FINAL PROJECT REPORT

Project Title: Non-nutritive sugar-based control strategy for spotted wing drosophila

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Other funding sources

Agency Name: USDA-ARS (Innovation-fund)
Amt. awarded: $25,000

Total project Funding: $82,720

Budget history

Item	2018	2019
[1]Salaries	$25,000	$25,750
[2]Benefits	$4,380	$4,510
[3]Wages	$2,880	$9,600
Equipment	$0	$0
[4]Supplies	$5,000	$4,000
[5]Travel	$800	$800
Plot Fees	$0	$0
Total	$38,060	$44,660

Footnotes: USDA ARS in-house fund supports for equipment, facilities and supplies for this project. [1]Salaries & [2]Benefit, 0.5 FTE Postdoc; [3]Wage, student assistant, $12/h x 10/w@4x6m; [4]Materialsand Supplies, non- & nutritive sugars; other supplies for bioassay, large potted blueberry plants, and Insect rearing materials and supplies; [5]Travel, PI and/or postdoc to attend and present results in the cherry commission or entomology meetings each year.

OBJECTIVES

Our approach for SWD control strategy is based on our current non-nutritive sugar study and previous research results. We recently discovered a variety of dosages of erythritol and other sugars in mixed or separate solutions had significantly reduced the survival of SWD adult flies, and suggested that erythritol alone or with sucrose had potential insecticidal activity. We have also found the impacts on fecundity and mortality from testing at a larger scale in greenhouse cages, and examined the nutritional pathway of ingested erythritol in the fly body. Undoubtedly, erythritol combined with sucrose reduced the survival and fecundity of SWD, which is caused by the physiological imbalance with the sugar osmolarity in the body. Based on those results, we proposed a possible mode of action of erythritol for insecticidal activity.

For practical applications, the erythritol formulation mixed with sucrose can be used as a potential insecticide or as a delivery agent combined with other biological insecticides such as RNAi (RNA interference) and microbial pathogen for SWD. To develop this new control method for cherry growers, we need to identify the mode of action of the erythritol formulation in SWD, evaluate the control efficacy from large scale tests, and investigate if negative impact(s) present on non-target insects. To achieve this goal, these specific objectives need to be accomplished in this project:

1. Test the efficacy of the erythritol formulation on SWD in a greenhouse
2. Test the efficacy of the erythritol formulation on SWD in a field
3. Evaluate the impact of the formulation applied on honeybees

SIGNIFICANT FINDINGS

- The erythritol formulation significantly reduced larval infestation and adult oviposition.
- The erythritol formulation reduced up to 90% larval infestation in the greenhouse trial.
- The erythritol formulation reduced infestations by SWD up to 96 % and overall by 49 % in the field.
- The erythritol formulation reduced up to 43% egg laying in the female fed the formulation.
- The erythritol formulation did not decrease survivorship of honeybees in a cage.

RESULTS & DISCUSSION

An erythritol formulation mixed with sucrose has reduced the lifespan and fecundity of SWD in the lab and greenhouse cages. The impacts of the formulation have been tested in the field or at a larger scale, and on honey bees. For these objectives, we conducted full arena greenhouse and field studies to evaluate the effectiveness of this non-toxic alternative. A part of the research results has been published in Journal of Economic Entomology, November 2018 entitled '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) (doi: 10.1093/jee/toy362).

1. Greenhouse trial

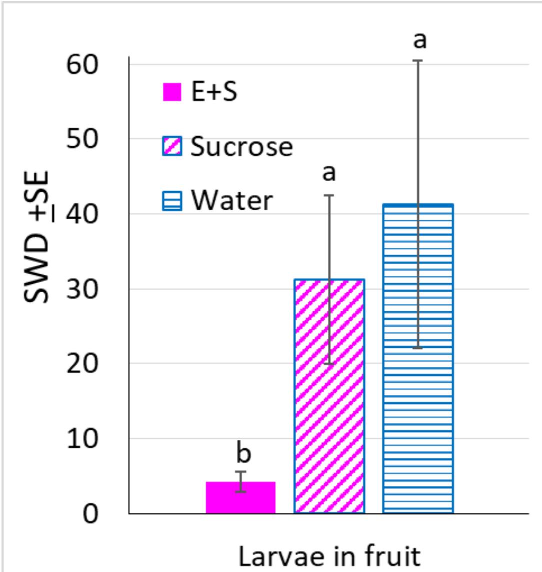

Figure 1. Numbers of SWD larvae infesting blueberries collected from bushes treated with E+S, sucrose or water.

We evaluated the erythritol formulation to reduce infestation by SWD exposed among blueberry bushes in the greenhouse. We found 86-90% reduction of SWD larvae infestation on blueberries collected from bushes treated with E+S than the bushes treated with the water or sucrose controls (P = 0.0003) (Fig. 2). The sucrose control showed that a sticky sugar formulation alone does not reduce SWD infestation, but the presence of erythritol does.

We also found fewer adults were trapped post-fruit removal in the greenhouse when bushes were treated with E+S than water, but there were also fewer adults with sucrose than water. We know that sucrose feeding extends the longevity of SWD (Tochen et al. 2016), and that satiated SWD are less likely to be trapped with fermenting odors than hungry SWD (Wong et al. 2018). Therefore, the lowered trap counts following sprays with sucrose-only may reflect fed SWD not being as attracted to the trap to get trapped.

2. Field trial

The E+S formulation was sprayed on blueberry bushes in the field, and evaluated the efficacy for SWD infestation.

2-1. Bag trial for Reka

The field trial in 'Reka' blueberries in June and July occurred before SWD infestation was prevalent yielding minimal data. So, we conducted trials where SWD were confined in mesh bags over Reka clusters to assess whether SWD laid on field-treated blueberries. The erythritol formulation was evaluated for reducing oviposition by SWD exposed to field conditions. We found about 72-78% fewer eggs were laid in berries treated with erythritol (Fig. 3).

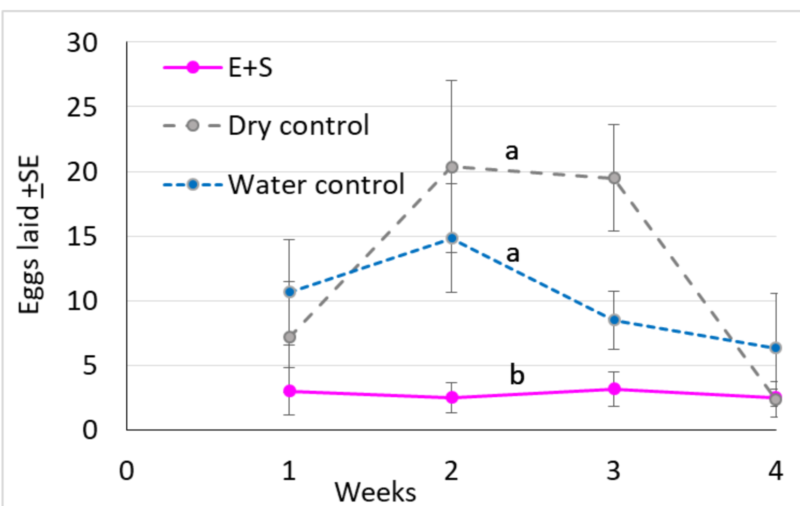

Figure 2. Numbers of eggs laid in blueberry fruits collected from plants treated with E+S, water control or dry control.

2-2. Field trial for Elliot

The E+S formulation was sprayed in 'Elliot' blueberry plants to reduce SWD infestation in the field. We found that SWD larvae was reduced by 49% overall among E+S plots. The difference was most pronounced at week 2 with a 96% reduction (Fig. 4).

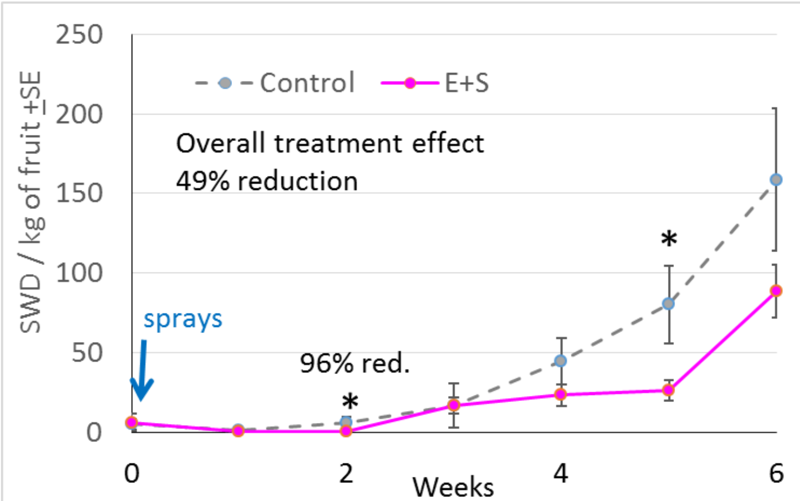

Figure 3. SWD infestation in blueberry fruits collected from plants treated with E+S or dry control.

3. Fly fecundity

To examine fecundity, flies were exposed to 5 blueberry fruits sprayed with E+S formulation, sucrose only or erythritol only for 48 h.

Eggs laid in the blueberries were counted under a microscope. When fed E+S or erythritol-only, females laid ~43% fewer eggs on blueberries than those fed sucrose in a 2 day period (Fig. 4A).

To clarify whether this reduction in fecundity was due to altered oviposition behavior or egg maturation, females were dissected to count ovarial eggs. Surprisingly, the number of ovarial eggs in E+S-fed flies was 37% or 45% more than sucrose- or erythritol-fed flies, with no difference between sucrose- and erythritol-fed flies (Fig. 4B). This suggests that females fed E+S have eggs available but exposure to erythritol reduces egg laying behavior.

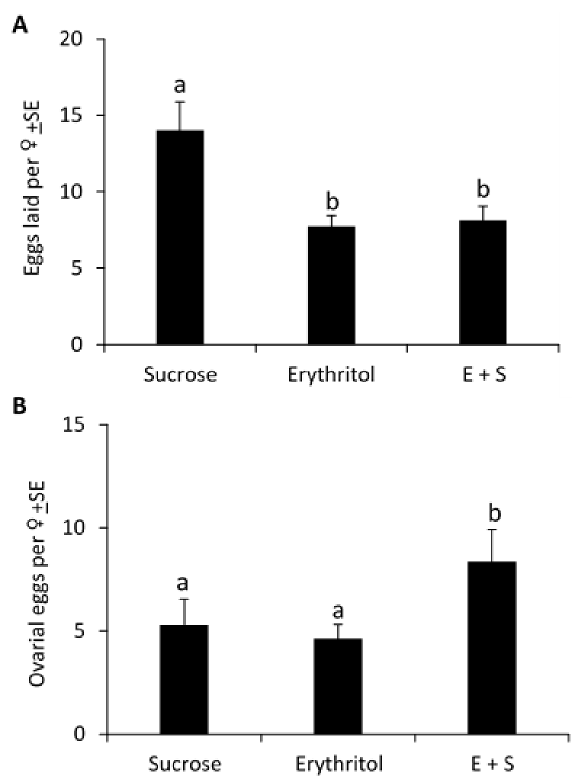

Figure 4. Fecundity (A) and ovarial eggs (B) of SWD females fed erythritol, E+S, or sucrose.

4. Impact on honeybee survivorship

The E+S formulation, erythritol, sucrose, or water were given to honeybees during foraging hours in a cage, and survival rates were similar among treatments at 74 – 85% (Fig. 5). If honeybees were exposed to erythritol for longer periods, differences may appear. But, the high exposure rate tested in the cage is not expected in the field. Honeybees are attracted to flowers via visual and odor cues. The attractiveness of the erythritol mixture is not known although sugars are odorless. Foraging workers can perceive sugar through the gustatory receptors. Future studies with erythritol sprays should monitor the presence of honeybees as well as other natural enemies to determine the extent of exposure, and test survivorship of other natural enemies with erythritol.

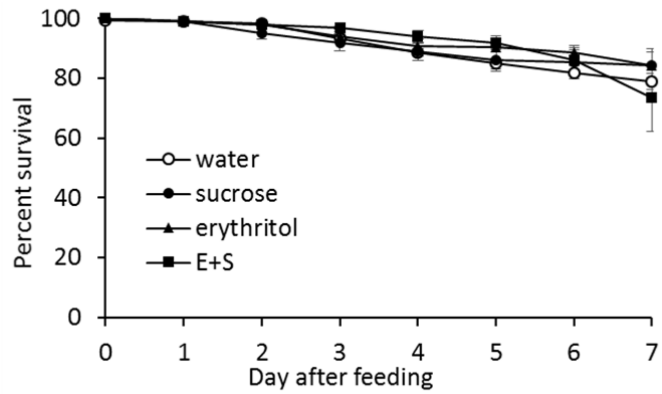

Figure 5. Survival rates of honeybees fed E+S, erythritol, sucrose, or water.

EXCUTIVE SUMMARY
Project Title: Non-nutritive sugar-based control strategy for spotted wing drosophila
Key Words: non-nutritive sugar, erythritol, non-toxic insecticide, *Drosophila suzukii*, *Aphis mellifera*

Spotted wing drosophila (SWD), *Drosophila suzukii*, is a destructive invasive pest, and attacks a wide range of ripening fruits including cherry crops. While numerous biological, cultural, mechanical, and chemical strategies are being developed for SWD control, currently the primary control methods rely on chemical pesticides despite human health and environmental risks. For insecticides to be part of a more sustainable program, efforts are underway to make insecticide applications more effective and reduce overall use, such as reduced spray programs and also to develop environmentally-friendly insecticides.

Sugars are used to stimulate pests to feed on insecticides, and thereby increase the effectiveness of the insecticide application. Sucrose as a feeding stimulant can be added to conventional or organic insecticides targeting the pest. Recently, the PIs investigated the effects of non-nutritive sugars and sugar alcohols on SWD by comparing the survivorship and fecundity of SWD. We found sucrose/erythritol formulations to have a potentially insecticidal effect on the fly. Our research results suggested that feeding caused mortality by the non-nutritive sugar increasing the osmotic pressure in the fly's blood system. The novel mode of action that we discovered represents a potentially useful strategy for a biological insecticide.

For practical applications, the erythritol formulation mixed with sucrose can be used as a potential insecticide or as a delivery agent combined with other biological insecticides. To develop this new control method for cherry growers, we proposed specific objectives: 1) Test the efficacy of the erythritol formulation on SWD in a greenhouse, 2) Test the efficacy of the erythritol formulation on SWD in a field, and 3) Evaluate the impact of the formulation applied on honeybees.

In this research, the significant outcomes are as follows. First, the erythritol formulation significantly reduced larval infestation and adult oviposition. Secondly, the erythritol formulation reduced up to 90% larval infestation in the greenhouse trial. Third, the erythritol formulation reduced infestation by up to 78 % and by 49 % overall in the fields. Fourth, the erythritol formulation reduced up to 43% egg laying in the female fed the formulation. Fifth, the erythritol formulation did not decrease survivorship of honeybees in a cage. In addition, we found the erythritol formulation reduced the longevity of SWD regardless of the presence of wounded blueberries as an alternative sugar source in a cup arena.

Besides causing death, erythritol ingestion appears to interfere with ovipositional behavior and/or physical process of egg laying. Since erythritol increases the osmotic pressure in the fly's hemolymph, this may impede physical movement needed for oviposition. It is also possible that erythritol could be an ovipositional deterrent, but this would need to be tested by applying erythritol to fruit and running no-choice and choice tests in future studies. The mixture did not decrease survivorship of honeybees within 7 days. Future studies should evaluate field applications, spray frequency and volume of erythritol mixture. While the present research focuses on *D. suzukii*, future research should investigate whether this tool can be applied to other Dipteran pests.