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# Evaluation of food baits to capture *Drosophila suzukii* in the southern of Brazil

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**Abstract:** Drosophila suzukii is one of the main pests of small fruits in the world. An effective monitoring is fundamental to detect the presence of the fly and to predict the infestation of fruits in new areas. We evaluated the food baits Ceratrap®, Torula®, Biofruit®, Suzukii Trap®, apple cider vinegar, and a homemade mixture comprising wine, vinegar and molasses (WVM) for D. suzukii attractiveness, and if reproductive stage affects females attraction and capture in the different baits. Bait selectivity of nontarget insects was assessed. The preference of adults between ripe blackberry fruit and the food baits was also evaluated. Adults showed a higher attraction (61.97% of the captured insects) to the WVM mixture than to Ceratrap® (1.32%), Torula® (0.52%), Biofruit® (13.15%), Suzukii Trap® (17.6%), and apple vinegar (5.4%). Considering the attractiveness to D. suzukii, Suzukii Trap® and apple vinegar were the most selective to non-target insects. In general, reproductively immature females showed a preference for Biofruit®, apple vinegar, and WVM, whereas mature females did not show bait preferences. Adults preferred ripe blackberry fruit over the WVM mixture. Understanding the field behavior of D. suzukii is of foremost importance to estimate insect population density and outline pest management strategies.

**Key words:** Invasive species, spotted wing drosophila, feeding attractant, bait, non-target insects.

## INTRODUCTION

Spotted wing drosophila (SWD), Drosophila suzukii Matsumura (Diptera: Drosophilidae), is considered the main insect pest of small fruit crops worldwide. It is widespread in several countries of North America, Europe (Calabria et al. 2012, Cini et al. 2014) and more recently has been observed in South America (Santos 2014a, Schlesener et al.

Correspondence to: Jutiane Wollmann E-mail: jutianewollmann@hotmail.com ORCid: http://orcid.org/0000-0002-6301-2004 2015, Andreazza et al. 2016, 2017, Garcia et al. 2017). In addition, it has the potential to colonize agricultural crops in countries in Oceania and Africa (Dos Santos et al. 2017). The rapid spread of the species is associated with its high polyphagia, biotic potential, and environmental adaptation. The economic impact of SWD has been recorded based on substantial losses in agriculture (Bolda et al. 2010, Lee et al. 2011, De Ros et al. 2015).

To better understand the behavior of the species, an intensive pest management program has been recommended using different food baits (Landolt et al. 2012, Lee et al. 2012, Iglesias et al. 2014, Burrack et al. 2015, Tonina et al. 2017). Among food baits, apple vinegar is the natural product most commonly used in SWD monitoring programs (Lee et al. 2012). In addition to being the standard bait to capture D. suzukii adults, vinegar allows a better visualization of the insects captured in the trap and is inexpensive (Lee et al. 2013). However, studies indicate that wine-based mixtures with apple cider vinegar (Landolt et al. 2012, Grassi et al. 2014, Tonina et al. 2017) or rice vinegar (Cha et al. 2012); compounds with yeast, granulated sugar, and water (Hamby et al. 2014, Iglesias et al. 2014); or formulations with yeast and wine (Huang et al. 2017) are highly effective and promising for use in SWD monitoring programs. Hydrolyzed protein-based products, such as BioAnastrepha<sup>®</sup>, Biofruit<sup>®</sup>, and Ceratrap<sup>®</sup>, are also promising (Lasa and Tadeo 2015).

Nevertheless, factors related to the local climate and crop type (Tonina et al. 2017), associated with the low selectivity of food baits, and the possibility of loss of effectiveness to capture adults in the event of ripe fruits can lead to erroneous conclusions and underestimation of pest population density (Iglesias et al. 2014, Hamby and Becker 2016, Huang et al. 2017).

Therefore, for a better understanding of the behavior of *D. suzukii* adults in the field, the present study aimed to (i) assess the attractiveness of food substrates to *D. suzukii* adults (target insect) and the selectivity of non-target insects; (ii) check the reproductive stage of *D. suzukii* females searching for food baits; and (iii) investigate the field preference of *D. suzukii* adults between ripe blackberry fruit and the food baits.

## MATERIALS AND METHODS

EFFICIENCY OF FOOD BAITS TO CAPTURE *D. suzukii* ADULTS AND THEIR EFFECTS ON NON-TARGET INSECTS

The capture efficiency of different food baits was investigated in the field, a commercial blackberry orchard (*Rubus* spp.) cv. Brasos (31°35′19″ S, 52°29′14″ W) with a history of *D. suzukii* infestation, in Pelotas, Rio Grande do Sul, Brazil from December 2015 (beginning of the harvest period) to January 2016 (end of the harvest period). Pest management was not performed in the experimental area during the 6-week evaluation period.

The food baits (treatments) were as follows: T1, Ceratrap® 1.5% (enzymatic hydrolyzed protein of animal origin) (Bioibérica S.A., Barcelona, Spain); T2, Torula® yeast (Isca Tecnologias Ltd., six 3 g tablets/L); T3, Biofruit® at 5% (hydrolyzed corn protein) (BioControle Métodos de Controle de Pragas Ltd., Indaiatuba, SP, Brazil); T4, Suzukii Trap® (Bioibérica, Barcelona, Spain); T5, Apple vinegar (undiluted pH = 4.40); and T6, a mixture comprising Merlot™ wine (60%) + apple vinegar (40%) + sugarcane molasses (20 g/L) (WVM) (adapted from Grassi et al. 2014). Distilled water traps were used as the control (T7).

The traps were made of plastic containers (500 mL) made of red polypropylene, with screw caps and 16 lateral holes (0.5 cm diameter each) in the middle of the containers (adapted from Wollmann et al. 2017). A bait solution was placed in each trap (150 mL). In all the baits an unflavored detergent drops (about 1 mL/L of bait) (Limpol Neutro, Bombril®) were added to break the surface tension. Subsequently, the traps were hung from branches of the inner crop lines, with nylon thread tied to the cap, approximately 1 m above the ground and spaced 5 m from each other. To avoid edge effects, the traps were set 5 m from the edge line of the orchard (Grassi et al. 2014).

The experimental design was a randomized complete block design, with seven treatments (food baits) and four replications (blocks). The traps were picked up on a weekly basis and immediately replaced by traps with new solutions. The insects captured were kept in glass containers (200 mL) and sent to the laboratory for screening. During

each weekly evaluation, the traps were randomly rotated within each block to avoid the effects of the trap position on insect behavior. In the laboratory, with the aid of a binocular stereomicroscope (40x), the captured insects were counted, and *D. suzukii* adults were sexed using specific taxonomic characters (Vlach 2013). Of the insects captured, only *D. suzukii* and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) were identified to species level. To determine the selectivity of the food baits by non-target insects, the remaining collected specimens were identified to the family level. After screening, all the specimens were placed in plastic microtubes (1.5 mL) and preserved in 70% ethanol.

REPRODUCTIVE MATURITY OF *D. suzukii* FEMALES CAPTURED IN THE TRAPS

Every week 10 captured females of each bait and block were removed randomly from the traps, accounting for 240 females per food bait during the 6-week evaluation period. When the number of females was below 10, all the females captured in the trap were analyzed. Determination of the reproductive maturity of females was performed through abdominal dissection in saline solution (0.5%) placed on a layer of Parafina<sup>®</sup> in a Petri glass dish (9-cm diameter). Subsequently, the females were separated into four groups using a binocular microscope (40x) according to the degree of ovarian development (Burrack et al. 2015): (i) with mature eggs only (eggs presenting fully formed respiratory filaments), (ii) with mature and immature eggs, (iii) with a mass of immature eggs, and (iv) with empty ovaries (without eggs).

NATURAL INFESTATION AND SEARCH PREFERENCE OF *D. suzukii* ADULTS FOR FRUIT AND/OR BAITS

Twenty ripe fruit of blackberry, apparently intact (without mechanical or insect damage), were randomly collected every week from each block, accounting for 80 fruit per week. In the laboratory,

the fruit were individually placed in transparent plastic pots (200 mL) containing a thin layer of vermiculite (1 cm) and sealed at the top with their lids, which were placed in a climate-controlled room  $[24^{\circ}C \pm 2^{\circ}C \text{ temperature}, 70\% \pm 10\%]$ relative humidity (RH), 12 h photoperiod] until adult emergence. The number of male and female of D. suzukii emerged per single fruit was counted daily and calculated the percentage of insects emerged per week in relation to the total emerged. After counting and screening, the adults were placed in plastic microtubes (1.5 mL) filled with 70% ethanol. To determine the preference of D. suzukii between ripe blackberry fruit and the baits, the number of insects emerging in the laboratory from the collected ripe fruit was compared with the number of adults captured in the food bait WVM (the most effective bait to capture D. suzukii) in the same period of evaluation.

## STATISTICAL ANALYSIS

Data on *D. suzukii* captures, non-target insects, and reproductive maturity of SWD females was submitted for residual analysis to confirm the assumptions of normality using the Shapiro–Wilk test and homoscedasticity using the Hartley test with PROC UNIVARIATE (SAS Institute 2000). When the assumptions were not confirmed, the raw data [Y] were transformed into [log (Y + 0.1)], [square root (Y + 1)], or [arcsine (square root Y)]. Subsequently, analysis of variance (ANOVA) was applied to the data, and the means were compared using the Tukey test ( $P \le 0.05$ ) (SAS Institute 2000).

The interaction of *D. suzukii* capture (random effect) between weeks of evaluation and food baits (fixed effects) as well as that between reproductive maturity (ovarian development stage groups) of *D. suzukii* females (random effect) and food baits (fixed effects) were subjected to bidirectional ANOVA with PROC GLM in SAS 9.1 (SAS Institute 2000).

To analyze the interaction and search preference of *D. suzukii* between blackberry fruit (fixed factor) and/or the WVM mixture (sources of variation), generalized linear models were tested with Poisson distribution (Demétrio et al. 2014) using the *hnp* package (Moral et al. 2016) of the R Core Team (2017); the treatment means were compared by the *glht* contrast function of the *multcomp* package of the R Core Team (2017).

#### RESULTS

EFFICIENCY OF FOOD BAITS TO CAPTURE *D. suzukii* ADULTS AND THEIR EFFECTS ON NON-TARGET INSECTS

A total of 9,011 specimens of D. suzukii were captured in the different treatments, with a significant interaction between food baits over time during the experiment both in males (F = 2.80; df = 3, 6; P < 0.0001) and females (F = 2.04; df =3, 6; P < 0.0036) of D. suzukii. Among the baits evaluated, the WVM mixture showed the highest attractiveness to D. suzukii adults (61.97% of the captured insects) during the evaluation period as well as in the weekly trap evaluations, showing a significant superiority (P < 0.05) over other baits (Table I, Fig. 1a). In general, during the evaluation period, the least attractive food baits to D. suzukii were apple cider vinegar (5.4%), Ceratrap<sup>®</sup> (1.32%), and Torula® (0.52%) (Table I). The percentage of captured insects in the control treatment ranged from 0 to 0.25% (Table I).

With respect to bait attractiveness to non-target insects, a total of 23,298 specimens were captured in the different treatments; these specimens belonged to the families Drosophilidae (18,996), Nitidulidae (3,517), Tephritidae (216), *Z. indianus* (47 insects), and Vespidae (21), and parasitoids belonging to the families Diapriidae and Figitidae (501 insects) were also observed (Table II). All the captured specimens were significantly more attracted to the WVM mixture (54.2% of the captured insects) (*P* 

< 0.05) than the other baits. The WVM mixture had lower selectivity by non-target insects than Biofruit® (26.5%), Vinegar® (10.6%), and Suzukii Trap® (7.97%) (Fig. 1b). The Ceratrap® (0.44%) and Torula® (0.29%) were the most selective, with a frequency equivalent to that shown by the control bait (distilled water). However, they were not efficient at attracting *D. suzukii*, so that we considered Suzukii Trap® and Vinegar® the most selective baits to non-target insects (Fig. 1b).

## OVARIAN MATURATION

A highly significant interaction between the food baits and degree of ovarian maturity of D. suzukii females was observed (F = 9.53; df = 6, 3; P < 0.0001). Bait attractiveness to females with mature eggs (P < 0.05) and to females with mature and immature eggs (P < 0.05) did not differ significantly among the baits, except with respect to the control (Table III). However, D. suzukii females with immature eggs had a greater preference for Biofruit® (3.5 females) than for the other baits (showing a statistically significant difference, P < 0.05) (Table III). Females without eggs had a significantly greater preference (P < 0.05) for the baits apple cider vinegar (2.07 females), WVM (2.36 females), and Suzukii Trap® (2.71 females) than Biofruit® (1.30 females), Ceratrap® (0.61 females), and Torula® (0.59 females) (Table III).

NATURAL INFESTATION AND SEARCH PREFERENCE OF *D. suzukii* ADULTS

The highest natural infestation of D. suzukii occurred in the first week of blackberry fruit sampling (44.6% of total of D. suzukii in infested berries), which was the period with the highest amount of ripe fruit in the field (Fig. 2). However, the infestation decreased significantly (F = 27.71, df = 3, 3; P < 0.0001) in the second (30.5%), third (18.0%), and fourth (6.94%) assessment weeks (Fig. 2). The analysis of the preference of D. suzukii adults between blackberry fruit and the

TABLE I Number (mean  $\pm$  standard error) of females and males of *Drosophila suzukii* captured with different food baits in a blackberry orchard.

E 11 . 24	Evaluated weeks							
Food bait	First	Second	Third	Fourth	Fifth	Sixth		
Female								
Biofruit <sup>®</sup>	$21.25 \pm 3.94 \text{ aC}$	$26.00 \pm 2.86 \\ bBC$	$18.25 \pm 2.87 \ bC$	$64.00 \pm 11.50 \\ bAB$	$76.00 \pm 19.82 \ bA$	$16.75 \pm 2.43 \ bC$		
Ceratrap <sup>®</sup>	$3.50\pm2.02~bAB$	$8.75 \pm 2.46 \text{ cA}$	$4.50 \pm 2.90 \text{ cAB}$	$3.75\pm1.03~\mathrm{cAB}$	$3.75 \pm 2.78$ $cdAB$	$1.75\pm1.75~\mathrm{cB}$		
Suzukii Trap <sup>®</sup>	$\begin{array}{c} 23.50 \pm 3.20 \\ aAB \end{array}$	$64.50 \pm 18.59 \text{ aA}$	$40.00 \pm 10.17 \ aA$	$49.75 \pm 5.10 \text{ bA}$	$63.00 \pm 20.28 \; bA$	$8.50\pm0.50\;bB$		
Torula <sup>®</sup>	$1.25\pm0.75\;bA$	$0.75 \pm 0.25 \; cdA$	$2.50 \pm 0.87 \; cA$	$1.50 \pm 0.64 \ cdA$	$2.25 \pm 1.60 \ cdA$	$0.75 \pm 0.75 \text{cA}$		
Vinegar	$4.75 \pm 2.21 \text{ bAB}$	$6.25 \pm 2.49 \text{ cAB}$	$3.00\pm1.29~\text{cB}$	$14.00 \pm 7.35 \\ bAB$	$14.75 \pm 3.70 \text{ bcA}$	$16.25 \pm 3.66 \text{ bA}$		
WVM*	$56.25 \pm 8.75 \text{ aC}$	$99.50 \pm 23.72$ aABC	$63.75 \pm 6.55$ aBC	$188.25 \pm 54.63$ aAB	$\begin{array}{c} 217.00 \pm 60.20 \\ aA \end{array}$	$90.75 \pm 44.32$ aBC		
Control (water)	$0.00\pm0.00~bA$	$0.25\pm0.25~dA$	$0.25 \pm 0.25 \text{ cA}$	$0.00\pm0.00~dA$	$0.00\pm0.00~dA$	$0.00 \pm 0.00 \; cA$		
Male								
Biofruit <sup>®</sup>	$7.25 \pm 4.38$ abAB	$3.50 \pm 0.87~bAB$	$1.25\pm0.63~bB$	$17.50 \pm 5.11 \text{ bA}$	$31.50 \pm 10.20 \text{ bA}$	$13.00\pm3.70~bA$		
Ceratrap®	$0.00 \pm 0.00 \; cA$	$0.50 \pm 0.29 \; bcA$	$0.75\pm0.75\;bA$	$0.50 \pm 0.50 \; cA$	$1.50 \pm 0.87 \; dA$	$0.50 \pm 0.29 \; cA$		
Suzukii Trap®	$8.75 \pm 2.21 \ abB$	$\begin{array}{c} 26.75 \pm 11.04 \\ \text{aAB} \end{array}$	$9.75 \pm 2.21 \text{ aB}$	$12.50 \pm 3.38 \text{ bB}$	$74.50 \pm 33.68 \text{ bA}$	$14.50 \pm 6.75 \text{ bB}$		
Torula <sup>®</sup>	$0.00 \pm 0.00 \; cA$	$0.00 \pm 0.00 \; cA$	$0.25\pm0.25\;bA$	$0.00 \pm 0.00 \; cA$	$1.50 \pm 0.64 \; cA$	$1.00 \pm 0.71~\text{cA}$		
Vinegar®	$2.75 \pm 1.03$ beBC	$3.00\pm1.73~\text{bBC}$	$1.00 \pm 0.41 \ bC$	$8.50 \pm 4.63$ bABC	$14.50 \pm 3.48$ cAB	$32.75 \pm 10.80$ bA		
WVM*	$18.50 \pm 5.92 \ aC$	$\begin{array}{c} 43.50 \pm 18.30 \\ aBC \end{array}$	$17.75 \pm 5.62 \text{ aC}$	$117.25 \pm 48.57$ aAB	$\begin{array}{c} 230.00 \pm 99.80 \\ aA \end{array}$	$253.50 \pm 152.32$ aA		
Control (water)	$0.25 \pm 0.25 \text{ cA}$	$0.00 \pm 0.00 \text{cA}$	$0.50\pm0.29~bA$	$0.00\pm0.00~\text{cA}$	$0.00\pm0.00~\text{dA}$	$0.25 \pm 0.25 \text{ cA}$		

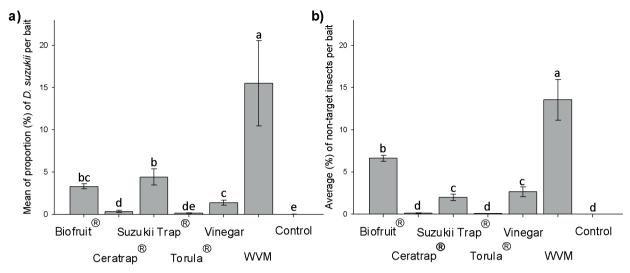
Means sharing the same lower case letter within a column or sharing the same capital letter within a row are not significantly different for females and males (Tukey's HSD,  $\alpha = 0.05$ ).

WVM mixture revealed a significant interaction (P < 0.05), with a higher search preference for the ripe fruit in the first, second, and third weeks of assessment (Fig. 2). However, in the fourth week, the period with the least amount of ripe fruit in the field, D. suzukii adults had a greater preference for the WVM mixture (Fig. 2).

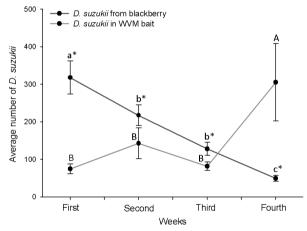
#### DISCUSSION

The WVM mixture was the most attractive food bait to *D. suzukii* male and female adults, which corroborate a number of studies indicating a high *D. suzukii* capture using combinations of wine and vinegar (Cha et al. 2012, Landolt et al. 2012, Grassi et al. 2014, Iglesias et al. 2014, Tonina et al. 2017) even without the addition of sugary substances. However, the addition of sugar to the bait solution improves the capture of *D. suzukii* due to increased

<sup>\*</sup>WVM: Wine + Vinegar + Molasses.



**Figure 1 - (a)** Means  $\pm$  standard error (%) of *Drosophila suzukii* insects (males and females) captured with different food baits in a blackberry orchard; (b) Means  $\pm$  standard error (%) of non-target insects captured with different food baits in a blackberry orchard. Means sharing the same lower case letter are not significantly different (Tukey's HSD,  $\alpha = 0.05$ ).



**Figure 2** - Means  $\pm$  standard error (count) of *Drosophila suzukii* adults emerged from blackberry fruit and captured with the bait mixture Wine + Vinegar + Molasses (WVM). Means sharing the same lower case letter or the same capital letter are not significantly different. Mean contrasts of the Generalized Linear Model following the Poisson distribution.

\* Significant interaction between the occurrences of *D. suzukii* in the WVM food bait and in infested blackberry fruit.

fermentation of the solution and consequently a higher release of chemical compounds such as acetoin, methanol, and ethanol, which better trigger the olfactory response of the searching adults (Landolt et al. 2012, Cha et al. 2014, Grassi et al. 2014). Moreover, a lower concentration of vinegar

in combination with wine enhances the appeal of the bait due to the reduction of the acidity in the mixture (Huang et al. 2017). In the present study, sugarcane molasses were added to the vinegar and wine-based bait because of the high availability of sugarcane molasses in the market and their low cost compared with that of granulated sugar.

Apple cider vinegar is the standard food bait used worldwide to monitor D. suzukii (Lee et al. 2013). In the present study, it was less enticing to D. suzukii than the WVM mixture, as was the case in other studies in which vinegar was compared against other fermented baits (Landolt et al. 2012, Iglesias et al. 2014, Burrack et al. 2015). Smaller catches of D. suzukii were recorded with the Ceratrap® and Torula® baits, which are products formulated from animal protein and yeasts, respectively, and are effective in monitoring the fruit fly species Anastrepha fraterculus (Wiedemann) and Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) (Nava and Botton 2010, Bortoli et al. 2016). The Biofruit® and Suzukii Trap® baits, which have animal protein and organic acids in their composition, respectively, combined with sugars lead to an average catch when compared to the WVM mixture, indicating

TABLE II
$Number \ (mean \pm standard \ error) \ of \ non-target \ insects \ captured \ with \ different \ food \ baits \ in \ a \ blackberry \ or chard.$
Food bait

	Food bait							
Specimens	Biofruit <sup>®</sup>	Ceratrap <sup>®</sup>	Suzukii Trap®	Torula <sup>®</sup>	Vinegar <sup>®</sup>	WVM*	Control (water)	
Drosophilidae	$1195.25 \pm 78.26 \\ b$	$7.25 \pm 5.30 \ d$	420.75 ± 93.10 c	$7.50 \pm 2.47 d$	360.00 ± 56.79 c	2757.00 ± 27.12 a	$1.25 \pm 0.75 \ d$	
Nitidulidae	$279.75 \pm 46.04$ a	$0.75\pm0.75~\text{c}$	$\begin{array}{c} 38.00 \pm \\ 5.05 \text{ b} \end{array}$	$1.00 \pm 1.00 \ b$	238.50 ± 91.89 a	321.00 ± 47.12 a	$0.25\pm0.25~\text{c}$	
Tephritidae	$2.50\pm0.87\;b$	$16.00 \pm 4.38$ ab	$2.00\pm1.41\;b$	$7.00\pm1.78\;b$	$1.00\pm1.00~\text{c}$	$25.25 \pm 10.02 \text{ a}$	$0.25\pm0.25~\text{c}$	
Z. indianus	$2.25 \pm 0.63 \ abc$	$0.00\pm0.00~\text{c}$	$1.25 \pm 0.48$ bc	$0.00\pm0.00~\text{c}$	$4.50\pm0.64~a$	$\begin{array}{c} 3.75 \pm 1.18 \\ ab \end{array}$	$0.00 \pm 0.00 \; c$	
Vespidae	$1.50\pm1.50\;ab$	$0.50\pm0.50\;b$	$0.00\pm0.00\;b$	$0.75\pm0.48\;b$	$0.25\pm0.25\;b$	$2.25\pm1.11\;a$	$0.00 \pm 0.00 \; b$	
Diapriidae/ Figitidae	$92.25 \pm 13.90$ a	$1.00 \pm 0.71 \text{ b}$	$2.00 \pm 1.68 \ b$	$0.75 \pm 0.75 \ b$	$13.75 \pm 1.97$ b	$81.00 \pm 20.24 a$	$0.25 \pm 0.25 \ b$	

Means sharing the same lower case letter within a row are not significantly different (Tukey's HSD,  $\alpha = 0.05$ ).

TABLE III

Number (mean ± standard error) of *Drosophila suzukii* females captured with different food baits according to their reproductive stage in a blackberry orchard.

F 11 4	Reproductive status						
Food bait	Mature	Mature/Immature	Immature	No eggs			
Biofruit <sup>®</sup>	$0.20 \pm 0.07~aC$	$0.91 \pm 0.16 \text{ aB}$	$3.50\pm0.18~aA$	$1.30\pm0.10~\text{bB}$			
Ceratrap <sup>®</sup>	$0.39 \pm 0.17 \; aA$	$0.86 \pm 0.27 \; aA$	$0.39 \pm 0.13 \; deA$	$0.61 \pm 0.35 \; bA$			
Suzukii Trap®	$0.42 \pm 0.12 \; aC$	$1.75\pm0.17~aAB$	$1.03 \pm 0.85~\mathrm{cB}$	$2.71 \pm 0.17 \; aA$			
Torula <sup>®</sup>	$0.10 \pm 0.06 \; abB$	$0.12 \pm 0.02 \; bAB$	$0.15 \pm 0.06 \ efAB$	$0.59 \pm 0.18 \; bA$			
Vinegar	$0.07 \pm 0.02 \; abC$	$0.91 \pm 0.31~aB$	$0.96 \pm 0.16 \ cdAB$	$2.07 \pm 0.34 \; aA$			
WVM*	$0.20 \pm 0.06~aC$	$1.28 \pm 0.17~aB$	$2.07 \pm 0.16 \ bAB$	$2.36 \pm 0.18 \; aA$			
Control (water)	$0.00\pm0.00\;bA$	$0.05 \pm 0.03~bA$	$0.00 \pm 0.00 \; fA$	$0.00 \pm 0.00 \; cA$			

Means sharing the same lower case letter within a column or sharing the same capital letter within a row are not significantly different (Tukey's HSD,  $\alpha = 0.05$ ).

that *D. suzukii* adults are attracted toward volatiles originating from fermented substances (Huang et al. 2017).

The WVM mixture, in addition to being the most attractive bait to *D. suzukii* adults, showed a lower selectivity by non-target insects, particularly species belonging to the families Drosophilidae, Nitidulidae, Tephritidae, and Vespidae and the microhymenoptera parasitoids Diapriidae and Figitidae. Selectivity of the food bait is of foremost importance to correctly characterize the population

density of the target pest in the field (Frewin et al. 2017), particularly that of small insects such as *D. suzukii* adults (4-6 mm) (Dreves et al. 2009). In this way, a food bait that is tempting only to the target species will facilitate the screening and visualization of the material collected and will help in the correct identification of the species present in the area (Iglesias et al. 2014, Burrack et al. 2015, Frewin et al. 2017). Species belonging to the families Nitidulidae (Fornari et al. 2013), Tephritidae (Nava and Botton 2010), and *Z.* 

<sup>\*</sup>WVM: Wine + Vinegar + Molasses.

<sup>\*</sup>WVM: Wine + Vinegar + Molasses.

indianus (Nava et al. 2015) and the naturally occurring parasitoids belonging to the families Diapriidae and Figitidae occur frequently in small fruit crops in southern Brazil (Wollmann et al. 2016). The capture of non-target species may be associated with their nutritional requirements for specific products (Burrack et al. 2015).

The attraction responses of D. suzukii to baits were proportional to the stage of ovarian development. Fully mature (with mature eggs) or partially mature (presence of mature and immature eggs) D. suzukii females did not distinguish between food baits and showed a lower demand for food baits than the females with immature eggs or empty ovaries (without eggs). This behavior may be related to the fact that females with a high number of mature eggs search for fruits to perform oviposition rather than traps that emit volatiles produced by fermentation (Burrack et al. 2015, Swoboda-Battarai et al. 2017). On the other hand, females with immature or developing eggs have a higher tendency to search for odors from fermentation due to higher nutritional requirements for egg maturation (Swoboda-Battarai et al. 2017). These trends were confirmed in the present study, in which reproductively immature females were more attracted to the baits Biofruit®, Suzuki Trap<sup>®</sup>, vinegar, and WVM. Food baits are not only important to monitor the incidence of the species in the orchard but also to act as pest control by capturing mature females before they have the opportunity to cause damage to the fruits.

The evaluation of the search preference of *D. suzukii* adults between ripe blackberry fruit and the WVM mixture revealed their greater preference for the ripe fruit. In this stage of fruit maturation volatiles are released at a higher rate, which favors the orientation of the insect toward the host (Abraham et al. 2015, Burrack et al. 2015, Swoboda-Battarai et al. 2017). In addition, there is a higher concentration of sugars in the fruits, setting the appropriate conditions for the nutritional balance between carbohydrates

and proteins required for larval development and consequently for the completion of the biological cycle (from egg to adult) (Jaramillo et al. 2015). This fact was confirmed by the higher occurrence of SWD in fruit of the first flowering, which were in the final stage of ripening and in a large quantity in the field during the study period. However, with the decrease in the number of fruit available in the orchard during the harvest period, there was a significant reduction in natural infestation and a greater search for the WVM mixture.

Although the WVM mixture was the most attractive to *D. suzukii* adults, it lost capture effectiveness during the peak production of ripe berries. This finding is of paramount importance in the monitoring and management programs of the pest because the lack of information on the biological and behavioral aspects of the insects may lead to erroneous conclusions and underestimation of the pest population, directly affecting the decision making regarding pest management.

Therefore, it is worth emphasizing the importance of daily monitoring of the orchard for D. suzukii incidence, not only via the analysis of the population of insects caught in the traps but also through visual inspection or fruit collection, to verify egg and larval infestation (Santos 2014b, Van Timmeren et al. 2017) or the emergence of insects from collected fruits, as was performed in the present study. These practices are fundamental for monitoring the population density of the pest in the field and define the best management strategies (Kogan 1998). The results obtained suggest that the attractiveness and effectiveness of the food bait to capture D. suzukii (the target insect) and non-target insects vary according to the bait used. In addition, D. suzukii female adults show a different behavior according to the degree of ovarian maturation and a greater search or preference for ripe fruit.

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## **AUTHOR CONTRIBUTIONS**

JW and FRMG conceived and designed research. JW, DCHS and JGAV conducted experiments. JW analyzed data and wrote the manuscript with DB contribution. FRMG, DB, DCHS and MSG reviewed the manuscript. All authors read and approved the manuscript.

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