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ORIGINAL ARTICLE

Response of *Drosophila suzukii* (Diptera: Drosophilidae) to non-host fruit volatile compounds

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Abstract

The spotted wing *Drosophila suzukii* (Diptera: Drosophilidae) is a polyphagous insect, considered one of the main pests of berries and other soft-skinned fruits worldwide. Unlike most of its sister species, such as *D. melanogaster*, *D. suzukii* prefers fresh fruit over rotting fruits for oviposition. Recent studies indicate that female *D. suzukii* are more attracted to fresh fruit volatiles, which trigger oviposition behavior. Because of this behavior change, a series of compounds extracted from fruits have been identified and evaluated for use as bait for better capture of the insect against those that had been commonly used before produced through processes of fermentation. Based on this, we analyzed two non-host fruits, banana (*Musa paradisiaca*) and orange (*Citrus sinensis*), as potential food bait to attract *D. suzukii* and compared them with merlot wine and apple cider vinegar. The results in the field showed greater *D. suzukii* capture by banana, orange and wine than by vinegar, which is commonly used in monitoring this pest. However, in the laboratory wine was statistically more attractive than the other baits. In addition, we identified a series of compounds that had not been reported in fruits, extracts or products of fermentation, indicating that there are compounds in non-host fruits that are potentially attractive with possible antennal activity. Our study can contribute to understanding which compounds are involved in attraction behavior of the spotted wing fly.

Keywords Spotted wing fly · Polyphagous insect · Food baits · Semiochemical · Monitoring · Trapping programs

Introduction

The spotted wing *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) is an invasive species from eastern Asia (Kanzawa 1935) that has emerged worldwide in fruit-producing regions (Walsh et al. 2011; Hauser 2011; Asplen et al. 2015; Klick et al. 2016; Benito et al. 2016; Evans et al. 2017). Its recent invasion of Europe and North America threatens the fruit industries of both continents (Walsh et al. 2011; Calabria et al. 2012; Cini et al. 2012; Rota-Stabelli et al. 2013). It was first detected in California, USA, in 2008 (Hauser 2011) and from 2012 on there are reports of its presence in the USA, Canada (Lee et al. 2011b), Mexico (SENASICA 2013), Europe (Calabria et al. 2012; Cini et al. 2012; Asplen et al. 2015) and South America (Deprá et al. 2014), a total of 52 countries affected worldwide (Orsted and Orsted 2019).

Drosophila suzukii is a polyphagous insect that, besides the many wild plants it uses as hosts, it also infests crops of high commercial value, such as stone fruits and soft-skinned fruits, such as cranberries, strawberries, raspberries, blackberries, plums, apricots and grapes, and potentially other crops (Walsh et al. 2011; Cini et al. 2012, Burrack et al. 2013; Abraham et al. 2015; Klick et al. 2016; Rice et al. 2016; Mazzi et al. 2017). Female oviposition frequently results in the presence of developing larvae that feed on fruit tissue, followed by rot due to secondary pathogenic infections that cause fruit abscission (Lee et al. 2011b;

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Cini et al. 2012; Ioriatti et al. 2015). They can attack and damage ripe fruits and without damaging close to harvest (Kaneshiro 1983; Mitsui et al. 2006; Lee et al. 2011a), a characteristic that differentiates *D. suzukii* from other drosophilids, which lay their eggs mainly in rotting fruit (Karageorgi et al. 2017). Due to its rapid development cycle and high polyphagia, this invasive species is causing severe damage to cultivated and non-cultivated species (Cini et al. 2012; Keesey et al. 2015; Kenis et al. 2016; Lee et al. 2015; Poyet et al. 2015). The damage caused by *D. suzukii* in commercial orchards can be observed before harvest when the emerged larvae feed on the infested fruit pulp, which tends to collapse and is invaded by a variety of fungi and bacteria that cause loss of their commercial value (Kanzawa 1939; Grassi et al. 2009; Walsh et al. 2011; Ioriatti et al. 2015). In Mexico, *D. suzukii* in blackberry plantations in Michoacán (Rebollar-Alviter 2014) and in commercial fig cultivars in the Morelos (Bautista et al. 2017), as well as in non-commercial orchards of guava in Veracruz (Lasa and Tadeo, 2015), illustrate its rapid dispersion and potential threat for production of berries and other commercially valuable fruit. In the United States alone infestations of *D. suzukii* have resulted in economic losses estimated at 324.3 million dollars yearly (Bolda et al. 2010; Walsh et al. 2011).

In monitoring with capture devices, several studies have demonstrated that the bait is the most important factor in attracting and capturing *D. suzukii* (Iglesias et al. 2014). Different types of baits and lures have been developed for D. *suzukii* in function of its associated olfactory preferences: both ripe and rotten fruit volatiles (Keesey et al. 2015). Those most commonly used in many monitoring programs are products of fermentation, such as apple cider vinegar, grape wine, mixtures of yeasts and sugar, acetic acid and ethanol (Cha et al. 2012, 2014, 2017; Landolt et al. 2012a, 2012b; Iglesias et al. 2014; Kleiber et al. 2014). Some commercial baits that use mixtures of compounds that are attractive for *D. suzukii* have also been developed (Cha et al. 2014). However, until now, the only viable tool for reducing economic damage caused by the fly in commercial orchards has been frequent application of chemical insecticides (Beers et al. 2011; Bruck et al. 2011; Van Timmeren and Isaacs 2013; Haye et al. 2016; Farnsworth et al. 2017), which frequently conflict with the required interval before harvest and with protocols of integrated pest management (Cini et al. 2012; Haye et al. 2016). In this context, the effectiveness of these chemical applications, as well as the eventual development of a successful integrated pest management program depends on a reliable monitoring program (Cini et al. 2012). Often, the most effective and lasting are those that integrate strategies based on olfactory communication of the insects (Heuskin et al. 2011).

Diverse authors have pointed out that volatiles from organic compounds, as well as from fruit fermentation, are used by drosophilid species to locate their hosts (Zhu et al. 2003; Becher et al. 2012; Bellamy et al. 2013). Recent studies have identified chemical signals that attract *D. suzukii* to their plant hosts, that elicit antennal activity and that are used by the flies for different behaviors: oviposition, aggregation, feeding, among others (Cloonan et al. 2018). The same author lists at least 72 compounds from different studies that elicit antennal activity in *D. suzukii* and are found in fruits, fruit extracts, foliage, and fermented products.

Apple cider vinegar is one of the most common baits used for monitoring *D. suzukii* (Cha et al. 2013) since it is easily acquired, economical and transparent, which facilitates observation of the captures, even though it is not the most attractive bait (Landolt et al. 2011). Wines and vinegars are used in capture programs in the US and Canada to detect and monitor the spotted wing fly (Walsh 2009). It has been demonstrated that the combination of merlot wine and apple cider vinegar synergizes attraction of *D. suzukii* (Landolt et al. 2011), while other species of drosophilids are attracted by corn flour yeast mixed with ethanol, acetic acid and 2-phenyl ethanol (Hutner et al. 1937; Reed 1938; Zhu et al. 2003). Behavioral and electrophysiological trials show that the olfactory sense plays an important role in *D. suzukii* host selection (Revadi et al. 2015). Females can infest a broad range of host fruits (Bellamy et al. 2013) where they oviposit and the larvae feed (Lee et al. 2011b; Walsh et al. 2011; Cini et al. 2012). It has also been demonstrated that *D. suzukii* is attracted by organic volatile compounds emitted by several ripening small fruit crops, such as cranberries, raspberries, strawberries, cherries and grapes (Abraham et al. 2015; Revadi et al. 2015). For *D. suzukii*, fresh fruit seems to be more associated with locating oviposition sites, while the odors of fermentation correlate more with feeding sites (Revadi et al. 2015; Keesey et al. 2015; Karageorgi et al. 2017; Mori et al. 2017). It is important to underline that Cloonan et al. (2018) report 31 species of *D. suzukii* plant hosts, belonging to 13 families, which share volatile compounds that elicit antennal activity.

Given this situation and the need to develop oviposition attractants different from the baits derived from fermentation for effective control and monitoring systems (Revadi et al. 2015), this study had three primary objectives. The first was to evaluate the attraction effect observed in two very common fruits in the vegetation of the study area (orange and banana), which have not been reported as hosts for *D. suzukii*. The second objective was to compare the level of attraction of these food baits against baits known as apple cider vinegar and wine, comparing their effect under laboratory and open field conditions in blackberry plantations. And the third objective was to identify the volatile compounds of each of the evaluated baits by gas chromatography and mass spectrometry with the aim of finding the volatiles involved in *D. suzukii* adult attraction to fresh fruits and in this way develop new control methods that contribute to integrated pest management.

Materials and methods

Biological material

Infested blackberry (*Rubus ulmifolius* S.) fruits were collected in February and March in the community of Xico, Veracruz, Mexico (19°25'23.60" N, 97°00'42.26" W, 1339 m altitude) to obtain *D. suzukii* adults. To establish the colony, parent flies recovered in the field were placed on a diet of corn flour agar as a medium for feeding and oviposition, following the method described by (Woltz et al. 2015). The adults that emerged from the diet were separated by age in small cages made from 1.5 L plastic recipients whose mouths were covered with an elastic (ELASTICA PONAN®) cloth to prevent the insects from leaving. In addition, in each cage water and food (3:1 mixture of sugar and hydrolyzed protein) was provided until they reached 10 and 15 days of age for use in the trials. The insect colony was kept in the laboratory at room temperature with a photoperiod of 12 h light: 12 h dark.

Bioassays in the laboratory

To conduct the behavioral tests in the laboratory, we designed cages made of two cylindrical transparent plastic bottles (food containers) with a capacity of 3.8 L (one gallon). A window was adapted at each end of the cover with cloth (teraglin) to permit even airflow in the interior and a hole in the upper half provided with a screw-on cover to introduce the flies (Fig. 1). A piece of cotton moistened with water was placed in the central part of the cage for the flies to hydrate. The baits evaluated were 35 mL apple vinegar (La burrita®, 5% acidity), 35 mL merlot wine (Don Simón®, 12% ethanol, Tempranillo selection, selected by J. García Carrión L.M, S.A., Guarnicioneros S/N, Daimiel, Ciudad Real, Spain), 35 mL orange juice (*Citrus sinensis* L.), 27 mL water + Tween, and 8 g banana (*Musa paradisiaca* L) (food baits that had not been evaluated previously), and water + Tween 20 (2 drops/L) as the control. To evaluate the attraction effect of the baits, we introduced 59.147 ml (2-ounce) capacity trap devices, similar to the bucket-type devices commonly used to monitor *D. suzukii* (SAGARPA-SENASICA 2016) (bait & control) into each cage at each end of the cage and rotating their position in each replication to avoid the effect of position. In each cage 10 females and 10 males between 10 and 15 days old were kept for 24 h. After this time, the uncaptured flies were removed from the cage and the capture devices were extracted to count the total number of flies captured and their sex. The assays were conducted in the laboratory under natural conditions of light and temperature. A total of six replications were done per evaluated bait.

Bioassays in the field

The field trials were conducted in a commercial blackberry plot in Tlalchy, municipality of Ixhuacán de los Reyes, Veracruz (19°22'55.80" N, 97°04'3.80" W, 1573 m altitude) during April and May 2019. In this case, we used transparent bucket-type traps, which have been used to monitor and trap *D. suzukii* in the field (SAGARPA-SENASICA 2016). Each trap was baited with 100 mL of each of the baits used in the laboratory assays and water + Tween 20 (2 drops/L) as the control. The traps were distributed along the rows of blackberry plants, separated by 3 and 4 m between rows, under a model of complete randomized blocks. The traps remained in the field for a week, after which they were removed and substituted to obtain a total of six replications. The insects captured were collected with a sieve and placed in jars with 70% alcohol, which were labeled and transported to the laboratory. The samples were examined under a dissection microscope (Celestron®) to separate the sexes and count the total number of *D. suzukii* adults.

Analysis and identification of volatile compounds

Headspace sampling

The samples of 5 g banana (*Musa paradisiaca* L), 5 mL orange juice (*Citrus sinensis* L), apple cider vinegar (La burrita®, 5% acidity), and merlot wine (Don Simon®, 12% ethanol, Tempranillo selection, selected by J. García Carrión L.M, S.A., Guarnicioneros S/N, Daimiel, Ciudad Real, Spain) were placed in a headspace vial, which were sealed with a PTFE/Teflon plug and heated to 85 °C for 15 min.

Gas chromatography GC-MS

For this analysis, we used 5%-phenyl-methylpolysiloxane (Agilent Technologies) and a 60 m column DB-5 with 0.25 mm internal diameter and 0.25 µm thick film. The temperature program for the GC-MS was 50 °C for 5 min, ramped up 10 °C/min to 250 °C, which was maintained 4 min, then heated 20 °C/min up to 280 °C. Helium was the carrier gas at a flow of 1 mL/min. Temperature of the injector was 250 °C, injection split with a split ratio of 15:1. Once the chromatogram was obtained, each of the peaks

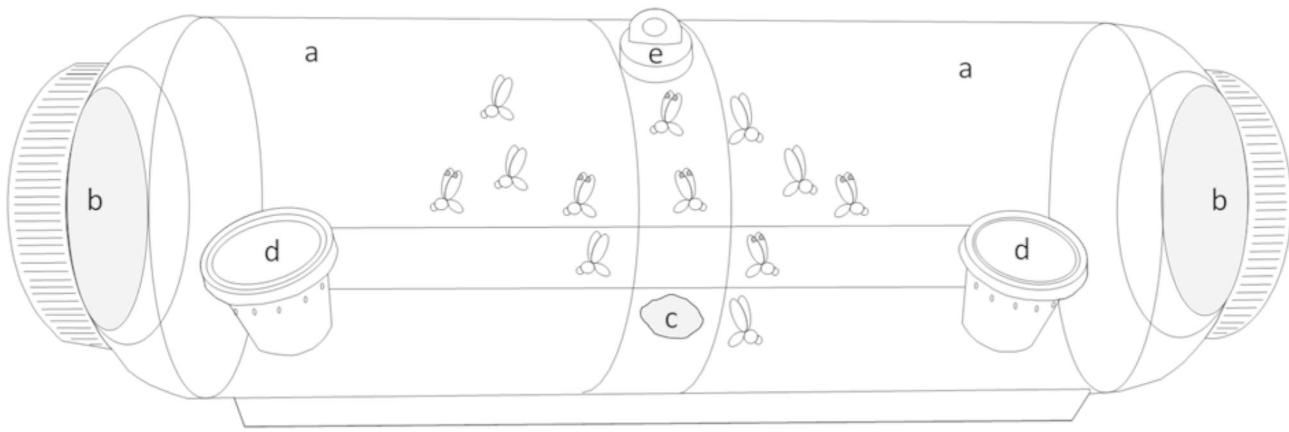

Fig. 1 Design of the experimental olfactometer for analysis of attraction in the laboratory: **a** body of the trap, **b** window vents, **c** cotton moistened with water, **d** devices that capture, and **e** orifice for introducing the flies

was identified by mass spectrometry using a mass spectrometer (Agilent Technologies model 5975 inert XL). The mass spectra were obtained by electronic impact ionization at 70 eV. For identification, the mass spectrum obtained for each compound was compared with a database (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0d).

Statistical analyses

For the laboratory tests, the captures recorded in the devices were analyzed with two-way ANOVAs, considering the capture number and sex of the captured organisms, while for the analysis of attraction effectiveness of the baits, the registered captures by replication were transformed to percentages and analyzed with a one-way ANOVA. The field trials were analyzed in a manner similar to the laboratory trials with two-way ANOVAs for the captures and considering the same parameters. The assumptions of data normality and homogeneity were verified with the Kolmogorov-Smirnov and Cochran C, Hartley and Bartlett tests; the data that did not satisfy these assumptions were transformed to ranges, following the methodology described by Conover and Iman (1981) to deal with values of zero obtained in the field captures, while enabling the combination of the non-parametric with the parametric statistic. The post-hoc tests were analyzed with the Tukey HSD test. We used the software Statistic 7 and SigmaPlot 10.0.

Results

Laboratory bioassays

The laboratory tests with device and trap trials permitted verification of the attraction effect of the different evaluated baits, as well as their effect on the sexes. The analyses indicated a positive attraction response to all of the baits (vinegar

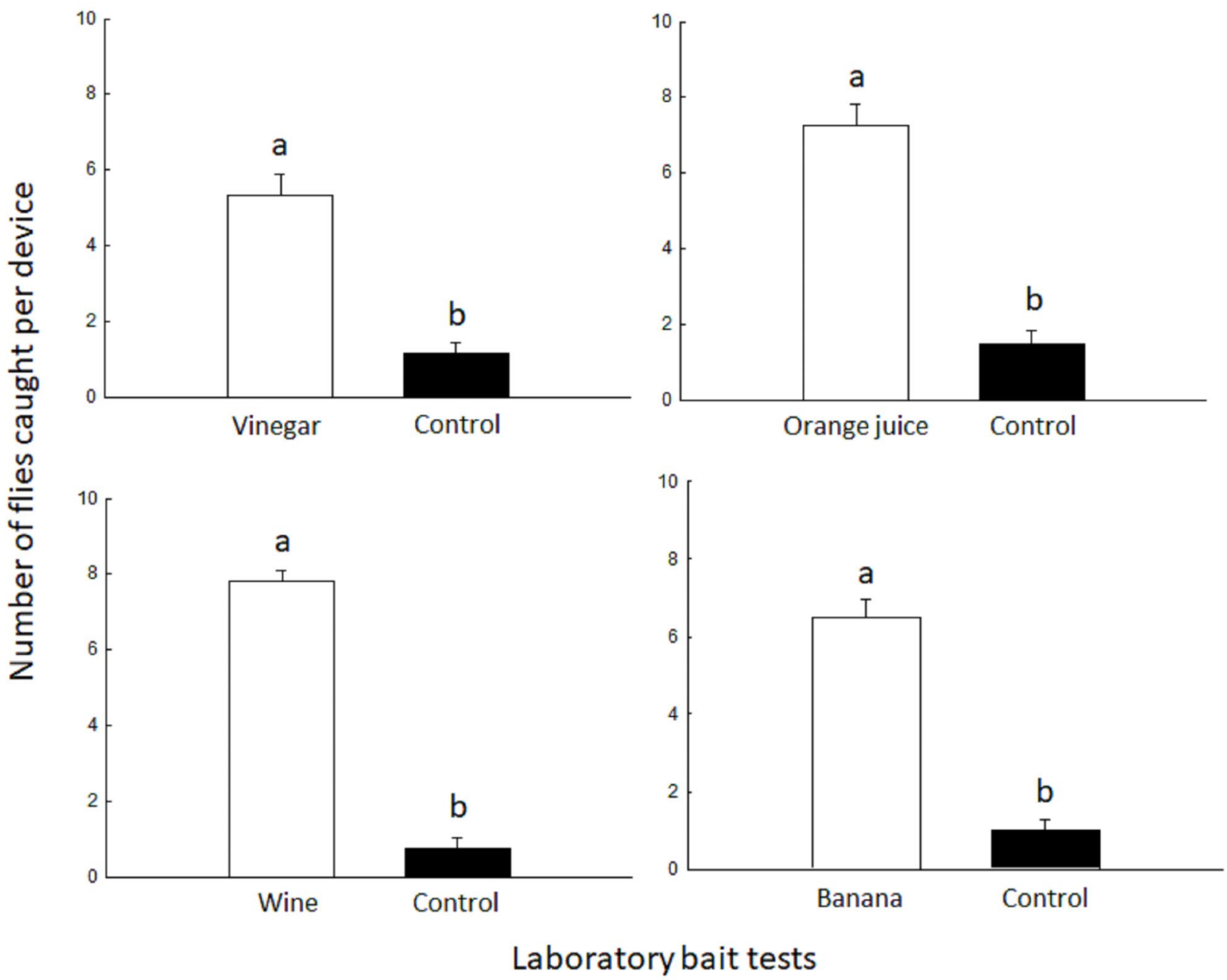

Fig. 2 Evaluation of the effect of attraction by the different baits evaluated under laboratory conditions versus a negative control. Columns represent the mean ± standard error of the total percentage of captures obtained by device and bait. Different letters in a column indicate statistical differences (α = 0.05)

$F(1, 21) = 44.795$, $p < 0.001$; banana $F(1, 21) = 103.480$, $p < 0.001$; orange juice $F(1, 21) = 79.793$, $p < 0.001$, and wine $F(1, 21) = 89.105$, $p < 0.001$), reflected in the larger number of *D. suzukii* fly captures compared with the devices baited with water + Tween 20 (Fig. 2). Regarding sex, there were no significant differences among the evaluated baits (vinegar $F(1, 21) = 0.645$ $p = 0.431$; banana $F(1, 21) = 0.095$, $p = 0.761$; orange juice $F(1, 21) = 2.028$, $p = 0.169$; and wine $F(1, 21) = 3.133$, $p = 0.091$).

Evaluation of bait attraction effectiveness by analysis of capture percentages revealed significant differences among the baits ($F(3, 20) = 3.283$, $p = 0.042$). The devices baited with apple cider vinegar obtained the lowest capture percentages (53.33 ± 7.92 SE), followed by those baited with banana (65.0 ± 5.0 SE), orange juice (75.5 ± 7.04 SE) and wine (78.33 ± 2.11 SE), these with capture levels statistically similar but different from that of vinegar (Fig. 3).

Field bioassays

The numbers of flies captured in field traps were significantly different among the evaluated baits ($F(3, 43) = 9.4064$, $p < 0.001$) (Fig. 4). More male and female *D. suzukii* were captured by traps baited with orange juice, wine and banana than by traps baited with vinegar. That is, the former three baits captured 12 to 14 times more females and males than the apple cider vinegar (orange juice: females = 6.67 ± 2.09 SE, males = 9.83 ± 4.82 SE; wine: females = 6.33 ± 3.13 SE, males = 8.67 ± 4.78 SE; banana: females = 3.33 ± 1.45 SE, males = 5.33 ± 3.23 SE). Moreover, there were no significant differences between sexes by evaluated bait (F(1,

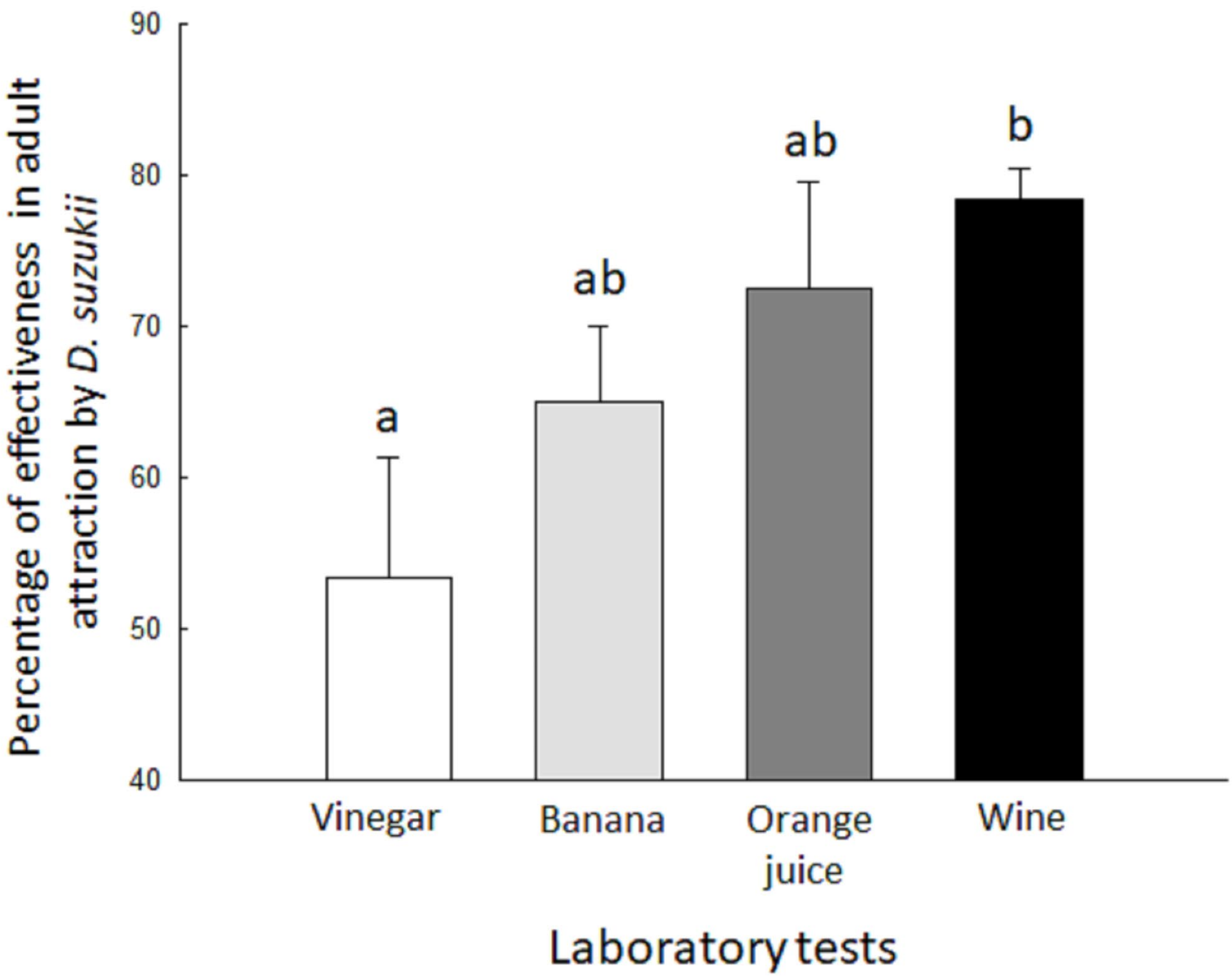

Fig. 3 Mean ± SE of the effectiveness percentage shown by the evaluated baits in attracting and capturing *D. suzukii*. The difference between treatments was determined with the HSD Tukey test. Different letters in the columns indicate statistical differences ($\alpha = 0.05$)

43) = 0.15102, $p = 0.699$); that is, we found no evidence of more attraction of females or males by any of the baits. Finally, we should highlight that these field results were similar to those obtained in the laboratory: orange juice, wine and banana baits had very similar capture levels, different from that of apple vinegar. The only difference between field and laboratory results was a higher capture index with orange juice than with wine in the field.

Analysis and identification of volatile compounds

Chemical analysis of the evaluated baits found a total of twenty-five compounds, among which were esters, alcohols, ketones, aldehydes, terpenes and monoterpenes. In vinegar, five compounds were found: acetic acid, three acetates (ethyl acetate, 2-pentyl and isopropyl) and ethyl butanoate. These same compounds, except for acetic acid, were also found in banana in different proportions, as well as five compounds more (four esters and 1 ketone). In wine, six volatile compounds were registered (one acid, three alcohols, and two esters); ethyl acetate is a volatile compound shared by vinegar and banana in a lower proportion. In banana, the largest number of volatile compounds was found, a total of nine. Finally, in orange juice six compounds (two esters, two terpenes, two monoterpenes, and one aldehyde) were also found, none of these was present in the other evaluated baits (Table 1).

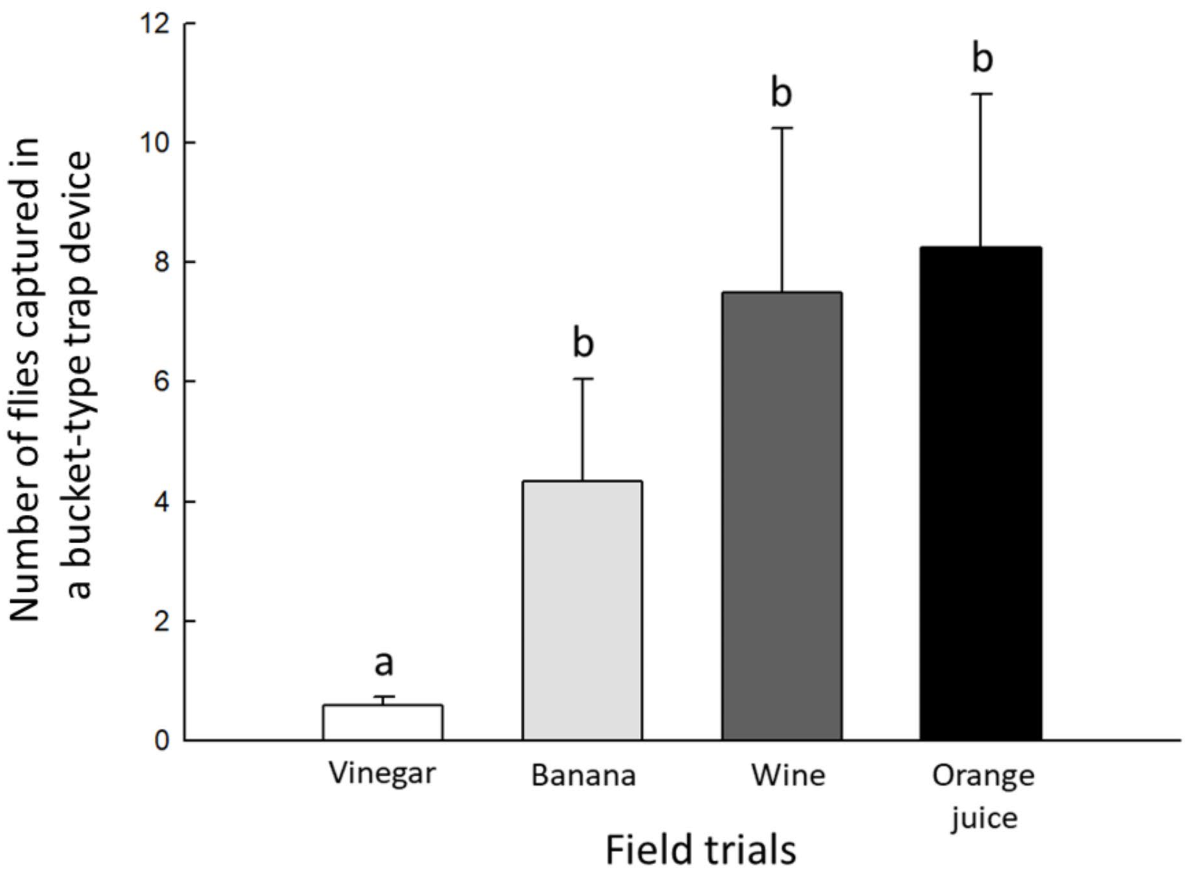

Fig. 4 Mean ± SE of the total number of flies captured and counted in capture devices (bucket-type) baited with the different attractants evaluated. The difference between treatments was determined with the post hoc Tukey HSD test. Different letters in the columns indicate statistical differences (α = 0.05)

Discussion

In this study we include the study and evaluation of food baits based on natural fruit in the attraction and capture of *D. suzukii* in both the laboratory and the field. It has been demonstrated that fruit volatiles trigger female oviposition behavior and can be used as a lure for more effective capture (Revade et al. 2015). Also, fitting the traps with specific oviposition attractants for this species can help to reduce capture of other drosophilids and beneficial insects, reduce erroneous identification of *D. suzukii* and increment effectiveness of the trap and control of this pest (Suran et al. 2007; Landolt et al. 2012a, b).

In this study, we corroborated greater attraction of *D. suzukii* to red wine than to apple cider vinegar (Landolt et al. 2011, 2012a). Nevertheless, we found a greater attraction effect with banana and orange juice, which had equal or greater levels of capture than those baited with apple cider vinegar or wine, considered the most effective food baits for *D. suzukii* capture (Walsh 2009; Cha et al. 2012, 2013).

Previous studies have reported that acetic acid is an important component of apple cider vinegar and is responsible for making it attractive for several species of drosophilids (Barrows 1907; Reed 1938; Dethier 1947; Becher et al. 2010; Landolt et al. 2012a), while in wine, ethanol is the substance considered the most important in making it attractive for *D. melanogaster* Meigen, 1830 (Reed 1938). The fact that we find acetic acid in vinegar and ethanol + acetic acid in the evaluated wine partly explains the captures recorded in the devices, similar to the report by Landolt et al. (2011, 2012a) and Cha et al. (2014), who mention that there is a positive integration of acetic acid and ethanol to lure *D. suzukii*, synergizing their effect, relative to their individual effect. Nevertheless, Landolt et al. (2011, 2012a) mention that *D. suzukii* adults were attracted by traps with acetic acid but not ethanol, suggesting that ethanol at different doses or

Table 1 Results of the chromatographic analysis performed on the four evaluated baits. The compounds and concentrations found in each are shown, as well as the volatiles that elicited antennal activity of *D. suzukii* in previous studies.

	VINEGAR	RED WINE	BANANA	ORANGE JUICE				
Compound	% AREA	% AREA	% AREA	% AREA	VAA-DS	Source	Compound	Reference
Ethyl alcohol		70.863			*	1,2	Alcohol	Revadi et al. (2015), Cha et al. (2012), Mazzetto et al. (2016).
Ethyl acetate	89.18	14.873	32.733		*	1,2	Esters	Revadi et al. (2015), Cha et al. (2012).
2-Pentanone			5.02		?	1	Ketone	
Acetic acid	9.03	0.604			*	1,2	Acid	Revadi et al. (2015).
3-Methoxy propanal		0.45			?	2	Aldehyde	
Isobutanol		0.416			?	2	Alcohol	
Isobutyl acetate			22.975		?	1	Esters	
Ethyl butanoate	0.59		1.724		*	1	Esters	Revadi et al. (2015).
Acetate of butilo			7.116		*	3	Esters	Abraham et al. (2015).
2-Pentyl acetate	0.48		3.771		?	2	Esters	
Isopentyl acetate (Isoamyl acetate)	0.73		22.819		*	1,2	Esters	Revadi et al. (2015), Cha et al. (2012).
Isopentanol (3-methyl-methane-1-ol)		12.47			*	3	Alcohol	Abraham et al. (2015).
α-Pinene				6.588	?	3	Terpene	
Isobutyl butyrate			1.634		?	1	Esters	
Sabineno				8.411	?	3	Monoterpene	
β-Myrcene				19.921	?	3	Terpene	
Octanal				4.325	?	3	Aldehyde	
D-Limonene				60.755	*	1,3	Monoterpene	Revadi et al. (2015).
Isopentyl isobutyrate			2.208		?	1	Esters	

* VAA-DS = Volatile with antennal activity in *D. suzukii*

1, Fruit; 2, Product of fermentation; 3, Fruit Extract

? = No antennal activity known

rates of release may have a different effect. For example, it has been found that *D. melanogaster* adults are attracted by high concentrations of ethanol produced by fermented fruit, while for *D. simulans* Sturtevant, 1919 and *D. immigrans* Sturtevant, 1921, high doses of ethanol were repulsive (Parsons and Spence 1981). However, the positive effect of the interaction of acetic acid and ethanol has also been reported for other insects in laboratory studies: *D. ampelophila* Loew, 1862 (Barrows 1907), *D. funebris* Fabricius, 1787 (Casana-Giner 1999), *D. melanogaster* Meigen, 1830 (Zhu et al. 2003) and members of the family Calliphoridae (Dethier 1947).

As mentioned above, the presence of acetic acid and ethanol in the analyzed wine can explain the efficiency of the latter in attracting *D. suzukii*. However, in the case of banana and orange juice, neither of these two compounds was found, contradicting the hypothesis that attraction of *D. suzukii* by the vinegar is due to the presence of acetic acid or by its combination with ethanol. This could lead us to believe that there are other compounds that can be equally attractive for *D. suzukii* and that there could be synergism among them (Landolt et al. 2012a). In this sense, our results show that devices baited with banana and orange juice had a higher number of captures than apple cider vinegar and that only orange juice was much better than any of the other baits evaluated, indicating the presence of other volatile compounds that are attractive for *D. suzukii* from non-host or not considered host plants. Like *D. suzukii*, several species of drosophilids respond to a large number of volatiles from fruits, extracts, fermented tissue and plant leaves (Zhu et al. 2003; Lee et al. 2011a; Becher et al. 2012; Cloonal et al. 2018). Here, the volatiles from banana and orange juice could have had an attraction effect as a food source since they attracted females and males equally. It is important to point out that several species of drosophilids respond to volatile compounds of their host plants. For example, Stökl et al. (2010) reported antennal activity in species of drosophilids in response to the volatile compounds hexanol and isoamyl acetate, which have been found in fruits such as banana, apple and pear (Zhu et al. 2003; Stökl et al. 2010).

In the case of *D. suzukii*, Revadi et al. (2015) state that compounds such as ethyl acetate have a fruity odor like ethyl hexanoate, and the flies in the laboratory tests had strong consistent electrophysiological response. Other studies with ethyl acetate report that ester is highly volatile but attractive for several insect species including various species of *Drosophila* (Larry and Lin, 1991; Stensmyr et al. 2003). Besides ethyl acetate, we found other components in vinegar and banana, such as 2-pentyl acetate, ethyl butanoate and isopentyl acetate; the latter two have been reported to elicit electrophysiological response in *D. suzukii* (Revadi et al. 2015), partly clarifying why *D. suzukii* was more attracted especially to banana, in which these compounds are found in greater proportion, than to vinegar (Landolt et al. 2012a). It has been demonstrated that the proportion and mixtures of pheromonal compounds or chemical volatiles influence insect attraction (Minks et al. 1973; Raguso 2008; Cha et al. 2011), and *D. suzukii* is a generalist insect. Moreover, butyl acetate present in banana and absent in vinegar could be another of the causes for more attraction to banana; electrophysiological response to this compound has been reported for *D. suzukii* (Abraham et al. 2015). Another clue to why banana is more attractive than vinegar is the presence in greater proportion of five esters in banana, compared with three in vinegar. These enable the volatiles from banana to remain for a longer time in the environment, like wine which loses less weight per day than vinegar (Cha et al. 2012).

Finally, perhaps the most unexpected finding is the effectiveness of orange juice in attracting *D. suzukii*. Our chemical analyses did not find any of the compounds that are potentially important in vinegar, wine or even in banana that could explain its unexpected attractiveness for *D. suzukii*. Aromatic volatiles, such as α-alpha pinene, β-myrcene and sabinene have been reported in citruses (Chisholm, et al. 2003; Choi 2003). Like our study, some of the compounds found in orange juice, such as α-alpha pinene and β-myrcene, attract *Bactrocera oleae* Rossi, 1790 (Scarpati et al. 1993), while the dipterans *Rhagoletis cerasi* Linnaeus, 1758 and *Atherigona soccata* Rondani, 1871 are attracted by octanal (Padmaja et al. 2010; Cavalloro et al. 1983), and d-limonene attracts both *R. cerasi* and *Anastrepha fraterculus* Wiedemann, 1830 (Raptopoulos et al. 1995; Milet-Pinheiro et al. 2015). According to Revadi et al. (2015), raspberry is the most attractive fruit and with which better larval behavior was observed in trials with *D. suzukii* in the laboratory. Moreover, it was the only fruit that contained several monoterpenes, which, even though they were found in relatively low concentrations, can be important in providing *D. suzukii* adults information on host quality (Maia and Moore 2011). Although the role of the volatiles from orange juice is not known, it is clear that they are attractive for both males and females and so could provide information indicating a food source. This points to orange as a potential host or site of reproduction and mating, as has been reported for other insects (Dicke and Van Loon 2000; Bruce et al. 2005; Hilker and McNeil 2007). Diverse studies on oviposition have demonstrated that *D. suzukii* can detect and locate several compounds from different fruits (Burrack et al. 2013), and recent studies with an olfactometer have demonstrated that gravid females and reproductively mature *D. suzukii* generally respond preferentially to signals from fruit (Clymans et al. 2019) whose released volatiles trigger oviposition behavior (Revadi et al. 2015). These findings indicate that *D. suzukii* is attracted by fermentation volatiles only when the flies are in search of protein-rich food, while they are attracted by fruit volatiles when they are in search of substrates for oviposition (Mitsui et al. 2006; Walsh et al. 2011; Keesey et al. 2015; Clymans et al. 2019). This could explain the case of the volatiles emitted by banana and orange juice. On the other hand, of the 25 compounds registered in the four baits, 11 have not been reported as compounds in fermented products or fruit extracts commonly used in monitoring that elicit antennal activity (Cha et al. 2012; Coonan et al. 2018). Nor are they present in fresh fruit (raspberry, strawberry, cranberry and cherry) (Abraham et al., 2015; Revadi et al. 2015; Coonan et al. 2018). But nine of these compounds are present in banana and orange juice, which are considered non-host fruits.

To date, even when important advances have been achieved in understanding the chemical signals involved in *D. suzukii* behavior and there are reports of around sixty compounds from both fermentation products and red fruits (berries) and even compounds from foliage that involve a list of compounds that includes acids, alcohols, aldehydes, ketones, norisoprenoids, isoprenoids, monoterpenes, sesquiterpenes, and aromatics (Cha et al. 2012; Revadi et al. 2015; Abraham et al. 2015; Keesey et al. 2015; Mazzetto et al. 2016), it is still not precisely known which compounds are implicated in oviposition, feeding and mating behaviors of this pest (Cloonan et al. 2018). Its sudden switch to fresh fruit and its oviposition behavior is still a daunting unknown (Mitsui et al. 2006; Walsh et al. 2011; Dekker et al. 2015). However, the detailed study of these changes may be key to help clarify peculiarities in oviposition behavior (Revadi et al. 2015) and in locating the different types of hosts (Burrack et al. 2013) shown by this singular pest.

In this study, we demonstrated that besides the already known berries, it is not at all absurd to explore the volatiles from other fruits present in the areas infested by this pest, especially because it is a polyphagous insect that easily adapts to different hosts (Burrack et al. 2013). Our results show that both banana and orange juice can be used as reliable baits for *D. suzukii* monitoring. According to Rice et al. (2016), it is necessary to develop oviposition attractants different from the fermentation baits commonly used, especially if we consider that gravid females use the volatiles from fresh fruit to locate potential substrates for oviposition (Karageorgi et al. 2017; Clymans et al. 2019) or feeding (Revadi et al. 2015). Moreover, our results strengthen the hypothesis of Landolt et al. (2012a) on the existence of other potentially important compounds that attract *D. suzukii*. Our results also leave a series of questions concerning what effects the compounds we reported could have in attracting the spotted wing fly. Clearly, it is necessary to conduct new studies that include electroantennographic tests and more detailed studies with identified compounds to improve doses and stability of the food baits evaluated. The use of baits that influence oviposition site search behavior is a good strategy when seeking to mitigate the damage caused by *D. suzukii* to the fruits and implementing an adequate integrated pest management program.

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Data Availability All relevant data supporting the findings of this study are available from the corresponding authors upon request.

Declarations

Conflict of interest The authors declare no conflicts of interest related to the work described in this manuscript.

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