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Sodium Chloride Added to Diluted Concord Grape Juice Prior to Fermentation Results in a Highly Attractive Bait for *Drosophila suzukii* (Diptera: Drosophilidae)

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At the interface of behavioral chemical ecology and pest management lays the evaluation of kairomones that can be used for either, monitoring or attract-and-kill of insect pests. Diluted Concord grape juice (DGJ) was previously reported to be highly attractive to male and female *D. suzukii*. Here, we conducted cage and field studies aimed at (1) comparing the attractiveness of fresh DGJ against that of commercial lures over a 3-year period in multiple locations, (2) quantifying the effects of DGJ aging for 1 and 2 weeks on *D. suzukii* attraction, and (3) ascertaining the effects of adding sodium chloride to DGJ prior to fermentation on the outcome. For all field studies, captures of non-target insects were recorded. Combined findings from the cage and field studies comparing DGJ and commercial lures indicate that fresh DGJ is as attractive or more attractive than the commercial lures that were evaluated. The results of the aging studies revealed that 7- and 14- day old DGJ baits are at least twice as attractive to female *D. suzukii* when compared to fresh DGJ. The response of other Drosophilids to the aged olfactory treatments mirrored that of *D. suzukii.* Remarkably, the addition of 2% sodium chloride to DGJ prior to aging resulted in a fourfold increase in attractiveness to male and female *D. suzukii* while reducing the number of non-target insects captured in traps. In conclusion, DGJ is an effective bait that can be deployed in traps and, when 2% sodium chloride is added, the resulting material outcompetes the performance of commercial lures and greatly reduces captures of non-target insects, thereby increasing bait selectivity. Overall, our findings increase our understanding of *D. suzukii* olfactory-driven behavior in response to fruit-based odor and demonstrate the potential use of a low-cost attractant for farmers who are not able to monitor for *D. suzukii* when commercially produced lures are expensive or less accessible.

Keywords: fermentation, behavioral response, bait selectivity, monitoring, bait accessibility

INTRODUCTION

At the interface of behavioral chemical ecology and pest management lays the evaluation of kairomones that can be used for either, monitoring or attract-and-kill of insect pests. The spotted-wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a vinegar fly native to southeast Asia and invasive to North America (Arriaga, 2011; Hauser, 2011; Lasa and Tadeo, 2015), South America (Deprá et al., 2014; Sandatino et al., 2015), and some regions of Europe (Calabria et al., 2012; Cini et al., 2012). *Drosophila suzukii* is primarily a pest of berry crops, including brambles [e.g., blackberries (*Rubus fruticosus* L.) and raspberries (*Rubus idaeus* L.)], strawberries (*Fragaria ananassa* Duchesne), blueberries (*Vaccinium corymbosum* L.), and currants (*Ribes* spp.). Soft-skinned grapes (*Vitis vinifera* L.) and tree fruits such as cherries (*Prunus cerasus* L.) and peaches [*Prunus persica* (L.) Batsch] are also hosts of this invasive pest.

Current pest management practices for *D. suzukii* require frequent insecticide applications, which come at a high environmental and economic cost to producers (Goodhue et al., 2011; Diepenbrock et al., 2016, 2017). In order to effectively time these sprays to mitigate damage, growers need to monitor *D. suzukii* populations. Commercial food-based lures are available for monitoring purposes (Cloonan et al., 2018). However, those lures are based on fermentation materials and consequently they also attract a comparatively high number of non-target insects (Cha et al., 2014). Captures of unwanted insects hinders trap performance and increases sorting time (Cha et al., 2015) and therefore, the development of more selective lures and baits is highly desirable (Larson et al., 2021).

Recent research has focused on the identification and evaluation of synthetic fruit- and foliage- based compounds tested either singly (e.g., Bolton et al., 2021; Dewitte et al., 2021; Little et al., 2021) or in blends (e.g., Feng et al., 2018; Liu et al., 2018; Bolton et al., 2019; Cloonan et al., 2019; Little et al., 2021; Urbaneja-Bernat et al., 2021) to develop more selective attractants for *D. suzukii*. Parallel research has evaluated natural sources of host plant odor, including volatiles emitted by intact blackberry, blueberry, cherry, raspberry, and strawberry fruit for attractiveness to *D. suzukii* (Abraham et al., 2015; Revadi et al., 2015). One low-cost and readily available fruit-based material that has been shown to be very attractive to male and female *D. suzukii* is diluted Concord grape juice (DGJ) (Piñero and Foley, 2018; Piñero et al., 2019a,b). When tested fresh, DGJ was found to be at least three times more attractive than some commercial lures, and about three times less attractive to non-target insects (Piñero et al., 2019b).

Fruit-based baits ferment over time after preparation, which potentially changes the odor chemistry and bait attractiveness to insects. For example, changes in attraction of tephritid fruit flies with bait age have been reported in tests of fermenting sugar baits (e.g., Castrejón-Gómez et al., 2004). While the effects of bait fermentation in Drosophilidae are known (Hunter et al., 1937), the specific effects of DGJ aging on *D. suzukii* have not been investigated. For tephritid fruit flies, borax (Epsky et al., 1993, 2014) and polypropylene glycol (Epsky et al., 2014) have been used as preservatives in aqueous food baits undergoing active fermentation. The presence of and choice of preservative may alter bait effectiveness either directly by adding additional volatile attractants, or indirectly by affecting rate and amount of fermentation (Epsky et al., 2015). Sodium chloride (= table salt) is a common inert ingredient in many insecticide formulations (Baker and Grant, 2018) and its behavioral effects when used it as a preservative have not been evaluated yet in *D. suzukii*.

Here, under both laboratory and field conditions we assessed (1) the attractiveness of DGJ relative to that of several commercial lures, (2) the effects of DGJ aging on captures of *D. suzukii* and non-target insects, and (3) the effects of adding sodium chloride to DGJ prior to fermentation on the outcome. For all field studies, we related captures of adult *D. suzukii* to those of other drosophilids, which represented the vast majority of non-target insects that were captured in traps. Our interest in DGJ stemmed from its local availability and comparatively low cost.

MATERIALS AND METHODS

Insects

Adult *D. suzukii* used for the cage experiments stemmed from a laboratory colony maintained at UMass Amherst. Flies were reared in 177 ml polypropylene square bottom bottles and fed Nutri-fly™ instant formulation diet (Genesee Scientific, San Diego, CA). Both the adults and the immature stages were maintained inside an insect growth chamber (Caron mod 7340-33-1; Caron Products Marietta, OH) with controlled photoperiod (16:8 L:D), temperature (25°C ± 0.5°C), and relative humidity (70%). Upon adult emergence, adults were transferred to 30 cm^3 screened cages. All flies tested were 3–5 days old.

Experiment 1: Attractiveness of Diluted Grape Juice vs. Commercial Lures Under Cage and Field Conditions

Cage Evaluations

The first series of cage bioassays were conducted from 5 to 25 June, 2019, and from 27 January to 19 March, 2020, using experimental cages (60 cm^3) made of nylon woven mesh (60 μm aperture) (BugDorm-6E610 Insect Rearing Cage, MegaView Science Education Services Co., Ltd., Taichung, Taiwan). Each cage had a single wire (15 cm in length) suspended from the center of the cage's ceiling. All olfactory treatments (described below) were evaluated using 1-L clear plastic containers (Highland Plastics Inc., Mira Loma, CA). Each trap was 7 cm in diameter, 15 cm in height, with 12 holes (4 mm in diam.) on the side to allow responding flies to enter the trap.

The first non-choice bioassay compared the attractiveness of DGJ (200 ml of a 1:3 dilution juice:water) against that of the Scentry® SWD lure (Scentry Biologicals Inc., Billings, MT) and AlphaScents® SWD lure (AlphaScents Inc., Syracuse, NY). The second bioassay compared the attractiveness of DGJ (200 ml) against that of Suzukii Trap® Max Captures (200 ml) (Bioiberica, Barcelona Spain). The third cage bioassay evaluated (1) DGJ (200 ml), (2) Trécé broad spectrum PEEL-PAK® multi-component lure, and (3) Trécé high selectivity 3-component lure (Trécé Inc., Adair, OK). Concord grape juice (Welch's Foods Inc., Concord, MA) was purchased from a local store. Except for the

Suzukii Trap® bait, which is a ready-to-use formulation, traps having commercial lures had 200 ml of unscented soapy water as a drowning solution.

For each observation day, 15 males and 15 females were released inside each cage between 0815 and 0830 h and they were allowed to acclimatize for 20 min. The observations were initiated immediately after introducing one trap with a given treatment inside a cage. One person quantified the number of males and females that were captured by traps at 4, 8, and 24 h after starting the experiment. All responding flies were removed with insect pinning forceps. During the observations, cages were rotated 90° every 15 min for the first 2 h to minimize the effects of position, then again at 4 and 8 h. Results show the percentages of males and females that were captured by traps over a 24-h period. Trials were replicated 4–7 times, depending on fly availability.

Field Evaluations

Over a 3-year period (2019–2021) and in multiple locations in Massachusetts, we compared the trap-capture ability of 1-L traps baited with fresh DGJ (1:3 dilution ratio) against traps baited with commercial lures. Emphasis was on early season captures of *D. suzukii*.

In 2019, three lure treatments were evaluated: (1) DGJ (200 ml), (2) Scentry® SWD lure, and (3) AlphaScents® SWD lure. On 10 May 2019, one set of three traps each was deployed at each of five Massachusetts locations: Deerfield (two locations), Belchertown, Whately, and Amherst. For the first three locations, traps were hung from cherry trees. In Whately, traps were deployed in a non-host area near (about 10 m away) commercial raspberries. In Amherst, traps were positioned 50 cm above elderberry plants using steel wire. Traps were removed on 17 June, 2019.

In 2020, five olfactory treatments were evaluated (1) DGJ (200 ml), (2) Scentry® lure, (3) AlphaScents® lure, (4) Trécé broad spectrum PEEL-PAK® multi-component lure, and (5) Trécé high selectivity 3-component lure. On 4 May, five traps were deployed at each of four locations: Deerfield, Belchertown, Whately, and Amherst. Traps were removed on 22 June, 2020.

In 2021, three treatments were evaluated: (1) fresh DGJ (200 ml), (2) 1-week old DGJ, and (3) Scentry® SWD lure. All traps were deployed on 5 May, at five locations (same four locations used in 2020 plus a blueberry block in a fruit farm located in Phillipston, MA). Traps were removed on July 22, 2021.

To minimize fermentation effects of DGJ, all traps were serviced twice a week. All captured insects were brought back to the laboratory for identification and sexing. At each trap inspection session, DGJ was replaced with fresh material. In 2019, the commercial lures were replaced on 27–29 May. In 2020 and 2021, commercial lures were replaced after 4 weeks.

Experiment 2: Effects of Diluted Concord Grape Juice Aging on *D. suzukii* Response Under Cage and Field Conditions

The relatively high attractiveness of fresh DGJ to *D. suzukii*, particularly females, has been established (Piñero et al., 2019a,b). This series of choice tests quantified the response of male and female *D. suzukii* to volatiles emitted by DGJ that had been aged in chambers, under controlled conditions, for either, 7 or 14 days. The subsequent response of *D. suzukii* was assessed under cage and field conditions. DGJ baits were prepared using 3.78 L containers with a gauze secured with rubber bands to allow for air exchange. DGJ was prepared three times a week and aged inside a chamber at 25°C and 65–75% r.h. The DGJ baits were prepared following a schedule that allowed for simultaneous treatment evaluations on a given date.

Cage Evaluations

Choice bioassays were conducted from 3 June to 20 July 2019. The treatments tested were (1) fresh DGJ, (2) 7-day old DGJ, (3) 14-days old DGJ, and (4) water as control. Four wires (15 cm in length) were suspended equidistantly at each of the four corners of the cages (same cages described above). All olfactory treatments were evaluated using 2 ml polypropylene microcentrifuge tubes (Eppendorf, Enfield CT). Prior to treatment application, the lids of the microcentrifuge tubes were removed, a 3 cm wire was wrapped around their neck, and a thin coating of Tangletrap insect coating (Tanglefoot Company, Grand Rapids, MI) was applied to the outer surface of the tubes to capture alighting flies (Piñero et al., 2019b).

On each observation day, 15 males and 15 females were released inside each cage between 0815 and 0830 h. Observations were initiated immediately after introducing the 2 ml Tangletrap-coated centrifuge tubes with the treatments. One person quantified the number of males and females that were captured by the Eppendorf tubes at 4, 8, and 24 h after starting the experiment. Results show the percentages of males and females that were captured by traps over a 24-h period. Each trial was replicated 12 times.

Field Evaluations

This study was conducted from 12 May to 30 June, 2020. The number of treatments evaluated and the bait-aging protocol were as described above. Each material was evaluated using 1-L traps in a commercial cherry block at the University of Massachusetts Cold Spring Orchard (Belchertown, MA). Five cherry trees of similar size (canopy diameter: 3–5 m) and fruit load were selected. Each tree served as a replicate. Four traps, each baited with one of the four different bait treatments, were placed at equidistant locations in the outer zone of each tree canopy. Traps were serviced twice a week, in order to prevent the bait treatments from deviating too much from the prescribed aging periods.

Experiment 3: Effects of Adding Sodium Chloride to Diluted Concord Grape Juice Prior to Bait Fermentation on *D. suzukii* Attraction in Cages and Trap Captures in the Field

In the second experiment we determined that fermentation of DGJ increases significantly the attractiveness of the bait to male and female *D. suzukii*. However, the same result applied to non-target insects. The third experiment assessed whether the response of adult *D. suzukii* and of other drosophilids could be manipulated by the addition of varying amounts of

sodium chloride (NaCl) in the form of table salt to DGJ prior to fermentation.

Cage Evaluations

Choice bioassays were as described in the second experiment. Bioassays were conducted from 3 June to 20 July 2021. The following four olfactory treatments were evaluated (1) fresh DGJ, (2) 1-week old DGJ with no NaCl added, (3) 1-week old DGJ with 2% NaCl (wt:vol), and (4) 1-week old DGJ with 4% NaCl. The experimental approach was as described for the second experiment (section "Experiment 2: Effects of Diluted Concord Grape Juice Aging on *D. suzukii* Response Under Cage and Field Conditions). Each bioassay was replicated 24 times.

Field Evaluations

In 2021, we conducted two field experiments. The first experiment compared the attractiveness of (1) fresh DGJ, (2) DGJ aged for 1 week in the absence of NaCl, (3) DGJ aged for 1 week with 2% NaCl, and (4) Scentry® SWD lure, to male and female *D. suzukii*, and to non-target insects. This study was conducted in a commercial cherry block at the University of Massachusetts Cold Spring Orchard (Belchertown, MA) from 1 June to 17 July 2021. Five cherry trees were used for this evaluation, and each tree served as a replicate. Traps were inspected twice a week.

The second field experiment was conducted in a commercial raspberry orchard in Whately, MA, from 26 July to 12 August 2021. The five olfactory treatments evaluated here were: (1) fresh DGJ, (2) DGJ aged for 1 week with 2% NaCl, (3) Scentry® SWD lure, (4) Trécé broad spectrum PEEL-PAK® multi-component lure, and (5) Trécé high selectivity 3-component lure. Each treatment was replicated six times. Traps were hung from the upper wire of the trellis system, along the perimeter of the block. Trap-capture data were collected twice a week.

Statistical Analysis

For the cage experiments, we calculated the proportion of males and females that responded in 24 h, from the total that were released. After arc-sin transformation, we then conducted preliminary analyses involving generalized linear mixed models assuming a Poisson distribution, which compared the effects of "treatment" (bait) and "fly sex" (fixed effects) and "replicate" (random factor), and the 2- and 3-way interactions among them. Overdispersion was tested by looking at deviance goodness of fit test using a log link function. Given that the interaction of "treatment" and "fly sex" was significant in two of the five cage studies, then one-way ANOVA or Bonferroni-corrected *t*-tests (for the cage study that compared only two treatments) were used to compare male and female responses across bait treatments. Data from the field evaluations were compared statistically among treatments with one-way ANOVA separately for males and females. For all analyses that involved ANOVA, data were transformed using $\sqrt{(x + 0.5)}$ prior to analysis to stabilize variances and means were separated, whenever appropriate, by Tukey HSD tests at the 5% probability level. All statistical analyses were performed using STATISTICA for WINDOWS (version 7.0; StatSoft Inc., Tulsa, OK, United States).

RESULTS

Experiment 1: Attractiveness of Fresh Diluted Grape Juice vs. Commercial Lures Under Cage and Field Conditions

Cage Evaluations

In the first cage bioassay conducted in the laboratory, the one-way ANOVA revealed a significant effect of treatment for males [ANOVA $F_{(2,9)} = 5.8$; $P = 0.027$] and females [ANOVA $F_{(2,9)} = 13.1$; $P = 0.002$] (**Figure 1A**). Captures of males and females over a 24-h period were significantly greater in traps baited with DGJ than in traps containing the Scentry® and AlphaScents® lures. In the second bioassay, the level of response of males to DGJ did not differ significantly from the response to the Suzukii Trap Max bait (*t*-test; $t = 0.03$, $P = 0.974$). In contrast, the response of females to DGJ was more than twice the level of response recorded in the Suzukii Trap Max bait (*t*-test *t*-value = 3.7, $P = 0.004$) (**Figure 1B**). In the third cage bioassay, no significant differences among treatments were noted for males [ANOVA $F_{(2,18)} = 2.1$; $P = 0.148$] (**Figure 1C**). Females responded in significantly greater numbers to fresh DGJ (48.6% of the females released in a 24 h period responded) than to the two Trécé lures (18 and 22.9% for the Trécé broad spectrum and selective lures, respectively) [ANOVA $F_{(2,18)} = 9.7$; $P = 0.001$].

Field Evaluations

In 2019, across all trapping dates captures of *D. suzukii* (males and females combined) were significantly greater in fresh DGJ-baited traps than in traps baited with the Scentry® SWD and AlphaScents® SWD lures [ANOVA $F_{(2,12)} = 9.5$; $P = 0.003$] (**Figure 2A**). Traps baited with DGJ captured 88.8% of all *D. suzukii* females, whereas each of the two commercial lures captured 5.6% of the total number of females. In terms of captures of non-target insects, in 2019, 21,119 insects belonging to the family Drosophilidae were captured by traps across all locations, bait treatments, and sampling dates. The two commercial lures attracted significantly more non-*D. suzukii* drosophilids than fresh DGJ [ANOVA $F_{(2,12)} = 9.5$; $P < 0.001$] (**Figure 2B**).

In the 2020 field evaluations, captures of male and female *D. suzukii* did not differ significantly among treatments [ANOVA $F_{(4,15)} = 0.9$; $P = 0.47$ and $F_{(4,15)} = 1.3$; $P = 0.32$, for males and females, respectively] during the 5-week trapping period (**Figure 3A**). In terms of captures of non-target insects, 3,874 drosophilids were captured by traps. Fresh DGJ and the Trécé selective lure attracted significantly fewer non-target insects [ANOVA $F_{(4,15)} = 3.1$; $P = 0.047$] than the Trécé broad spectrum lure, whereas captures in traps baited with the Scentry® SWD and AlphaScents® SWD lures were intermediate (**Figure 3B**).

Experiment 2: Effects of Diluted Grape Juice Aging on *D. suzukii* Response Under Cage and Field Conditions

Cage Evaluations

The cage results revealed a significant effect of DGJ aging on the response of male [ANOVA $F_{(3,44)} = 10.6$; $P < 0.001$] and female [ANOVA $F_{(3,44)} = 9.9$; $P < 0.001$] *D. suzukii*. DGJ aged for 1 and

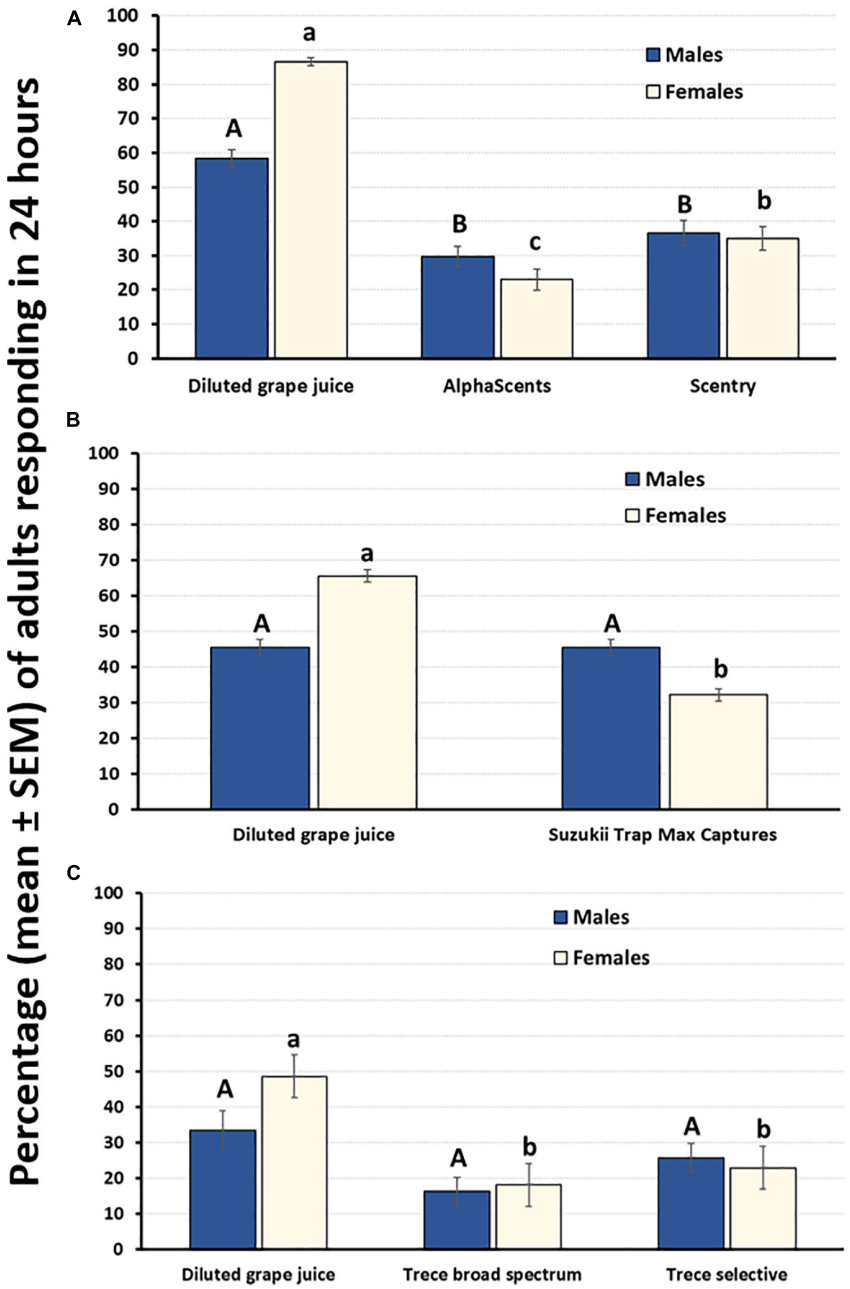

FIGURE 1 | Response, expressed as the mean percentage (± SEM) of male and female *D. suzukii* captured over a 24-h period in cage tests, to fresh diluted Concord grape juice and commercial *D. suzukii* lures and baits: **(A)** Scentry® SWD and AlphaScents® SWD, **(B)** Suzukii Trap Max Captures bait, and **(C)** Trécé broad spectrum and selective lures. For each sex, means superscribed by different letters (uppercase = treatment comparison among males; lowercase = treatment comparison among females) indicate significant differences according to ANOVA and Tukey HSD tests and *t*-tests at $P = 0.05$.

2 weeks attracted significantly more male and female *D. suzukii* than fresh DGJ, and this treatment showed to be significantly more attractive to females, but not to males, when compared to water control (**Figure 4**).

Field Evaluations

The statistical analyses revealed a significant effect of treatment for both males [ANOVA $F_{(3,16)} = 22.4$; $P < 0.001$] and females [ANOVA $F_{(3,16)} = 22.6$; $P < 0.001$]. While female *D. suzukii* showed a significant preference for 1- and 2-week-old DGJ compared to fresh DGJ, no significant differences among fresh and fermented DGJ were recorded in the case of males (**Figure 5A**). Captures of non-*D. suzukii* drosophilids were significantly greater in traps with the two aged treatments, which were similar between them, than in traps with fresh DGJ [ANOVA $F_{(3,16)} = 242.5$; $P < 0.001$] (**Figure 5B**).

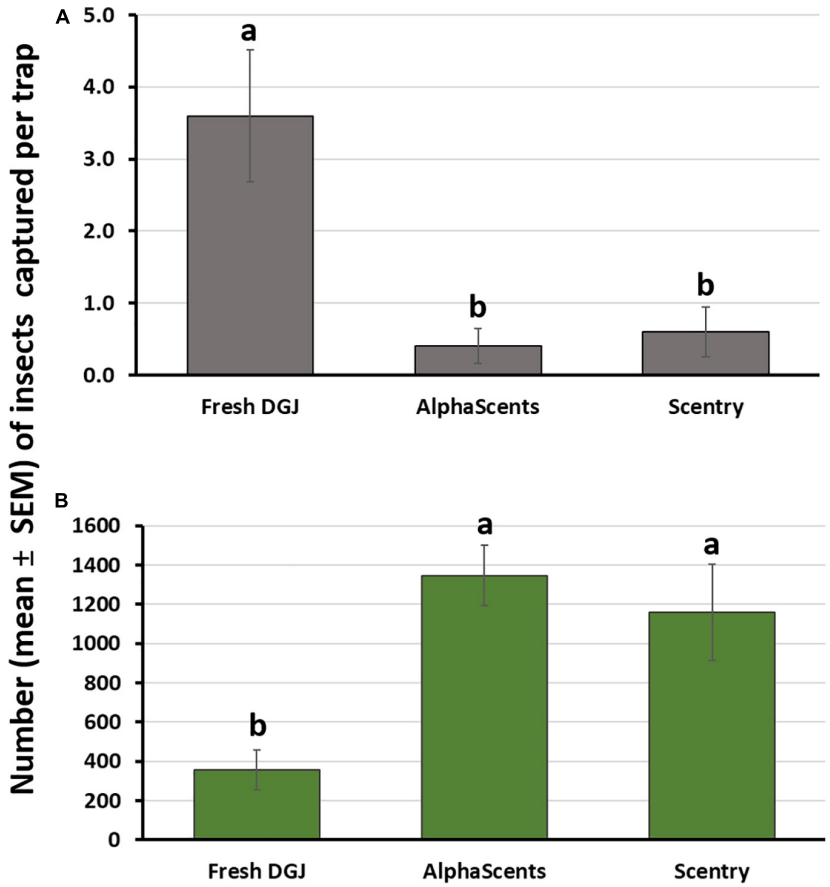

FIGURE 2 | Early season captures (means ± SEM) of **(A)** adult *D. suzukii* (male and females combined) and **(B)** other drosophilids in traps baited with fresh diluted Concord grape juice (DGJ), and two commercial lures. Study conducted in five Massachusetts locations from 17 May to 17 June 2019. Means superscribed by different letters denote significant differences according to ANOVA and HSD Tukey tests at $P = 0.05$.

Experiment 3: Effects of Adding Sodium Chloride to Diluted Concord Grape Juice Prior to Bait Fermentation on *D. suzukii* Attraction in Cages and Trap Captures in the Field

Cage Evaluations

The ANOVAS revealed a significant effect of treatment for both males [ANOVA $F_{(3,92)} = 15.8$; $P < 0.001$] and females [ANOVA $F_{(3,92)} = 37.9$; $P < 0.001$]. DGJ aged for 1 week in the presence of 2% NaCl was significantly more attractive to males and females than any other treatment (**Figure 6**). Increasing the NaCl concentration to 4% resulted in decreased attraction, which was comparable to that recorded for the 0% NaCl treatment. Each of the aged materials was significantly more attractive to males and females than fresh DGJ.

Field Evaluations

Figure 7 presents the results of the comparison of fresh DGJ, DGJ aged for 1 week in the absence and presence of 2% NaCl, and the Scentry® SWD lure. For both males [ANOVA $F_{(3,16)} = 4.3$; $P = 0.02$] and females [ANOVA $F_{(3,16)} = 4.6$; $P = 0.016$] there were significant differences in response levels among treatments. For both males and females, 1-week old DGJ that was aged in the presence of 2% NaCl attracted significantly more *D. suzukii* than any other treatment. For males, the response to 1-week old DGJ with no NaCl added was intermediate, and fresh DGJ was not attractive when compared to water control. For females, the response to DGJ that was aged for 1 week in the absence of NaCl did not differ statistically from that recorded to fresh DGJ, and both materials were significantly more attractive than water control (**Figure 7A**). Captures of other drosophilids were significantly greater [ANOVA $F_{(3,16)} = 3.6$; $P = 0.036$] in Scentry® SWD lure-baited traps than in traps containing other treatments, which were statistically similar (**Figure 7B**).

In the second field study, a significant effect of treatment was noted for males [ANOVA $F_{(4,20)} = 7.5$; $P < 0.001$] and females [ANOVA $F_{(4,20)} = 9.8$; $P < 0.001$]. DGJ that included NaCl at 2% concentration and aged for 1 week outperformed the fresh DGJ and the three commercial lures. Fresh DGJ was as attractive to males as the Trécé selective and the Scentry® SWD lures. Fresh DGJ was significantly more attractive than the Trécé broad spectrum lure. The response of females to fresh DGJ was comparable to that shown to the Trécé selective and Scentry®

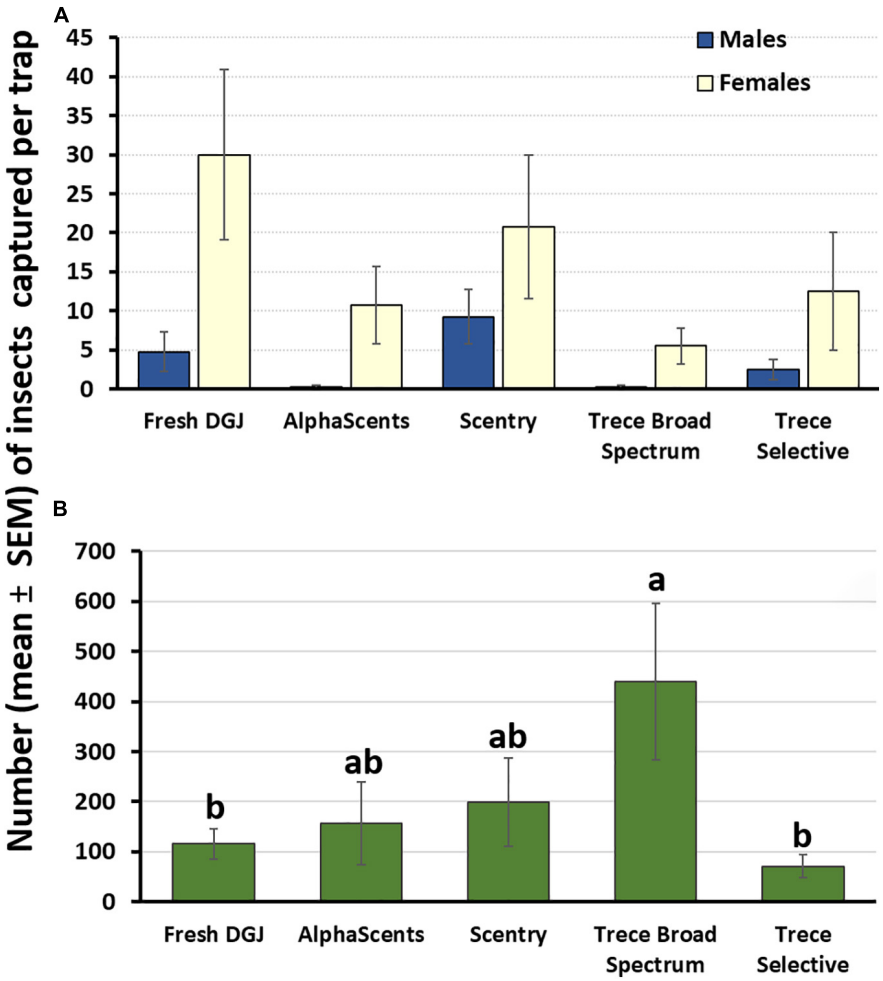

FIGURE 3 | Early season captures (means ± SEM) of **(A)** male and female *D. suzukii* and **(B)** other drosophilids, in traps baited with fresh diluted Concord grape juice (DGJ) and four commercial lures. Study conducted in four Massachusetts locations from 11 May to 7 July 2020. For non-target insects, means superscribed by different letters denote significant differences according to ANOVA and HSD Tukey tests at $P = 0.05$.

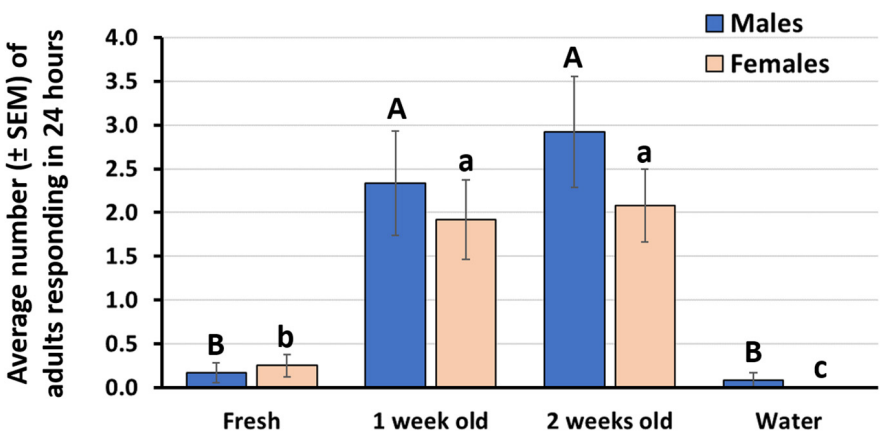

FIGURE 4 | Response, expressed as the mean percentage (± SEM) of male and female *D. suzukii* captured over a 24-h period in cage tests, to diluted grape juice either, fresh or aged for 1 and 2 weeks. For each fly gender, means superscribed by different letters indicate significant differences according to ANOVA and HSD Tukey tests at $P = 0.05$.

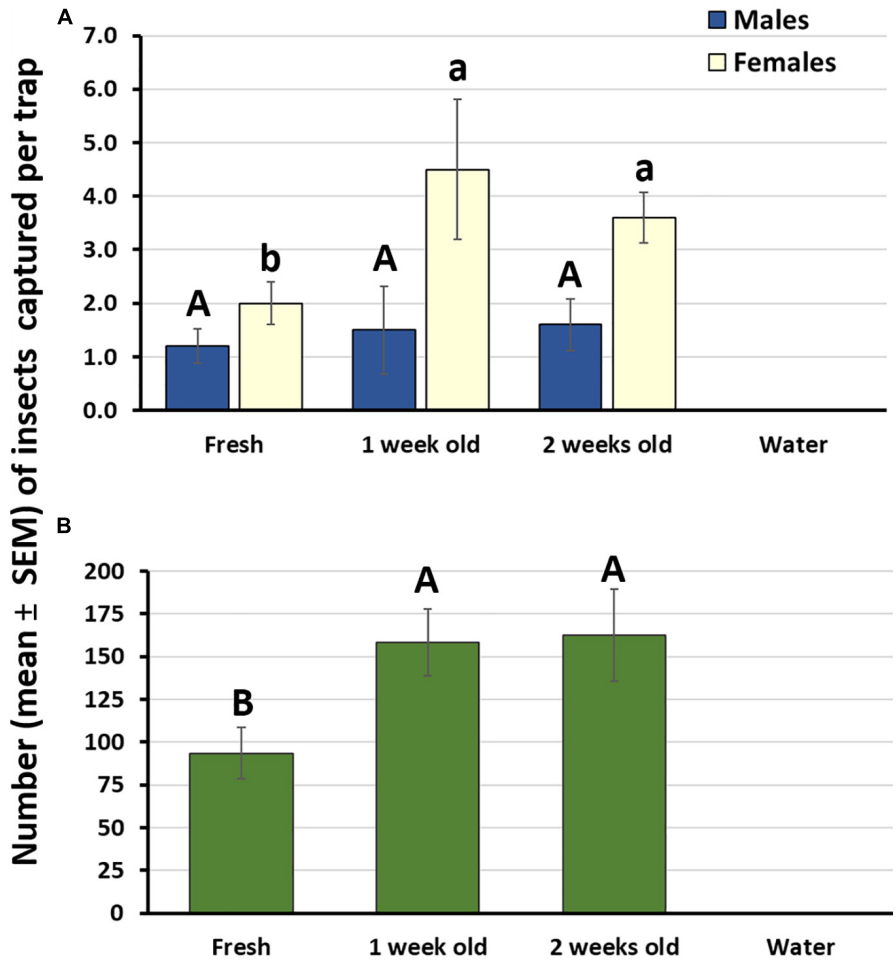

FIGURE 5 | Captures (means ± SEM) of **(A)** male and female *D. suzukii* and **(B)** other drosophilids in traps baited with diluted Concord grape juice according to its level of fermentation. Study conducted in commercial cherry block at the University of Massachusetts Cold Spring Orchard (Belchertown, MA). For each sex of *D. suzukii*, different letters indicate significant differences according to ANOVA and HSD Tukey tests at $P = 0.05$.

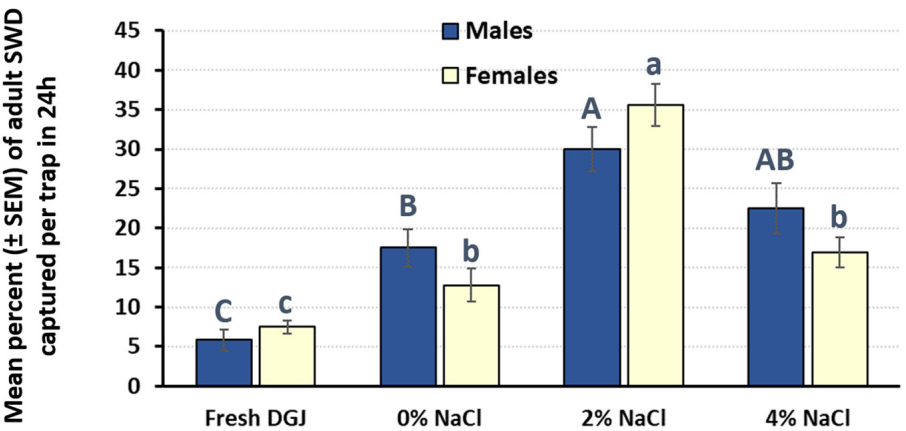

FIGURE 6 | Response, expressed as the percentage of male and female *D. suzukii* captured over a 24-h period in cages, to diluted grape juice either, fresh or aged for 7 days with 0, 2, and 4% of NaCl added prior to fermentation. For each fly gender, means superscribed by different letters indicate significant differences according to ANOVA and HSD Tukey tests at $P = 0.05$.

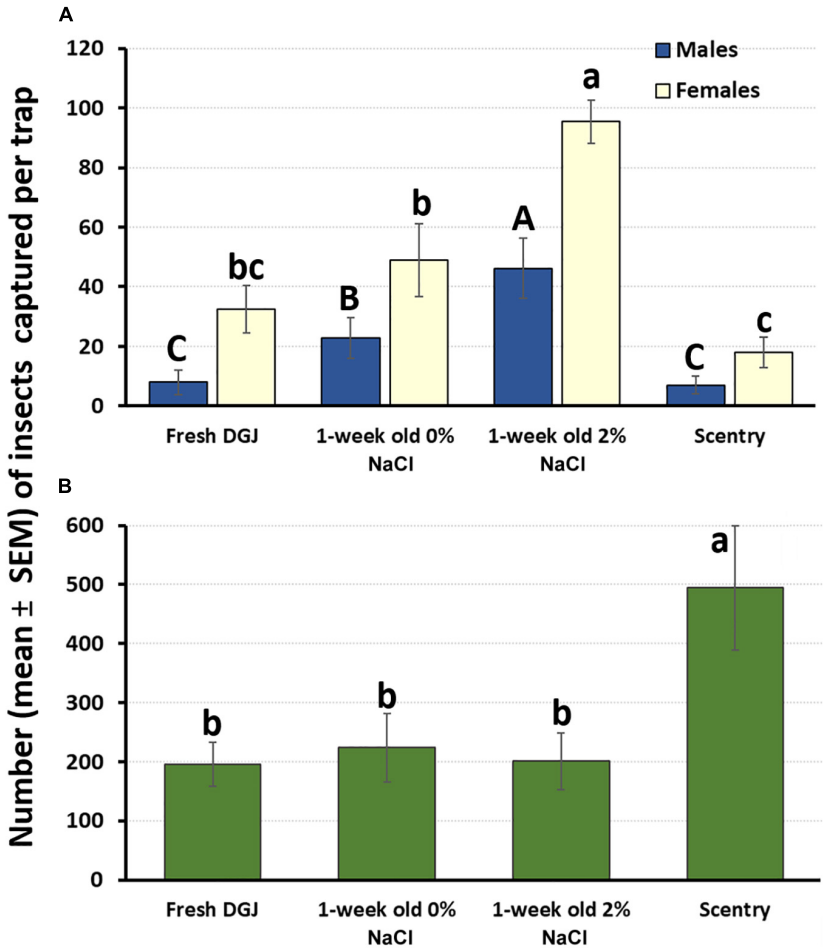

FIGURE 7 | Captures (means ± SEM) of **(A)** male and female *D. suzukii* and **(B)** other drosophilids in traps baited with diluted Concord grape juice (DGJ) either, fresh or aged for 7 days with 0 and 2% of NaCl added prior to fermentation vs. Scentry® SWD lures. Study conducted in a commercial cherry block at the University of Massachusetts Cold Spring Orchard (Belchertown, MA) from 1 June to 17 July 2021. For each sex of *D. suzukii*, different letters indicate significant differences according to ANOVA and Tukey HSD tests at $P = 0.05$.

SWD lures, but greater than that recorded to the Trécé broad spectrum lure (**Figure 8A**).

In terms of captures of non-target insects, the Trécé selective lure attracted the fewest number of drosophilids [ANOVA $F_{(4,20)} = 3.8$; $P = 0.019$] whereas the Scentry® SWD lure attracted significantly more insects than any other treatment except for the Trécé broad spectrum lure (**Figure 8B**).

DISCUSSION

Improved efficacy of *D. suzukii* lures is desirable to improve monitoring systems and potentially for the development of more effective semiochemically-based management tactics. From an applied research perspective, the development and/or improvement of food-based baits targeting female fruit flies need to take into consideration fly behavior, safety, cost, availability of ingredients, and performance under controlled and field conditions, among other factors. In this study, we compared the attractiveness of fresh DGJ against that of various commercial lures, tested the effects of DGJ aging on the outcome, and quantified the effects of adding NaCl to DGJ prior to fermentation.

Our combined results from the cage studies revealed a significantly greater level of response of adult *D. suzukii*, primarily females, to fresh DGJ within a 24-h period when compared to commercial lures. Our field studies showed similar results except for the 2020 evaluation, which revealed no significant differences in trap captures of male and females among the treatments that were evaluated. Female-biased captures could be explained in part by either, a preference of females over males for fruit-based materials, as reported by Piñero et al. (2019a) using cherry juice, or due to effects of microbial (e.g., yeasts) metabolic volatiles acting as protein cues for females (Cloonan et al., 2018). It is important to note that preference for volatile odors may also differ between males and females depending on the flies' physiological state (e.g., feeding history, female mating status) (Wong et al., 2018; Clymans et al., 2019).

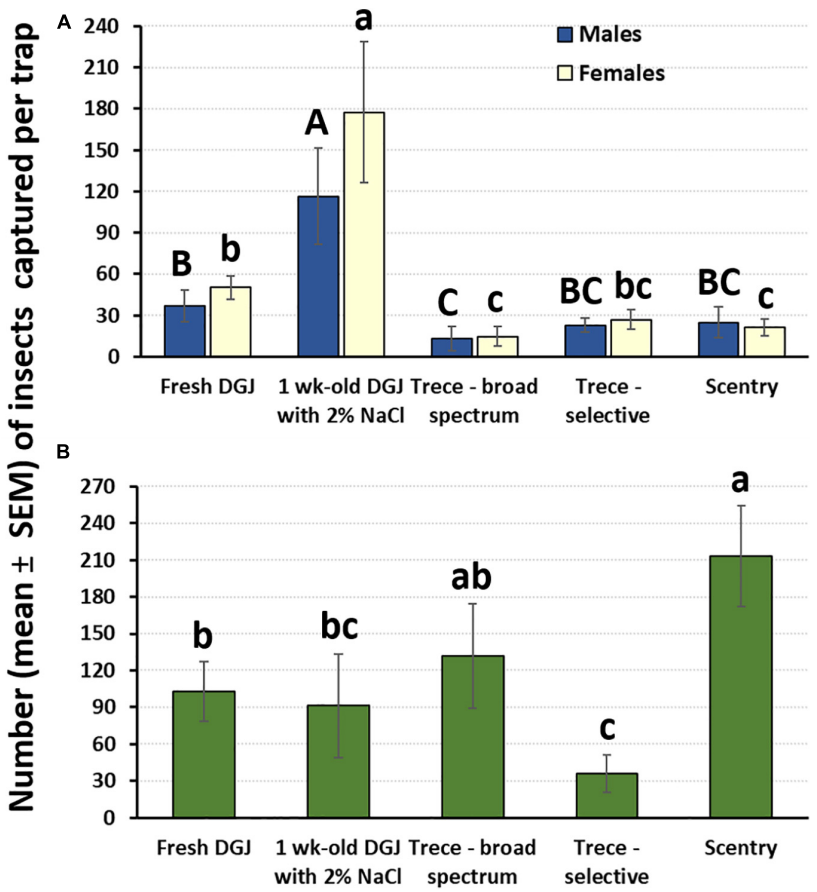

FIGURE 8 | Captures (means ± SEM) of **(A)** male and female *D. suzukii* and **(B)** other drosophilids in traps baited with diluted Concord grape juice (DGJ) either, fresh or aged for 7 days with 2% of NaCl added prior to fermentation vs. three commercial lures. Study conducted in a commercial raspberry orchard in Hatfield, MA, from 26 July to 12 August 2021. For each sex of *D. suzukii*, different letters indicate significant differences according to ANOVA and Tukey HSD tests at $P = 0.05$.

All previous research conducted with DGJ (e.g., Piñero et al., 2019a,b; Piñero and Godoy-Hernandez, 2020; Ware et al., 2021; Wen et al., 2021) was conducted using fresh material. For research purposes, all previous field evaluations were conducted servicing traps twice a week in order to minimize fermentation effects. Because those procedures were not meant to lead to integrated pest management (IPM) recommendations for growers, then we subsequently conducted studies to determine the impact of DGJ aging on *D. suzukii* attraction.

Fruit-based baits ferment over time after preparation, which potentially changes the odor chemistry and bait attractiveness to insects. It is well known that drosophilids are highly attracted to products of fermentation (Hunter et al., 1937). We did not compare the attractiveness of DGJ against that of fermented food-type lures and baits such as wine, vinegar, beer, and fermenting fruit/sucrose/yeast because those materials are broadly attractive to a diversity of insects (Cha et al., 2015; Cloonan et al., 2018). While our previous research (e.g., Piñero and Foley, 2018; Piñero et al., 2019a,b; Piñero and Godoy-Hernandez, 2020) showed that fresh DGJ is more selective than commercial lures, in the second experiment we found that both *D. suzukii* and other Drosophilids showed increased responses to 1- and 2-week old DGJ when compared to fresh material, thus resulting in reduced DGJ bait selectivity. In other fruit fly systems, preservatives are added to aqueous solutions to preserve bait and insects captured, as well as to reduce bait evaporation and insect escape (e.g., Epsky et al., 2014). Preservatives used for trapping tephritid fruit flies include polypropylene glycol, borax (Epsky et al., 2014), proxel (Moreno and Mangan, 2002), and polyethylene glycol (Mangan and Thomas, 2014). The presence and choice of preservative may alter bait effectiveness either directly by adding additional volatile attractants, or indirectly, by affecting rate and amount of fermentation (Epsky et al., 2015). For example, polypropylene glycol was found to contribute to attraction when used with protein baits in some tests of *Anastrepha ludens* (Loew) and *A. suspensa* (Loew) (Tephritidae) (Thomas et al., 2001). Our behavioral results under cage and field conditions indicated that the addition of 2% NaCl to DGJ prior to the 1-week aging of the bait exerted a strong positive effect on the response of male and female *D. suzukii*. To our surprise, the very same treatment reduced the response of other drosophilids. Such a reduced response of non-target insects has an olfactory basis since all experiments were designed to investigate the insects' behavioral response via olfaction, in the absence of

visual cues. This is a striking example of a single factor influencing the fermentation processes, leading to contrasting behavioral responses in Drosophilidae. From an applied perspective, this finding means that DGJ with 2% NaCl has a high level of *D. suzukii* specificity, comparable to that of fresh DGJ.

The specific microbe's physiological and fermentation mechanisms underlying the observed behavioral differences in *D. suzukii* are unknown, but they may include osmotic stress (Tamás and Hohmann, 2003; Dakal et al., 2014), potentially resulting in different volatile profiles. The presence of NaCl in DGJ may affect the ability of yeast and/or bacteria to undertake fermentation and malolactic fermentation, respectively. As discussed by Cloonan et al. (2018), it is conceivable that interactions between fruit-based compounds and yeast volatiles modulate adult *D. suzukii* behavioral responses to food and mate- resources.

In summary, the level of attractiveness of fresh DGJ to male and female *D. suzukii* was enhanced when DGJ was aged for up to 2 weeks, a result that was mirrored by the captures of other drosophilids. Such an increased level of DGJ attractiveness to *D. suzukii* was further augmented when DGJ was aged in the presence of 2% NaCl, relative to DGJ aged in the absence of NaCl and the captures of non-target insects was reduced drastically, increasing bait selectivity. Our findings increase our understanding of (1) male and female *D. suzukii* olfactory responses to fruit-based materials, and (2) the influence of NaCl, a household compound, on the distinct level of response of *D. suzukii* and non-targets to aged DGJ. Taken together, these results when combined with its low cost and accessibility make DGJ a feasible monitoring option for small-scale growers around the globe, who are not able to monitor or manage *D. suzukii* populations because commercially available baits are too expensive or are inaccessible. It is important to assess potential yeast metabolic volatile compounds which appear to mediate *Drosophila* attraction in the context of fruit-based materials.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JP designed the study and did the statistical analysis. XW, HG-H, and AG carried out the study. JP wrote the manuscript with contributions from XW. All authors contributed to the article and approved the submitted version.

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