

Antifungal Properties of Spotted Wing Drosophila (*Drosophila suzukii*) Larvae

by
Ann Bernert

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirement for the
degree of

Honors Baccalaureate of Science in Bioresource Research
(Honors Scholar)

Honors Baccalaureate of Arts in International Studies
(Honors Scholar)

Presented May 28, 2015
Commencement June 2015

AN ABSTRACT OF THE THESIS OF

Ann Bernert for the degrees of Honors Baccalaureate of Science in Bioresource Research and Honors Baccalaureate of Arts in International Studies presented on May 28, 2015.

Title: Antifungal properties of spotted wing drosophila (*Drosophila suzukii*) larvae.

Abstract approved:

Ken Johnson

Spotted wing drosophila, *Drosophila suzukii*, is a recently introduced, invasive pest in Oregon. It oviposits in near-mature and mature fruit, and thus is an important concern for the small fruit industry. A crop consultant observed that mature raspberry fruit with *D. suzukii* larvae do not show symptoms of molds as readily as fruit without larvae. We attempted to replicate these observations in the laboratory. A cage of *D. suzukii* was reared and then used for exposing raspberry fruit to ovipositing adults. Fruit were randomly assigned to control or exposed groups. Exposed fruit were placed in the cage for 1 hr and then incubated individually in 6-ml plastic cups with ventilated lids for 7 days (16-hr photoperiod) at 25°C. After incubation, fruit were assessed for number of fungal colonies and mold severity. Fruit with larvae had significantly fewer fungal colonies (paired t-test, $P = 0.02$). A well diffusion test of the supernatant from *D. suzukii* infested raspberry fruit was likewise capable of significant radial growth inhibition in a saprobic fungus (paired t-test, $P < 0.05$). Future research could determine the mechanism of fungal inhibition. The implications of this study include a potential new antifungal agent as well as a better understanding of the *D. suzukii* life strategy.

Key Words: spotted wing drosophila, *Drosophila suzukii*, fungi, larvae

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Presented May 28, 2015
Commencement June 2015

Honors Baccalaureate of Science in Bioresource Research and Honors

Baccalaureate of Arts in International Studies project of Ann Bernert presented on May 28, 2015.

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ACKNOWLEDGEMENTS

I would like to acknowledge my incredible mentor, Dr. Ken Johnson for supporting me throughout the thesis process. I would also like to acknowledge my amazing committee members, Dr. Vaughn Walton and Todd Temple, for teaching me so much about laboratory work, writing, and the research process. A huge thank you to the USDA lab for providing spotted wing stock for my colonies. Also thanks to Amy Dreves and Amanda Lake for providing insight to spotted wing biology and rearing. The Walton laboratory members likewise provided important advice for the maintenance of the colonies. My academic advisor, Wanda Crannell, was invaluable in helping me pursue my research interests and guiding me through my undergraduate curriculum.

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Antifungal Properties of Spotted Wing Drosophila (*Drosophila suzukii*) Larvae

INTRODUCTION

The spotted wing drosophila, *Drosophila suzukii*, ((Matsumura) (Diptera: Drosophilidae)) is native to Asia and since 1980 has become invasive in a number of other countries. In the United States, the fly was first reported to invade Hawaii in 1980 and then California in 2008 (Hauser, 2011; Walsh *et al.*, 2011). This pest was found in Oregon in 2009 and has since concerned the agricultural industry as a significant pest. The common name of the insect is due to the black spots located on the tips of the male's wings. Unlike other *Drosophila* species that lay eggs in rotting fruit, female *D. suzukii* lays its eggs in ripening fruit with the aid of a serrated ovipositor (Rota-Stabelli *et al.*, 2013). Since it is difficult to identify if fruit is infested with *D. suzukii* eggs, fruit can be sent to market and become infested with larvae a few days after harvest. Preferred food sources of this fly include blueberries, cherries, plums, peaches, and raspberries. All these crops are produced in the Willamette valley of Oregon, which makes understanding the biology of this pest pertinent to a diverse and sizable number of fruit producers (Lee *et al.*, 2011). In west coast states, the potential economic damage posed by *D. suzukii* in blackberries, raspberries and cherries is estimated at 860 million USD annually (Walsh *et al.*, 2011).

Research has revealed that there are many interspecific interactions between *Drosophila* species and fungi (Hodge *et al.*, 1999; Trienens *et al.*, 2010; Rohlf, 2005). *Drosophila melanogaster*, a close relative of *D. suzukii*, are attracted to the presence of

yeasts volatiles to identify appropriate egg laying environments instead of the aromatic compounds of fruit (Becher *et al.*, 2012). *D. suzukii* has also been shown to be attracted to yeast volatiles, also opposed to fruit volatiles, in order to locate appropriate egg laying substrate (Hamby *et al.*, 2012). While there appears to be interesting and potentially symbiotic associations with unicellular fungi like yeasts, *Drosophila* species compete with filamentous fungi for the same food source (Trienens *et al.*, 2010). In addition, an antifungal compound named Drosomycin is produced by adult *Drosophila melanogaster* flies as a response to fungal infection (Fehlbaum *et al.*, 1994). *D. melanogaster* has also been documented to produce cecropin proteins, another antifungal agent (Ekengren & Hultmark, 1999). Due to the close relation of *D. suzukii* to *D. melanogaster*, it is plausible that *D. suzukii* also produces antifungal peptides. While the composition of bacterial communities and yeast communities associated with *D. suzukii* has been explored (Hamby *et al.*, 2012; Chandler *et al.*, 2014) there has yet to be research studying *D. suzukii* interactions with filamentous fungi and possible antifungal compound production.

Antifungal compounds are medically and agriculturally significant. However, it is sometimes difficult to identify pharmaceutically and medically applicable antifungal compounds that do not cause excess detrimental side effects (Butts & Krysan, 2012). Part of this is due to fungal and mammal phylogeny. Fungi are much more closely related to humans than other microbial pathogens like bacteria, which makes it difficult to identify compounds that will effectively decimate the fungal pathogen without detrimental side effects. The rate of incidence of invasive fungal infections has also been increasing in the last 30 years in the United States and European countries (McNeil *et al.*, 2001; Maschmeyer 2004; Bitar *et al.*, 2014). Incidences of fungal infections are particularly

high in immune-compromised patients and can be fatal (McNeil, *et al.*, 2001; Maschmeyer 2004).

The agricultural field can also benefit from the discovery of new antifungal compounds. Globally, fungal plant pathogens are some of the most economically significant microbes. To manage these fungal pathogens, approximately 70 million pounds of fungicides are applied in the United States annually according to the US Environmental Protection Agency (EPA). Extensive measures are taken in agriculture to prevent fungal plant pathogens from devastating a crop. In certified organic farming, the use many of these fungicides is not permissible creating an even higher demand for biologically sourced, organic compounds. Thus, if *D. suzukii* is producing an antifungal compound, it may have future application to certified organic pathogen control practices.

Interestingly, a crop consultant observed that mature raspberry fruit with *D. suzukii* larvae do not show symptoms of fungal colonization as readily as fruit without larvae (J. Todd, personal interview). This observation suggests a potential interspecific interaction with filamentous fungi that may provide important information into the *D. suzukii* life cycle. As an ideal meal for molds, it is unlikely that small fruits would not be colonized with filamentous fungi. We hoped to repeat these results in the laboratory to scientifically identify if *D. suzukii* larvae are indeed capable of significant fungal inhibition.

The objective of this study was replicate these initial field observations under controlled laboratory conditions in order to determine whether fungal inhibition was due to the physical disruption of mycelial growth by the larvae or some other means. First we assessed the fungal colonization and fruit fly larvae and pupae on wild harvested fruit.

From this we found an association between the presence of larvae or pupae and a lack of fungal colonization. We then infected raspberries with *D. suzukii* larvae under controlled, laboratory settings and compared the fungal colonization on these fruits to fruits free of *D. suzukii* larvae. Once it was demonstrated that the presence *D. suzukii* larvae in raspberry fruits decreased the amount of fungal colonization, we performed a well diffusion test using a supernatant collecting from a fruit and *D. suzukii* larvae mixture and measured radial of the fungus towards the supernatant and away from the supernatant.

MATERIALS AND METHODS

Wild fruit assessment

Fungal colonization on wild collected blackberry fruits was observed in order to identify if antifungal effects of spotted wing drosophila larvae were evident in fruit naturally infested with *D. suzukii*. The initial observation was conducted in summer 2013 which displayed high *D. suzukii* pressure (J. Klick, personal interview; Wiman *et al.*, 2014). Thus, it was assumed most wild thickets of berries were infested with spotted wing drosophila. Thirty five *Rubus armeniacus* blackberry fruits were collected in late August from a wild area in Corvallis, Oregon during clear and sunny weather conditions. These blackberry thickets were not treated with pesticides. Fruits were brought back to the lab and placed individually in 30 mL plastic cups (Figure 1), capped with hole-punched lids, and placed in growth chamber incubator at 25° C with a 16:8 (L:D) photoperiod. One week later, fruits were assessed for fungal diversity and decay and presence of *D. suzukii* pupae and larvae.

Establishing *D. suzukii* colonies

Two colonies of *D. suzukii* were established inside BioQuip Bug Dorm 2 insect rearing cages (Figure 2). Initial stock of *D. suzukii* was provided by United States Department of Agriculture (USDA), Agriculture Research Services, Horticultural Crops Research Laboratory in Corvallis, Oregon. The USDA growth medium for rearing and maintaining *D. suzukii* was utilized with the omission of propionic acid and methyl paraben. The purpose of these compounds is to reduce fungal contamination in the feed but as we were interested in the natural ability of the larvae to inhibit fungal growth we did not want these materials to potentially confound the results.

Laboratory fruit observation

Raspberry fruits were acquired from local grocery store and inspected under a dissecting microscope at 80x magnification for presence of insects (Figure 3A). Ten fruits that passed the inspection as insect-free were placed in open 30 mL cups. These cups were then placed in the *D. suzukii* rearing cage with ovipositing females for 35 minutes (Figure 3). After 35 minutes, fruits were removed and capped with hole-punched lids. Ten fruit also passing insect-free inspection remained unexposed to *D. suzukii* were individually placed in 30 mL cups as control fruit. All fruits were incubated in growth chamber for one week at 25 ° C with a 16:8 (L:D) photoperiod. Fruits were then removed and assessed for presence of larvae, pupae, fungal diversity, and fungal coverage of fruit. This same experiment was repeated with twice the original sample size and twice the length of exposure to ovipositing *D. suzukii* females. Thus, in this replication, fruits in individual cups were exposed to ovipositing female *D. suzukii* for 48 hours.

Collection of supernatant from fruit and larvae mixture

Raspberry fruit were infested with *D. suzukii* larvae and incubated in the same manner outlined in the above methods section. Incubated raspberry fruit cups were then uncapped and filled with 1.5 mL of sterile deionized water (Figure 4A). The contents of each cup was mixed by glass stir rod to suspend the fruit solids, larvae, and pupae (Figure 4B). This mixture was then placed in 2 mL centrifuge tubes filled to the 1.5 mL mark (Figure 4C). Filled tubes were then centrifuged for 2 minutes at 9,000 RPM. After this first cycle of centrifugation, complete separation of the supernatant from the solids was not attained, so another round of centrifugation was completed for 4 minutes at 10,000 RPM. The supernatant was then collected using a sterile micropipette and transferred to a new, sterile 2 mL centrifuge tube for storage.

Assessment of antifungal properties of fruit supernatant

The antifungal properties of the collected supernatant were determined using a well-diffusion assay. Two wells were created with a 6-mm cork-borer in 9-cm diameter petri plates filled with 60 mL of non-amended Difco Potato Dextrose Agar (PDA). The wells were located 4-cm apart, with each well 2-cm from a perpendicular center-line that bisected the plates. A mycelial plug of a filamentous fungus growing on a decaying raspberry was isolated on PDA was used for the source of mycelial plug inoculum. This mycelial plug inoculum was then placed directly in the center of the. One well was filled with 50 μ L of the supernatant and the other well was filled with 50 μ L of sterile DI water (Figure 4D). The radial growth of the fungus toward the control well and toward the supernatant filled well was measured 72 hours after inoculation. Radial growth towards the supernatant and towards the control well was then compared using a 2-sample t-test to compare means.

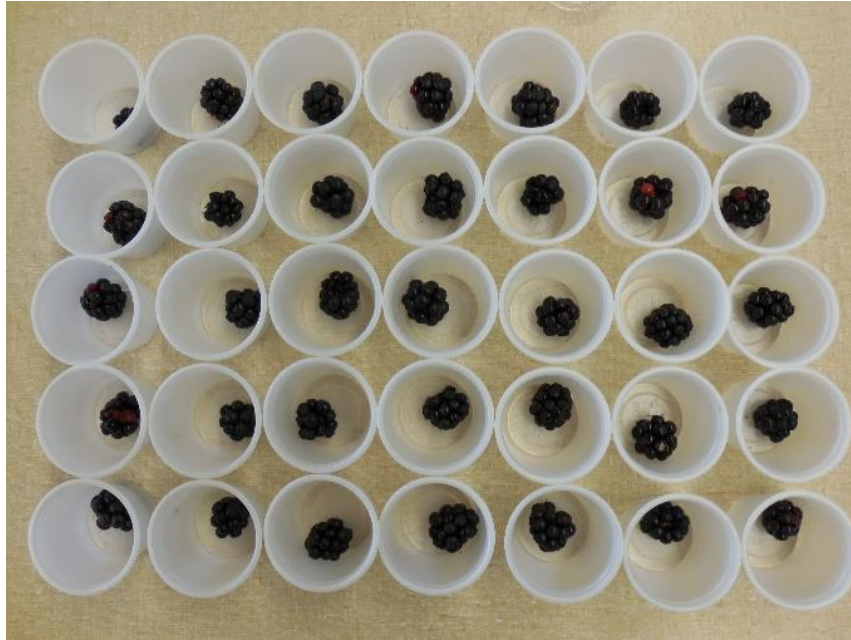


Figure 1. Wild *Rubus armeniacus* fruits were harvested and placed in 30 mL ventilated cups to monitor for fungal colonization and fruit fly larvae and pupae.

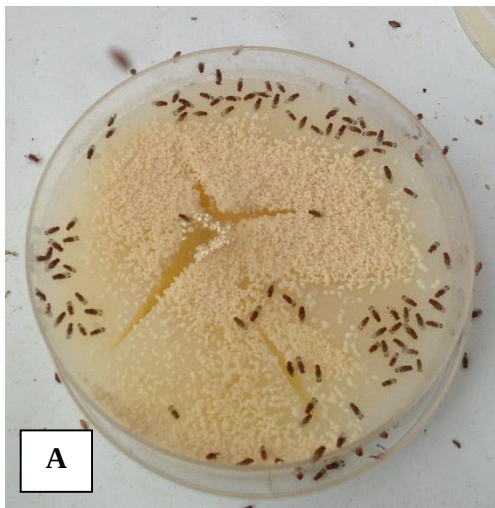


Figure 2. Establishing a *Drosophila suzukii* colony requires a rearing cage, diet, and water source. Diet is sprinkled with baker's yeast to attract egg laying females to oviposit (B). Eggs hatch in the diet turning to larvae and eventually pupae (A).



Figure 3. Store-bought raspberry fruit (A) was inspected and exposed to egg laying *Drosophila suzukii* females (B&C) in order to attain larval infested fruit samples.

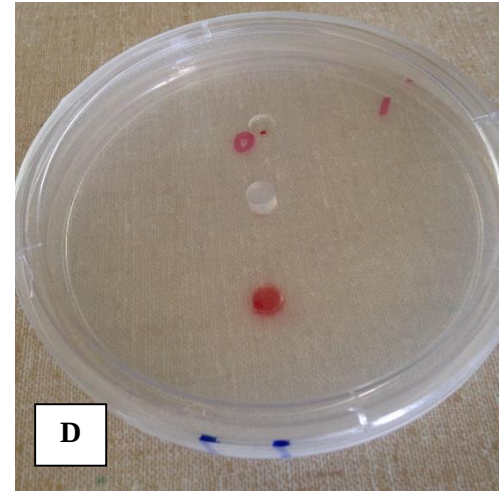
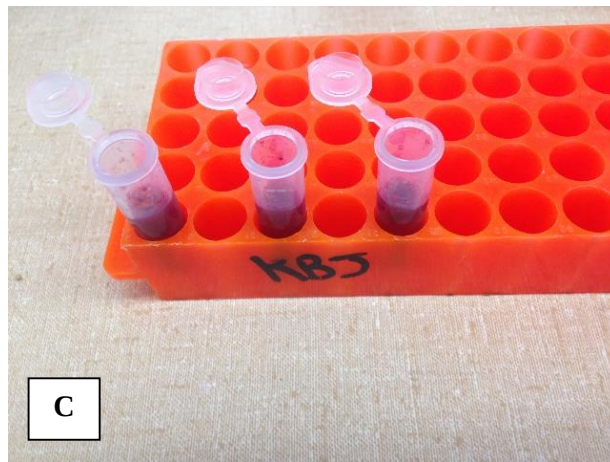


Figure 4. In order to collect a supernatant from the larvae infested fruit, 1 mL of distilled, sterile water was added to fruit infested with larvae in order (A). The mixture was stirred to suspend solids (B) and put into centrifuge tubes to separate solids from supernatant (C). The collected supernatant was then used in a well diffusion test against a fungus isolate (D).

RESULTS

Wild harvest *Rubus armeniacus* fruits, presence of *D. suzukii*, and amount of fungal decay

Wild harvest blackberry fruits contained an average of 4.1 pupae and larvae. Only 1 of the 35 fruits collected and measured began to mold over while none of the other fruit had any signs of filamentous fungi colonization (Figure 5). This single fruit with mold sample only had one pupae present. The highest number of pupae/larvae in one sample was 10 while the low was 0. Of all fruits sampled, 91% contained at least one pupae/larvae, while 29% had greater than 5 pupae/larvae.

Laboratory fruit results

Fruit without *D. suzukii* larvae feeding contained three times the fungal diversity compared to raspberry fruit with *D. suzukii* larvae. Fungal decay in experimental samples occurred only in treated fruit where no pupae or larvae were able to found suggesting that lack of larval infestation was associated with fungal colonization in the fruit. In all experimental samples where signs of larvae and pupae were visible, there was no fungal coverage and no signs of fungi present (Figure 9). The fruit structure was also completely deteriorated by samples with a high presence of larvae and pupae (Figure 10).

Well diffusion test

Radial growth was reduced by 65% when the fungus was exposed to the larval fruit juice supernatant (Figure 11). The difference between radial growth of the fungus towards the supernatant and towards the water control was statistically significant at $p < 0.001$ according to a 2-sample t-test.

Relevant observations

Experimental fruit samples infested with *D. suzukii* smell strongly of vinegar. Also, spotted wing drosophila larvae acidify their environment as was determined by pH test strip on diet media infested with larvae and diet media free of larvae.

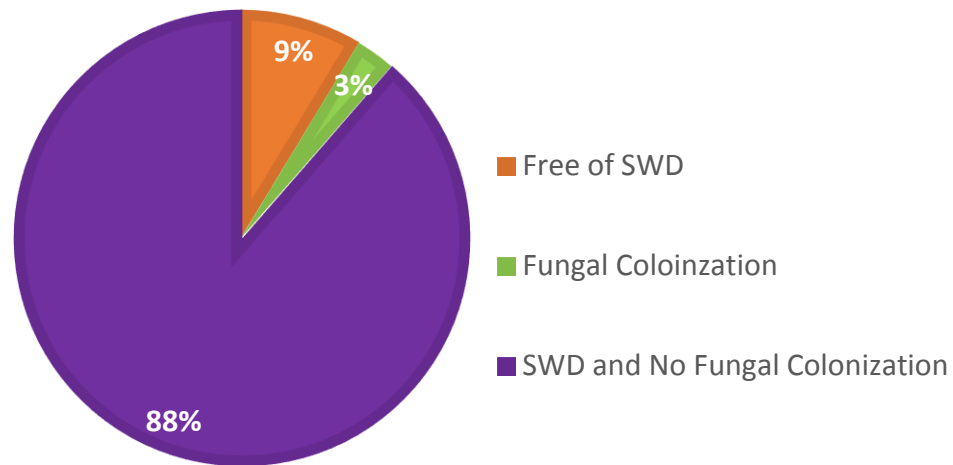


Figure 5. Observations of the proportion of wild harvested blackberries with *D. suzukii* larvae and fungal colonization. The majority of the fruit collected were observed to contain fruit fly larvae and had no fungal colonization.

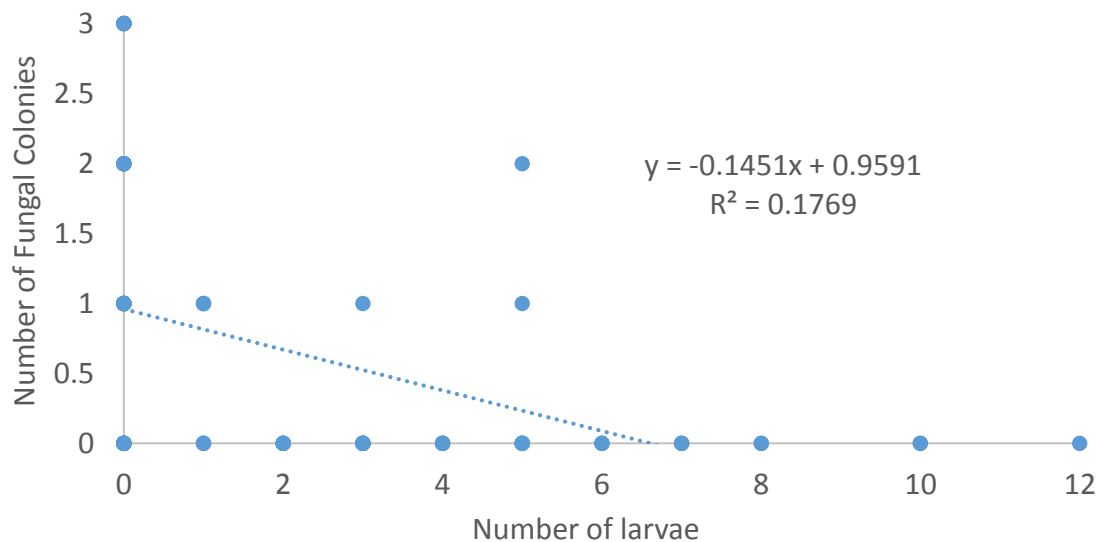


Figure 6. A scatter plot of the number of *D. suzukii* larvae and the resulting number of fungal colonies on individual fruits. Includes data points from the wild harvested blackberry fruit and the preliminary and replication of the laboratory raspberry fruit experiment.

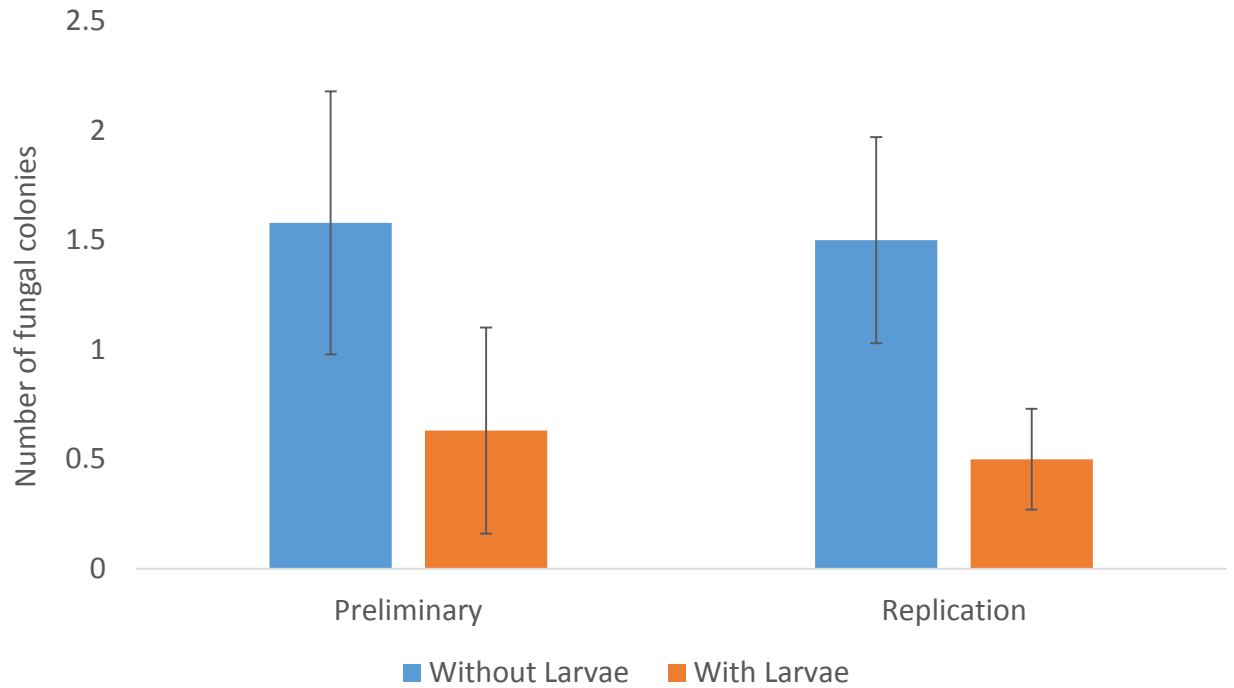


Figure 7. The number of different fungi colonizing on raspberry fruit with and without *D. suzukii* larvae. According to a 2-sample t-test, both are statistically significant with p -value < 0.002 for both experiments.

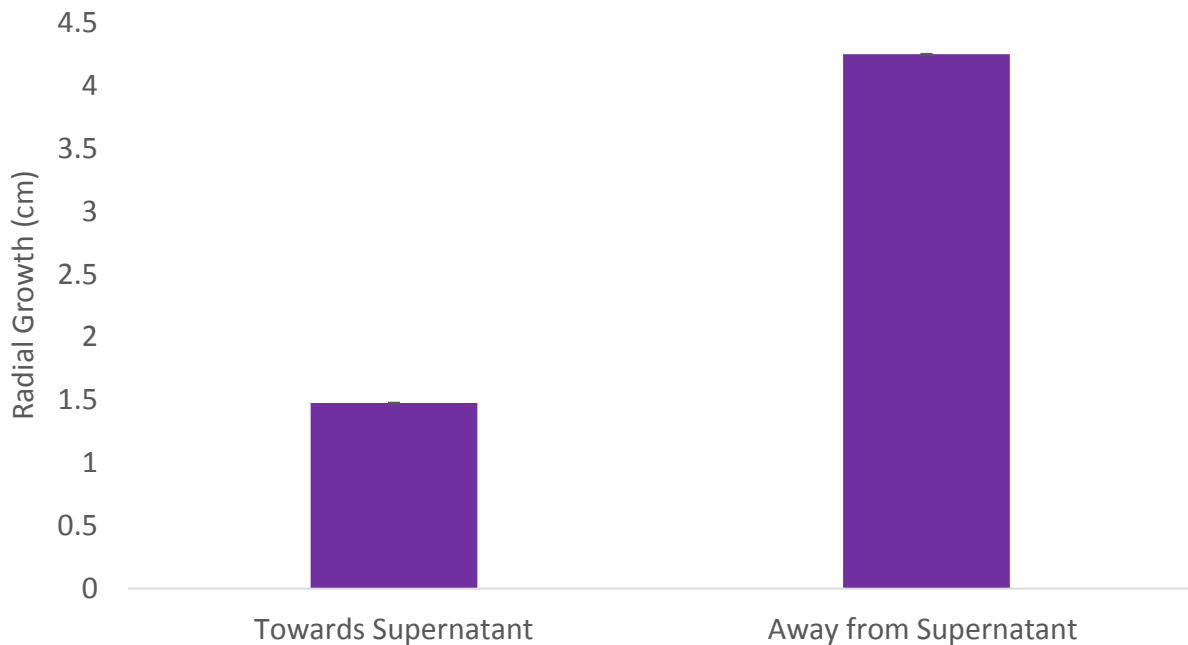


Figure 8. The radial growth of a saprobic fungus towards and away from larval supernatant. The difference between radial growth was statistically significant using a 2-sample t-test where p -value < 0.001 .



Figure 9. Visual results of fungal colonization on raspberry fruit with *D. suzukii* larvae (A), and control fruit clean of larvae (B).

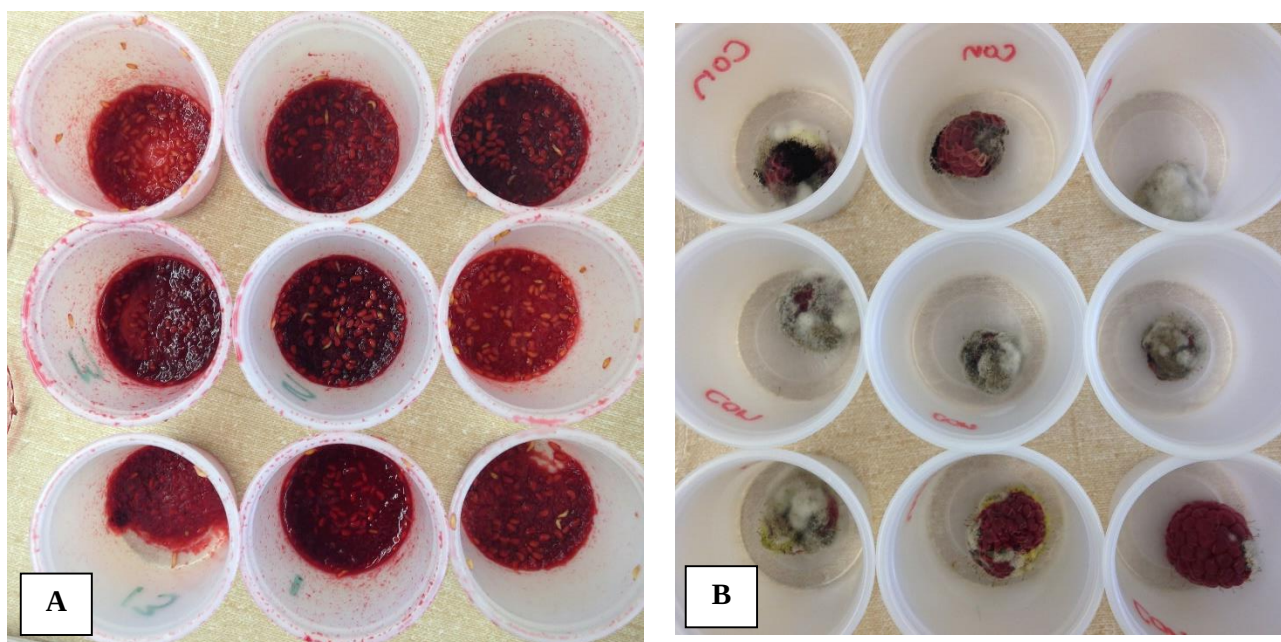


Figure 10. Replication results of fungal colonization on raspberry fruit with *D. suzukii* larvae (A), and control fruit clean of larvae (B). For the replication experiment, fruits were exposed to egg laying females for twice as long and a sample size of fruit was twice as large.



Figure 12. *Drosophila suzukii* were observed to acidify their environment. The fly diet, pictured on left, is prepared to be at relatively neutral pH. However, diet that has been used by *Drosophila suzukii* larvae (petri plate on right) drops to a pH of 3.2.

DISCUSSION

There was a pattern observed in wild harvested, ripe, *Rubus armeniacus* fruits between presence of *Drosophila* larvae and an almost nonexistent fungal colonization (Figure 5). There was a negative correlation between fungal colonization on raspberries and *Drosophila suzukii* larvae under laboratory settings (Figure 6). Also, in laboratory settings, the presence of *Drosophila suzukii* larvae significantly reduced the number of fungal colonies that developed on raspberry fruits (Figures 7, 9 & 10). The supernatant extracted from larval infested raspberry fruits caused significant growth inhibition in a filamentous fungus suggesting that the antifungal properties of *D. suzukii* larvae are biochemical (Figure 8 & 11). Another interesting observation is that *D. suzukii* larvae acidify their substrate (Figure 12) and fruits infested with them smell strongly of acetic acid.

This observation can be connected to the fact that *D. suzukii* has also recently been demonstrated to vector wine spoilage bacteria in vineyards. These wine spoilage bacteria exacerbate the economic damage caused by this fly (Ioriatti *et al.*, 2015). Most prominently, the bacterial phyla vectored by *D. suzukii* include by *Acetobacter* and *Gluconobacter* which both are acetic acid producers. *Acetobacter* and *Gluconobacter* also produce the compounds of acetaldehyde and ethyl acetate (Bartowsky, 2008). The unfavorable sensory descriptors of all these compounds include bruised apple, vinegar, sour, pungent, and nail polish remover (Bartowsky, 2008). Interestingly, *Acetobacter* is the second most prolifically associated genus of bacteria residing in *D. suzukii* (Chandler *et al.*, 2014). It is plausible that acetic acid bacteria may play a critical role in the *D.*

suzukii life cycle. Speculating farther, perhaps it is not the larvae themselves producing an antifungal compound, but a bacterial symbiont associated with the larvae.

A significant impact of this research is the potential discovery of a new antifungal substance. Antifungal metabolites are in high demand in the medical field as effective antifungal compounds are difficult to find (Butts & Krysan, 2012). Fungal pathogens are much more closely related to humans making it difficult to identify compounds that will effectively suppress a fungal pathogen without significantly harming the host. Antifungal compounds are also important agriculturally to treat fungal plant pathogens.

In addition, an outcome of this research is to gain a better understanding of the life strategies of the spotted wing drosophila. With a better understanding of this new pest, hopefully better control of it will be possible. In comparison to other *Drosophila* species, *D. suzukii* is not as well researched. When investigating potential microbial biological control agents of this economically significant pest, knowledge of *Drosophila suzukii* and microbial interspecific interactions should be useful. To our knowledge, this is the first documentation of antifungal activity in *D. suzukii* larvae.

REFERENCES

Bolda, M. P., Goodhue, R. E., and Zalom, F. G. 2010. Spotted Wing *Drosophila*: potential impact of a newly established pest. *Agricultural and Resource Economics Update*. 13:5-9.

Becher, P., Flick G., Rozpedowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M., Hansson, B., Piskur, J., Witzgall, P., and Bengtsson, M. 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Funct Ecol* 26, 822-828.

Butts, A. and Krysan, D. 2012. Antifungal Drug Discovery: Something Old and Something New. *PLOS Pathogen Collections*.

Bitar, D., Lortholary, O., Strat, Y., Nicolau, J., Coignard, B., Tattevin, P., Che, D., Dromer, F. 2014. Population-Based Analysis of Invasive Fungal Infections, France, 2001-2010. *Emerging Infectious Diseases* 20 7 1149-1155.

Chandler, J., James, P., Jospin, G., and Lang, J. 2014. The bacterial communities of *Drosophila suzukii* collected from undamaged cherries. *PeerJ* 2:e474.

Ekengren, S. and Hultmark, D. 1999. *Drosophila* cecropin as an antifungal agent. *Insect Biochemistry and Molecular Biology* 29:965-972.

Fehlbaum, P., Bulet, P., Michaut, L., Lagueux, M., Broekaert, W., Hetru, C., and Hoffmann, J. 1994. Septic injury of *Drosophila* induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. *J Biol Chem* 269: 33159-33163.

Hamby, K., Hernández, A., Boundy-Mills, K., Zalom, F. 2012. Associations of Yeasts with Spotted Wing *Drosophila* (*Drosophila suzukii*; Diptera: Drosophilidae) in Cherries and Raspberries. *Appl Environ Microbiol* 78:4869-4873.

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*. 67:1352-1357. 10.1002/ps.2265.

Hodge, S., Mitchell, P., Arthur, W. 1999. Factors affecting the occurrence of facilitative effects in interspecific interactions: an experiment using two species of *Drosophila* and *Aspergillus niger*. JSTOR Wiley 87:166-174.

Ioriatti, C., Walton, V., Dalton, D., Anfora, G., Grassi, A., Maistri, S., and Mazzoni V. 2015. *Drosophila suzukii* (Diptera: Drosophilidae) and its Potential Impact to Wine Grapes During Harvest in Two Cool Climate Wine Grape Production Regions. J Econ Entomol DOI: 10.1093/jee/tov042.

Lee, J., Bruck, D., Curry, H., Edwards, D., Haviland, D., Van Steenwyk, R., and Yorgey, B. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Management Science 67:11 1358-1367

McNeil, M., Nash, S., Hajjeh, R., Phelan, M., Conn, L., Plikaytis, B., Warnock, D. 2001. Trends in Mortality Due to Invasive Mycotic Diseases in the United States, 1980-1997. Clinical Infectious Diseases 33 5 641-647.

Maschmeyer, G. 2006. The changing epidemiology of invasive fungal infections: new threats. International Journal of Antimicrobial Agents 27:3-6.

Rota-Stabelli, O., Blaxter, M., and Anfora, G. 2013. *Drosophila suzukii*. Current Biology 23:R8-R9.

Rohlf, M. 2005. Clash of kingdoms of why *Drosophila* larvae positively respond to fungal competitors. Frontiers in Zoology. 2:2.

Trienens, M., Keller, N., Rohlf, M. 2010. Fruit, flies and filamentous fungi: experimental analysis of animal-microbe competition using *Drosophila melanogaster* and *Aspergillus* mould as a model system. Nordic Society Oikos. 119:1765-1775.

Walsh, D.B., Bolda, M. P., Goodhue, R.E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O'Neal, S. D., and 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:G1-G7.

Wiman, N.G., Walton, V.M., Dalton, D.T., Anfora, G., Burrack, H.J., Chiu, J.C., Daane, K.M., Grassi, A., Miller, B., Tochen, S., Wang, X., Ioriatti, C. (2014) Integrating Temperature-Dependent Life Table Data into a Matrix Projection Model for *Drosophila suzukii* Population Estimation. PLoS ONE 9(9): e106909.

