An assessment of the oviposition behavior of spotted-wing drosophila (*Drosophila suzukii*) in the presence of a novel management tool in Pinot noir grapes

by

Rachel Ann Blood

A THESIS

submitted to
Oregon State University
Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Zoology
(Honors Associate)

Presented May 24, 2019
Commencement June 15, 2019

AN ABSTRACT OF THE THESIS OF

Rachel Ann Blood for the degree of Honors Baccalaureate of Science in Zoology presented on May 24, 2019. Title: An assessment of the oviposition behavior of spotted-wing drosophila (Drosophila suzukii) in the presence of a novel management tool in Pinot noir grapes

Abstract approved:____

Vaughn Walton

Spotted-wing drosophila (SWD, *Drosophila suzukii*) is an invasive fruit fly species native of Southeast Asia. In vineyards, SWD lays eggs in damaged and intact fruit of the most soft-skinned varieties, and feeds on damaged fruits during the harvest period. Feeding and oviposition activities increase the likelihood of vectoring spoilage bacteria, particularly when fruit integrity is negatively impacted due to cracking, diseases, hail injury, and bird damage. The aim of this study was to evaluate the efficacy of a novel SWD management tool, a pesticide-free behavioral disruptor (BD), in vineyard systems. Data were collected from a series of laboratory and field trials on Pinot noir grapevines. Intact and damaged single fruits or clusters were exposed to the pest either in presence or absence of the BD. The laboratory trials resulted in a reduction in oviposition in both compromised and intact grape fruits. The field trials resulted in a similar reduction in egg laying in commercial-standard conditions. These findings indicate that the BD has the potential to significantly impact SWD oviposition and feeding activities in commercial vineyard settings.

Key Words: Spotted-wing drosophila, behavior disruptor, grape, oviposition

Corresponding e-mail address: bloodr@oregonstate.edu

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APPROVED:

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Vaughn Walton, Mentor, representing the Department of Horticulture

Jadwiga Giebultowicz, Committee Member, representing the Department of Integrative Biology

Marco Valerio Rossi-Stacconi, Committee Member, representing the Department of Horticulture

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

Rachel Ann Blood, Author

ACKNOWLEDGEMENTS

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INTRODUCTION

The spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a highly invasive pest endemic to Southeast Asia. Over the years, SWD presence has been reported across an increasing number of countries and continents. The first sign of its expansion was reported in various farmland areas throughout Europe and North America in 2008. Consequentially, that same year, it was reported that a significant amount of damage was done to crops due to SWD presence. Eventually, SWD made their way to Oregon in 2009 (Walsh et al. 2011). The pest is known to develop in many economically important fruit crops, e.g. blueberries, cherries, blackberries, raspberries, strawberries, and grapes. The pest was discovered in various fruit crops in 13 of Oregon 36 counties (Dreves et al. 2009). The prevalence of their destructive behaviors increases greatly as soon as the fruit penetration resistance drops below a certain threshold, females SWD lay eggs in healthy, ripening fruits using a serrated ovipositor. SWD is just one of two *Drosophila* species known to penetrate ripening fruits (Walsh et al. 2011; Cini et al. 2012). In addition to the direct damage caused by larval feeding, fruit puncturing provides a pathway for colonization of fungi and bacteria, which may contribute to further fruit decay leading to even greater economic devastations. The damage this causes to the crops can range from seemingly negligible to an 80% crop loss (Lee et al. 2011).

One of the fruit crops in Oregon susceptible to the consequences of SWD activity is wine grapes. Previous studies have reported that SWD lay less eggs and are less likely to fully develop on grape fruits relative to other small fruits (Lee et al. 2011, Ioriatti et al. 2015, Ioriatti et al 2017). Nonetheless, it has been observed that SWD can be found feeding on wine grapes even more so when they are negatively impacted by cracking, disease, and bird damage during the harvest period (Barata et al. 2012; Ioriatti et al. 2015). When the fruits are compromised as previously described, SWD populations are expected to survive for longer periods, to lay more eggs, and to be a vector of various spoilage bacteria in wine grapes during the latter portion of the season (Ioriatti et al. 2015). In fact, regardless if they lay eggs or not, they can still be a vector of spoilage bacteria (Ioriatti et al. 2017). In particular, they can cause increased levels of *Acetobacter spp.* related to SWD activity alone or in combination with other drosophila species negatively impact the quality of high-value wines, as well as increase production costs (Ioriatti et al. 2015). Increased levels of both microbiota, also vectored by SWD, and *Acetobacter spp*. resulted in the production of several volatiles that contribute to the spoilage process and induced fermentation of the wine grapes (Ioriatti et al. 2017).

Controlling SWD on farms is difficult and several approaches to reduce its populations have been attempted in different cropping systems. Typical control methods consist of insecticides and behavioral control, such as trapping, but their control is mainly dependent on the use of insecticides. Due to their rapid generation turn over, SWD control requires many applications of various insecticides (Haye et al. 2016; Wallingford et al. 2016; Renkema et al. 2016; Kirkpatrick et al. 2017). Behavioral controls offer an alternative to the dependency of growers on insecticides. Nonetheless, fruit produces blends of semiochemicals which most of the times outcompete the synthetic lures, leading to the failure of mass trapping technique efforts. Moreover, several lures focus on food attraction, while little attention has been given to the manipulation of oviposition behavior (Haye et al. 2016; Cloonan et al. 2019).

There are several technologies making use of behavior manipulation to control insect pests (Lee et al. 2011; 2013; Iglesias et al. 2014; Evans et al. 2017; Kirkpatrick et al. 2017), including a recently developed novel, pesticide-free behavioral disruptor (BD) product, which can compete with ripening fruit in modulating the SWD oviposition behavior (Tait et al. 2018). Previous laboratory and field tests on cherry and soft-skin fruits showed that the BD causes an alteration of the SWD behavior that ultimately resulted in a substantial diversion of egg load from the fruit towards the product itself. Currently, it is unknown how the BD affects the behavior of SWD in a commercial vineyard environment.

This study had two main goals. The first goal of this study is to evaluate the efficacy of the BD for reducing the impact of SWD on wine grape fruits during the harvest period in commercial vineyard settings. Another goal is to evaluate the efficacy of the BD against wounded fruit skins (compromised fruits). The hypothesis is that the presence of the BD will result in a reduction of SWD egg infestations in wine grapes.

MATERIALS AND METHODS

Mass Rearing of *D. suzukii*. A colony of SWD has been maintained in the laboratory since 2009 from adults and pupae provided by the Horticultural Crops Research Laboratory (USDA-ARS, Corvallis, OR, USA). This colony has been periodically supplemented with wild-caught adults and pupae from the Willamette Valley and Columbia River Gorge of Oregon. The adults were kept in a BugDorm mesh cage measuring 30x30x30 cm (MegaView Science, Taichung, Taiwan). Adults were provided with a sponge soaked in a cup of deionized water, as well as a Petri dish (9x1.5 cm) containing standard cornmeal medium (Daniel et al. 2011). Dishes containing newly laid eggs were transferred every other day to pint plastic freezer containers measuring 8.5x8.5x5 cm (Arrow Plastic; United States Plastic Corp®, Model #: 04201, Lima, OH, USA). The Petri dishes remained uncovered, allowing larvae to wander freely within the limits of the pint container. Pint containers were covered with a screened lid to allow air exchange and prevent larval escape. Both adults and juveniles were kept in the laboratory at 23± 2°C, 65% RH and a photoperiod of 16:8 (L:D).

Experimental Design - Laboratory Trials. Oviposition trials were conducted on organically managed Pinot noir grapes (Planted in 2018 at location (GPS) of 44°34'04.7"N 123°14'40.1"W) collected between the third week of September and the second week of October. The grapes were collected with approximately 3 cm of the stem still attached to the fruit. The grapes were managed by the Oregon State University Botany Plant Pathology Farm off Highway OR 34, Corvallis, OR, USA. The grapes were collected in Ziploc® bags and stored in a 4-degree Celsius climatic chamber. These trials were carried out on October 31st, November 1st, and November 2nd using ventilated arenas (Figure 1) in the laboratory to determine the efficacy of the BD. There were two treatments, water and the new BD formula, that were paired against either intact or compromised fruits. 5 g of the treatments were added to a small petri dish. Each dish was added to a separate arena. In total, there were 10 control and 10 treated replicates per trial. Approximately three grape berries were added to another petri dish so that the surface area of the grapes exposed was equivalent to the surface area of the treatments. In half of the replicates per trial, approximately half the total number of grapes in each arena were artificially wounded to simulate cracking. The grapes were artificially wounded by piercing and cutting 2 - 3 cm on the side of the berry with a scalpel . A total of 10 (5 females and 5 males) 7-15 day old SWD were released within each arena. All treated and control replicates were started between 10 am and 12 pm. Fruit was collected after an exposure period of 24 hours. Experiments were repeated twice more for a total of three dates of measurement.

Experimental Design - Field Trials. Oviposition trials were conducted between September 25th – 28th and October 8th – 11th on organically managed Pinot noir grapes (Planted in 2018 at location (GPS) of 44°34'04.7"N 123°14'40.1"W). The grapes were managed by the Oregon State University Botany Plant Pathology Farm off Highway OR 34, Corvallis, OR, USA. Trials were carried out using isolated bunches of approximately 20-25 grapes per cluster to determine efficacy of the two formulas of the BD. The treatments were distributed throughout the vines as follows: a third of the bunches with one of the formulas of the BD, considered the old formula, the second third of the bunches had the new formula of the BD, and the last third was without either formula. 14g of both formulas of the BD were placed in a Petri dish (9x1.5 cm) and placed at the bottom of the mesh sleeve that surrounded the cluster. In half of the 20 replicates in each of all three treatments, approximately half the number of grapes on each bunch were artificially wounded to simulate cracking. Those grapes were pierced with a scalpel as described above. Entire bunches and treatments were covered using a netting sleeve (Figure 2). A total of 12 SWD (6 females and 6 males) that were 7-15 days old were released within each sleeve. Both treated and control treatments were initiated between 10 am and 12 pm and fruit was collected after 72 hours exposure period. Experiments were repeated once more during the harvest period therefore obtaining two sample dates.

Statistical Analysis To determine whether the oviposition behavior was affected by the presence or absence of the BD from the laboratory experiment, data sets were analyzed using a multifactorial analysis of variance (ANOVA) test. To determine whether the oviposition behavior was affected by the presence or absence of the BD from the field experiment, the data sets were analyzed using a multifactorial analysis of variance (ANOVA) test after a $\log_{10}$ + 1.5

transformation. The presence or absence of wounds and date of trial were analyzed as covariates for testing the null hypothesis (ß = 0). All tests were performed in Statistica. All mean values are presented as averages ± standard deviation (SD).

RESULTS

Laboratory Experiment. For those replicates in the presence of the BD, there was significantly less oviposition per fruit on average compared to the replicates were the BD was absent ($F = 8.0123$, $df = 1$, $P < 0.01$) (Fig. 3). The proportion of infested fruits was significantly less in the presence of the BD than it was in the absence of the BD ($F = 4.9437$, $df = 1$, $P < 0.05$) (Fig. 3). In intact fruits, when the BD was present, replicates overall displayed a 43.4% reduction in oviposition per fruit compared to those treatments where the BD was absent (Fig. 3). Likewise, in the compromised fruits, the presence of the BD, treatments overall displayed a 62.5% reduction in oviposition per fruit compared to those with an absence of the BD (Fig. 3). The treatment, presence or absence of the BD, was the only factor that resulted in significant differences in fruit oviposition (Table 1). For fruit infestation, both treatment, presence or absence of the BD, and the dates of the trials factors resulted in significant differences (Table 1).

Field Experiment. In the presence of the BD, a strong overall numerical trend of less oviposition per fruit was observed compared to the treatments where no BD was used (Fig. 4). There was also a strong trend toward the reduction of the fruit infestation levels in the presence of the BD compared to the absence of the BD (Table 3; Fig. 5). In intact fruits, the presence of the old and new BD formula resulted in a 21.3% and 30.8% reduction in oviposition. Likewise, in compromised fruits, the presence of the old and new BD formula resulted in a 17.4% and

52.6% reduction in oviposition, respectively, compared to its absence. Date had a significant effect on fruit infestation and oviposition levels (Table 3; Table 4; Table 5). Treatment, the presence or absence of the BD, or the presence or absence of wounds did not significantly affect oviposition and fruit infestation (Table 3; Table 4; Table 5).

DISCUSSION

Our study indicates that BD treatments generally result in a reduction of both oviposition and fruit infestation levels by SWD in wine grapes. This reduction trend was seen regardless if the fruit was compromised or not, suggesting that the BD is attractive enough to SWD to draw them to the BD and away from the vulnerable fruit. This attractiveness in such unfavorable conditions is important to SWD management in commercial vineyard settings. The reason being that previous studies have found that SWD are more likely to be found on compromised fruits during the ripening and harvest periods. This is when the grapes typically have a lower pH and a lesser penetration force is required for oviposition (Ioriatti et al. 2015). In previous studies, compromised (incised) fruit typically resulted in increased oviposition in those fruits and increased survivorship (Ioriatti et al. 2015, 2017). Our study contradicted this previous work in that we did not see a significant difference of oviposition and fruit infestation dependent on the presence or absence of wounds. Another observation stemming from this study was the variance of the egg laying. This was primarily seen between the two trials dates of the field experiment. One possible reason for the strong trend in the data rather than any significance seen in the field experiment was ripeness, and consequentially the firmness, of the fruits in the first field trial. The first field trial was conducted at the very beginning of the typical harvest period of the Pinot noir grapes. It is possible that the penetration force was too great of the SWD females, which resulted in a lack oviposition that occurred in that trial. This trend occurred across all the treatments in that trial. However, it is unlikely that the lack of oviposition in the first field trial could be related to the weather and sun exposure during that period due to the little variation seen in the climatic conditions during those dates. Also, while date was shown to be a significant parameter between the two trials, it was shown that it did not have a significant impact on oviposition and fruit infestation across treatments within those two trials (Table 2). Therefore, there are no significant differences in oviposition and fruit infestation among treatments when separated by dates.

Within the field trials, we observed a trend between the two BD formulas. The old BD formula was used as a negative control based on previous knowledge of its ability to reduce infestation levels in soft-skinned fruits (Tait et al. 2018). There is an observed trend of lesser oviposition and fruit infestation in the presence of the new BD formula than that of the old. This suggests the new BD formula is possibly more effective in a field environment than the old formula is. This provides knowledge to the creators of the BD as the development of the product proceeds.

Another observation of our study is that the BD can promote oviposition activity overall. On average, treated replicates had similar oviposition, in both the fruit and the BD, as control replicates did in the laboratory experiments. This suggests that not only are the SWD attracted to the BD more than the fruits, but they also find it a suitable and comparable substrate for oviposition. Although, the scope of BD persistence under field conditions was not assessed in this study, but it warrants further investigation. This also suggests that the presence of the BD is disrupting normal oviposition behavior in typical crop substrates. A reduction in activity and oviposition on the fruit could potentially decrease the negative effects of larval feeding and the vectoring of spoilage bacteria in commercial vineyard settings by creating a disruption in the normal activities of SWD.

CONCLUSION

SWD is invasive pest species that warrants a more sustainable control method. In the presence of the BD, there was a reduction in the oviposition activity in both compromised and intact grapes in the laboratory experiment. Also, in the presence of the BD, there was a similar reduction in oviposition activity in the field experiment. These findings indicate that the BD has the potential to significantly impact SWD feeding and oviposition activities in commercial vineyard settings. This could likely reduce the vectoring spoilage bacteria by SWD to the grape fruits.

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FIGURES AND TABLES

Table 1. The numerical values from the multifactorial analysis of variance (ANOVA) tests examining the effects of the parameters on *Drosophila suzukii* oviposition (n° eggs per berry) and fruit infestation (%) from the laboratory experiments.

Effect of Parameters	Univariate Tests of Significance for laboratory experiments				
	SS	Degree of Freedom	MS	F	p
OVIPOSITION					
Treatment	166.41	1	166.41	8.01	0.01
Cracking	49.98	1	49.98	2.40	0.12
Date	65.02	2	32.51	1.56	0.21
Treatment*Cracking	2.32	1	2.32	0.11	0.73
Treatment*Date	3.03	2	1.51	0.07	0.92
Cracking*Date	45.67	2	22.83	1.09	0.34
Treatment*Cracking*Date	27.40	2	13.70	0.65	0.52
FRUIT INFESTATION					
Treatment	5913.60	1	5913.60	4.94	0.03
Cracking	1127.70	1	1127.70	0.94	0.33
Date	8366.00	2	4183.00	3.49	0.03
Treatment*Cracking	6.70	1	6.70	0.01	0.94
Treatment*Date	450.50	2	225.20	0.18	0.82
Cracking*Date	4137.80	2	2068.90	1.72	0.18
Treatment*Cracking*Date	1430.40	2	715.20	0.59	0.55

Table 2. The numerical values from the multifactorial analysis of variance (ANOVA) tests examining the effects of the parameters on *Drosophila suzukii* oviposition (n° eggs per berry) and fruit infestation (%) from the field trials.

Effect of Parameters	Univariate Tests of Significance for field trials				
	SS	Degree of Freedom	MS	F	p
OVIPOSITION					
Treatment	0.99	2	0.49	3.78	0.02
Cracking	0.07	1	0.07	0.55	0.45
Date	14.96	1	14.96	113.52	0.00
Treatment*Cracking	0.10	2	0.05	0.38	0.67
Treatment*Date	0.07	2	0.03	0.29	0.74
Cracking*Date	0.17	1	0.17	1.35	0.24
Treatment*Cracking*Date	0.07	2	0.03	0.30	0.73
FRUIT INFESTATION					
Treatment	1.03	2	0.51	2.37	0.09
Cracking	0.07	1	0.07	0.34	0.55
Date	26.08	1	26.08	119.69	0.00
Treatment*Cracking	0.01	2	0.01	0.04	0.96
Treatment*Date	0.07	2	0.03	0.16	0.84
Cracking*Date	0.33	1	0.33	1.53	0.21
Treatment*Cracking*Date	0.02	2	0.01	0.06	0.93

Figure 1. The experimental setup of the laboratory trials. Ventilated arenas were constructed from plastic beakers that contained ventilation holes and a plastic tube connected to a vacuum pump to ensure air flow (Tait et al. 2018). A total of 12 adult *Drosophila suzukii* (6 males and 6 females) were released into each arena for a 24-hour period. Each arena contained 3 Pinot noir grapes and either 5 mL of water (control treatment) or 5 g of the behavior disruptor). There were 10 replicates per each treatment per trial for all three trials.

Figure 2. The experimental setup of the field trials. Trials were conducted at the OSU Botany and Plant Pathology research farm on Pinot noir wine grapes during September 25th – 28th and October 8th – 11th 2018. Grape clusters consisting of 20 – 25 fruits were exposed to 12 adult *Drosophila suzukii* (6 males and 6 females) for a 72-hour period into each replicate. There were 20 replicates per treatment. The three treatments were the untreated control, the old behavior disruptor formula (negative control), and the new behavior disruptor formula. There were two trials in total.

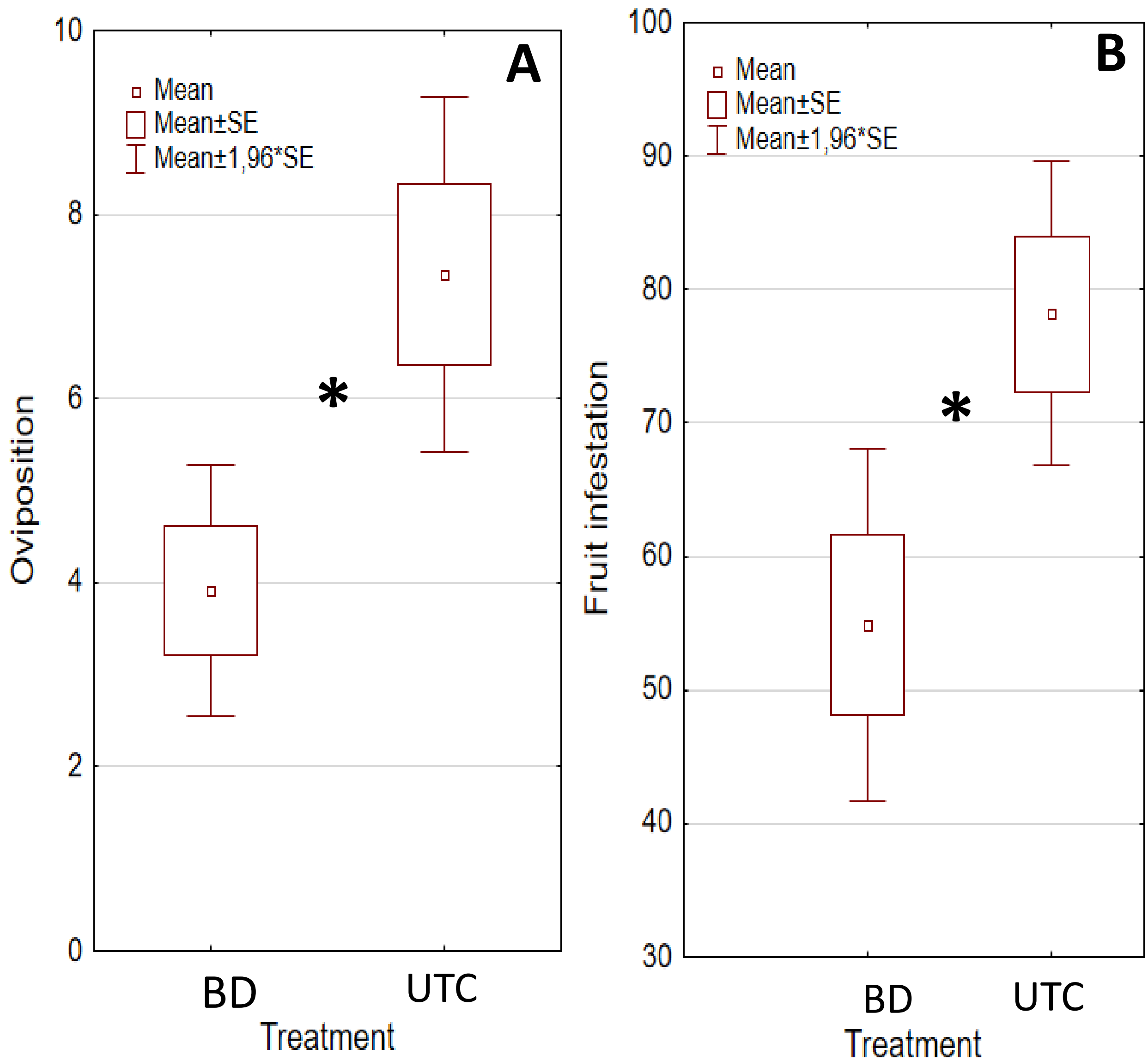

Figure 3. Laboratory experiments. A) Average oviposition (eggs per berry) and B) fruit infestation (%) by *Drosophila suzukii* between treated groups. BD: behavior disruptor; UTC: untreated control. Asterisks indicate significant difference between treatments.

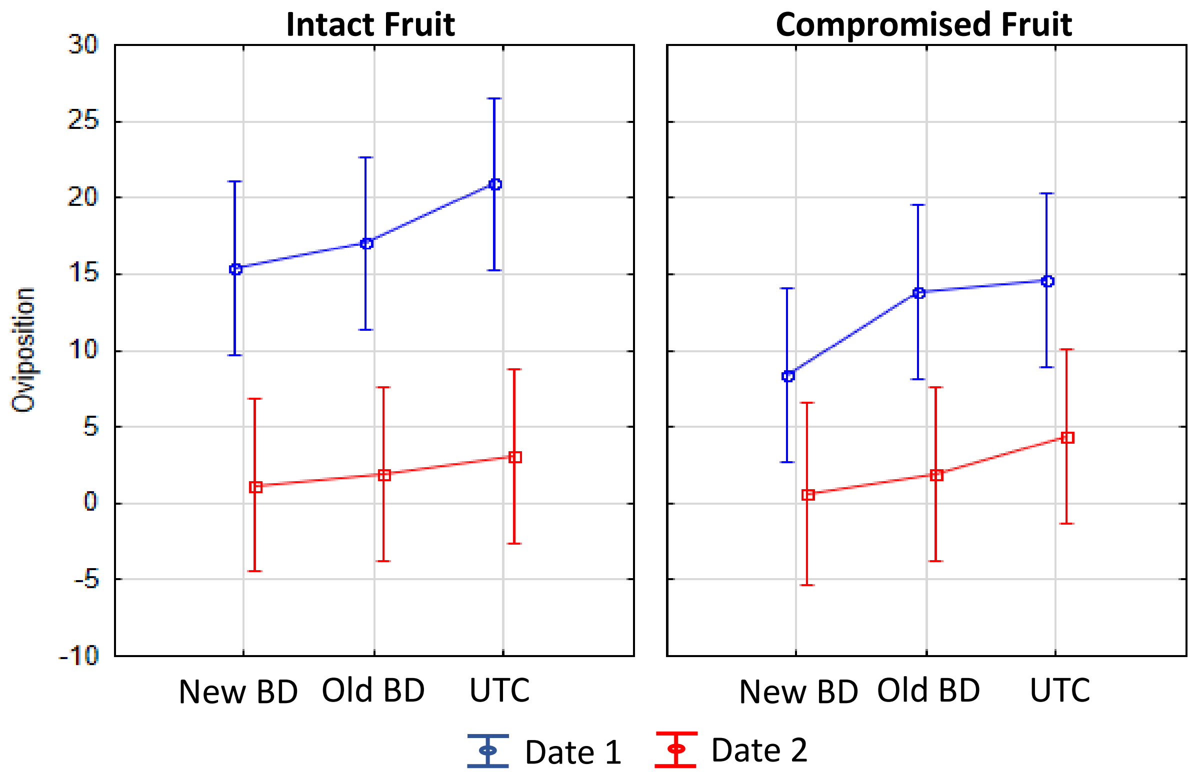

Figure 4. Mean (±SD) oviposition by *Drosophila suzukii* per fruit per treatment in the field experiment on Pinot Noir winegrape in Corvallis, Oregon during 2018. Treatments consisted of new behavior disruptor (New BD) formula, old behavior disruptor (Old BD) formula, and untreated control (UTC).

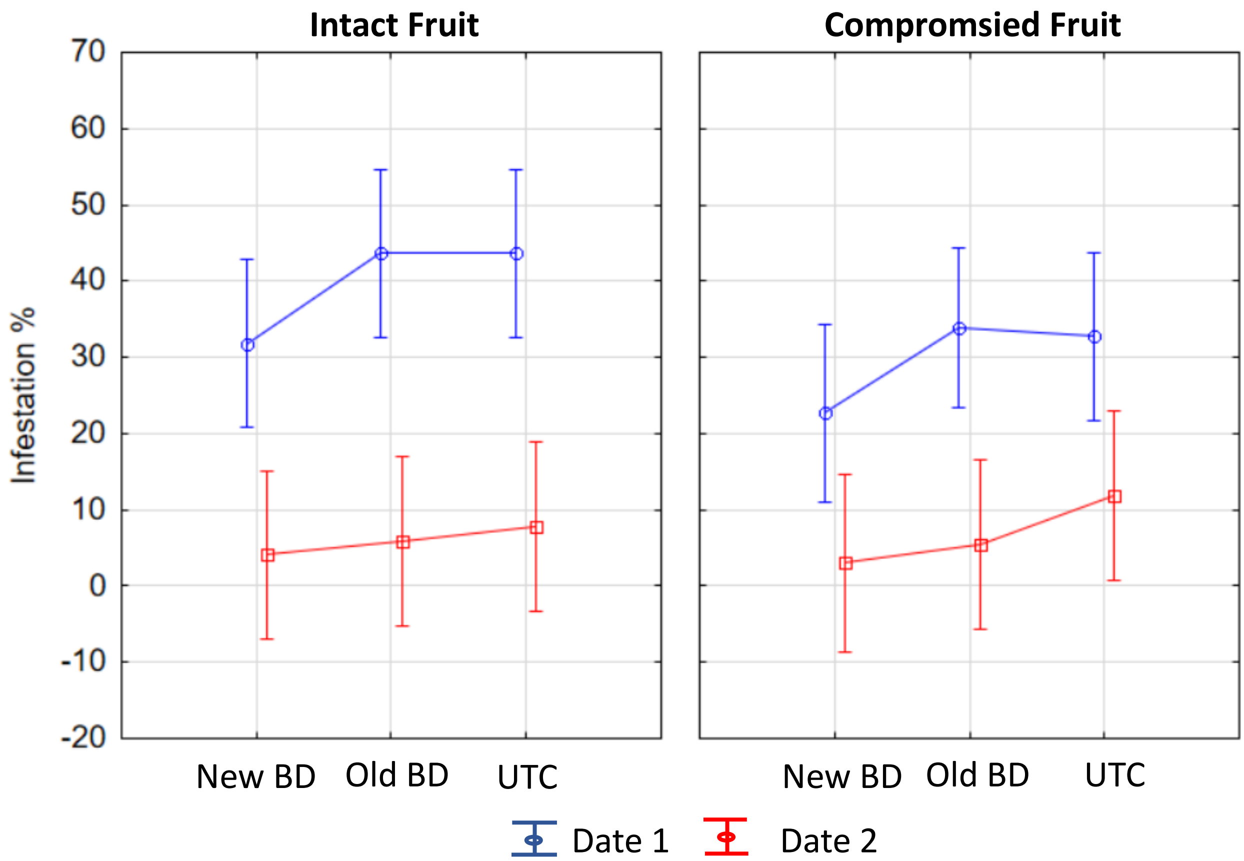

Figure 5. Mean (±SD) fruit infestation percentage by *Drosophila suzukii* in the field experiment on Pinot Noir winegrape in Corvallis during 2018. Treatments consisted of new behavior disruptor (New BD) formula, old behavior disruptor (Old BD) formula, and untreated control (UTC).