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Efficacy of entomopathogenic fungal products for biological control of spotted wing drosophila (Diptera: Drosophilidae) under laboratory conditions

Elena M. Rhodes[1,*], *Pasco B. Avery*[2], *and Oscar E. Liburd*[1]

The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a serious pest of thin-skinned fruits and is native to Southeast Asia (Kanzawa 1939). *Drosophila suzukii* was first reported in mainland USA from Santa Cruz County, California, in 2008 (Hauser 2011) and has since spread throughout the United States (Walsh et al. 2010; Burrack et al. 2012). It has a wide host range, which includes crop hosts such as blackberries, blueberries, cherries, raspberries, and strawberries (Bellamy et al. 2013) and numerous wild host plants (Lee et al. 2015). Female *D. suzukii* possess a serrated ovipositor that allows them to oviposit in ripening and ripe fruit (Hauser 2011). The larvae develop in the fruit, which renders the fruit unmarketable. In fact, a single larva can cause an entire shipment of fruit to be rejected.

Current management practices rely on frequent applications of broad-spectrum insecticides, particularly organophosphates and pyrethroids, and the reduced-risk spinosyns (Bruck et al. 2011; Haviland & Beers 2012; Van Timmeren & Isaacs 2013). Organic growers have few options and rely heavily on applications of Entrust®, the OMRI (Organic Materials Review Institute)-approved formulation of spinosad (Van Timmeren & Isaacs 2013). A potential addition to this limited arsenal of insecticides is the use of entomopathogenic fungi.

Several entomopathogenic fungal genera, including *Beauveria* and *Metarhizium*, have been evaluated for effectiveness for a variety of insect pests (Vega et al. 2009). Strains of *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae), *Isaria fumosorosea* (Wize) (= *Paecilomyces fumosoroseus*) (Cordycipitaceae), and *Metarhizium anisopliae* (Metschnikoff) (Clavicipitaceae) have shown efficacy for several species of tephritid fruit flies (Beris et al. 2013; Ibrahim et al. 2014; Qazzaz et al. 2015; Rashad et al. 2015). Strains of these fungal species also have shown some efficacy for *D. suzukii* when infested blueberries are treated (Cuthbertson & Audsley 2016), in cherries (Gargani et al. 2013), and by directly spraying adults under laboratory conditions (Woltz et al. 2015).

The purpose of this study was to compare the effectiveness of the commercially available entomopathogenic products BotaniGard® ES and PFR-97™ 20% WDG for control of *D. suzukii* in blueberries under laboratory conditions at low and high field label rates. Both adult mortality and percent emergence from fruit after exposure to the fungal products were assessed.

Blueberries were not in season locally at the time of this study, so USDA certified organic blueberries (Sunnyridge Farms, Codigua, Melipilla, Chile) were purchased from Publix Supermarket in Gainesville, Florida, USA, the day each trial was started. Berries were washed with Fit Fruit & Vegetable Wash (HealthPro Brands Inc., Cincinnati, Ohio, USA) using the directions on the label. After washing, fruit were allowed to air dry on a paper towel before use for about 10 min.

A colony of *D. suzukii* was maintained in the University of Florida Small Fruit and Vegetable IPM Laboratory in Gainesville, Florida, USA. Flies were reared on Formula 4-24® Instant Drosophila Media (Carolina Biological Supply Company, Burlington, North Carolina, USA). The colony was kept in a Percival environmental chamber (Percival Scientific Inc., Perry, Iowa, USA) at 23 °C and 65% RH under a 14:10 h L:D photoperiod.

The fungal products tested in this study were BotaniGard® ES (*Beauveria bassiana* strain GHA, Laverlam International Corporation, Butte, Montana, USA) and PFR-97™ 20% WDG (*I. fumosorosea* Apopka strain 97, Certis USA, LLC, Columbia, Maryland, USA). Viability of each fungal product, determined by counting CFUs formed on an agar plate, was > 84% during the experiments.

There were 3 experimental trials. In each trial, there were 4 replicates of 5 treatments in a completely randomized design: (1) deionized water treated control, (2) BotaniGard ES (BotaniGard) low rate, (3) BotaniGard high rate, (4) PFR-97 20% WDG (PFR 97) low rate, and (5) PFR 97 high rate. For the low and high rates, 0.1 and 1.0 mL of BotaniGard and 0.1 and 1.0 g of PFR 97, respectively, were mixed in 100 mL of deionized water. The control consisted of 100 mL of deionized water. Each treatment fungal suspension was prepared in a 150 mL glass beaker. Blueberries were dipped into the treatments using blunt forceps, placed onto dry filter paper in 6 cm plastic Petri dishes, and allowed to air dry for about 10 min before dishes were placed into experimental arenas. Nine blueberries were dipped and placed onto each Petri dish. Fresh suspensions were mixed for each of the 3 experimental trials.

Experimental arenas consisted of 1 L plastic deli containers with 6 cm diam holes cut in the lids and covered with fine mesh. The Petri dishes containing the treated berries, as described above, were placed on the bottom of each arena. Twelve *D. suzukii* (6 females and 6 males) were released into each arena. A food source for the adult flies, a 1.5 mL centrifuge tube filled with sucrose solution with a dental wick inserted in the top of each tube, was placed in each arena. Mortality was assessed at 24, 48, 72, and 168 h (1 wk) post-application.

After 1 wk, dead flies were surface sterilized by dipping in 70% ethanol for 5 to 10 s, rinsing briefly (about 1–2 s) in deionized water, dipping in 1% bleach solution for 1 min, and then rinsing in 3 changes of deionized water for 1 to 2 s. After surface sterilization, flies were placed on moistened filter paper in 10 cm Petri dishes. Flies from each treatment were placed into separate Petri dishes, sealed with Parafilm and then placed in the environ-

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mental chamber described above for 1 wk to allow for mycosis of the flies and confirm the fungal phenotype contained in the product.

Berries that were exposed to the *D. suzukii* adults in the arenas were placed into 59 mL deli cups and kept in the environmental chamber described above for 2 wk. After this duration, the number of emerged adult male and female *D. suzukii* were counted and recorded.

Data from all 3 trials was combined in the analysis. Neither mortality nor emergence data met the assumptions of ANOVA even with transformation, so nonparametric statistics were used to analyze the data. The Kruskal-Wallace test ($\alpha = 0.05$) for general alternatives, and the Dwass, Steel, Critchlow-Fligner multiple comparisons test ($\alpha = 0.05$), were used to analyze the data and to determine significance amongst the treatments (Hollander & Wolf 1999).

After 24 h, there was significantly higher mortality in the BotaniGard high-rate treatment compared with control, PFR 97 low-rate, and PFR 97 high-rate treatments (Fig. 1; $H' = 10.2$; df = 5; $P = 0.04$). There were no differences among treatments at any other sampling time (all $H' \leq 6.4$; df = 5; $P \geq 0.2$).

Significantly higher numbers of *D. suzukii* emerged from the control, PFR 97 high-rate, and PFR 97 low-rate treatments compared with the BotaniGard high-rate treatment (Fig. 2; $H' = 17.5$; df = 5; $P = 0.002$). Emergence was significantly lower in the BotaniGard low-rate treatment compared to both PFR treatments, but not compared to the control.

No flies from the untreated control showed signs of fungal sporulation other than that from saprophytic fungi. An average of 93 ± 2% of flies from the BotaniGard high rate treatment showed mycosis. Mycosis was lower in the other treatments at 42 ± 19%, 37 ± 20%, and 20 ± 5% in the BotaniGard low-rate, PRF 97 high-rate, and PRF 97 low-rate treatments, respectively.

There was an average mortality of 39.5 ± 11.2% in the high-rate BotaniGard treatment after 24 h that increased to 87 ± 8% by 1 wk post treatment. Cuthbertson & Audsley (2016) found > 40% mortality of *D. suzukii* within 7 d of exposure to both *I. fumosorosea* and *M. anisopliae*. Similarly, Ibrahim et al. (2014) found that *B. bassiana* caused the highest mortality in *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) flies after 6 d and in *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) after 5 d. There were no significant differences in mortality of adult *D. suzukii* after 24 h in this study, likely due to the high natural mortality that occurred.

However, the BotaniGard high rate treatment reduced the number *D. suzukii* flies emerging from dipped berries below levels found in the control. Falchi et al. (2014) found that *B. bassiana* conidia-based preparations applied to oranges acted as an oviposition deterrent to *C. capitata* females. The fruit dip method used in this study may have produced a similar deterrent effect in *D. suzukii*. In another study, Gargani et al. (2013) dipped berries after exposure to *D. suzukii* and did not find a reduction in the number of emerging flies. Therefore, it is unlikely that the fungi affect larvae developing in fruit.

The results from this study indicated that BotaniGard at the high treatment rate demonstrated the best efficacy for *D. suzukii*. BotaniGard and other strains of *B. bassiana* often have shown efficacy for *D. suzukii* (Cuthbertson et al. 2014; Gargani et al. 2013). However, Woltz et al. (2015) found that *M. anisopliae* caused significant mortality in *D. suzukii* while *B. bassiana* and *I. fumosoroseus* did not. Cuthbertson & Audsley (2016) found that both *I. fumosoroseus* and *M. anisopliae* reduced the emergence of *D. suzukii* from infested berries. The differences in this study are likely due to the use of other strains and assay methods. Also, natural variation in *D. suzukii* populations could play a role as well (Robertson et al. 1995).

From these results, it does not appear that either of the products could be used as a stand-alone treatment. However, BotaniGard could be useful as a rotation option with other effective insecticide compounds. Further research is needed to determine if BotaniGard causes mortality of *D. suzukii* in the field, and if it produces an oviposition deterrent effect in the field.

Summary

Spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a major pest of bushberries, caneberries, and other thin-skinned fruits around the world. Current control programs rely solely on the use of frequent insecticide applications. Entomopathogenic fungi have

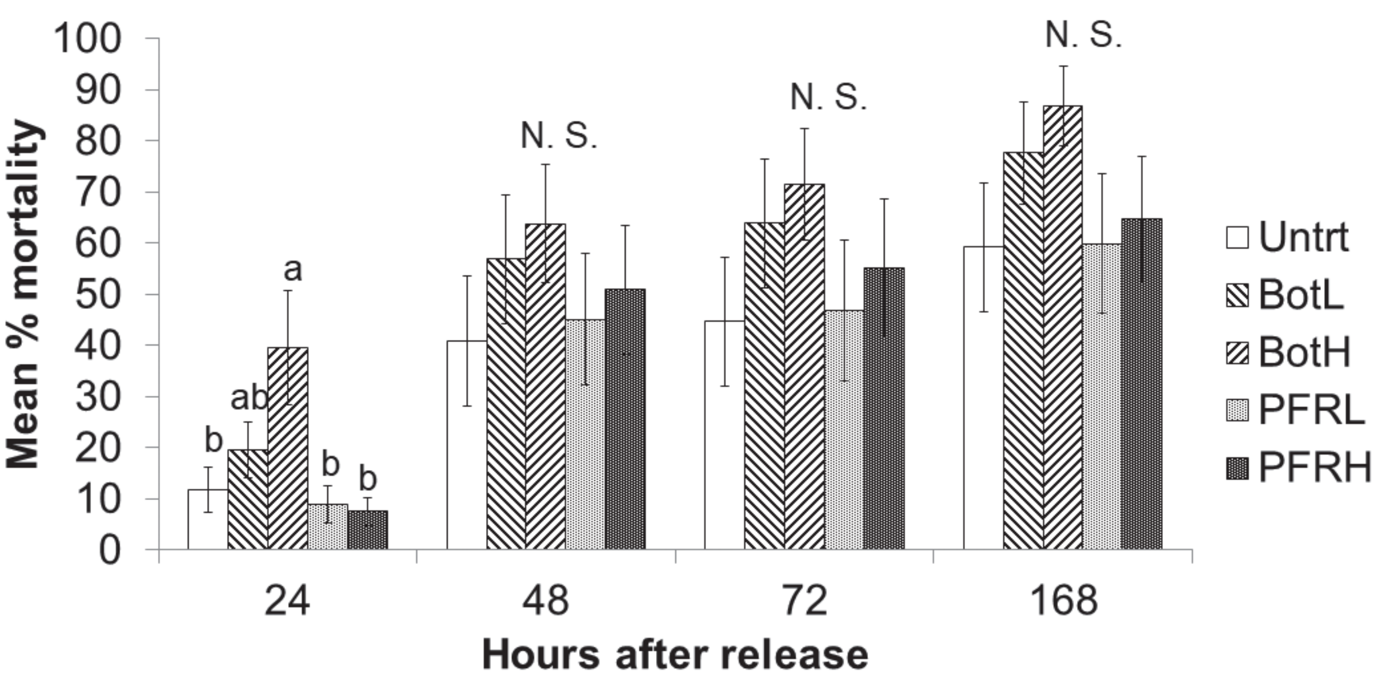

Fig. 1. Percent mortality of spotted wing drosophila after 24, 48, 72, and 168 h in each treatment (Untrt = deionized water treated control, BotL = BotaniGard low rate, BotH = BotaniGard high rate, PFRL = PFR 97 low rate, and PFRH = PFR 97 high rate). Bars with the same letter are not significantly different from each other ($P > 0.05$). N. S. = no significant differences ($P > 0.05$). Error bars represent standard error of the mean.

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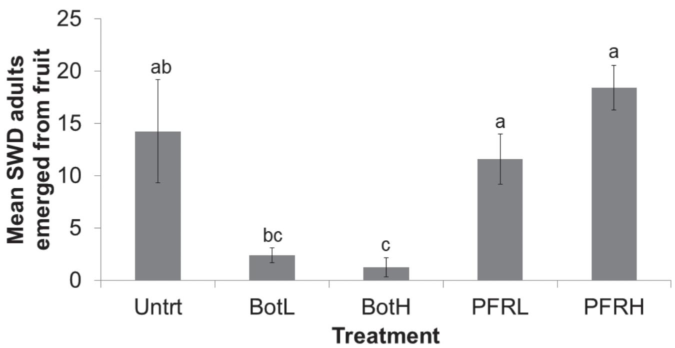

Fig. 2. Adult spotted wing drosophila emergence from berries 2 wk after removal from the arenas. Bars with the same letter are not significantly different from each other ($P > 0.05$). Error bars represent standard error of the mean.

shown some efficacy for tephritid fruit flies and *D. suzukii*. In this study, formulated products BotaniGard ES (*Beauveria bassiana*), and PFR-97 20% WDG (*Isaria fumosorosea*) were evaluated for efficacy on adult *D. suzukii* in laboratory fruit-dip studies. Blueberry fruits were dipped in high and low label rates of each fungal suspension before exposure to *D. suzukii*. Mortality was assessed at 24, 48, 72, and 168 h (1 wk) after *D. suzukii* release. After 1 wk exposure, the remaining live flies, dead flies, and fruit were removed from containers. The live flies were discarded whereas dead flies were placed in separate containers and checked for mycosis after 1 wk. Fruits were placed in small solo cups with lids and checked after 2 wk for emergence of *D. suzukii*. The high rate of BotaniGard ES caused significant mortality after 24 h and both rates of BotaniGard ES reduced emergence from fruit compared with the control. BotaniGard ES may be useful in rotation with other effective compounds, but further research under field conditions is needed to confirm this finding.

Key Words: *Drosophila suzukii*; spotted wing drosophila; entomopathogenic fungi; blueberries

Sumario

La mosca drosophila del ala manchada, *Drosophila suzukii* Matsumura, es una plaga importante de arbustos de bayas, cañas de bayas y otras frutas de piel fina por todo el mundo. Los programas de control actuales dependen únicamente del uso de aplicaciones frecuentes de insecticidas. Los hongos entomopatógenos han demostrado cierta eficacia contra las moscas de la fruta tefrítidas y *D. suzukii*. En este estudio, se evaluaron los productos formulados BotaniGard ES (*Beauveria bassiana*) y PFR-97 20% WDG (*Isaria fumosorosea*) para determinar su eficacia contra los adultos de *D. suzukii* en estudios de inmersión de frutas en el laboratorio. Se sumergió la fruta del arándano en cada suspensión de hongo según la tasa alta y baja indicada en la etiqueta antes de la exposición a *D. suzukii.* Se evaluó la mortalidad a las 24, 48, 72 y 168 horas (1 semana) después de la liberación de *D. suzukii*. Después de una semana de exposición, se eliminaron los restos las moscas vivas, moscas muertas y frutas de los contenedores. Se eliminaron las moscas vivas mientras las moscas muertas se colocaron en recipientes separados que fueron revisados para la micosis después de 1 semana. Las frutas se colocaron en tasas pequeñas individuales con tapas que fueron revisadas después de 2 semanas para la emergencia de *D. suzukii*. La alta tasa de BotaniGard ES causó una mortalidad significativa después de 24 horas y ambas tasas de BotaniGard ES redujeron la emergencia de la fruta en comparación con el control. BotaniGard ES puede ser útil en rotación con otros compuestos efectivos, pero se necesita más investigación en condiciones de campo para confirmar este hallazgo.

Palabras clave: *Drosophila suzukii*; hongos entomopatógenos; arándanos

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