A food-grade gum as a management tool for Drosophila suzukii

Gabriella Tait¹,²,³, Clive Kaiser³, M. Valerio Rossi-Stacconi³, Daniel T. Dalton³, Gianfranco Anfora¹,⁴, Vaughn M. Walton³

¹Research and Innovation Centre, Fondazione Edmund Mach, San Michele all’Adige, Italy
²Department of Agricultural and Environmental Sciences, University of Udine, Italy
³Department of Horticulture, Oregon State University, Corvallis, Oregon, USA
⁴Center of Agriculture Food and Environment, University of Trento, San Michele all’Adige, Italy

Abstract

Drosophila suzukii is an insect pest of worldwide distribution on soft-skinned fruit. This species is able to utilize different habitats and substrates for nutrition and reproduction, a capacity that can be attributed to olfactory cues. The first aim of the current study was to create and evaluate a novel gum matrix as a management tool for D. suzukii in a commercial cropping system. Next, we identified a biologically important volatile from an important ingredient within the matrix. The efficacy of the proof of concept matrix as a management tool was assessed in laboratory and semi-field conditions. The detection of active volatile compounds was performed using gas chromatography (GC) coupled with electroantenna detection (EAD) techniques. Volatiles significantly modified response of D. suzukii in controlled electrophysiology and orientation studies, resulting in 46.7% mean oviposition reduction in controlled laboratory trials on five susceptible fruit types. Field trials were thereafter conducted over periods of 72 ± 2 to 96 ± 2 hours on commercial-standard blueberry bushes. Fruit on bushes exposed to predetermined numbers of D. suzukii displayed 50 to 76% reductions of fruit infestation and total eggs laid, respectively, in gum treatments. Up to 40% of fruit on untreated plants were targeted by D. suzukii, whereas less than 20% of fruit on treated bushes were targeted. These results indicate that the insecticide-free gum matrix significantly reduces D. suzukii damage under commercial production conditions. This reduction may be due to a combination of altered behaviour and the division of reproductive resources. The current work will likely expand integrated pest management options to control D. suzukii populations in commercial field settings.

Key words: Spotted-wing drosophila, oviposition, insect bait, behaviour manipulation, Integrated Pest Management.

Introduction

The spotted-wing drosophila, Drosophila suzukii (Matsumura), indigenous to Southeast Asia, is an invasive pest species causing direct damage to soft-skinned fruits (Lee et al., 2011; Cini et al., 2012). The first description of the economic damage caused by this species was recorded in Japan in 1916 (Kanzawa, 1939). D. suzukii has since spread to become a pest of worldwide importance (Hauser et al., 2011; Asplen et al., 2015). Following its detection in 2008 in both Europe and USA, its damage caused estimated economic losses of €3 million in Trentino province (Italy) (Cini et al., 2012) and can cause an estimated $511 million in losses annually in western production regions of North America (Bolda et al., 2010; Farnsworth et al., 2017). Current integrated pest management (IPM) practices for D. suzukii include trapping and fruit monitoring (Lee et al., 2011; 2013; Evans et al., 2017; Kirkpatrick et al., 2017), biological control (Chabert et al., 2012; Kasuya et al., 2013; Rossi-Stacconi et al., 2013; 2017; Gabarra et al., 2015; Nomano et al., 2015; Woltz et al., 2015; Daane et al., 2016), chemical control (Beers et al., 2011; Bruck et al., 2011; Van Timmeren and Isaacs, 2013; Wise et al., 2015; Murphy et al., 2015), and cultural control (Lee et al., 2015; Tochen et al., 2016a; 2016b). The majority of these control methods significantly increases production costs. Pesticides controls result in 1-7 days of effect, needing up to twenty applications per season.

Oviposition levels of D. suzukii are affected by multiple factors, including environmental conditions (Kinjo et al., 2014; Tochen et al., 2014; 2016b; Enriquez and Colinet, 2017), sexual maturity (Zerulla et al., 2015; Rossi-Stacconi et al., 2016; Ryan et al., 2016; Wiman et al., 2016; Grassi et al., 2018), presence of essential food resources (Mitsui et al., 2006; Briem et al., 2016; Tochen et al., 2016a), and fruit susceptibility (Bellamy et al., 2013; Burrack et al., 2013; Ioriatti et al., 2015; Lee et al., 2015). Pest detection is one of the foundations of effective IPM. To this end, several attractants have been tested and are used to bait D. suzukii traps (Landolt et al., 2012; Cha et al., 2013; 2017; Tonina et al., 2018). Several of these baits are composed of a mix of volatiles of red wine, cider wine and sugars and are focused on attraction (Landolt et al., 2012; Cha et al., 2017), but little focus to date has been given to manipulation of oviposition. In particular, work has been done on oviposition deterrents, whereas none tried to encourage oviposition on non-crop substrates (Renkema et al., 2016; Wallingford et al., 2016a, 2017). The oviposition behaviour of D. suzukii is not well characterized (Mitsui et al., 2006). Oviposition includes multiple steps during which the insect tests the quality of fruit (Karageorgi et al., 2017). One of the important steps includes probing of the substrate with the proboscis based on the volatiles associated with ripening fruit (Karageorgi et al., 2017; Tait, unpublished), and a decision is made whether to feed and oviposit or not. It is believed that leaves may contribute to volatile signals resulting in D. suzukii ovi-
position (Keesey et al., 2015). Currently, however, little information is available as to the specific role that each of these aspects plays in oviposition by D. suzukii.

Behavioural manipulation of insects has historically been an important component of the IPM toolkit (Dent, 1995). Volatiles emitted from the plant surface are received by olfactory receptor systems in phytophagous insects and act as a chemical message that helps them orient toward hosts (Visser, 1986; Agelopoulos et al., 1999). This concept is often used in ‘push-pull’ control tactics. Such a strategy may also hold potential for the control of Drosophila species. Many studies have examined the odour preference of D. suzukii which may be of use in push-pull tactics (Cha et al., 2012; 2014; Hamby et al., 2012; Keesey et al., 2015; Abraham et al., 2015; Revadi et al., 2015; Hamby and Becher, 2016; Kirkpatrick et al., 2016, 2017; Renkema et al., 2016; Wallingford et al., 2016a; 2016b; 2017; Frewin et al., 2017; Huang et al., 2017; Rice et al., 2017). It is possible that D. suzukii may maximize survival of offspring by selecting optimal hosts for oviposition (Minkenberg et al., 1992; Gripenberg et al., 2010) by having the ability to select the best option from a myriad of acceptable ones (Yang et al., 2008). Females should be more willing to accept alternative hosts, however, as egg load and search time for an oviposition site increases (Jaenike, 1990). By providing attractive alternative non-fruit hosts, it may be possible that D. suzukii may divert resources toward these alternate resources.

The goal of this work was to develop proof of concept. We believe that a behaviour disruptor will ultimately modulate the D. suzukii oviposition in commercial crops. Based on results of previous field trials on D. suzukii aggregation behaviour (Tait, unpublished), we identified novel compounds that ultimately affect oviposition. Starting from these ingredients, two attractive food-grade and biodegradable matrices were produced by varying the water content. The one with less water, the solid matrix (SM), had a gelatinous consistency, which allowed spotted applications without dripping, even on sloped surfaces. The one with more water, the liquid matrix (LM), had a creamy consistency and could be applied using standard spray equipment on wider areas. Here we describe a series of experiments aimed to: 1) evaluate the matrices as a management tool for D. suzukii in a commercial-standard cropping system and 2) verify the presence of active volatile compounds. The efficacy of the matrices to reduce oviposition was assessed in both laboratory and field conditions. The detection of active volatile compounds was performed using gas chromatography (GC) coupled with electroantenna detection (EAD) techniques.

Materials and methods

Insects

D. suzukii used for the laboratory and field trials were direct offspring of individuals collected in Oregon, USA (Willamette Valley, 44°55’58.2”N 123°03’50.6”W) in 2009. A second D. suzukii population originating from individuals collected in northern Italy (Trentino Province, 46°04’24.3”N 11°07’17.2”E) was used for GC-EAD experiments in addition to Oregon D. suzukii. Laboratory colonies from both geographic locations were regularly supplemented with locally collected flies to prevent inbreeding. Flies were reared in a 24 × 24 cm cage (Bugdorm-1, MegaView Science Co., Ltd., Taichung, Taiwan) and provided with a water wick and an artificial corneal diet that served as both an oviposition medium and food source. Insects were maintained in the laboratory at 22 °C, 65% RH and a photoperiod of 8:16 (L:D). In all tests, D. suzukii were circa 8 days old and previously mated.

Laboratory choice assays

Constant and uniform airflow was created within each of the containers using a vacuum at 1.5 L min⁻¹ from the base and through the upper portion of each of the respective containers. Attraction to the active ingredient A was verified in double-choice behavioural experiments (Figure 1). Arenas were prepared using 2-L transparent Griffin-style graduated low-form plastic beakers (Nalgene, Rochester, NY). For each beaker, 9 ventilation holes (1 cm diameter) were cut along the circumference approximately 6 cm from the base. The holes were covered with fine white mesh in order to prevent D. suzukii individuals from escaping. The top of each beaker was drilled and connected to a 0.5 cm diameter plastic tube providing a vacuum in order to create a constant and uniform air flow (1.5 L min⁻¹) within the containers. Beakers were placed upside down on a flat work surface covered by white paper sheets. Two 25 mL plastic cups (Dart Container Corporation, Mason, MI) containing ~10 mL of water and 5 g of the selected ingredient A were placed at the base of each beaker. Both cups were covered with a wax film (Parafilm® M, Pechiney, 296

![Figure 1. Schematic representation of the setup used for the laboratory double-choice assays.](image)
Chicago, IL). Three holes (~0.3 cm diameter) were cut on the film coverings in order to allow the flies to enter the cups. Twenty mated females and males were released within each arena, allowing them to orient and eventually enter the cups. After 24 h, the number of *D. suzukii* caught within each cup was counted, as well as flies that did not make any choice.

**Oviposition tests**

The principal ingredient A was mixed at a 1.5% rate with other proprietary food-grade components (Kaiser et al., 2018) under laboratory conditions at 22 °C, 65% RH. The matrix also contained between ~84% (SM) and 89% (LM) deionized water. The resultant matrices were used for *D. suzukii* efficacy trials within 1-2 hours of preparation in both laboratory experiments and field trials.

**Laboratory oviposition experiments**

Experiments were performed on blackberries, blueberries, cherries, strawberries, and raspberries under controlled conditions. Two 25-ml Deli cups (Dart Container Corporation, Mason, MI) were placed inside each arena, one containing circa 6 ± 0.1 g of the SM, and the other containing fresh fruit in treated repetitions. The control treatments only contained fruit. For each fruit type, we exposed an approximate surface area of 32 mm², corresponding to the exposed area of the matrix within the Deli cup in the treated repetitions. Fifteen *D. suzukii* individuals, 10 females and 5 males, were released into each arena. After 24 h, the number of eggs laid on the berries and into the matrix was counted. The experiment for each fruit type was replicated ten times. Initial trials looking at increase in cherry fruit per arena did not result in a significant reduction in the number of eggs laid per berry between treatments (single cherry = 36.5 eggs/cherry; three cherries = 49.25 eggs/cherry; F4,15 = 4.18, *P* = 0.82).

**Field oviposition trials**

Oviposition trials were conducted from 8 August to 17 October 2017 at the Lewis-Brown Farm at Oregon State University (44°33′13″N 123°13′07″W) in an organic 11-year-old drip irrigated cv. Elliott blueberry field. Plants were spaced approximately 0.76 m apart within rows with 3.05 m between rows and were not treated with insecticides. The width and height of plants were approximately 1 m and 1.5 m, respectively. Two drip irrigation lines, one on either side of the blueberry plant, were placed under sawdust mulch cover within standard raised beds and provided irrigation at levels of 100% (255 ± 5 mm water per growing season), of the estimated crop evapotranspiration (ETc) requirement (Bryla et al., 2011).

Two series of trials were performed. In the first, single fruit clusters were isolated in mesh bags, while the second trial was carried out using isolated whole bushes. Field oviposition trials including fruit were conducted by covering fruit clusters (10-23 berries) with 20 cm × 30 cm white organza mesh bags (Uline, Pleasant Prairie, WI). All mesh bags were placed approximately 1 m apart on the north side of the bush within the shade of the canopy and 0.2 to 1.3 m above ground level. Each mesh bag contained 10 *D. suzukii* adults, 5 females and 5 males. Either SM or LM were added to the treatment mesh bags. Solid matrix was provided at 40 ± 0.5 mL in 10 cm diameter petri dishes (figure 2a) on 8 and 31 August; 13, 15 and 25 September; and 11 October 2017. Liquid gum matrix was applied by spraying 5 mL on the surface of the mesh bags using an all-purpose spray bottle (Home Depot, Atlanta, GA) (figure 2b) on 13 September, 15 September, and 11 October 2017. Every trial date contained 10 replicates for treated and control clusters each. All trials were started between 15:00 and 17:00 and were collected 72 ± 2 hours later to determine the levels of oviposition on fruits and within the gum matrix. All damaged berries were excluded when assessing the egg-laying levels in the laboratory under a dissecting microscope. Experiments based on these treatments were replicated 10 times on each of the treatment dates and were conducted on 10 separate days.

Whole-bush *D. suzukii* exposure trials using both SM and LM were conducted in order to determine efficacy when the matrix is applied at the base of the bush. Entire bushes were covered using 80 g Tek-Knit netting (Berry Protection Solutions, Stepheightown, NY) which extended to the soil surface (figure 2c). In total, 100 *D. suzukii* (50 females and 50 males) were released within each netted bush. Solid and liquid gum matrices were respectively trialled using 100 ± 2 mL SM placed within 5 Petri dishes (10 cm diameter), or by spraying 100 ± 2 mL of LM at the base of the bush on a 15 × 30 cm microfiber cloth (Costco, Kirkland, WA). Replicates of both treated and control plants were exposed between 15:00 and 17:00 and fruit were collected 72 ± 2 to 96 ± 2 hours after initial placement of flies. Solid matrix experiments were replicated twice, i.e. on 22 and 24 September 2017. Liquid matrix experiments were repeated 3 times, i.e. on 27 September, 3 October and 9 October 2017. On each date, 5 replicates (bushes) per treatment were set. After the *D. suzukii* exposure period, 20 firm berries were collected from each area of the plant, designated as the top (~1.3 m above soil level), middle (~0.8 m above soil level) and bottom (~0.3 m above soil level) for a total of 60 berries collected per bush. Assessments of oviposition were determined by calculating the number of eggs laid per berry and percent of infested berries.

**Weather data**

Weather data including temperature (°C), humidity (%) and rainfall (mm) were obtained from the Corvalis, Oregon Agrimet weather site (Oregon State University Hyslop Farm 44°38′03″N 123°11′24″W) (https://www.usbr.gov/pn/agrimet/agrimetmap/crvoda.html). In this way it was possible to verify daily the weather conditions during the field trials.

**Volatile collection**

To extract volatiles from the principal ingredient A, 50 mL of water were mixed with 2 g of it in order to obtain a paste. 10 g of paste were placed into a glass tube...
Figure 2. *D. suzukii* blueberry exposure trials using 20 cm by 30 cm white organza mesh bags and covering fruit clusters containing 10-23 berries (a). Solid and liquid gum matrices were respectively trialed using ~40 mL solid gum within 10 cm diameter petri dishes (b), or by spraying 5 mL of liquid gum on the surface of the mesh bags. Whole-bush *D. suzukii* exposure trials using both solid and liquid gum were conducted by covering whole bushes using netting, which extended to the soil surface (c).

(length 25 cm, diameter 3 cm) acting as a collection chamber. Air from the headspace of the tube was extracted at 150 mL min⁻¹ and forced through a sorbent cartridge (75 mg Super Q; Sigma-Aldrich, Germany) by means of a vacuum pump (DC 12/16 FK, Aersistem, Milan, Italy). Charcoal-filtered air was pushed simultaneously into the tube by the same pump to maintain constant pressure. Multiple replications of volatile collection were carried out over 72 h in a climatic chamber at 25 ± 2 °C and 70 ± 5% RH. Trapped volatiles were eluted from the sorbent cartridge using 1 mL of dichloromethane (>99% purity, Sigma-Aldrich) at room temperature. Collections were reduced to 200 µL via solvent evaporation under a low-velocity air stream. The final extract was then kept at ~20 °C until use in GC-EAD trials.

**GC-EAD analysis**

Adult individuals of *D. suzukii* were anesthetized by refrigeration at ~20 °C for 2 min. The response of the insects was recorded with an EAG apparatus (Syntech, Hilversum, NL) connected to the antennae through microcapillary glass electrodes filled with Kaisling solution [NaCl (7.5 gL⁻¹); CaCl₂ (0.21 gL⁻¹); KCl (0.35 gL⁻¹);
NaHCO₃ (0.2 g L⁻¹) containing 5 g L⁻¹ polyvinylpyrrolidone (PVP, Sigma-Aldrich). The protocol called for the head of the insect to be mounted on a microcapillary tube connected to a grounded silver electrode, while the recording silver electrode was brought in contact with the distal tip of one antenna. Two µL of the concentrated principal ingredient A extracts were injected into a Clarus 500 GC (Perkin Elmer Inc., Waltham, MA) in splitless mode, with a polar Elite-VMS column (30 m × 0.32 mm; Perkin Elmer Inc., Waltham, MA) programmed from 60 °C (hold 3 min) at 8 °C min⁻¹ to 250 °C (hold 10 min) with hydrogen as the carrier gas and interfaced with the EAG apparatus (Revadi et al., 2015). The GC column effluent was combined with nitrogen make-up gas and then a 1:1 ratio between the flame ionization detector (FID) and an antenna of a D. suzukii female. Compounds eluted from the capillary column were delivered to the antenna through a glass tube (12 cm × 8 mm) via a constant humidified air stream (0.5 L min⁻¹) filtered with charcoal. The air tube was located 4-5 mm from the antenna. Both the antennal and the FID signals were amplified and recorded simultaneously using GC-EAD Syntech software (Ockenfels Syntech GmbH, Buchenbach, Germany).

Headspace extracts were tested on 2 different D. suzukii populations, one originating from individuals collected in northern Italy and one from individuals collected in Oregon. From each population, 5 females were tested. The graphical area under the peaks was used for analysing the chromatograms. The relative quantity of each compound was calculated in relation to the most abundant compound, which was set at a value of 100. The EAD responses were calculated by Autospike software (Syntech) measuring the maximum amplitude of negative deflection (mV) elicited by D. suzukii antennae.

Statistical analysis
Data from laboratory double-choice experiments and oviposition trials were analysed using one-way ANOVA, and the Tukey’s HSD test was applied to separate difference at α < 0.05. Mesh bags field trials data were analysed using one-way ANOVA tests. Whole-bush field trials data were analysed using factorial ANOVA test followed by Tukey’s HSD test. Differences in volatile perception between Oregon and Italian D. suzukii were tested with the Mann-Whitney U-test (Mann and Whitney, 1947). All analyses were run using Statistica 12 (StatSoft Inc., Tulsa, OK) (Hill and Lewicki, 2007).

Results

Laboratory choice assays
The number of flies selecting Deli cups containing attractant ingredient A was significantly higher (F₂, 3₂ = 188.99, P < 0.001, figure 3) than both flies selecting deionized water and flies not making any choice.

Laboratory oviposition experiments
Overall, the presence of gum matrix resulted in a significant reduction in egg laying in all fruit types compared to untreated control treatments under controlled laboratory conditions (mean reduction = 48.3%, F₁, ₉₈ = 19.13, P < 0.001). When flies were presented a choice between gum matrices and fruit, a reduction of 46.5%, 46.28%, 51.5%, 43.5% and 48.5% of eggs in fruits compared to the control treatments were recorded for blackberry, blueberry, cherry, raspberry and strawberry respectively (table 1). The numbers of eggs laid in fruit

### Table 1. Mean number of D. suzukii eggs in control compared to berries with liquid matrix (LM). Laboratory oviposition experiments included a choice test between a water control and fruit type within an inverted modified 2-L plastic beaker. Numbers of deposited eggs were recorded after 24 hours of exposure to D. suzukii. For each F-value the degrees of freedom are (1, 19).

<table>
<thead>
<tr>
<th>Fruit type</th>
<th>Eggs in control fruit</th>
<th>Eggs in the LM</th>
<th>Eggs in fruit next to LM</th>
<th>F-value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberries</td>
<td>11.6 ± 2.0</td>
<td>0 ± 0</td>
<td>6.2 ± 1.2</td>
<td>5.42</td>
<td>0.032</td>
</tr>
<tr>
<td>Blueberries</td>
<td>17.2 ± 1.1</td>
<td>11.8 ± 3.12</td>
<td>8.4 ± 1.1</td>
<td>32.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cherries</td>
<td>58.1 ± 8.9</td>
<td>2 ± 0.66</td>
<td>28.2 ± 6.2</td>
<td>7.60</td>
<td>0.013</td>
</tr>
<tr>
<td>Raspberries</td>
<td>46.0 ± 4.7</td>
<td>10.2 ± 3.82</td>
<td>26.0 ± 2.6</td>
<td>13.73</td>
<td>0.002</td>
</tr>
<tr>
<td>Strawberries</td>
<td>46.4 ± 4.8</td>
<td>1.1 ± 0.48</td>
<td>23.9 ± 4.1</td>
<td>12.93</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Figure 3. The number of D. suzukii flies selecting Deli cups containing ingredient A compared to flies selecting deionized water under controlled laboratory conditions within ventilated choice arenas.
were statistically higher than in the matrices ($F_{1, 98} = 32.85, P < 0.001$), with $18.54 \pm 2.0$ eggs in fruit compared to $5.02 \pm 1.2$ eggs in the matrices.

Field oviposition trials

Field experiments using mesh bags to cover branches containing fruit indicated a consistent reduction of eggs laid using both SM and LM, with an overall reduction of eggs laid on fruits of $51.2\%$ ($F_{1, 99} = 20.76, P < 0.001$). The fruit exposed to *D. suzukii* in the mesh bag field experiments showed a significant reduction of eggs laid on berries for the solid gum formulation on 5 out of 6 of the dates when this formulation was trialled (mean eggs in fruit in absence of the matrix = $28.7 \pm 3.7$, mean eggs in fruit in presence of the SM = $14 \pm 1.7$, $F_{1, 99} = 20.76, P < 0.001$, figure 4a). The reduction of oviposition ranged from 41.5 to 72.2% during the *D. suzukii* exposure periods, which ranged from 72 to 96 ± 2 hours. On 31 August 2017, the reduction of egg-laying was not statistically significant at 41.5% reduction in egg laying.

The fruit exposed to *D. suzukii* in the mesh bag field experiments displayed a significant reduction of eggs laid on berries for the LM on all 3 dates when this formulation was tested (mean eggs of control = $26.8 \pm 3.7$, mean eggs in fruit in presence of LM = $13.2 \pm 3.0$, $F_{1, 53} = 17.56, P < 0.001$, figure 4b). The reduction of egg-laying ranged from 43.8 to 58.1% over each of the ~86 h exposure periods to *D. suzukii*.

The whole-bush field experiments using the solid formulation resulted in a significant reduction (61.1%) of eggs laid on berries on both of the dates (figure 5a). The reduction of egg-laying ranged from 39 to 75% over the exposure periods to *D. suzukii*. Treatment, location, interaction between treatments, date and interaction between the treatment and the location within blueberry bushes significantly affected the *D. suzukii*

![Figure 4](image-url)
Figure 5. Effect of solid (a) and liquid gum (b) on D. suzukii egg laying in fruit on whole plants on eleven-year-old drip irrigated ‘Elliott’ blueberry in Corvallis Oregon during 2017. Bars with an asterisk indicate a significant reduction in egg laying.

oviposition and also the fruit infestation level (table 2). When the liquid formulation was used, we observed a significant oviposition reduction (60.1%) on all the dates when the experiment was performed (table 2, figure 5b). The reduction of egg-laying ranged from 44 to 75% over the exposure periods to D. suzukii. The treatment, the date and the location significantly affected the D. suzukii oviposition and the fruit infestation level (table 2).

Weather data

The temperature, humidity and precipitation from 15 August to 15 October varied significantly during the respective trial events. The days during which the field experiments were performed are indicated (figure 6). The hottest day with the lowest humidity on which experiments were conducted occurred on 1 September, (Tmax 34.2 °C, mean 49.5% RH) with no precipitation recorded. The coldest day with the highest humidity on

Table 2. Results of the factorial ANOVA analysis performed on the whole-bush field trials data.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Wilks value</th>
<th>F-values</th>
<th>df</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solid matrix</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.547</td>
<td>19.43</td>
<td>2, 47</td>
<td>***</td>
</tr>
<tr>
<td>Date</td>
<td>0.932</td>
<td>1.71</td>
<td>2, 47</td>
<td>0.193</td>
</tr>
<tr>
<td>Location</td>
<td>0.350</td>
<td>16.20</td>
<td>4, 94</td>
<td>***</td>
</tr>
<tr>
<td>Treatment*Date</td>
<td>0.854</td>
<td>4.03</td>
<td>2, 47</td>
<td>*</td>
</tr>
<tr>
<td>Treatment*Location</td>
<td>0.731</td>
<td>3.99</td>
<td>4, 94</td>
<td>**</td>
</tr>
<tr>
<td>Date*Location</td>
<td>0.867</td>
<td>1.73</td>
<td>4, 94</td>
<td>0.15</td>
</tr>
<tr>
<td>Treatment<em>Date</em>Location</td>
<td>0.930</td>
<td>0.86</td>
<td>4, 94</td>
<td>0.488</td>
</tr>
<tr>
<td><strong>Liquid matrix</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.697</td>
<td>15.46</td>
<td>2, 71</td>
<td>***</td>
</tr>
<tr>
<td>Date</td>
<td>0.846</td>
<td>3.10</td>
<td>4, 142</td>
<td>*</td>
</tr>
<tr>
<td>Location</td>
<td>0.610</td>
<td>9.95</td>
<td>4, 142</td>
<td>***</td>
</tr>
<tr>
<td>Treatment*Date</td>
<td>0.904</td>
<td>1.83</td>
<td>4, 142</td>
<td>0.126</td>
</tr>
<tr>
<td>Treatment*Location</td>
<td>0.898</td>
<td>1.95</td>
<td>4, 142</td>
<td>0.105</td>
</tr>
<tr>
<td>Date*Location</td>
<td>0.816</td>
<td>1.90</td>
<td>8, 142</td>
<td>0.064</td>
</tr>
<tr>
<td>Treatment<em>Date</em>Location</td>
<td>0.929</td>
<td>0.67</td>
<td>8, 142</td>
<td>0.718</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.005, *** P < 0.001.
Figure 6. Daily minimum and maximum temperatures and precipitation during the experimental period on ‘Elliott’ blueberry in Corvallis, Oregon during 2017. Arrows indicate the days during which field experiments were initiated. Which experiments were conducted occurred on 12 October, \((T_{\text{max}} 13.7 \, ^\circ C, \text{a mean of } 91.3\% \text{ RH})\) with 18.9 mm precipitation. Generally, it appeared as if more eggs were laid by \(D. \text{suzukii}\) during periods when temperatures were below 30 \(^\circ C\) and above 20 \(^\circ C\).

Electrophysiological response to attractive volatiles
GC-EAD analyses from the headspace extracts of the principal ingredient A revealed the presence of 2 distinct peaks (figure 7) named from here onward as Compound 1 (CP1) and Compound 2 (CP2). Both compounds and

Figure 7. Coupled gas chromatographic-electroantennographic (GC-EAD) analysis of headspace extracts tested on two different \(D. \text{suzukii}\) populations, one northern Italy and one from individuals collected in Oregon. The upper traces are the antennal response from a \(D. \text{suzukii}\) female antenna, the lower trace is the chromatogram monitored with flame ionization detector (FID). The EAD responses were calculated by Autospike software (Syntech) measuring the maximum amplitude of negative deflection (mV) elicited by \(D. \text{suzukii}\) antennae. The headspace extracts were collected from the principal ingredient A.
their relative quantities of the corresponding volatiles were 14.47 and 100 for the first CP1 and the second peak CP2 respectively. *D. suzukii* individuals elicited consistent antennal response only to CP2. No difference in the antennal response amplitude was observed between Italian (0.062 ± 0.096 mV; mean ± SD) and Oregon (0.058 ± 0.094 mV) *D. suzukii* females (U = 11; P = 0.834).

**Discussion**

The data presented provide a novel and proof of concept approach to the management of *D. suzukii* egg-laying behaviour through behavioural manipulation. We demonstrated significant reductions of egg-laying on blueberries, cherries, strawberries, raspberries and blackberries under controlled laboratory conditions. Field experiments were carried out on blueberry clusters, each containing a predetermined number of *D. suzukii*. Egg-laying was significantly reduced on 8 out of 9 experimental dates. These results were obtained using both SM and LM formulations over periods of 72 to 96 ± 2 hours. Then, whole-bush field experiments exposing fruit to *D. suzukii* populations over 3-4 days included the location within the experimental bush as an additional factor in the experimental design. Here, oviposition was significantly reduced on all 5 of the experimental dates. The location on the bush had the largest impact on the reduction of egg-laying, with the largest reductions occurring in the middle and lower portions of the blueberry bush. Overall, the reduction of the absolute number of eggs and the number of infested berries was largely similar. In all cases, when egg-laying was reduced, there was also a reduction in the number of infested berries.

The data were collected under varying environmental conditions over a 2-month period for the field experiments on blueberry. Considering weather conditions, it appeared as if egg-laying was higher under conditions where temperatures were above 20 °C and below 30 °C; however, it appeared that temperature, humidity and rainfall had minimal overall impact on the efficacy of the applied treatments. Finally, the data generated from the EAG trials indicate a similar response in both the Italian and Oregon populations of *D. suzukii* using volatiles originating from carbohydrates within the matrix. Subsequent experiments (Rossi-Stacconi et al., in preparation) conducted under controlled laboratory conditions showed significant attraction to the key volatile identified in the EAG trials.

The fact that the treated fruit consistently displayed significant reductions of *D. suzukii* damage provides a strong impetus of future commercial implementation. Research on the control of *D. suzukii* has predominantly focused on attractants (Landolt et al., 2012; Cha et al., 2017; Frewin et al., 2017; Tonina et al., 2018). Alternative methods looked at the use of sugar, a phagostimulant mixed with pesticides and sprayed on the canopy to improve efficacy of insecticides (Cowles et al., 2015). Push-pull strategies proposing aversive and attractive stimuli in order to modify *D. suzukii* pest distribution in the crop resulted in encouraging results (Renkema et al., 2016; Wallingford et al., 2016a; 2016b; 2017; 2018). In the current study, the attractive gum matrices for *D. suzukii* shows comparatively favourable results, where treatments using the substrate lead to significant reductions of fruit infestation over relatively extended periods. It is possible that these reductions are the result of altered egg-laying behaviour toward susceptible fruit. These data indicate a strong, consistent and competitive attraction of the substrate when presented together with berries. The current study provides evidence that *D. suzukii* crop damage can be reduced by using attractive volatiles in commercial field settings. The compound as tested contains no conventional insecticides and provides an alternative and environmentally friendly management tool for *D. suzukii*. Since *D. suzukii* has become an economically important pest in both Americas and Europe (Cini et al., 2012; Asplen et al., 2015; Tait et al., 2017), the number of tools and techniques to manage *D. suzukii* commercially has increased. Currently, several trap designs (Lee et al., 2011; 2013), synthetic volatiles (Cha et al., 2014) and different types of baits (Landolt et al., 2012; Cha et al., 2013; Renkema et al., 2014; 2017; Grassi et al., 2015) are available, aiding in the control of this important pest. It is acknowledged that the currently available tools have many limitations (Haye et al., 2016). Moreover, the prevailing problem with synthetic volatile compositions is that they do not mimic natural circumstances. Even with all available options, effective management of the pest is a challenge and requires a more efficient method of control.

The matrices described in this study focuses on manipulation of pest behaviour. In addition to be an alternative ovipositional medium, this matrix may be providing *D. suzukii* a substrate where several additional activities of biological significance may be observed (Tait, unpublished) including feeding and mating. Our findings support the use of the matrices as a biologically based product to handle problematic *D. suzukii* infestations in an environmental friendly way (Almajar et al., 2017; Lanouette et al., 2017; Woltz and Lee, 2017). Despite this, it must be considered that trials show between 50% and 76% reduction in eggs laid in fruit that received the treatments, suggesting a pairing of this method with additional approaches available in an IPM program (Cossentine and Ajanath, 2017; Frewin et al., 2017). One of the characteristics of the gum is that it is water-soluble on account of its organic composition and can be applied without limitations of time or quantity. Considering the work of Tochen (2014; 2016a) and Grassi et al. (2018), a consistent application of the substrate could be particularly useful during the late dormant period, and when fly activity increases. This is because during these 2 key bottleneck periods of the year, few ovipositional sites and limited food sources are available, with the exception of berries on secondary plant species including *Hedera helix* L. and *Viscum album* L. berries during winter/early spring (Grassi et al., 2018), and *Sarcococca* species (Lee et al., 2015; Kenis et al., 2016). Providing flies with an alternative substrate for oviposition could possibly reduce the chances.
of infestation of early-ripening susceptible commercial fruits at the beginning of the growing season (Little et al., 2017). Moreover, larvae do not survive because of the dehydrating effect of both matrix formulations (Tait, unpublished), providing a constant reduction of the population. Future avenues of research are needed to address these concerns.

Additional laboratory experiments should also focus on understanding the mechanisms of the gum, including Gas Chromatography-Mass Spectrometry (GC-MS), Electoroantennography (EAG) dose-response curve, olfactometer tests, wind tunnel experiments, longevity studies in the field and primarily find a natural attractant to include into the matrix in order to reduce the natural host plant population. Future goals may include testing the product during early and late winter conditions with the aim to assay the efficacy of attraction of the lure using winter morph D. suzukii.

Although our data provide information on a non-commercial proof of concept, we believe that the matrix provides an alternative to conventional control techniques and is not directly applied onto fruit, potentially resulting in the opportunity of reductions of pesticide residues. Ultimately a market-ready product would be similar to multiple other commercially available behaviour disruptors also in the border areas where the surrounding vegetation offers refuge to the pest (Ometto et al., 2013; Kenis et al., 2016; Enriquez and Colinet, 2017). Specifically, this lure is able to attract both females and males of D. suzukii and induces females to lay eggs on the substrate. All field trials were conducted for a total of 3 to 4 days, within the time span of field longevity periods of many currently used conventional pesticides (Van Timmeren and Isaacs 2013; Wise et al., 2015). We observed no phytotoxicity associated with the matrices on plant parts. Additional studies regarding the persistence of the substrate in different climatic regions are suggested.

Acknowledgements

We thank Linda Brewer, Jessica Buser, Gabriella Boyer and Kyoo Park for technical assistance. We thank Dave Bryla and Scott Robbins for support at the OSU Lewis-Brown Experimental Farm.

References


MANN H. B., WHITNEY D. R., 1947.- On a test of whether one of two random variables is stochastically larger than the other.- *Annual Mathematical Statistics*, 18 (1): 50-60.


Authors’ addresses: Vaughn M. WALTON (corresponding author: vaughn.walton@oregonstate.edu), Department of Horticulture, Oregon State University, Corvallis, Oregon, USA; Gabriella TAIT (gabriella.tait@fmach.it); Clive KAISER (clive.kaiser@oregonstate.edu); M. Valerio ROSSI-STACCONI (marco.stacconi@oregonstate.edu); Daniel T. DALTON (daniel.dalton@oregonstate.edu); Gianfranco ANFORA (gianfranco.anfora@fmach.it).

Received August 4, 2018. Accepted October 29, 2018.