Host Status and Fruit Odor Response of \textit{Drosophila suzukii} (Diptera: Drosophilidae) to Figs and Mulberries

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ABSTRACT \textit{Drosophila suzukii} Matsumura (Diptera: Drosophilidae) is an agricultural pest with a wide host range. It is known to infest fruit that are still ripening on the plant, as well as rotting and damaged fruit. Our study sought to determine whether \textit{D. suzukii} use mulberries (\textit{Morus} spp.) and figs (\textit{Ficus carica} (L.)) as hosts, as their host status was ambiguous. Accordingly, we collected 25 field-infested fruit and counted the numbers of \textit{D. suzukii} emerging from them. We also sought to determine whether female \textit{D. suzukii} would respond to olfactory cues from ripe figs and mulberries. As the host population has been known to impact host odor response, flies from mulberry, fig, and cherry origins were tested in “one-choice” olfactometry studies. Our results show that mulberries and figs can serve as hosts for \textit{D. suzukii} and that female flies will respond to their odors. The host population did affect response to fruit odors, although further studies are necessary to determine habitat fidelity. This has implications for management of this pest, especially in backyard and mixed fruit orchard situations, which commonly occur in the current range of \textit{D. suzukii}, and fig and mulberry may serve as a pest reservoir for other hosts and cultivated crops.

KEY WORDS “one-choice,” habitat fidelity, 4-way olfactometer, Spotted-wing \textit{Drosophila}
made as to whether *D. suzukii* females respond to fig and mulberry odors. It may be that female *D. suzukii* exhibit odor preferences for their host fruit, and even exhibit habitat fidelity as seen in other *Drosophila* species. We quantified field infestation using field-collected fruit and used “one-choice” olfactometry trials to assess the response of female *D. suzukii* to ‘Illinois Everbearing’ mulberry (*Morus alba x rubra*) and ‘Mission’ fig (*F. carica*) odor in terms of host finding and fidelity. Further classification of the host status of mulberry and fig fruits is important in terms of predicting potential backyard and commercial damage as well as to assess the potential pest reservoir expected from landscape fig and mulberry trees.

**Materials and Methods**

**Host Fruit Emergence Study.** Twenty-five fruits (as close to peak ripeness as possible) from Illinois Everbearing mulberry (17 August 2011), Mission fig (7 September 2011), and Brown Turkey fig (24 August 2011) were randomly collected off of trees from orchards with known *D. suzukii* pest pressure at the U.S. Department of Agriculture Wolfskill Germplasm Repository in Winters, CA. The fruit had no visible signs of exterior damage, although the ostiole on the fig may serve as an access point for oviposition. Each individual fruit was placed in a plastic cup covered with organza mesh fabric (held in place with a rubber band) and held for 16 d at constant 22°C with a photoperiod of 16:8 (L:D) h. All emerging *Drosophila* were collected and identified as *D. suzukii* or other *Drosophila* spp.

**Host Fruit Populations.** We established host populations in May–June 2012. Mulberries and cherries were collected from the USDA Wolfskill Germplasm Repository and placed in separate plastic emergence cages. Figs were not collected because fig season had not yet begun. All emerging *D. suzukii* were kept and used for our study.

Flies were also collected May–June 2012 using sliced strawberry (to prevent host odor preference bias in the collected flies); fruit-baited traps (Ziploc Smart Snap [SC Johnson, Rancine, WI] 591-ml containers with nine 4-mm holes in the lid) were placed in the fig (∼156 m from mulberry, ∼393 m from cherry), mulberry (∼223 m from cherry), and cherry orchards at USDA Wolfskill Germplasm Repository. Adult flies in the traps were collected, and the strawberries were kept in emergence chambers to collect any emerging *D. suzukii*. These orchard trapped flies were combined with the flies emerging from infested host fruit, and host populations were established and maintained in separate Fisherbrand square, polyethylene, six oz. stock bottles (Fisher, Pittsburgh, PA) containing 50 ml of Applied Scientific Jazz-Mix® *Drosophila* Food (Fisher, Pittsburgh, PA). Colonies were kept at 22°C in a cabinet incubation chamber (Percival Scientific, Inc., Perry, IA) with a photoperiod of 16:8 (L:D) h, and olfactory experiments were performed two to five generations after establishment.

**‘One-choice’ Olfactometer Bioassay.** A 4-choice olfactometer and air delivery system with vacuum control (Olfactometer Product No. OLFM-4C-2440PE, Air Delivery System Product No. OLFM-4C-ADS+V, Analytical Research Systems, Inc., Gainesville, FL) was used to test olfactory response of starved female *D. suzukii* (aged 2–7 d). The olfactometer was based on a design by Vet et al. (1983) and Kalule and Wright (2004). Air is drawn through four evenly spaced channels into a central chamber. This air was blown through 175 ml of deionized water in a PYREX 250 ml Heavy Wall Filtering Flask with Sidearm Tubulation (Corning Incorporated, Tewksbury, MA) to humidify the air, creating four distinct odor fields in the central chamber (see Fig. 1). The air is drawn out of the olfactometer by a vacuum pressure pump (insect inlet adaptor [Fig. 1]) attached to an open channel located in the floor of the central chamber, where mixing of odors also occur. Females were sexed by visual inspection and collected by aspiration from their stock bottles 20–24 h before trials were to occur and starved in a Dry Keeper Desiccator cabinet (Sanpateccorp, Osaka, Japan). The desiccant was removed, and relative humidity was maintained at 60–80% by placing a water-filled tray at the bottom of the cabinet. Flies were kept separate by host fruit population within the cabinet in 25-ml Falcon Tubes (BD Biosciences, San Jose, CA) that were cut at both ends and covered with organza mesh fabric held in place with a rubber band. The desiccator cabinet was then placed within the previously described cabinet incubation chamber.

Trials were conducted under a 120V fluorescent light placed in a reflector dish (UL LLC, Northbrook, IL). The light was suspended 56 cm above the olfac-
terometer and was covered with wax paper to evenly disperse the light. A Logitech C200 webcam (Newark, CA) was also suspended above the olfactometer to record the activity of the flies during the entirety of the trials. A brown cardboard box surrounded the olfactometer to prevent interference by visual stimuli. Before conducting the trials, airflow was visualized by conducting a smoke test. Airflow was set at 300 ml/min for each arm to allow flies to move about normally inside the olfactometer.

Each fruit odor was individually tested. Fresh, intact Illinois Everbearing mulberries and Mission figs (commercial varieties that are also commonly planted in urban landscapes) were collected the day of or the day preceding the trial. Fruit was randomly collected from trees in the USDA Wolfskill Germplasm Repository as close to peak ripeness as possible using aluminum foil to prevent the fruit from absorbing foreign odors and contamination by human hands. The fruit had no visible signs of exterior damage. The fruit was stored at room temperature wrapped in two layers of aluminum foil after being returned to the laboratory. Two cut (using sterilized [rinsed with 80% ethanol that was allowed to evaporate] stainless steel scissors) mulberries were used for each mulberry odor trial, whereas one fig slice of approximately equivalent size and mass of the mulberry samples was used for each fig odor trial. The cut fruit was then randomly placed inside one of the olfactometer’s four internal odor source adaptors (Fig. 1). The other three were empty to serve as null control odors.

Flies were introduced into the central chamber using a plunger (slowly pushed upward to coax the flies into the central chamber) made from a modified Tampax Pearl Compak tampon applicator (Proctor & Gamble, Cincinnati, OH). The outer tube of the applicator was wrapped in construction paper to form a tighter seal in the olfactometer’s open channel. The center hole in the inner tube was covered with tape to prevent flies from escaping. Each trial lasted 20 min and began when all flies entered the exposure chamber of the olfactometer. At the end of each trial, the position of all flies was recorded and flies were marked as within the trap bottle or nonresponsive.

Clean trap bottles were used each trial, and all the olfactometer components were cleaned at the end of each trial period (three to five trials). All glassware and acrylic and polyethylene components were cleaned using a 1% Alconox Powdered Precision Cleaner (Alconox Inc., White Plains, NY). Glassware was then rinsed with acetone followed by deionized water and baked overnight at 160°C in a Stabil-therm Gravity Oven (Blue M Electric Co., Blue Island, IL). All acrylic and ethylene components were rinsed with 80% ethanol followed by deionized water and air dried.

**Statistical Analysis.** A nonparametric Friedman-analysis of variance (ANOVA) was performed using Proc Freq in SAS 9.2 (SAS Institute 2008) to test the null hypothesis that flies were evenly distributed in each of the olfactometer trap bottles for the one-choice olfactometry trials. To compare the response to the different fruit treatments by the *D. suzukii* host populations in these trials, a logistic regression model was used, as the data were binomial. This was performed using Proc Glimmix in SAS 9.2 (SAS Institute 2008) to allow us to include the trial number in the analysis as a random effect to control for between trial variation within each fruit and population (trial number [fruit population]). The fixed effects were fruit, population, and fruit × population with the response variable being the number of flies responding to the fruit over the total number of flies. An LS means statement was included to generate pairwise differences in response.

**Results**

*D. suzukii* emerged from field-collected Illinois Everbearing mulberries, Mission figs, and Brown Turkey figs. Mulberries had the highest emergence, followed by Brown Turkey and Mission figs, respectively (Table 1). Other *Drosophila* spp. were prevalent in both fig varieties, with the most other *Drosophila* found in Brown Turkey variety. No other *Drosophila* emerged from mulberries (Table 1).

Fruit and the interaction of fruit × population were found to have a significant impact on *D. suzukii* response (fruit $F_{1,32} = 5.01, P = 0.0323$; population $F_{2,32} = 0.05, P = 0.9557$; fruit × population $F_{2,32} = 3.78, P = 0.0338$). Twenty-four independent trials were performed using Mission fig as the fruit attractant and 23 were performed using Illinois Everbearing mulberry. Overall, *D. suzukii* females responded more strongly to Mission fig than to Illinois Everbearing mulberry (Fig. 2), although the significant fruit × population interaction indicates that response varied by host population.

Within the host populations, the Friedman-ANOVA rejected the null hypothesis that the flies were evenly distributed in each of the four olfactometer trap bottles with $P < 0.05$ for all one-choice fruit odor experiments (fig–fig [host population–fruit odor]: $F_{3,6} = 9.780, P = 0.0205$; fig–mulberry: $F_{3,5} = 10.8571, P = 0.0125$; mulberry–fig: $F_{3,10} = 19.4231, P = 0.0002$; mulberry–mulberry: $F_{3,10} = 9.0882, P = 0.0281$; cherry–fig: $F_{3,8} = 14.2917, P = 0.0025$; cherry–mulberry: $F_{3,8} = 19.4231, P = 0.0002$; mulberry–cherry: $F_{3,8} = 19.4231, P = 0.0002$).
10.0667, P = 0.0180); therefore, we can conclude that flies were not evenly distributed among each of the four trap bottles (Fig. 3). Higher percentages of mulberry flies were observed in the Fig trap bottles than mulberry trap bottles (mulberry–Fig vs. mulberry–mulberry t(37) = 3.11, P = 0.0036) (Fig. 3). A similar trend was seen in Figs, though this was not statistically significant (Fig–Fig vs Fig–mulberry t(29) = 0.92, P = 0.3668). For our control population, the percentage of cherry flies was higher in the Fig trap bottles than in the mulberry trap bottles (cherry–Fig vs. cherry–mulberry t(31) = 2.18, P = 0.0366) (Fig. 3). Mulberry flies were most attracted to the fig fruit (LS Means estimate = –1.0881, t(27) = –3.10, P = 0.0045), followed by cherry flies (LS Means estimate = –1.3393, t(26) = –3.45, P = 0.0019) then fig flies (LS Means estimate = –2.1538, t(32) = –4.57, P < 0.0001). Fig flies were most attracted to the mulberry odor (LS Means estimate = –1.5286, t(27) = –3.10, P = 0.0045), followed by cherry flies (LS Means estimate = –2.6037, t(36) = –6.06, P < 0.0001) then mulberry flies (LS Means estimate = –2.7994, t(41) = –6.62, P < 0.0001) (Fig. 3).

Discussion

We found that D. suzukii can complete a full generation in mulberries and figs and that female D. suzukii respond to both odors; thus, these fruits can serve as hosts. From mulberries, an average of two to three adult D. suzukii emerged per fruit, whereas three to four flies per fruit emerged on average from fig cultivars. To put this in perspective, Burrack et al. (2013) found 1.17 ± 0.14 D. suzukii larvae and pupae per fruit from field-collected ripe intact blackberries (Rubus spp.) and 2.90 ± 0.34 larvae and pupae from ripe intact raspberries (Rubus idaeus (L.)) grown without tunnels in North Carolina. Raspberries and blackberries are known to experience heavy commercial infestation. No other Drosophila spp. emerged from Illinois Everbearing mulberry, although other Drosophila composed the vast majority of the fig emergence for the Brown Turkey variety. According to Takahashi and Kimura (2005), interspecific larval interactions are essentially competitive. It has been suggested that D. melanogaster may be a formidable competitor for...
resources in some hosts (Dean et al. 2012), which may explain the species’ proportions of the emerging adult Drosophila in figs.

D. suzukii respond to mulberry and fig odors and appear to more likely select novel fruit hosts as opposed to fruit in which they originated. This may have been influenced by our host population collecting methods or laboratory rearing methods. The host populations were started from D. suzukii trapped in strawberry-baited traps, as well as flies emerged from mulberry and cherry fruit, but not fig fruit. This is because fig season did not begin until after we began establishing host populations. However, trapping was performed at that time as preliminary trapping data had indicated presence of D. suzukii before the beginning of fig season. Field populations may be highly mobile, and may intermix within these blocks. All flies were reared on Jazz Mix, which is much different in quality than the fruit resources to which they were responding, may vary from batch to batch, and genetic differences in the host populations may be lost because of being reared on lab medium (Hoffmann and McKechnie 1991). Starvation may have also forced the flies to accept a more novel food source. According to Turelli and Hoffmann (1988), stressed flies may be less discriminating of available food. Dispersers assess time available for search by monitoring physiological factors, including fat stores and endurance (Stamps and Davis 2006), which are decreased when starved. This may have also led to the preference trends we observed. Despite efforts to ensure fig slices and mulberries were of similar mass and volume, variation in quantity of volatiles produced would skew the results of the olfactory assay. Although more research is necessary to validate this behavior, it may be an important consideration in developing attractants.

Although the results of our study do not resolve D. suzukii habitat fidelity or odor preference, they are still useful in assessing the host range of this pest species. From our host emergence and one-choice olfactometry studies, we conclude that D. suzukii are attracted to and can develop on figs and mulberries. From these results, not only should commercial fruit growers be wary, but backyard growers as well. Figs and mulberries have a long fruiting interval that often overlaps with more valuable crops, such as cherries. Figs and mulberries are common backyard and landscape trees in California, and may serve as a reservoir for D. suzukii populations. Commercial growers, especially mixed orchard growers, and backyard growers may want to monitor local figs and mulberries for presence of D. suzukii and take necessary measures to prevent their spread.

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