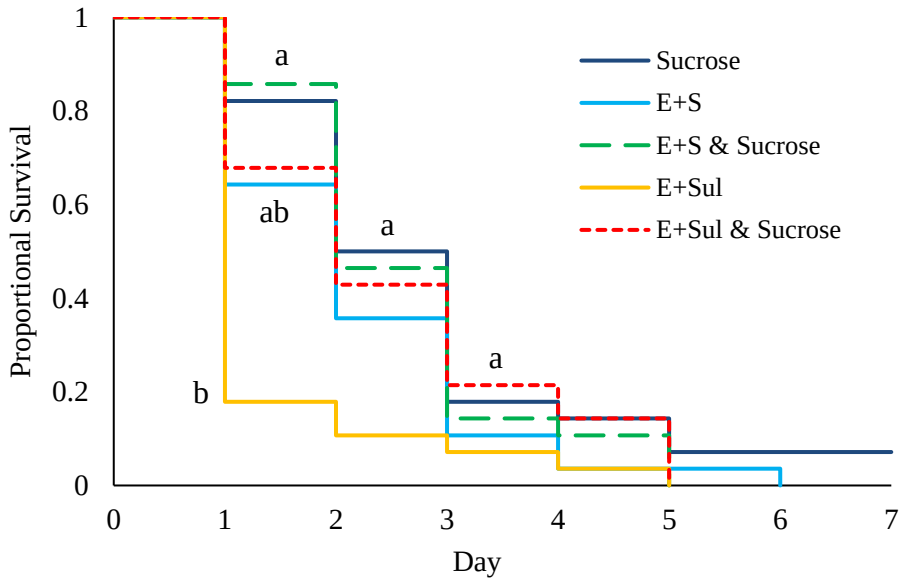

Figure 5. Kaplan-Meier survival curves of yellow jacket adults with availability to feed on erythritol treatments with and without choice of sucrose available. All treatments had available water to drink. E+S E+S= (1.5M Erythritol: 0.5M Sucrose) and E+Sul (1.5M Erythritol: 0.1M Sucralose). Different letters indicate statistical significance by adjusted sidak (P<0.05).

3.4 *Performance of erythritol for spotted-wing drosophila control in field setting*

Larval infestation in collected fruit. During both years, there was no difference in levels of larval infestation amongst treatments. Overall, wild *D. suzukii* infestation was minimal. In 2020, nearly 1 kg of fruit was collected from each treatment by end of the season, with only seven flies emerging from control, 16 from E+S treated berries, and 17 from E+Sul treated berries over a six-week period. In the outdoor 2021 trial with Bluecrop, nearly 3.5-4 kg of fruit was collected

from each treatment, from both beginning and end dates since the inner plot was sprayed with insecticides. Roughly 0.5 kg of fruit was collected from each treatment in ORUS-235-3 clones by the end of the season. In both cases, there was zero larval infestation. In the hoop house, a total of 9.5-11 kg of fruit was collected per treatment by the end of the season; only 1-2 adults emerged in all treatments over a seven-week period.

Adult trapping. In 2020, the number of adults in baited traps significantly differed by treatment overall ($F_{2, 15}$=4.86, P=0.02), especially for three weeks after spraying (treatment*week $F_{10,75}$=2.2, P=0.024) (Fig. 6). Traps near E+S treated bushes had the most flies, then control, and E+Sul having the least flies. In contrast, in the outdoor 2021 trial, minimal adults were trapped per week per plot with no difference detected between treatments ($F_{6,30}$=0.85, P=0.54), (Table 2).

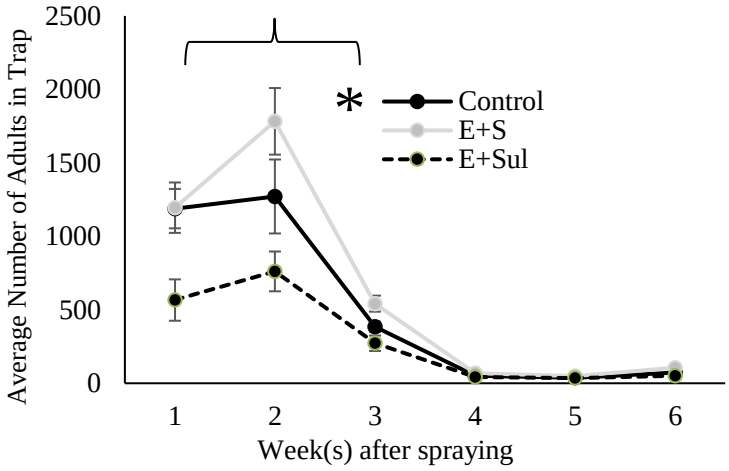

Figure 6. Average number ± standard error of adult *D. suzukii* caught in apple cider vinegar:wine traps per week during Outdoor 2020 trial. Asterisk indicates strong statistical difference between week 1-3. E+S (1.5M Erythritol: 0.5M Sucrose) and E+Sul (1.5M Erythritol: 0.1M Sucralose).

Oviposition in field trials. During both years, a similar number of eggs were laid among treatments (2020: $F_{2, 45}$=0.25, P=0.77, 2021: $F_{2, 51}$=0.66, P=0.52). Averages per treatment are reported in Table 2.

Table 2. Summary of results from field trials: adult *D. suzukii* trapping, oviposition bag study, and fruit quality assessments during 2020-2021.

		D. suzukii		**Fruit Quality Assessment**			
Trial	**Treatment**	**Average Adults in Traps ± SE**	**Average Eggs Laid in Bags ± SE**	**Firmness (g/mm) ± SE**	**Diameter ± SE**	**Penetration Force (cN) ± SE**	**°Brix ± SE**
Outdoor 2020	Control	500 ± 99.87	16.77 ± 3.72	-	-	-	-
	E+S	624 ± 119.53	14.22 ± 3.81	-	-	-	-
	E+Sul	287 ± 57.27	18.16 ± 3.74	-	-	-	-
Outdoor 2021	Control	1.33 ± 0.45	8.25 ± 4.27	207.06 ± 9.82	14.87 ± 0.47	13.92 ± 3.35	13.74 ± 0.36
	E+S	2.22 ± 0.81	12.25 ± 3.28	201.15 ± 5.42	14.82 ± 0.55	12.97 ± 3.14	13.55 ± 0.37
	E+Sul	1 ± 0.51	11.6 ± 4.77	202.61 ± 9.83	15.23 ± 0.57	13.20 ± 2.22	14.21 ± 0.62
Hoop House 2021	Control	-	-	164.37 ± 3.56	12.33 ± 0.62	13.91 ± 3.35	13.74 ± 0.36
	E+S	-	-	158.67 ± 3.88	12.03 ± 0.40	12.96 ± 3.13	13.55 ± 0.37
	E+Sul	-	-	161.34 ± 4.52	11.99 ± 0.49	13.2 ± 2.22	14.2 ± 0.62

3.5 *Plant health and fruit quality assessment during field trials*

Leaf damage. During the outdoor 2020 trial, sprayed bushes had more damage in comparison to control bushes during the end assessment ($F_{2, 15}$=12.21, P<0.001, Fig. 7c). There was no difference in sun-scalding damage between treatments ($F_{2, 15}$=1.42, P=0.27, data not shown).

During the outdoor 2021 trial, control plots had no signs of damage, while E+S and E+Sul sprayed plots had increasing damage as the weeks progressed (treatment $F_{2,175}$=46.7, P<0.001, Fig. 7b). One week after the spray application, there was no difference between treatments. During weeks 2, 3 and 4, there was a significant difference from control bushes and experimental bushes (treatment*week $F_{6,531}$=16.6, P<0.001). The upper canopy showed most severe damage, followed by mid-canopy, and minimal damage in lower canopy ($F_{2, 531}$=15.32, P<0.001; 17%, 14%, and 5% damage, respectively).

During the hoop house 2021 trial, potential phytotoxicity was recorded by randomly choosing ten branches in each plot and counting the proportion of leaves showing damage 7 wk after spraying. Sprayed bushes had a significant increase in damage in comparison to control bushes; E+Sul sprayed bushes had 6% damage while E+S had 1% damage ($F_{2, 8}$=21.7, P<0.001, Fig 7d).

Mold presence. In 2021, bushes were scanned for visible signs of *Botyrtis* mold and powdery mildew. We referenced symptoms of these as described in Polashock et al. (2017); there was 1.4% appearance of *Botyrtis* mold on E+S sprayed fruit after 4 wk in our outdoor trial, less than 1% on control, and none on E+Sul sprayed. There was no appearance of these on any treatments in the hoop house trial.

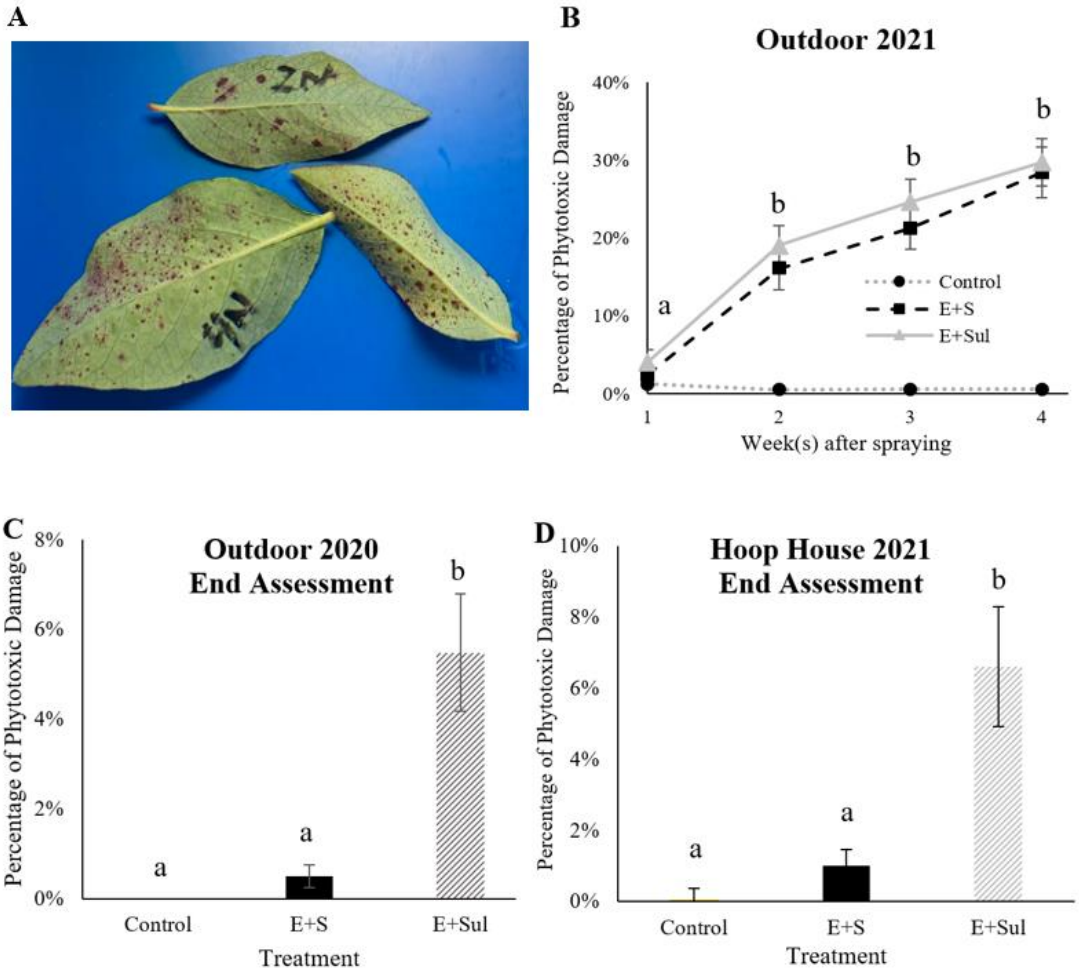

Figure 7.

Figure 7. Visual example of damage on E+Sul sprayed leaves (a), percentage of blueberry leaves showing phytotoxicity over time ± standard error (SE) during Outdoor 2021 trials (b), percentage showing damage ± SE during end assessment in Outdoor 2020 trial (c) and Hoop House 2021 trial (d). Control=no spray, E+S= 1.5M Erythritol: 0.5M Sucrose, E+Sul = 1.5M Erythritol: 0.1M Sucralose. Different letters denote statistical significance (*P <0.05) by Tukey HSD.

Fruit quality assessment. All fruit quality tests showed that the erythritol sprays were not detrimental when sprayed on bushes as their fruit began to color. During 2021 field trials, fruit was collected to measure fruit firmness, size, penetration force of the fruit skin, and °Brix. Table 3 summarizes these results. In the outdoor 2021 trial, fruit firmness and diameter were similar among treatments (Firmness: $F_{2, 11}$=0.27, P = 0.76, Diameter: $F_{2, 11}$=1.03, P=0.38). Penetration force to puncture fruit skin was also similar among treatments ($F_{2,11}$=3.87, P=0.053) as well as °Brix ($F_{2, 11}$=0.49, P=0.62). In the hoop house 2021 trial, there was also no detectable difference in the firmness, size, penetration force nor °Brix of berries between treatments (Firmness: $F_{2, 12}$=1.29, P = 0.31, Diameter: $F_{2,12}$=0.21, P=0.81, Penetration force: $F_{5,9}$=0.9, P=0.51, °Brix: $F_{2, 12}$=0.78, P=0.48).

4. Discussion

In this study we examined non-target effects and field performance of two prospective human-safe insecticide options targeted toward *D. suzukii* control: erythritol+sucrose and erythritol+sucralose. This work explored non-target effects on honeybees (*Apis mellifera*) since they are important in providing agricultural pollination services, which are valued near US$6 billion annually (Southwick & Southwick, 1992). Although yellow jackets are often considered nuisance pests, western yellow jackets (*Vespula pensylvanica*) were commonly seen visiting sprayed areas, thus we also explored non-target effects to adult yellow jackets. We also noted other non-target insects visiting our experimental plots: syrphidae and muscidae flies, lady bird and cucumber beetles, bumblebees, solitary bees and hornets or wasps not identified by plain sight. No experimental tests were done to examine non-target effects to these groups, and this should be explored further. In our outdoor 2020-2021 trials, honeybee visitation was similar among all treatments which indicates that honeybees are not considerably lured toward the sugar sprays. Total visitation by honeybees was markedly lower in 2021 compared to 2020, this could have been due to insecticide sprays, unusually hot weather (Parsons, Seasonal Climate Forecast Verification) or that the 2021 field was further away from established hives. Our additional trial conducted in late August-September 2021 examined whether honeybees preferentially visit erythritol-sprayed vegetation with varying distances from hives. Our findings indicated that honeybee visitation was similar between sprayed and unsprayed bushes, regardless of bushes being near or away from hives. On average, less than two bees were observed per plot per sample period. Since our sprays were sprayed on fruiting bushes (non-flowering), these results confirm that honeybees are not particularly attracted towards either spray.

Choi et al. (2019) showed that the erythritol+sucrose combination had no significant effects on adult honeybee survivorship. Although there was relatively scarce honeybee visitation to sprayed bushes, investigating toxicity to developing brood is important as foraging adults may consume and bring back erythritol residues to the hive. In contrast to other studies that have tested insecticide toxicity to laboratory reared honeybee brood *in vitro* (Doublet et al. 2015), we tested our erythritol formulations *in vivo* in established 8-frame Langstroth hives. Developing honeybee brood release pheromones to signal feeding needs to nurse bees and stimulate worker bees to forage (Conte 1995). Honeybee adults produce royal jelly in mandibular and

hypopharyngeal glands, located in their heads, and feed brood royal jelly during the first few days of development, then a combination of pollen, honey, and other glandular secretions throughout their development until pupation (Crailsheim 1990). Due to the complexity of cooperative brood care within a honeybee hive, our methods did not investigate assimilation of erythritol or sucralose residues into hypopharyngeal or mandibular glands or consider varying food demands or size between young and old larvae. Instead, our methods of dripping erythritol solutions directly into brood cells was to test mortality against exposure to an unrealistic volume and concentration of erythritol-residues. Our high-dose exposure results show no acute toxicity of either erythritol+sucrose or erythritol+sucralose combinations in comparison to control (distilled water), although, the number of emerged adult bees from the young larvae group were 35-40% less than old larvae group which suggests young larvae were more sensitive to this methodology. Additionally, overall survivorship decreased by 20-25% amongst young and old larvae within the first three days in all treatments; this reaction could be related to a disturbance in larval pheromone signaling. Pheromones are used by larvae to signal a need for more nutrition (He et al. 2016) and when our erythritol formulations were dripped into the larval brood cells, the sugars or polyols could have altered or blocked normal physiology involved in pheromone production or signaling. Another alternative explanation for the quick reduction in survivorship in the first few days could be due to chemical cues from immuno-stimulation where adult honeybees may remove sickened larvae from brood cells (Swanson et al. 2009). Overall, our results for honeybee visitation and brood survival are encouraging because adults do not preferentially forage on erythritol sprayed plants, and there was no detectable toxicity by erythritol formulations when exposed to brood at a high, unrealistic concentration. Since this experiment was conducted during a single field season, we plan to conduct another repeated trial during an upcoming field season to confirm the trend or alter methodology to account for the variable sizes of different ages of larvae.

Given that yellow jackets are considered a nuisance pest to growers during harvest season because of their nest-defense aggression and tendency to frequent crop fields with carbohydrate resources (Akre et al. 1980) and a significantly high visitation rate to our erythritol formulations during field trials, we conducted a simple survivorship experiment. Yellow jackets were fed field formulations directly, with and without a separate sucrose source to test if erythritol and sucralose is toxic or non-nutritive to yellow jackets. Unlike studies that have measured

insecticide toxicity to adult western yellow jackets through topical drop application (Lewallen, 1968, Johansen & Davis, 1972), we tested erythritol toxicity via consumption, since that is the mode of action that has been found to be insecticidal to the pests mentioned above. Our results show little to no acute toxicity of erythritol or sucralose to yellow jackets, but yellow jackets do starve if they cannot feed on a carbohydrate source. When fed only non-nutritive erythritol+sucralose, wasps died in 24 h, likely from starvation. When fed erythritol+sucrose, wasps had a source of nutrition and fared slightly longer than erythritol+sucralose. Survivorship of sucrose-fed wasps with and without erythritol formulations available were similar but relatively low. This is likely due to the conditions of the experiment; yellow jackets are a carnivorous *Vespula* spp. and may not fare well in captivity being fed only a carbohydrate.

During both years of field trials, larval infestation results were inconclusive in the Elliot variety (outdoor 2020), Blue Crop and ORS-235-3 clones (outdoor 2021), and Elliot variety in hoop house (hoop house 2021). In 2020, although adult traps were abundant with spotted-wing drosophila, blueberry infestation rates were minimal. This could have to do with more favorable host plants near our chosen plot; as spotted-wing drosophila prefer caneberries, raspberries and blackberries over blueberries (Bellamy et al. 2013). In 2021, three cultivars were utilized but treated differently due to location, availability, and whether chemical insecticides were applied to the inner plots. Infestation was also extremely low in the hoop house where laboratory-reared flies were released weekly. The low infestation rates could be due to flies being released in the hoop house during an unusual heat wave in Corvallis, OR 2021 (Parsons, Seasonal Climate Forecast Verification). It may have been too hot inside and flies could have died before laying eggs. If replicated in the future, flies would be released in evening, so they have 12 h of cool night temperatures to lay eggs since optimal egg laying temperature is between 18-22 °C (Tochen et al. 2014). Although our field efficacy trials were inconclusive given the low rate of infestation in all the treatments including the control, we intend to continue this work. Promising results were obtained from Sampson et al. (2019), who field tested erythritol alone on blueberries and found a 64-82% reduction in *D. suzukii* infestation.

During trials to directly examine oviposition rates with *D. suzukii* placed on blueberry clusters in mesh bags, oviposition rates were similar in all treatments in 2020-2021. We ensured that the blueberry clusters we used were viable hosts by confirming any laid eggs developed into

adults. In Price et al. (2021), erythritol coated berries were shown to be an ovipositional deterrent with flies showing a clear preference to lay eggs on control berries rather than erythritol coated; with 70% (erythritol+sucrose) and 44% (erythritol+sucralose) fewer eggs laid than the control. With supporting evidence of erythritol to deter egg laying, it is uncertain why we did not see a difference in oviposition rates among treatments.

By quantifying the proportion of leaves damaged following erythritol sprays, we observed noticeable leaf damage from the erythritol+sucralose spray, and little to no detriment from the erythritol+sucrose spray. Our sprays were applied upon ripening of the blueberry fruits and showed no detriment to fruit quality. It is unknown whether erythritol is toxic to blueberry bush or fruit development like in Scanga et al. (2018), where erythritol was harmful to corn and tomato seed germination and root growth (Scanga et al. 2018). Additionally, since the sugar sprays are water soluble, it could be assumed that the residues on leaves could be washed off after harvest. Amy-Sagers et al. (2017) previously hypothesized that sucralose in aquatic systems would negatively affect photosynthesis because it is an unmetabolizable chlorinated sugar molecule. They showed that sucralose acts as a carbon source and increases photosynthesis capacity in *Lemna minor,* or common duck weed. To our knowledge this is the first study to observe phytotoxic damage by sucralose on a terrestrial cultivated plant. Since our erythritol+sucralose spray affected 5-9% of blueberry leaves/branches, and erythritol+sucrose affected ~1% after 6-7 weeks during outdoor 2020 and hoop house 2021 trials, sucralose appears problematic for the plants. Although, both sprays led to 20-30% leaf damage in the outdoor 2021 trial, one difference in these trials is that outdoor 2021 was conducted during a heat surge (Parsons, Seasonal Climate Forecast Verification); the high temperatures and sun exposure could have increased phytotoxic effects. Since our plots were different each year, we cannot make an inference as to how the sprays effect the blueberry bush or fruit yield in following years. More research on how erythritol and sucralose affect blueberry plant development, leaf tissue analysis and other cultivated *D. suzukii* host plants should be conducted. To conclude, our two field formulations containing erythritol, sucrose or sucralose cause no detectable non-target damage to honeybees, yellow jackets, and blueberry fruit in comparison to control groups. Given the potential of our two erythritol formulations in killing *D. suzukii* in laboratory conditions in published mortality experiments, additional trials could be conducted in areas with higher wild *D. suzukii* infestation or different host crop type. Due to erythritol being water soluble, a humid

environment may yield different results than what we observed during the dry summer conditions of the Willamette Valley of Oregon. After the sprays dry on the plant, the flies may have difficulty feeding on the formulation. Further exploration into field efficacy in other climates is crucial in the development of a human-safe insecticide alternative for globally distributed pest *Drosophila suzukii*.

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References

Amy-Sagers, Cherisse, Keith Reinhardt, and Danelle M. Larson. (2017). Ecotoxicological assessments show sucralose and fluoxetine affect the aquatic plant, *Lemna Minor*. *Aquatic Toxicology (Amsterdam, Netherlands)* 185: 76–85. https://doi.org/10.1016/j.aquatox.2017.01.008.

Akre, R.D., A. Greene, J.F. MacDonald, P.J. Lan-dolt, and H. G. Davis. (1980). Yellowjackets of America North of Mexico. U.S. Department of Agriculture, Agriculture Handbook No. 552, 102 pp.

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*(6), e98949. https://doi.org/10.1371/journal.pone.0098949

Bellamy, D. E., Sisterson, M. S., & Walse, S. S. (2013). Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii. PLOS ONE, 8*(4), e61227. https://doi.org/10.1371/journal.pone.0061227

Binns, N. M. (2003). Sucralose—All sweetness and light. *Nutrition Bulletin, 28*(1), 53–58. https://doi.org/10.1046/j.1467-3010.2003.00307.x

Burgess, E. R., & Geden, C. J. (2019). Larvicidal potential of the polyol sweeteners erythritol and xylitol in two filth fly species. *Journal of Vector Ecology*, *44*(1), 11–17. https://doi.org/10.1111/jvec.12324

Burgess, E. R., & King, B. H. (2017). Insecticidal potential of two sugar alcohols to *Musca domestica* (Diptera: Muscidae). *Journal of Economic Entomology*, *110*(5), 2252–2258. https://doi.org/10.1093/jee/tox190

Choi, M.-Y., Lucas, H., Sagili, R., Cha, D. H., & Lee, J. C. (2019). Effect of erythritol on *Drosophila suzukii* (Diptera: Drosophilidae) in the presence of naturally-occurring sugar sources, and on the survival of *Apis mellifera* (Hymenoptera: Apidae). *Journal of Economic Entomology*, *112*(2), 981–985. https://doi.org/10.1093/jee/toy362

Choi, M.-Y., Tang, S. B., Ahn, S.-J., Amarasekare, K. G., Shearer, P., & Lee, J. C. (2017). Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii. Journal of Insect Physiology*, *99*, 86–94. https://doi.org/10.1016/j.jinsphys.2017.04.001

Conte, Y. L. (1995). Brood pheromone can modulate the feeding behavior of *Apis mellifera* workers (Hymenoptera: Apidae). *JOURNAL OF ECONOMIC ENTOMOLOGY*, *88*(4), 7.

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*(2), 640–653. https://doi.org/10.1093/jee/tou100

Crailsheim, K. (1990). The protein balance of the honeybee worker. *Apidologie*, *21*(5), 417–429. https://doi.org/10.1051/apido:19900504

Doublet, V., Labarussias, M., de Miranda, J. R., Moritz, R. F. A., & Paxton, R. J. (2015). Bees under stress: Sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honeybee mortality across the life cycle: Pesticide-pathogen interactions in honeybees. *Environmental Microbiology*, *17*(4), 969–983. https://doi.org/10.1111/1462-2920.12426

Duarte, C., Guerra, M., Daniel, P., Camelo, A. L., & Yommi, A. (2009). Quality changes of highbush blueberries fruit stored in CA with different CO2 levels. *Journal of Food Science*, *74*(4), S154–S159. https://doi.org/10.1111/j.1750-3841.2009.01118.x

Ehlenfeldt, M. K., & Martin, R. B. (2002). A survey of fruit firmness in highbush blueberry and species-introgressed blueberry cultivars. *HortScience*, *37*(2), 386–389. https://doi.org/10.21273/HORTSCI.37.2.386

Fisher, M. L., Fowler, F. E., Denning, S. S., & Watson, D. W. (2017). Survival of the house fly (Diptera: Muscidae) on Truvia and other sweeteners. *Journal of Medical Entomology*, *54*(4), 999–1005. https://doi.org/10.1093/jme/tjw241

Fry, J. C. (2012). Natural low-calorie sweeteners. In *Natural Food Additives, Ingredients and Flavourings* (pp. 41–75). https://doi.org/10.1533/9780857095725.1.41

Gilkey, P. L., Bolshakov, D. T., Kowala, J. G., Taylor, L. A., O'Donnell, S., Marenda, D. R., & Sirot, L. K. (2018). Lethal effects of erythritol on the mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae). *Journal of Applied Entomology*, *142*(9), 873–881. https://doi.org/10.1111/jen.12538

Goffin, J., Gallace, N., Berkvens, N., Casteels, H., De Ro, M., Bylemans, D., & Beliën, T. (2017). Toxicity of erythritol, a sugar alcohol and food additive, to *Drosophila suzukii* (Matsumara). *Acta Horticulturae*, *1156*, 843–848. https://doi.org/10.17660/ActaHortic.2017.1156.124

Hallmann, C. A., Foppen, R. P. B., van Turnhout, C. A. M., de Kroon, H., & Jongejans, E. (2014). Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature*, *511*(7509), 341–343. https://doi.org/10.1038/nature13531

Haviland, D. R., & Beers, E. H. (2012). Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. *Journal of Integrated Pest Management*, *3*(2), 1–6. https://doi.org/10.1603/IPM11034

He, X. J., Zhang, X. C., Jiang, W. J., Barron, A. B., Zhang, J. H., & Zeng, Z. J. (2016). Starving honeybee (*Apis mellifera*) larvae signal pheromonally to worker bees. *Scientific Reports*, *6*(1), 22359. https://doi.org/10.1038/srep22359

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Jaffé, R., Jensen, A., Köhler, A., Magyar, J., Özkyrym, A., Pirk, C., Rose, R., Strauss, U., Retschnig, G., Tarpy, D., & Zheng, H.-Q. (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research*, *52*. https://doi.org/10.3896/IBRA.1.52.4.10

Jarau, S., & Hrncir, M. (2009). *Food Exploitation By Social Insects: Ecological, Behavioral, and Theoretical Approaches*. CRC Press.

Johansen, C. A., & Davis, H. G. (1972). Toxicity of nine insecticides to the western yellowjacket. *Journal of Economic Entomology*, *65*(1), 40–42. https://doi.org/10.1093/jee/65.1.40

Kuroda, K., Hirakawa, S., Suzuki, M., Shinji, K., Ogasa, K., Uraji, T., Amachi, T., & Ueda, M. (2008). Growth acceleration of plants and mushroom by erythritol. *Plant Biotechnology*, *25*(5), 489–492. https://doi.org/10.5511/plantbiotechnology.25.489

Lee, J. C., Dalton, D. T., Swoboda-Bhattarai, K. A., Bruck, D. J., Burrack, H. J., Strik, B. C., Woltz, J. M., & Walton, V. M. (2016). Characterization and manipulation of fruit susceptibility to *Drosophila suzukii. Journal of Pest Science*, *89*(3), 771–780. https://doi.org/10.1007/s10340-015-0692-9

Lee, S.-H., Choe, D.-H., & Lee, C.-Y. (2021). The impact of artificial sweeteners on insects. *Journal of Economic Entomology*, *114*(1), 1–13. https://doi.org/10.1093/jee/toaa244

Lewallen, L. L. (1968). Preliminary toxicity studies on the yellowjacket *Vespula pensylvanica* (Saussure) in California. *California Vector Views*, *15*(1). https://www.cabdirect.org/cabdirect/abstract/19701001242

Munro, I. C., Bernt, W. O., Borzelleca, J. F., Flamm, G., Lynch, B. S., Kennepohl, E., Bär, E. A., & Modderman, J. (1998). Erythritol: An interpretive summary of biochemical, metabolic, toxicological and clinical data. *Food and Chemical Toxicology*, *36*(12), 1139–1174. https://doi.org/10.1016/S0278-6915(98)00091-X

O'Donnell, S., Baudier, K., Fiocca, K., & Marenda, D. R. (2018). Erythritol ingestion impairs adult reproduction and causes larval mortality in *Drosophila melanogaster* fruit flies (Diptera: Drosophilidae). *Journal of Applied Entomology*, *142*(1–2), 37–42. https://doi.org/10.1111/jen.12409

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). *Journal of Insect Science, 16*(47), Article 47. https://doi.org/10.1093/jisesa/iew031

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos, 120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Price, B. E., Lee, J. C., & Choi, M.-Y. (2021). Erythritol combined with non-nutritive sucralose increases feeding by Drosophila suzukii, quickens mortality and reduces oviposition. *Crop Protection*, 105812. https://doi.org/10.1016/j.cropro.2021.105812

Rombaut, A., Guilhot, R., Xuéreb, A., Benoit, L., Chapuis, M. P., Gibert, P., & Fellous, S. (2017). Invasive *Drosophila suzukii* facilitates *Drosophila melanogaster* infestation and sour rot outbreaks in the vineyards. *Royal Society Open Science*, *4*(3), 170117. https://doi.org/10.1098/rsos.170117

Roubos, C. R., Gautam, B. K., Fanning, P. D., Van Timmeren, S., Spies, J., Liburd, O. E., Isaacs, R., Curry, S., Little, B. A., & Sial, A. A. (2019). Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). *Journal of Applied Entomology*, *143*(6), 609–625. https://doi.org/10.1111/jen.12620

Sampson, B. J., Easson, M. W., Stringer, S. J., Werle, C. T., Magee, D., & Adamczyk, J. J. (2019). Laboratory and field assessments of erythritol derivatives on the survival, reproductive rate, and control of *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, *112*(1), 173–180. http://dx.doi.org.ezproxy.proxy.library.oregonstate.edu/10.1093/jee/toy274

Sampson, B. J., Werle, C. T., Stringer, S. J., & Adamczyk, J. J. (2017). Ingestible insecticides for spotted wing drosophila control: A polyol, Erythritol, and an insect growth regulator, Lufenuron. *Journal of Applied Entomology*, *141*(1–2), 8–18. https://doi.org/10.1111/jen.12350

Sánchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, *232*, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Scanga, S. E., Hasanspahič, B., Zvorničanin, E., Koženjić, J. S., Rahme, A. K., & Shinn-Thomas, J. H. (2018). Erythritol, at insecticidal doses, has harmful effects on two common agricultural crop plants. *PLOS ONE*, *13*(4), e0192749. https://doi.org/10.1371/journal.pone.0192749

Schmidt-Jeffris, R. A., Beers, E. H., Smytheman, P., & Rehfield-Ray, L. (2021). Erythritol, an artificial sweetener, is acaricidal against pest mites and minimally harmful to a predatory

mite. *Journal of Economic Entomology*, *114*(4), 1701–1708. https://doi.org/10.1093/jee/toab101

Shackleton, K., Balfour, N. J., Toufailia, H. A., Gaioski, R., Barbosa, M. de M., Silva, C. A. de S., Bento, J. M. S., Alves, D. A., & Ratnieks, F. L. W. (2016). Quality versus quantity: Foraging decisions in the honeybee (Apis mellifera scutellata) feeding on wildflower nectar and fruit juice. *Ecology and Evolution*, *6*(19), 7156–7165. https://doi.org/10.1002/ece3.2478

Southwick, E. E., & Southwick, L. (1992). Estimating the economic value of honeybees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology*, *85*(3), 621–633. https://doi.org/10.1093/jee/85.3.621

Swanson, J. A. I., Torto, B., Kells, S. A., Mesce, K. A., Tumlinson, J. H., & Spivak, M. (2009). Odorants that induce hygienic behavior in honeybees: identification of volatile compounds in chalkbrood-infected honeybee larvae. *Journal of Chemical Ecology*, *35*(9), 1108–1116. https://doi.org/10.1007/s10886-009-9683-8

Tang, S. B., Lee, J. C., Jung, J. K., & Choi, M.-Y. (2017). Effect of erythritol formulation on the mortality, fecundity and physiological excretion in *Drosophila suzukii. Journal of Insect Physiology*, *101*, 178–184. https://doi.org/10.1016/j.jinsphys.2017.07.015

Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, *43*(2), 501–510. https://doi.org/10.1603/EN13200

Wade, M. R., Zalucki, M. P., Wratten, S. D., & Robinson, K. A. (2008). Conservation biological control of arthropods using artificial food sprays: Current status and future challenges. *Biological Control*, *45*(2), 185–199. https://doi.org/10.1016/j.biocontrol.2007.10.024

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O'Neal, S. D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, *2*(1), G1–G7. https://doi.org/10.1603/IPM10010

Wentz, K., Cooper, W. R., Horton, D. R., Kao, R., & Nottingham, L. B. (2020). The artificial sweetener, erythritol, has insecticidal properties against pear psylla (Hemiptera:

Psyllidae). *Journal of Economic Entomology*, *113*(5), 2293–2299. https://doi.org/10.1093/jee/toaa124

Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honeybees. *Annual Review of Entomology*, *63*(1), 327–344. https://doi.org/10.1146/annurev-ento-020117-043423

Zheng, C., Zeng, L., & Xu, Y. (2016). Effect of sweeteners on the survival and behaviour of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Effect of sweeteners on *Bactrocera dorsalis*. *Pest Management Science*, *72*(5), 990–996. https://doi.org/10.1002/ps.4079

CHAPTER 5
General Conclusions

Briana Price

Spotted-wing drosophila, *Drosophila suzukii*, is a destructive invasive pest from Asia that attacks a wide range of ripening small fruits. Primary fruit injury is due to larval feeding and damage from egg laying also leads to mold growth and attracts secondary pests. Current management significantly involves of revolving insecticide application despite detriment to the environment, human health, and beneficial insects. A prospective environmentally-friendly and human-safe insecticide, erythritol has been researched in prior years (Choi et al. 2017, 2019; Goffin et al. 2017; Sampson et al. 2017, 2019; Tang et al. 2017) and has shown great success in decreasing fecundity and killing spotted-wing drosophila quickly. With confirmation of non-nutritive sugar, erythritol, having insecticidal properties to *Drosophila suzukii*, this thesis aimed to investigate effects of a sweet additional non-nutritive sugar, sucralose, on *D. suzukii* oviposition, survival, feeding preference, and physiology, examine field efficacy of two erythritol formulations: 1.5M erythritol+ 0.5M sucrose (E+S) and 1.5M erythritol+ 0.1M sucralose (E+Sul) on blueberry crop as well as non-target effects of those two formulations on blueberry crop, honeybee brood and adult yellow jacket survival.

We found that when sucralose is combined with erythritol, there was reduced oviposition rates, enhanced feeding, and quickened mortality, in comparison to erythritol+sucrose combination. Our three oviposition experiments supported that when erythritol formulations are given to flies in some form, there is a reduction in oviposition due to the sugar texture on the berry surface, meaning there is a deterrent effect. Our results suggest that berries coated with erythritol did not reduce survival of *D. suzukii* developing inside and the erythritol applied may not leach into the blueberry fruit skin enough to substantially affect developing larvae. In choice tests between erythritol alone vs. erythritol+sucralose formulations, flies preferred to feed on formulations containing 0.1M sucralose. Consumption of sucralose was comparable to water, so it not enticing alone, it elicits more feeding when combined with erythritol. Additionally, our mortality assays revealed that erythritol formulations with 0.1M sucralose were quickly detrimental and flies fed E+Sul died quicker than by starvation on water. These feeding and mortality results supported our hypothesis of sucralose being enticing, or phagostimulative, and a 0.1M sucralose was a suitable concentration to use for field trials.

We also investigated if *D. suzukii* metabolizes sucralose because it is a chlorinated form of sucrose. We performed various physiological experiments to investigate whether sucralose can be converted to a usable carbohydrate in the fly body. By examining glycogen, lipid and

sugar levels in flies fed sucralose through anthrone and vanillin tests, and quantifying amount of sugars in frass and hemolymph of flies through gas chromatography-mass spectrometry, we confirmed that *D. suzukii* do not metabolize sucralose to any substantial extent and sucralose is slowly accumulated in the hemolymph of flies. Much like erythritol, sucralose consumption leads to starvation, and hyperosmotic pressure that negatively effects fly survival. Flies fed only non-nutritive sugar combination erythritol+sucralose also become greatly dessicated in 24 h. The desiccation is likely due to flies using their own fluids to attempt restoration of physiological homeostasis. In field settings, flies would likely have access to water sources, so we also tested if our E+S or E+Sul formulations are still effective in killing spotted-wing drosophila when water is present. Several experiments showed that, while still effective, erythritol formulations kill *D. suzukii* slower when water is available; the E+S formulation is not as effective in killing *D. suzukii* as the zero-calorie E+Sul formulation.

During field trials, efficacy of managing wild spotted-wing drosophila populations using our E+S and E+Sul formulations was explored as well as non-target effects. During both years of field trials, larval infestation results were inconclusive which could have been due to presence of a more favorable host berry near our chosen plots. There was presence of phytotoxic damage to the various blue berry cultivars that we tested during both years. During the second year, we quantified the proportional phytotoxicity from our erythritol sprays and found noticeable phytotoxic damage from our E+Sul spray, and little to no detriment from our E+S spray. Our sprays were applied upon ripening of the blueberry fruits and showed no detriment to fruit quality (firmness, size, penetration force to pierce fruit epidermis and °Brix).

Additionally, during field trials, honeybee and yellow jacket visitation was monitored even though treatments would be sprayed on fruiting bushes (non-flowering); honeybee visitation frequency was similar among all treatments which would indicate that they are not lured toward the sugar sprays, even when bushes in closer proximity to hives are sprayed. Although scarce visitation, the impacts of foraging adults feeding erythritol to honeybee brood was previously unknown and our methods of dripping erythritol solutions directly into brood cells was our attempt to replicate forager bees regurgitating extremely high doses of erythritol-residuals to developing brood. Our results show that there is minimal toxicity of both erythritol and sucralose in comparison to control (distilled water). On the other hand, yellow jacket visitation was greater in both types of erythritol formulations so additionally tested erythritol

toxicity to adult yellow jackets. Our results show little to no toxicity by erythritol or sucralose to yellow jackets. When fed only non-nutritive E+Sul, wasps died in 24 h, likely dying from starvation. When fed E+S, wasps had a source of nutrition and fared slightly longer than E+Sul.

To conclude, our two field formulations,1.5M erythritol+ 0.5M sucrose (E+S) and 1.5M erythritol+ 0.1Msucralose (E+Sul), kill and reduce oviposition by spotted-wing drosophila efficiently in lab settings and do not cause detectable non-target damage to honeybees, yellow jackets, and blueberry fruit. Although our field infestation data was inconclusive, previous studies have more promising results of erythritol field performance for spotted-wing drosophila control. Given the potential of these formulations, this thesis work provides well-rounded information to further develop erythritol as a practical management product for growers to use.

Bibliography

Abrieux, A., & Chiu, J. C. (2016). Oral delivery of dsRNA by microbes: Beyond pest control. *Communicative & Integrative Biology*, *9*(6), e1236163. https://doi.org/10.1080/19420889.2016.1236163

Ahn, S.-J., Donahue, K., Koh, Y., Martin, R. R., & Choi, M.-Y. (2019). Microbial-Based double-stranded rna production to develop cost-effective RNA interference application for insect pest management. *International Journal of Insect Science*, *11*, 1179543319840323. https://doi.org/10.1177/1179543319840323

Allan, S. A. (2011). Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, *36*(1), 59–67. https://doi.org/10.1111/j.1948-7134.2011.00141.x

Amrein, H., & Thorne, N. (2005). Gustatory perception and behavior in *Drosophila melanogaster*. *Current Biology*, *15*(17), R673–R684. https://doi.org/10.1016/j.cub.2005.08.021

Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., Gibert, P., Gutierrez, A. P., Hoelmer, K. A., Hutchison, W. D., Isaacs, R., Jiang, Z.-L., Kárpáti, Z., Kimura, M. T., Pascual, M., Philips, C. R., Plantamp, C., Ponti, L., Vétek, G., … Desneux, N. (2015). Invasion biology of spotted wing drosophila (*Drosophila suzukii)*: A global perspective and future priorities. *Journal of Pest Science*, *88*(3), 469–494. https://doi.org/10.1007/s10340-015-0681-z

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., & Kopp, A. (2014). The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1781), 20132840. https://doi.org/10.1098/rspb.2013.2840

Barmina O, Kopp A. (2007). Sex-specific expression of a HOX gene associated with rapid morphological evolution. *Dev Biol* **311**: 277–286.

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*(6), e98949. https://doi.org/10.1371/journal.pone.0098949

Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., & Roberts, J. (2007). Control of

coleopteran insect pests through RNA interference. *Nature Biotechnology*, *25*(11), 1322–1326. https://doi.org/10.1038/nbt1359

Beardsley, J., K. Arakaki, G. Uchida, B. Kumashiro, and W. Perreira. 1999. New records for Diptera in Hawaii, Records Hawaii Biol. Survey for 1998, Part 1: 51-57.

Bernt, W. O., Borzelleca, J. F., Flamm, G., & Munro, I. C. (1996). *ARTICLE NO. 0098 Erythritol: A Review of Biological and Toxicological Studies*.

Bezerra Da Silva, C. S., Price, B. E., Soohoo-Hui, A., & Walton, V. M. (2019). Factors affecting the biology of Pachycrepoideus vindemmiae (Hymenoptera: Pteromalidae), a parasitoid of spotted-wing drosophila (*Drosophila suzukii)*. *PLOS ONE*, *14*(7), e0218301. https://doi.org/10.1371/journal.pone.0218301

Bezerra Da Silva, C. S., Price, B. E., & Walton, V. M. (2019). Water-Deprived Parasitic Wasps (Pachycrepoideus vindemmiae) Kill More Pupae of a Pest (*Drosophila suzukii*) as a Water-Intake Strategy. *Scientific Reports*, *9*(1), 3592. https://doi.org/10.1038/s41598-019-40256-8

Bing, X., Gerlach, J., Loeb, G., & Buchon, N. (2018). Nutrient-dependent impact of microbes on *Drosophila suzukii* development. *MBio*, *9*(2). https://doi.org/10.1128/mBio.02199-17

Binns, N. M. (2003). Sucralose—All sweetness and light. *Nutrition Bulletin*, *28*(1), 53–58. https://doi.org/10.1046/j.1467-3010.2003.00307.x

Bolda, M. P., Goodhue, R. E., & Zalom, F. G. (n.d.). *Spotted Wing Drosophila: Potential Economic Impact of a Newly Established Pest*. 4.

Bruck, D. J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., Gerdeman, B., & Spitler, H. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. *Pest Management Science*, *67*(11), 1375–1385. https://doi.org/10.1002/ps.2242

Burgess, E. R., Johnson, D. M., & Geden, C. J. (2018). Mortality of the house fly (Diptera: Muscidae) after exposure to combinations of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) With the polyol sweeteners erythritol and xylitol. *Journal of Medical Entomology*, *55*(5), 1237–1244. https://doi.org/10.1093/jme/tjy083

Burke, C. J., & Waddell, S. (2011). Remembering nutrient quality of sugar in Drosophila. *Current Biology*, *21*(9), 746–750. https://doi.org/10.1016/j.cub.2011.03.032

Chabert, S., Allemand, R., Poyet, M., Eslin, P., & Gibert, P. (2012). Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, *Drosophila suzukii*. *Biological Control*, *63*(1), 40–47. https://doi.org/10.1016/j.biocontrol.2012.05.005

Chiu, J. C., Jiang, X., Zhao, L., Hamm, C. A., Cridland, J. M., Saelao, P., Hamby, K. A., Lee, E. K., Kwok, R. S., Zhang, G., Zalom, F. G., Walton, V. M., & Begun, D. J. (2013). Genome of *Drosophila suzukii*, the spotted wing drosophila. *G3 Genes|Genomes|Genetics*, *3*(12), 2257–2271. https://doi.org/10.1534/g3.113.008185

Choi, M.-Y., Lucas, H., Sagili, R., Cha, D. H., & Lee, J. C. (2019). Effect of erythritol on *Drosophila suzukii* (Diptera: Drosophilidae) in the presence of naturally-occurring sugar sources, and on the survival of *Apis mellifera* (Hymenoptera: Apidae). *Journal of Economic Entomology*, *112*(2), 981–985. https://doi.org/10.1093/jee/toy362

Choi, M.-Y., Tang, S. B., Ahn, S.-J., Amarasekare, K. G., Shearer, P., & Lee, J. C. (2017). Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii*. *Journal of Insect Physiology*, *99*, 86–94. https://doi.org/10.1016/j.jinsphys.2017.04.001

Cini, A., Ioriatti, C., & Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology*, *65*, 149–160.

Cloonan, K. R., Abraham, J., Angeli, S., Syed, Z., & Rodriguez-Saona, C. (2018). Advances in the chemical ecology of the spotted wing drosophila (*Drosophila suzukii)* and its applications. *Journal of Chemical Ecology*, *44*(10), 922–939. http://dx.doi.org.ezproxy.proxy.library.oregonstate.edu/10.1007/s10886-018-1000-y

Corti, A. (1999). *Low-calorie Sweeteners: Present and Future*. Karger Medical and Scientific Publishers.

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*(2), 640–653. https://doi.org/10.1093/jee/tou100

Deprá, M., Poppe, J. L., Schmitz, H. J., De Toni, D. C., & Valente, V. L. S. (2014). The first records of the invasive pest *Drosophila suzukii* in the South American continent. *Journal of Pest Science*, *87*(3), 379–383. https://doi.org/10.1007/s10340-014-0591-5

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of “lure and kill” in long-term pest management and eradication of invasive species. *Journal of Economic Entomology*, *102*(3), 815–835. https://doi.org/10.1603/029.102.0301

FAO - News Article: Keeping plant pests and diseases at bay: experts focus on global measures. (n.d.). Retrieved July 2, 2021, from http://www.fao.org/news/story/en/item/280489/icode/

Farnsworth, D., Hamby, K. A., Bolda, M., Goodhue, R. E., Williams, J. C., & Zalom, F. G. (2017). Economic analysis of revenue losses and control costs associated with the spotted wing drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry: Economic analysis of spotted wing drosophila in California raspberries. *Pest Management Science*, *73*(6), 1083–1090. https://doi.org/10.1002/ps.4497

Garcia, F. R. M. (2020). *Drosophila suzukii Management*. Springer Nature.

Githiomi, C., Muriithi, B., Irungu, P., Mwungu, C. M., Diiro, G., Affognon, H., Mburu, J., & Ekesi, S. (2019). Economic analysis of spillover effects of an integrated pest management (IPM) strategy for suppression of mango fruit fly in Kenya. *Food Policy*, *84*, 121–132. https://doi.org/10.1016/j.foodpol.2019.03.006

Goffin, J., Gallace, N., Berkvens, N., Casteels, H., De Ro, M., Bylemans, D., & Beliën, T. (2017). Toxicity of erythritol, a sugar alcohol and food additive, to *Drosophila suzukii* (Matsumara). *Acta Horticulturae*, *1156*, 843–848. https://doi.org/10.17660/ActaHortic.2017.1156.124

Goodhue, R. E., Bolda, M., Farnsworth, D., Williams, J. C., & Zalom, F. G. (2011). Spotted wing drosophila infestation of California strawberries and raspberries: Economic analysis of potential revenue losses and control costs. *Pest Management Science*, *67*(11), 1396–1402. https://doi.org/10.1002/ps.2259

Green, J. E., Cavey, M., Médina Caturegli, E., Aigouy, B., Gompel, N., & Prud’homme, B. (2019). Evolution of Ovipositor Length in Drosophila suzukii Is Driven by Enhanced Cell Size Expansion and Anisotropic Tissue Reorganization. *Current Biology*, *29*(12), 2075-2082.e6. https://doi.org/10.1016/j.cub.2019.05.020

Gress, B. E., & Zalom, F. G. (2019). Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. *Pest Management Science*, *75*(5), 1270–1276. https://doi.org/10.1002/ps.5240

Grotz, V. L., & Munro, I. C. (2009). An overview of the safety of sucralose. *Regulatory Toxicology and Pharmacology*, *55*(1), 1–5. https://doi.org/10.1016/j.yrtph.2009.05.011

Gullickson, M. G., Rogers, M. A., Burkness, E. C., & Hutchison, W. D. (2019). Efficacy of organic and conventional insecticides for *Drosophila suzukii* when combined with erythritol, a non-nutritive feeding stimulant. *Crop Protection*, *125*, 104878. https://doi.org/10.1016/j.cropro.2019.104878

Hamby, K. A., E. Bellamy, D., Chiu, J. C., Lee, J. C., Walton, V. M., Wiman, N. G., York, R. M., & Biondi, A. (2016). Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii. Journal of Pest Science, 89*(3), 605–619. https://doi.org/10.1007/s10340-016-0756-5

Hampton, E., Koski, C., Barsoian, O., Faubert, H., Cowles, R. S., & Alm, S. R. (2014). Use of early ripening cultivars to avoid infestation and mass trapping to manage *Drosophila suzukii* (Diptera: Drosophilidae) in *Vaccinium corymbosum* (Ericales: Ericaceae). *Journal of Economic Entomology*, *107*(5), 1849–1857. https://doi.org/10.1603/EC14232

Hauser, M. (2011). A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science*, *67*(11), 1352–1357. https://doi.org/10.1002/ps.2265

Haviland, D. R., & Beers, E. H. (2012). Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. *Journal of Integrated Pest Management*, *3*(2), 1–6. https://doi.org/10.1603/IPM11034

Jarau, S., & Hrncir, M. (2009). *Food Exploitation By Social Insects: Ecological, Behavioral, and Theoretical Approaches*. CRC Press.

Kaneshiro, K. Y. 1983. *Drosophila* (Sophophora) *suzukii* (Matsumura). Proceedings of the Hawaiian Entomology Society 24: 179.

Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. *Studies on Drosophila suzukii Mats.* https://www.cabdirect.org/cabdirect/abstract/19410501073

Karageorgi, M., Bräcker, L. B., Lebreton, S., Minervino, C., Cavey, M., Siju, K. P., Grunwald Kadow, I. C., Gompel, N., & Prud'homme, B. (2017). Evolution of multiple sensory systems drives novel egg-laying behavior in the fruit pest *Drosophila suzukii. Current Biology*, *27*(6), 847–853. https://doi.org/10.1016/j.cub.2017.01.055

Kenis, M., Tonina, L., Eschen, R., van der Sluis, B., Sancassani, M., Mori, N., Haye, T., & Helsen, H. (2016). Non-crop plants used as hosts by *Drosophila suzukii* in Europe. *Journal of Pest Science*, *89*(3), 735–748. https://doi.org/10.1007/s10340-016-0755-6

Kuroda, K., Hirakawa, S., Suzuki, M., Shinji, K., Ogasa, K., Uraji, T., Amachi, T., & Ueda, M. (2008). Growth acceleration of plants and mushroom by erythritol. *Plant Biotechnology*, *25*(5), 489–492. https://doi.org/10.5511/plantbiotechnology.25.489

Lee, J. C. (2019). What we can learn from the energetic levels of insects: a guide and review. *Annals of the Entomological Society of America*, *112*(3), 220–226. https://doi.org/10.1093/aesa/say051

Lee, J. C., Dreves, A. J., Cave, A. M., Kawai, S., Isaacs, R., Miller, J. C., Van Timmeren, S., & Bruck, D. J. (2015). Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). *Annals of the Entomological Society of America*, *108*(2), 117–129. https://doi.org/10.1093/aesa/sau014

Lee, J. C., Wang, X., Daane, K. M., Hoelmer, K. A., Isaacs, R., Sial, A. A., & Walton, V. M. (2019). Biological control of spotted-wing drosophila (Diptera: Drosophilidae)—current and pending tactics. *Journal of Integrated Pest Management*, *10*(1), 13. https://doi.org/10.1093/jipm/pmz012

Lemaitre, B., & Miguel-Aliaga, I. (2013). The digestive tract of *Drosophila melanogaster*. *Annual Review of Genetics*, *47*(1), 377–404. https://doi.org/10.1146/annurev-genet-111212-133343

Mamta, B., & Rajam, M. V. (2017). RNAi technology: A new platform for crop pest control. *Physiology and Molecular Biology of Plants*, *23*(3), 487–501. https://doi.org/10.1007/s12298-017-0443-x

Mao, Y.-B., Cai, W.-J., Wang, J.-W., Hong, G.-J., Tao, X.-Y., Wang, L.-J., Huang, Y.-P., & Chen, X.-Y. (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology*, *25*(11), 1307–1313. https://doi.org/10.1038/nbt1352

Miller, B., Anfora, G., Buffington, M., Daane, K. M., Dalton, D. T., Hoelmer, K. M., Stacconi, M. V. R., Grassi, A., Ioriatti, C., Loni, A., Miller, J. C., Ouantar, M., Wang, X., Wiman, N. G., & Walton, V. M. (2015). Seasonal occurrence of resident parasitoids associated with *Drosophila suzukii* in two small fruit production regions of Italy and the USA. 9.

Mitchell, H. (2008). *Sweeteners and Sugar Alternatives in Food Technology*. John Wiley & Sons.

Moon, H., Jeya, M., Kim, I., & Lee, J. (2010). Biotechnological production of erythritol and its applications. *Applied Microbiology and Biotechnology; Heidelberg*, *86*(4), 1017–1025. http://dx.doi.org.ezproxy.proxy.library.oregonstate.edu/10.1007/s00253-010-2496-4

Morse, R. A., & Calderone, N. W. (2000). *National Agricultural Statistics Service of the USDA, in-*.

Munro, I. C., Bernt, W. O., Borzelleca, J. F., Flamm, G., Lynch, B. S., Kennepohl, E., Bär, E. A., & Modderman, J. (1998). Erythritol: An interpretive summary of biochemical, metabolic, toxicological and clinical data. *Food and Chemical Toxicology*, *36*(12), 1139–1174. https://doi.org/10.1016/S0278-6915 (98)00091-X

Murphy, K. A., Tabuloc, C. A., Cervantes, K. R., & Chiu, J. C. (2016a). Ingestion of genetically modified yeast symbiont reduces fitness of an insect pest via RNA interference. *Scientific Reports*, *6*(1), 1–13. https://doi.org/10.1038/srep22587

Murphy, K. A., West, J. D., Kwok, R. S., & Chiu, J. C. (2016b). Accelerating research on spotted wing drosophila management using genomic technologies. *Journal of Pest Science*, *89*(3), 631–641. https://doi.org/10.1007/s10340-016-0741-z

Nelson S., 2008. Sooty mold. Plant Dis. 52 www.stahr.hawaii.edu/oc/freepubs/pdf/PD-52.pdf

Nishida, G. 1997. Hawaiian Terrestrial Arthropod Checklist, Bishop Museum Technical Report. Bishop Museum, Honolulu, Hawaii. pg 263.

O'Grady, P., J. Beardsley, and W. Perreira. 2002. New records for introduced Drosophilidae (Diptera) in Hawai'i. Bishop Mus Occ Pap. 69: 34–35

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Olson, D. a. W. N. M., Fadamiro, H., Lundgren, J. O. nathan G., & Heimpel, G. E. (2000). Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology*, *25*(1), 17–26. https://doi.org/10.1046/j.1365-3032.2000.00155.x

Plantamp, C., Estragnat, V., Fellous, S., Desouhant, E., & Gibert, P. (2017). Where and what to feed? Differential effects on fecundity and longevity in the invasive *Drosophila suzukii*. *Basic and Applied Ecology*, *19*, 56–66. https://doi.org/10.1016/j.baae.2016.10.005

Poyet, M., Le Roux, V., Gibert, P., Meirland, A., Prévost, G., Eslin, P., & Chabrerie, O. (2015). The wide potential trophic niche of the asiatic fruit fly *Drosophila suzukii* : the key of its invasion success in temperate Europe? *PLoS ONE, 10*(11). https://doi.org/10.1371/journal.pone.0142785

Price, B. E., Yoon, J.-S., Choi, M.-Y., & Lee, J. C. (2021). Effects of nonnutritional sugars on lipid and carbohydrate content, physiological uptake, and excretion in *Drosophila suzukii.* Archives of Insect Biochemistry and Physiology, e21860. https://doi.org/10.1002/arch.21860.

Price, Briana E., Jana C. Lee, and Man-Yeon Choi. (2021). Erythritol combined with non-nutritive sucralose increases feeding by *Drosophila suzukii*, quickens mortality and reduces oviposition. *Crop Protection* 105812. https://doi.org/10.1016/j.cropro.2021.105812.

Prud'homme, B., Gompel, N., Rokas, A. *et al.* Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene. *Nature* **440,** 1050–1053 (2006). https://doi.org/10.1038/nature04597

Qiu, W., Wang, Z., Nie, W., Guo, Y., & Huang, L. (2007). GC–MS Determination of Sucralose in Splenda. *Chromatographia*, *66*(11), 935–939. https://doi.org/10.1365/s10337-007-0422-4

Raspi, A., Grassi, A., & Benelli, G. (2014). *Zaprionus tuberculatus (Diptera Drosophilidae):* First records from the European mainland. 4.

Rendon, D., Hamby, K. A., Arsenault-Benoit, A. L., Taylor, C. M., Evans, R. K., Roubos, C. R., Sial, A. A., Rogers, M., Petran, A., Van Timmeren, S., Fanning, P., Isaacs, R., & Walton, V. (2020). Mulching as a cultural control strategy for Drosophila suzukii in blueberry. *Pest Management Science*, *76*(1), 55–66. https://doi.org/10.1002/ps.5512

Rendon, D., Walton, V., Tait, G., Buser, J., Lemos Souza, I., Wallingford, A., Loeb, G., & Lee, J. (2019). Interactions among morphotype, nutrition, and temperature impact fitness of an invasive fly. *Ecology and Evolution*, *9*(5), 2615–2628. https://doi.org/10.1002/ece3.4928

Roderick, G. K., Hufbauer, R., & Navajas, M. (2012). Evolution and biological control. *Evolutionary Applications*, *5*(5), 419–423. https://doi.org/10.1111/j.1752-4571.2012.00281.x

Roubos, C. R., Gautam, B. K., Fanning, P. D., Van Timmeren, S., Spies, J., Liburd, O. E., Isaacs, R., Curry, S., Little, B. A., & Sial, A. A. (2019). Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). *Journal of Applied Entomology*, *143*(6), 609–625. https://doi.org/10.1111/jen.12620

Rzechonek, D. A., Dobrowolski, A., Rymowicz, W., & Mirończuk, A. M. (2018). Recent advances in biological production of erythritol. *Critical Reviews in Biotechnology*, *38*(4), 620–633. https://doi.org/10.1080/07388551.2017.1380598

Sampson, B. J., Easson, M. W., Stringer, S. J., Werle, C. T., Magee, D., & Adamczyk, J. J. (2019). Laboratory and field assessments of erythritol derivatives on the survival, reproductive rate, and control of *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, *112*(1), 173–180. http://dx.doi.org.ezproxy.proxy.library.oregonstate.edu/10.1093/jee/toy274

Sampson, B. J., Werle, C. T., Stringer, S. J., & Adamczyk, J. J. (2017). Ingestible insecticides for spotted wing drosophila control: A polyol, Erythritol, and an insect growth regulator, Lufenuron. *Journal of Applied Entomology*, *141*(1–2), 8–18. https://doi.org/10.1111/jen.12350

Scanga, S. E., Hasanspahič, B., Zvorničanin, E., Koženjić, J. S., Rahme, A. K., & Shinn-Thomas, J. H. (2018). Erythritol, at insecticidal doses, has harmful effects on two common agricultural crop plants. *PLOS ONE*, *13*(4), e0192749. https://doi.org/10.1371/journal.pone.0192749

Schmidt-Jeffris, R. A., E. H. Beers, P. Smytheman, and L. Rehfield-Ray. 2021. Erythritol, an artificial sweetener, is acaricidal against pest mites and minimally harmful to a predatory mite. Journal of Economic Entomology 114: 1701-1708.

Schöneberg, T., Lewis, M. T., Burrack, H. J., Grieshop, M., Isaacs, R., Rendon, D., Rogers, M., Rothwell, N., Sial, A. A., Walton, V. M., & Hamby, K. A. (2021). Cultural Control of Drosophila suzukii in Small Fruit—Current and Pending Tactics in the U.S. *Insects*, *12*(2), 172. https://doi.org/10.3390/insects12020172

Shackleton, K., Balfour, N. J., Toufailia, H. A., Gaioski, R., Barbosa, M. de M., Silva, C. A. de S., Bento, J. M. S., Alves, D. A., & Ratnieks, F. L. W. (2016). Quality versus quantity: Foraging decisions in the honeybee (*Apis mellifera* scutellata) feeding on wildflower

nectar and fruit juice. *Ecology and Evolution*, *6*(19), 7156–7165. https://doi.org/10.1002/ece3.2478

Shindou, T., Sasaki, Y., Eguchi, T., Euguchi, T., Hagiwara, K., & Ichikawa, T. (1989). Identification of erythritol by HPLC and GC-MS and quantitative measurement in pulps of various fruits. *Journal of Agricultural and Food Chemistry*, *37*(6), 1474–1476. https://doi.org/10.1021/jf00090a004

Steck, G. J., & Steck, G. (2005). *Zaprionus indianus Gupta (Diptera: Drosophilidae), A Genus and Species New to Florida and North America*. 2.

Steffan, S. A., Lee, J. C., Singleton, M. E., Vilaire, A., Walsh, D. B., Lavine, L. S., & Patten, K. (2013). Susceptibility of cranberries to *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, *106*(6), 2424–2427. https://doi.org/10.1603/EC13331

Tang, S. B., Lee, J. C., Jung, J. K., & Choi, M.-Y. (2017). Effect of erythritol formulation on the mortality, fecundity and physiological excretion in *Drosophila suzukii. Journal of Insect Physiology*, *101*, 178–184. https://doi.org/10.1016/j.jinsphys.2017.07.015

Taning, C. N. T., Christiaens, O., Berkvens, N., Casteels, H., Maes, M., & Smagghe, G. (2016). Oral RNAi to control *Drosophila suzukii* : Laboratory testing against larval and adult stages. *Journal of Pest Science*, *89*(3), 803–814. https://doi.org/10.1007/s10340-016-0736-9

Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. *Environmental Entomology*, *43*(2), 501–510. https://doi.org/10.1603/EN13200

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*(3), 793–802. https://doi.org/10.1007/s10340-016-0762-7

Toxopeus, J., Jakobs, R., Ferguson, L. V., Gariepy, T. D., & Sinclair, B. J. (2016). Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii. Journal of Insect Physiology*, *89*, 37–51. https://doi.org/10.1016/j.jinsphys.2016.03.006

USDA APHIS | Fruit Flies. (2020). Retrieved July 2, 2021, from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/fruit-flies/fruit-flies-home

Van der Linde, K., Steck, G., Hibbard, K., Birdsley, J., Alonso, L., & Houle, D. (2009). First records of *Zaprionus indianus* (Diptera: Drosophilidae), a pest species on commercial fruits from Panama and the United States of America. *Florida Entomologist*, *89*, 402–404. https://doi.org/10.1653/0015-4040(2006)89[402:FROZID]2.0.CO;2

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. https://doi.org/10.1016/j.cropro.2013.08.003

Wallingford, A. K., Cha, D. H., & Loeb, G. M. (2018). Evaluating a push-pull strategy for management of *Drosophila suzukii* Matsumura in red raspberry: Push-pull for *D. suzukii* management. *Pest Management Science*, *74*(1), 120–125. https://doi.org/10.1002/ps.4666

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O'Neal, S. D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, *2*(1), G1–G7. https://doi.org/10.1603/IPM10010

Wang, X., & Messing, R. H. (2004). The ectoparasitic pupal parasitoid, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), attacks other primary tephritid fruit fly parasitoids: host expansion and potential non-target impact. *Biological Control*, *31*(2), 227–236. https://doi.org/10.1016/j.biocontrol.2004.04.019

Wang, X.-G., Kaçar, G., Biondi, A., & Daane, K. M. (2016). Foraging efficiency and outcomes of interactions of two pupal parasitoids attacking the invasive spotted wing drosophila. *Biological Control*, *96*, 64–71. https://doi.org/10.1016/j.biocontrol.2016.02.004

Wang, X.-G., Serrato, M. A., Son, Y., Walton, V. M., Hogg, B. N., & Daane, K. M. (2018). Thermal performance of two indigenous pupal parasitoids attacking the invasive *Drosophila suzukii* (Diptera: Drosophilidae). *Environmental Entomology*, *47*(3), 764–772. https://doi.org/10.1093/ee/nvy053

Wentz, K., Cooper, W. R., Horton, D. R., Kao, R., & Nottingham, L. B. (2020). The artificial sweetener, erythritol, has insecticidal properties against pear psylla (Hemiptera: Psyllidae). *Journal of Economic Entomology*, *113*(5), 2293–2299. https://doi.org/10.1093/jee/toaa124

Woltz, J. M., & Lee, J. C. (2017). Pupation behavior and larval and pupal biocontrol of *Drosophila suzukii* in the field. *Biological Control*, *110*, 62–69. https://doi.org/10.1016/j.biocontrol.2017.04.007

Wong, J. S., Cave, A. C., Lightle, D. M., Mahaffee, W. F., Naranjo, S. E., Wiman, N. G., Woltz, J. M., & Lee, J. C. (2018). *Drosophila suzukii* flight performance reduced by starvation but not affected by humidity. *Journal of Pest Science*, *91*(4), 1269–1278. https://doi.org/10.1007/s10340-018-1013-x

Wong, J. S., Wallingford, A. K., Loeb, G. M., & Lee, J. C. (2018). Physiological status of *Drosophila suzukii* (Diptera: Drosophilidae) affects their response to attractive odours. *Journal of Applied Entomology*, *142*(5), 473–482. https://doi.org/10.1111/jen.12497

Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honeybees. *Annual Review of Entomology*, *63*(1), 327–344. https://doi.org/10.1146/annurev-ento-020117-043423

Yousef, M., Aranda-Valera, E., & Quesada-Moraga, E. (2018). Lure-and-infect and lure-and-kill devices based on *Metarhizium brunneum* for spotted wing drosophila control. *Journal of Pest Science*, *91*(1), 227–235. https://doi.org/10.1007/s10340-017-0874-8

Zhu, F., Xu, J., Palli, R., Ferguson, J., & Palli, S. R. (2011). Ingested RNA interference for managing the populations of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Pest Management Science*, *67*(2), 175–182. https://doi.org/10.1002/ps.2048

Appendix A: Preliminary observation of phytotoxic damage on Elliot variety blueberry leaves

Objective: To observe if and/or when sprayed erythritol formulations because noticeable phytotoxicity on blueberry leaves.

Rationale: During 2020 field trial, noticeable discoloration was observed on E+S and E+Sul sprayed blueberries. Since phytotoxicity could be signs of detriment to plant health or influence changes in blueberry yield, we questioned when and to what degree phytotoxicity is present by spraying single branches in various sections of the canopy and creating a photo-log to visually capture presence of damage.

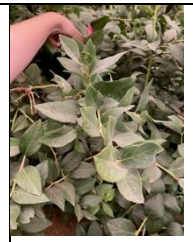
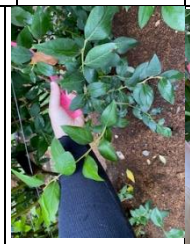
Methods: Blueberries of the Elliot variety at Lewis Brown Farm, Corvallis, OR were used in this trial. Eastern and western facing branches in upper and lower canopy were randomly assigned treatments 1) no spray (control), 2) 1.5 M erythritol + 0.5 M sucrose (E+S), or 3) 1.5 M erythritol + 0.1 M sucralose (E+Sul). Branches were sprayed with a standard household spray bottle 4-5 times, tagged, and photographed weekly for six weeks. Branches were resprayed during week 2 after a rainstorm. During week 4, Oregon wildfire smoke was heavy.

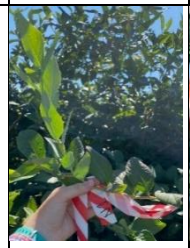
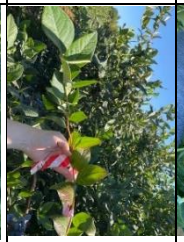
Results:

Some flagging was accidently removed by helpers, losing the data so replicate numbers are not consecutive. Little to no phytotoxicity (PT) seen in control treatments. Some damage seen in E+S and E+Sul sprayed bushes. Appearance of PT seems to be more prevalent in upper canopy with more sun exposure. More experiments in 2021 are needed to measure proportional damage seen on a week-to-week basis.

Table 1. Photolog of Elliot variety blueberry leaf damage after erythritol sprays from 2020 preliminary phytotoxic damage observational study.

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control 1 West side, lower canopy Did not see PT damage							
Control 2 West side, upper canopy Did not see PT damage							
Control 4 East side, upper canopy Did not see PT damage							

E+S 1 West side, upper canopy Did not see PT damage							
E+S 2 West side, lower canopy Damage seen starting wk 6							 

E+S 3 East side, upper canopy Did not see PT damage							
E+S 4 East side, lower canopy Did not see PT damage							
E+Sul 1 West side, upper canopy Damage seen starting wk 5						 	 

E+Sul 2 West side, lower canopy Did not see PT damage							
E+Sul 3 East side, upper canopy Damage seen starting wk 3