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Fly responses to food color, orientation, and toxic bait composition in *Drosophila suzukii*.

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Abstract

Toxic baits comprising a combination of food attractants and a toxicant could contribute to the control of the spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), a major invasive pest of soft fruit and berries. Laboratory cage experiments revealed that flies of both sexes were significantly more attracted to dried red droplets of 0.3% sucrose solution and were more likely to consume red-colored droplets compared to blue, green or colorless droplets. Flies of both sexes showed a tendency to feed on dried droplets placed on the floor of the cage rather than droplets presented upside-down on the roof or on the cage side-wall. When offered commercial insecticides (5—50 parts per million active ingredient) in dried sucrose solution, fly mortality of both sexes was highest in the spinosad treatment, lowest in abamectin and intermediate in deltamethrin and spinetoram based products. Male flies had significantly higher mortality than females. A mixture of 25 ppm spinosad with 1.3% sucrose + 1.3% corn syrup + 1.3% glycerol (named 4% mixture) was consumed by female flies more than any of the components alone. Addition of 1% apple juice to the 4% mixture resulted in an additional increase in spinosad-induced mortality. In contrast, the addition of apple cider vinegar, increasing concentrations of apple juice (5—10%), or the addition of the yeasts *Saccharomyces cerevisiae* or *Hanseniaspora uvarum* (5 × 10^6 cells/mL) did not increase fly mortality for reasons that were unclear. We conclude that the combination of 4% mixture + 1% apple juice could prove to be a useful bait for the delivery of spinosad or other biorational insecticides for *D. suzukii* control, although this requires field testing in commercial fruit production settings.

KEYWORDS bait color, feeding position, insecticide, spinosad, apple juice, yeast

1 INTRODUCTION

Drosophila suzukii (Diptera: Drosophilidae), the spotted wing drosophila, is a devastating invasive agricultural pest of various fruit crops, including cherries, strawberries, raspberries and numerous others in Asia, Europe and the Americas (Little et al., 2020; Tait et al., 2021). Current control methods rely heavily on the application of synthetic insecticides, which can result in increased production costs, elevated pesticide residues in fruit produce and an increased risk of pest resistance (Isaacs et al., 2022; Shawer 2020). Modern integrated pest management practices focus on the use of trap technologies for pest population monitoring (Lee et al., 2013; Lasa et al., 2017; Burrack et al., 2020), physical barriers (netting) to exclude adult flies (Kuesel & Gonthier 2020), cultural control practices (Liburd & Rhodes 2020; Schöneberg et al., 2021) and the use of selective biorational compounds and organic-approved compounds that conserve insect natural enemy populations and contribute to pest control (Sial et al., 2019; Lisi et al., 2023).

Attract-and-kill strategies, in which a pest attractant is combined with a killing agent (insecticide), have proved to be valuable contributions to the integrated management of this pest and is an active area of research. Such strategies have advantages over conventional spray applications because they can greatly reduce the quantities of insecticides required for pest control, reduce the presence of pesticide residues in fruit and minimize the adverse effects of pesticides on natural enemy populations (Noble et al., 2021, 2023). As a result, toxic baits have been developed that can significantly increase the efficacy of conventional compounds such as pyrethroids and organophosphate insecticides (Gullickson et al., 2019; Fanning et al., 2021), or modern selective compounds such as cyantraniliprole and spinosyn-based formulations (Andreazza et al., 2017; Roubos et al., 2019).

The main attractant used in these baits has been sucrose at concentrations in the range 1.2—3.6 g/L (Cowles et al., 2015; Knight et al., 2016; Fanning et al., 2021). Feeding on sucrose is triggered by contact with a sweet surface and is regulated by taste receptors in the fly's tarsi (Thoma et al., 2016). In addition, sources of proteins have been evaluated with positive results, particularly yeasts such as *Saccharomyces cerevisiae* and *Hanseniaspora uvarum*. Indeed, *H. uvarum* plays an important role in the nutritional physiology, host attraction behavior, oviposition and survival of *D. suzukii* (Scheidler et al., 2015; Mori et al., 2017; Bellutti et al., 2018; Tungadi et al., 2023). The presence of yeasts or yeast culture media in insecticide spray applications can result in an increase in the efficacy of several different classes of insecticides (Bianchi et al., 2020; Rehermann et al., 2021; Spitaler et al., 2022).

Fruit products such as apple juice, apple nectar and mixtures of apple-derived volatile compounds have been reported to be highly attractive to *D. suzukii* and more selective than apple cider vinegar, which is commonly used in traps for pest monitoring (Feng et al., 2018; Lasa et al., 2020; Larson et al., 2021). Previous studies on fly responses to traps of different colors and designs (Burrack et al., 2020), have identified positive responses to red-colored visual stimuli (Lee et al., 2013; Kirkpatrick et al., 2018), although attraction to different colored foods has not been tested to our knowledge.

Similarly, some fly species, such as the tephritid *Anastrepha suspensa* (Diptera: Tephritidae) show a clear preference to feed in certain body orientations (Nigg et al., 2004). The feeding responses of *D. suzukii* to food offered in different spatial orientations have been largely overlooked, despite the relevance of this behavior in identifying the most suitable physical orientation of toxic baits applied to crop plants.

The present study built on these results to examine the influence of color and physical orientation on attraction and consumption of an experimental bait in each sex. Following this, sex-specific fly mortality following consumption of different biorational insecticides was then compared to a reference pyrethroid treatment. As females are responsible for oviposition on crops, finally we examined whether female consumption of sucrose based bait could be improved by the addition of apple products, yeasts, or other sugars or the polyol glycerol that target gustatory receptors in *Drosophila* spp. (Wisotsky et al., 2011; Freeman et al., 2014). The overall aim was to assess the attraction and feeding of *D. suzukii* on toxic baits and whether this might contribute to increased pest control under laboratory conditions.

2 MATERIALS AND METHODS

2.1 Insect colony and baits

A laboratory colony of *D. suzukii* was started in the Instituto de Ecología AC, Xalapa, Veracruz, Mexico, with adults obtained from the Centro Nacional de Referencia Fitosanitaria, Tecámac, Estado de México, in August 2018. Adults were allowed to oviposit in a cornmeal-based artificial diet (Dalton et al., 2011), dispensed into 300 ml plastic cups and covered with a fine nylon gauze under laboratory conditions of 24 ± 1 °C, 60 ± 10 % relative humidity (RH) and 12:12 h light-dark photoperiod (L:D) provided by 20 W LED light strips placed 5 cm above the cages (1150-1300 lux). Following emergence, adult males and females were collected every day using a glass tube aspirator and sexed by observation of the wing spots (males) and ovipositor (females). Flies were kept together in acrylic cages (30 x 30 x 30 cm) with nylon mesh sides until required for experiments. Each cage contained a dish of sugar and torula yeast (*Cyberlindnera jadinii*) as food and a tube of water sealed with a cotton pad. All experiments were performed under the same laboratory conditions used for rearing *D. suzukii*. Flies were not starved prior to use in experiments as, in our experience, starved flies tend to feed on the first acceptable food item that they encounter and tend to be less selective than non-starved insects.

Experimental baits were based on sucrose (raw cane sugar, Chedraui, Mexico), high fructose corn syrup (HFCS; Karo®, ACH Foods México, Mexico), apple juice (Jumex, Sabormex, Mexico), apple cider vinegar (ACV) (Conservas La Costeña, Mexico) and glycerol (99.5% purity, Sigma Aldrich, Mexico). In all cases, edible food dye (McCormick Food Color, Mexico) was added at a concentration of 0.5% (vol/vol) to change the color of experimental baits, unless otherwise specified.

2.2 Bait color

In the first experiment, sucrose solutions of different colors were evaluated under choice conditions in a 30 x 30 x 30 cm acrylic cage. Four different 0.3% (wt/vol) sucrose solutions were evaluated: i) a colorless control, ii) red solution, iii) blue solution and iv) green solution by the addition of food coloring at a concentration of 0.5% (vol/vol). Each solution was distributed in twenty 10-μL-droplets, in the lid of a 90 mm diameter Petri dish and allowed to dry overnight. Dried droplets were used to avoid variation from flies attracted to a potential water source when the droplets were wet. Four Petri dish lids, each with dried droplets of a different color, were placed in a random sequence at the corners of the acrylic cage (Supplemental figure S1A). A moist cotton wool wick was present in cages during each experiment as a water source. A group of 40 non-starved, 4-d-old flies (20 females and 20 males) was released in the center of each cage. The number of flies that were present on each Petri dish lid was counted at 30 min intervals over a 4 h period (for a total of eight counts). The percentage of flies observed to visit each Petri dish over the 4 h period was subjected to analysis. A total of ten replicates were performed by rotating the position of the treatments in each replicate. Different batches of flies collected from different colony cages were used over the course of the experiment.

A second experiment was conducted under non-choice conditions to compare the response of adult *D. suzukii* flies to dried droplets of 0.3% sucrose solution of three different colors: red, blue and green that were prepared as mentioned in the first experiment. The experiment was performed inside cylindrical 0.6 L plastic cages covered with a nylon mesh. For each treatment, a 55 mm diameter Petri dish lid containing five droplets of 10 μL of colored solution was placed on the base of the plastic cage (Supplemental figure S1B). A moist cotton wool wick was present during each experiment as a water source. A group of 40 non-starved, 4-d-old flies (20 females and 20 males), was released and allowed to feed on the sucrose solution. At five hours later, all the individuals were collected and placed in 70% ethanol to be sorted by sex and examined for gut color. Preliminary tests indicated that 70% ethanol did not affect the integrity or color properties of any of the food dyes. The flies with a colored gut were counted. A total of ten replicates were performed independently using different batches of flies on different days.

2.3 Feeding position

To determine the preferred feeding position of adult *D. suzukii* flies, a non-choice test was performed using dried red colored 0.3% sucrose solution, as this color elicited the highest feeding response in the previous experiments. A rectangular sheet of parafilm (30 x 50 mm), holding ten droplets of 10 μL of colored sucrose solution was placed at one of three different positions inside a 30 x 30 x 30 cm acrylic cage. The parafilm sheet was placed i) at the center of the cage floor or, ii) at the center of a cage side wall or, iii) at the center of the cage roof (Supplemental figure S1C). Colorless double-sided adhesive tape was used to fix the position of the parafilm in each case. A group of 40 non-starved, 2—5-d-old flies (20 females and 20 males) was released inside each cage. A moist cotton wool wick was present in cages as a water source. The number of flies present on each parafilm sheet was counted at 30 min intervals over a 4 h period (eight counts in total). After that time, all the individuals were collected and placed in 70% ethanol, sorted by sex and examined for evidence of red gut coloration. The total number of flies (males and females), at each position (cage floor, wall or roof) was calculated as the sum of the eight counts and was subjected to analysis. A total of 15 replicates were performed for each bait position using different batches of flies.

2.4 Insecticide selection

To identify a suitable biorational insecticide to kill *D. suzukii* adult flies, a non-choice test was performed using four different commercial products, namely deltamethrin (Decis Forte 10 EC, Bayer, Mexico) as a reference synthetic insecticide treatment, and three biorational insecticides based on spinosad (Spintor 12 SC, Dow AgroSciences, Mexico), spinetoram (Exalt 6 SC, Dow AgroSciences, Mexico) and abamectin (Abactin 1.8 CE, Proveedora Agroindustrial de Sinaloa, Mexico). Insecticides were mixed with 0.3% sucrose solution and 0.5% red food coloring at three different concentrations, namely 5, 25 or 50 mg a.i. of insecticide/L of sucrose solution, equivalent to 5, 25 and 50 parts per million (ppm). This range of concentrations were selected as they resulted in desirable levels of mortality (ca. 10 – 90%) in previous toxicological studies involving *D. suzukii* (deltamethrin: Civolani et al., 2012; Shaw et al., 2019; spinosad: Andreazza et al., 2017; Mori et al., 2017; spinetoram: Mermer et al., 2019; Fanning et al., 2021; abamectin: Morais et al., 2021).

Twenty droplets of 10 µl of each mixture were placed on the lid of a 90 mm diameter Petri dish and allowed to dry overnight. Each Petri dish lid was then placed inside a 30 x 30 x 30 cm acrylic cage (Supplemental figure S1D). A moist cotton wool wick was present as a water source. A group of 40 non-starved, 2-5-d-old flies (20 females and 20 males) was released inside each cage. The mortality of flies was counted in each cage after 2, 4, 8 and 24 h. Flies that did not respond when gently touched with a fine paintbrush were considered dead. A total of five replicates was performed for each insecticide with different batches of flies. A group of 40 flies was also offered 0.3% sucrose solution and 0.5% red food color without insecticide as a control in each experiment.

2.5 Toxic bait evaluation

2.5.1 Formulation

In a first experiment, eight different baits were evaluated under non-choice conditions: i) 0.3% sucrose solution as the reference treatment, ii) 4% sucrose solution, iii) 4% glycerol solution, iv) 4% high fructose corn syrup (HFCS) solution, v) 4% solution of a mixture of sucrose, glycerol and HFCS in equal proportions (1.3% each), hereafter named "4% mixture", vi) 4% mixture plus 1% (vol/vol) apple cider vinegar, vii) 4% mixture plus 1% (vol/vol) apple juice and viii) 4% mixture plus 1% apple cider vinegar plus 1% apple juice. All baits included 25 ppm of spinosad and 0.5% red food coloring. A control solution was also prepared involving a mixture of 0.3% sucrose solution and red coloring, without spinosad. As in the previous experiment, 20 drops of 10 µl of each solution were placed on the lid of a 90 mm Petri dish and allowed to dry overnight. A single Petri dish lid was placed inside a 3 L capacity acrylic cage (25 x 13 x 13 cm) with nylon mesh walls. A moist cotton wool wick was present in cages as a water source. As females are responsible for oviposition on the crop, a group of 20 non-starved, 2-5-d-old female flies was released inside each cage. Male flies were not tested. Baits were exposed to flies for an 8 h period from 10.00 to 18.00 hours. Baits were then removed from the cages and discarded. The number of dead females was recorded at 24 h after being released. A total of 28 replicates were performed for the 0.3% sucrose treatment and the 4% mixture treatment, as these were the main reference treatments in this experiment, whereas 14 replicates were performed for the other bait mixtures and the control without spinosad. Each experimental run involved all treatments and was replicated over time using different batches of insects.

2.5.2 Apple juice concentration

A second experiment was performed based on the high mortality response observed in the 4% mixture + 1% apple juice treatment in the previous experiment. The second non-choice experiment was conducted to compare the effect of different concentrations of apple juice on fly mortality; i) 4% mixture, ii) 4% mixture + 1% apple juice iii) 4% mixture + 5% apple juice and iv) 4% mixture + 10% apple juice. All treatments included red food coloring and 25 ppm of spinosad except for a 4% mixture without spinosad as a control. The experiment methodology was similar to that of the previous experiment. A group of 20 non-starved, 2-5-d-old female flies was released inside each cage. Female mortality was measured at 23 hours after flies were released. Ten replicates were performed for each treatment.

2.5.3 Formulation with apple juice + yeasts

A third experiment was conducted to assess whether the presence of yeasts affected the mortality of *D. suzukii* females. Two yeast species were used: *Saccharomyces cerevisiae,* that was growth from a commercial yeast strain (Tradi-Pan, Safmex, Mexico), and a strain of *Hanseniaspora uvarum* named RiM1 previously isolated from raspberry (*Rubus idaeus* L.) fruits (Lasa et al. 2019). Prior to use, yeasts were grown in 250 ml Erlenmeyer flasks with 50 ml of yeast-peptone-dextrose (YPD) broth (10 g yeast extract, 20 g casein peptone, 20 g dextrose in 1 L sterile distilled water) under agitation at 200 rpm and 25 °C for a period of 48 h. Yeast cells were then titrated using a hemocytometer (Neubauer Improved, Lancing, UK) under an optical microscope (400x). Five different treatments were compared: i) 0.3% sucrose solution as a reference treatment, ii) 4% mixture plus 1% apple juice, iii) 4% mixture plus 1% apple juice plus 5 × 10^6 cells/ml of *S. cerevisiae*, iv) 4% mixture plus 1% apple juice plus 5 × 10^6 cells/ml of *H. uvarum*, v) 4% mixture plus 1% apple juice plus 5 × 10^6 cells/ml of *H. uvarum* plus 5 × 10^6 cells/ml of *S. cerevisiae*. Red food coloring and 25 ppm spinosad were included in all treatments except for the control. The control comprised the 4% mixture and red food coloring plus 1% apple juice plus 5 × 10^6 cells/ml of *H. uvarum* plus 5 × 10^6 cells/ml of *S. cerevisiae* but without spinosad. A group of 20 non-starved, 2-5-d-old female flies was released inside each cage. Female mortality was measured 24 hours after flies were released. Twelve replicates were performed for each treatment.

2.6 Statistical analysis

Prior to analyses the normality and homoscedasticity of data were examined by Shapiro-Wilk test and Levene's test, respectively. A non-parametric Kruskal-Wallis (KW) test was used to compare the number of *D. suzukii* adults present on Petri dishes containing different colored solutions, followed by Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons. The prevalence of *D. suzukii* adults that consumed the different color baits was compared by analysis of variance (ANOVA) with color and sex as factors. The number of flies that visited droplets on Parafilm sheets on the floor, roof or side-wall of cages were $\sqrt{(x+0.5)}$ transformed to stabilize variance, whereas the percentages of flies that consumed droplets on Parafilm sheets and the percentage of mortality of female flies that consumed preparations containing 25 ppm spinosad were normalized by arcsine transformation prior to ANOVA. Cumulative mortality values at 24 h in the experiments involving insecticides at concentrations of 5, 25 and 50 ppm were normally distributed and were analyzed by ANOVA with insecticide, concentration and sex as factors. Following ANOVA, means were compared by Tukey test ($P < 0.05$). Mortality at earlier time points (2, 4 and 8 h) was generally low in all insecticide treatments and was therefore examined graphically but was not subjected to statistical analysis (Supplemental Figure S2). All analyses were performed using the R-based package Jamovi v.2.3.21 (Jamovi 2023).

3 RESULTS

3.1 Bait color

Under choice conditions, the percentage of flies that visited Petri dish lids over the observation period, differed significantly among colors (KW: H = 15.87; df = 3; P = 0.001).

Red droplets were preferred over colorless (P = 0.012), green (P = 0.004) or blue droplets (P = 0.024) (Fig. 1A).

Under non-choice conditions, the mean percentage of flies that consumed bait droplets differed significantly among colors (F = 505.0; d.f. = 2, 54; P = 0.005). Red droplets were consumed more frequently than blue (P = 0.048) or green droplets (P = 0.004) (Fig 1B), a behavior that did not differ significantly between the sexes (F = 140.1; d.f. = 1, 54; P = 0.075).

3.2 Feeding position

Under non-choice conditions, the mean number of *D. suzukii* flies that were observed on the parafilm sheet over the 4 h observation differed significantly for the floor, side-wall and roof positions (F = 5.053; df = 2, 84; P = 0.008) (Fig. 2A). The main effect of sex was not significant (F = 0.467; df = 1, 84; P = 0.496), but the position×sex interaction was significant (F = 3.283; df = 2, 84; P = 0.042). Both sexes visited droplets on the floor the most; females visited droplets on the floor significantly more frequently than droplets on the side-wall (P = 0.043), whereas males visited droplets on on the floor significantly more than those on the roof (P = 0.045) (Fig. 2A).

These findings were reflected in the patterns of consumption of droplets that also differed significantly by position (F = 13.519; df = 2, 84; P < 0.001) and by the interaction of position × sex (F = 3.473; d.f. = 2, 84; P = 0.036) (Fig. 2B). Female consumption of droplets was significantly higher on the floor compared to the side-wall (P =0.010), whereas for males, droplet consumption was highest on the floor and significantly lower on the roof (P < 0.001) and side-wall (P = 0.029).

3.3 Insecticide selection

When offered commercial insecticides in 0.3% sucrose solution, overall fly mortality (both sexes) in cages increased over time in all treatments, but was generally low (<30%) until 24 h after the start of the experiment (Supplemental Figure S2). For this reason, cumulative mortality at 24 h was subjected to statistical analysis. Fly mortality at 24 h varied significantly with insecticide type (Fig 3A-D). Spinosad was significantly more toxic than any of the other compounds whereas deltamethrin and abamectin were the least toxic and spinetoram had intermediate toxicity (F = 13.526; d.f. = 3, 128; P < 0.001). Mortality increased significantly with insecticide concentration (F = 73.526; d.f. = 3, 128; P < 0.001), and with the interaction insecticide × concentration (F = 3.654; d.f. = 9, 128; P < 0.001). Sex had a significant effect as males experienced ~10% higher mortality than females in all insecticide treatments (F = 11.589; d.f. = 1, 128; P < 0.001). To examine this effect in greater detail the responses of each sex were plotted against insecticide concentration for each insecticide separately (Fig. 3A-D). The higher mortality of male flies over female flies was evident in all insecticides tested, although this effect was not always significant at a given concentration. As the treatment involving 25 ppm spinosad resulted in high mortality in both sexes with little variation in mortality response at 24 h, this treatment was selected to test fly consumption of toxic baits in the following experiments.

3.4 Toxic bait evaluation

3.4.1 Formulation

In the first non-choice experiment, the mortality of *D. suzukii* females differed significantly among the different preparations in mixtures with spinosad (F = 10.038; d.f. = 7, 132; $P < 0.001$). Compared to the reference treatment of 0.3% sucrose with spinosad (mean ± SE: 34.6 ± 2.5% mortality), flies were not killed in higher proportions when offered sucrose, HFCS or glycerol individually at a concentration of 4%, whereas when these substances were combined in equal proportions to produce the 4% mixture, female mortality increased significantly to 51.1 ± 3.5% (Table 1). The addition of 1% apple juice to the 4% mixture resulted in an additional significant increase in spinosad-induced fly mortality (67.2 ± 2.8%). In contrast, 1% ACV had no significant effect on spinosad-induced mortality, either when present in the 4% mixture or when mixed with apple juice (Table 1). No mortality was observed in the groups of flies offered control preparations without spinosad.

3.4.2 Apple juice concentration

The addition of apple juice to the mixture of sucrose + HFCS + glycerol resulted in a significant increase in spinosad-induced mortality of female flies (F = 5.837; d.f. = 3, 36; $P = 0.002$) (Figure 4). Increasing the concentration of apple juice from 1% to 10% had no significant effect on fly mortality due to spinosad, suggesting that flies were not more attracted to, or did not consume greater quantities of preparations containing higher concentrations of apple juice (Tukey, $P > 0.05$). No mortality was observed in any of the control groups of flies offered experimental mixtures without spinosad.

3.4.3 Formulation with apple juice + yeasts

As observed in the previous experiment, compared to the 0.3% sucrose treatment, the prevalence of spinosad-induced mortality was significantly increased in preparations containing 1% apple juice (Fig. 5) (F = 10.959; d.f. = 4, 55; $P < 0.001$), whereas the presence of yeasts, *S. cerevisiae* or *H. uvarum*, or both yeasts together, did not result in an additional increase in spinosad-induced mortality. None of the flies in the control group (without spinosad) died during the experiment.

4 DISCUSSION

Laboratory experiments revealed color and positional preferences for *D. suzukii* attraction and consumption of dried droplets of sucrose bait. Our results demonstrated a clear attraction to red colored droplets of sucrose solution over blue, green or colorless droplets. Red droplets were also consumed more frequently than blue or green droplets. Experiments on visual attraction to colored targets have revealed preferences for red, black and yellow targets. This, despite a limited visual sensitivity to longer wavelengths at the red end of the visible spectrum and higher visual sensitivity to shorter wavelengths in the green and blue part of the spectrum (Little et al., 2019). As a result, monitoring traps for *D. suzukii* are often red, or employ a combination of red and black (Lee et al., 2013; Lasa et al., 2017; Kirkpatrick et al., 2018). There is also evidence that the contrast between the target color and the background has a strong effect on *D. suzukii* attraction in both sexes (Kirkpatrick et al., 2016; Little et al., 2019).

Bait color has not been tested previously as far as we know, although this has likely been overlooked as toxic baits are usually applied to foliage (Noble et al., 2021; Fanning et al., 2021), which is why we allowed the droplets to dry before performing experiments with flies. The addition of blue dye to a sucrose and yeast extract preparation did not affect feeding of *D. melanogaster* in a laboratory assay (Deshpande et al., 2014). For *D. suzukii*, the use of red plastic spheres with an impregnated wax, sucrose and spinetoram cap contributed to pest control in raspberry and blueberry crops (Nixon et al., 2021). On a note of caution, we cannot discount the possible effect of social facilitation in these results as both sexes of *D. suzukii* are attracted to pheromonal compounds released by females, so that testing flies in groups in cages might have introduced additional variation due to pheromonal communication within each experimental group (Lima et al., 2023).

Both sexes of *D. suzukii* tended to prefer to visit and feed on droplets that were placed horizontally on the floor of the laboratory cage (Fig 2AB), although the difference between feeding on droplets on the floor (right side up) and the roof (upside down) was statistically significant only for males. This suggests that baits targeted at *D. suzukii* should be applied to the upper surface of foliage or to a horizontal surface device, protected from rainfall.

These findings may have implications for the use of the capillary feeding assay (CAFE) used to quantify food intake in *Drosophila* spp. (Ja et al., 2007). In the CAFE assay liquid food is offered through a vertical capillary tube from which flies feed in an inverted (upside-down) position. For *D. melanogaster*, this feeding position may be familiar as they feed on hanging fruit or climb over rotten fallen fruit (Diegelmann et al., 2017). This was not the case for *D. suzukii* that tended to feed right-side up. As we did not quantify the amount of droplet consumed in each orientation, it is unclear whether *D. suzukii* consumption varied with the feeding posture or position, although for *D. melanogaster* feeding position can affect the volume consumed (Deshpande et al., 2014).

Bioassays of mixtures of red colored sucrose solution with three biorational products and one conventional pyrethroid insecticide as a reference treatment revealed two main findings. First, *D. suzukii* mortality responses varied significantly among insecticides, with spinosad as the most toxic and abamectin as the least toxic compound at the concentrations tested. The high susceptibility of *D. suzukii* to spinosad has been observed on multiple occasions under laboratory and field conditions.

Second, for all insecticides, male flies suffered ~10% higher mortality than females. This may be related to the larger body size of females which are almost 60% heavier than males (Valtierra-de-Luis et al., 2019). The higher susceptibility of males is unlikely to be due to the quantity (dose) of spinosad consumed, as males consume a ~30% lower volume of spinosad + sucrose droplets than females (Valtierra-de-Luis et al., 2019). In a detailed study, *D. suzukii* susceptibility to malathion was consistently ~2-fold higher in males compared to female flies aged 2 – 8 days, whereas this difference varied by up to 8-fold in *D. suzukii* flies exposed to spinosad or cypermethrin, indicating that both sex and insect age affect insecticide responses in this pest (Smirle et al., 2017).

Subsequent experiments focused on the responses of female flies to red colored solutions of sugars, glycerol and apple-derived products, in which spinosad was present as a toxic indicator of fly feeding behavior (Table 1). Mortality results indicated that female flies did not consume 4% solutions of sucrose, HFCS or glycerol any more than the reference treatment of 0.3% sucrose, whereas a 4% mixture of each of these components together resulted in a significant increase in spinosad-induced mortality. The attraction of *D. suzukii* to low concentrations of sucrose has been well established (Cowles et al. 2015). Fructose is an important component of floral nectars (Gill & Walters 2023) and floral feeding markedly increased survival of *D. suzukii* compared to insects that consumed only water (Tochen et al., 2016). Adult feeding on sucrose or nectar increases carbohydrate reserves in flies (Tochen et al., 2016). We therefore expected a strong response of *D. suzukii* to HFCS treatment, but this was not observed. Similarly, application of various bioinsecticides with 12.5% corn syrup did not improve control of *D. suzukii* in raspberry crops (Fanning et al., 2018). Incidentally, glycerol has a low insecticidal activity in *D.*

suzukii but only at concentrations much higher than those used in the present study (Díaz-Fleischer et al., 2019).

The addition of 1% apple juice to the 4% mixture resulted in a ~30% increase in mortality of *D. suzukii* females (Table 1). This pest is attracted to fruit volatile compounds, even those originating from fruit species that are not commonly used for oviposition (Tadeo et al., 2022). In the case of apple, fermented apple juice had a higher diversity of volatiles and higher concentrations of selected volatiles compared to fresh apple juice (Feng et al., 2018). These volatiles were identified and used to produce artificial blends that were highly attractive to *D. suzukii* when used in traps placed in blueberry and raspberry crops (Feng et al., 2018; Larson et al., 2021). In the present study, however, increasing concentrations of apple juice (5-10%) did not result in higher spinosad-induced mortality compared to the 1% concentration (Fig. 4), which is a promising finding as it would reduce the cost of applying an insecticidal bait containing an apple juice component.

Like other drosophilids, *D. suzukii* feeds on yeast, which is a critical nutritional source during the adult and larval stages. The addition of *H. uvarum* or *S. cerevisiae* to the 4% mixture + apple juice preparation failed to significantly increase spinosad-induced mortality (Fig. 5). Previous studies have observed increased attraction, adult feeding and oviposition by *D. suzukii* in the presence of these yeasts at concentrations in the range 10^7 – 10^9 cells/mL (Bellutti et al., 2018; Noble et al., 2019; Bianchi et al., 2020; Spitaler et al., 2022), somewhat higher than the 5 x 10^6 cells/ml that we tested. However, this lower concentration of yeast was sufficient to produce fermentation of the 4% mixture as evidenced by the development of turbidity and a distinct yeasty odor during the 24 h experiment. However, it is possible that an interaction between the yeast and the 4% mixture components or the diluted spinosad formulation could have influenced the production of fermentation volatile compounds that promote attraction in *D. suzukii* (Kumokita et al., 2023). It is also possible that the prevalence of unmated females in experimental groups or the growth medium used to produce yeasts affected female responses to yeasts, as both mating and growth media can influence yeast attraction and feeding behavior in this species (Mori et al., 2017; Lasa et al., 2019).

We conclude that the combination of the red colored 4% mixture + 1% apple juice could prove to be an attractive and useful bait for the delivery of biorational insecticides such as spinosad that act by ingestion, for control of *D. suzukii*. This requires validation by field testing applications directly to foliage or colored panel devices in commercial fruit production settings.

AUTHOR CONTRIBUTIONS

Rodrigo Lasa: Conceptualization; investigation; funding acquisition; methodology; writing – review and editing; visualization; project administration; supervision; data curation; resources. **Saide Aguas-Lanzagorta**: Conceptualization; investigation; methodology; data curation; visualization. **Trevor Williams**: Conceptualization; visualization; formal analysis; writing – original draft; data curation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Raw data are available at: https://data.mendeley.com/datasets/8byv7bbgk9/1

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Figure legends:

FIGURE 1 Responses of flies to droplets of sucrose solution (0.3%) of different colors (colorless, red, green, blue). (A) Box-whisker plot of the percentage of flies that visited Petri dishes with colored droplets over a 4-h period in a laboratory choice experiment. Figure shows median (line), mean (cross), data points (circles), interquartile range (box) and data range (bars). Different letters above boxes indicate significant differences (DSCF, $P < 0.05$). (B) Mean (± SE) percentage of male and female flies that consumed colored droplets (red, green, blue) over a 5 h period under non-choice conditions. Different letters above columns indicate significant differences for both sexes together (ANOVA, Tukey, $P < 0.05$).

FIGURE 2 Responses of male and female flies to red colored droplets of sucrose solution (0.3%) placed on the floor, roof or side-wall of a laboratory cage under non-choice conditions (A) mean (±SE) number of flies observed visiting droplets, (B) mean (± SE) percentage of flies that consumed droplets over a 4-h period. Different letters above columns indicate significant differences for comparisons of feeding position and sex (two-way ANOVA, Tukey, $P < 0.05$).

FIGURE 3 Mean (± SE) percentage of mortality of male and female flies offered red colored sucrose solution containing 5—50 ppm of commercial formulations based on (A) deltamethrin, (B) spinosad, (C) spinetoram, (D) abamectin. Different letters above columns indicate significant differences for comparisons of insecticide concentrations and sex for each insecticide separately (two-way ANOVA, Tukey, $P < 0.05$).

FIGURE 4 Mean (± SE) percentage of mortality of female flies offered 25 ppm spinosad in a 4% mixture (sucrose + HFCS + glycerol) alone, or in combination with apple juice (1—10%), under non-choice conditions. Different letters above columns indicate significant differences (ANOVA, Tukey, $P < 0.05$).

FIGURE 5 Mean (± SE) percentage of mortality of female flies offered 25 ppm spinosad in 0.3% sucrose solution, or a 4% mixture alone or in combination with 1% apple juice and the yeasts *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* under non-choice conditions. Different letters above columns indicate significant differences (ANOVA, Tukey, $P < 0.05$).

Fig. 1

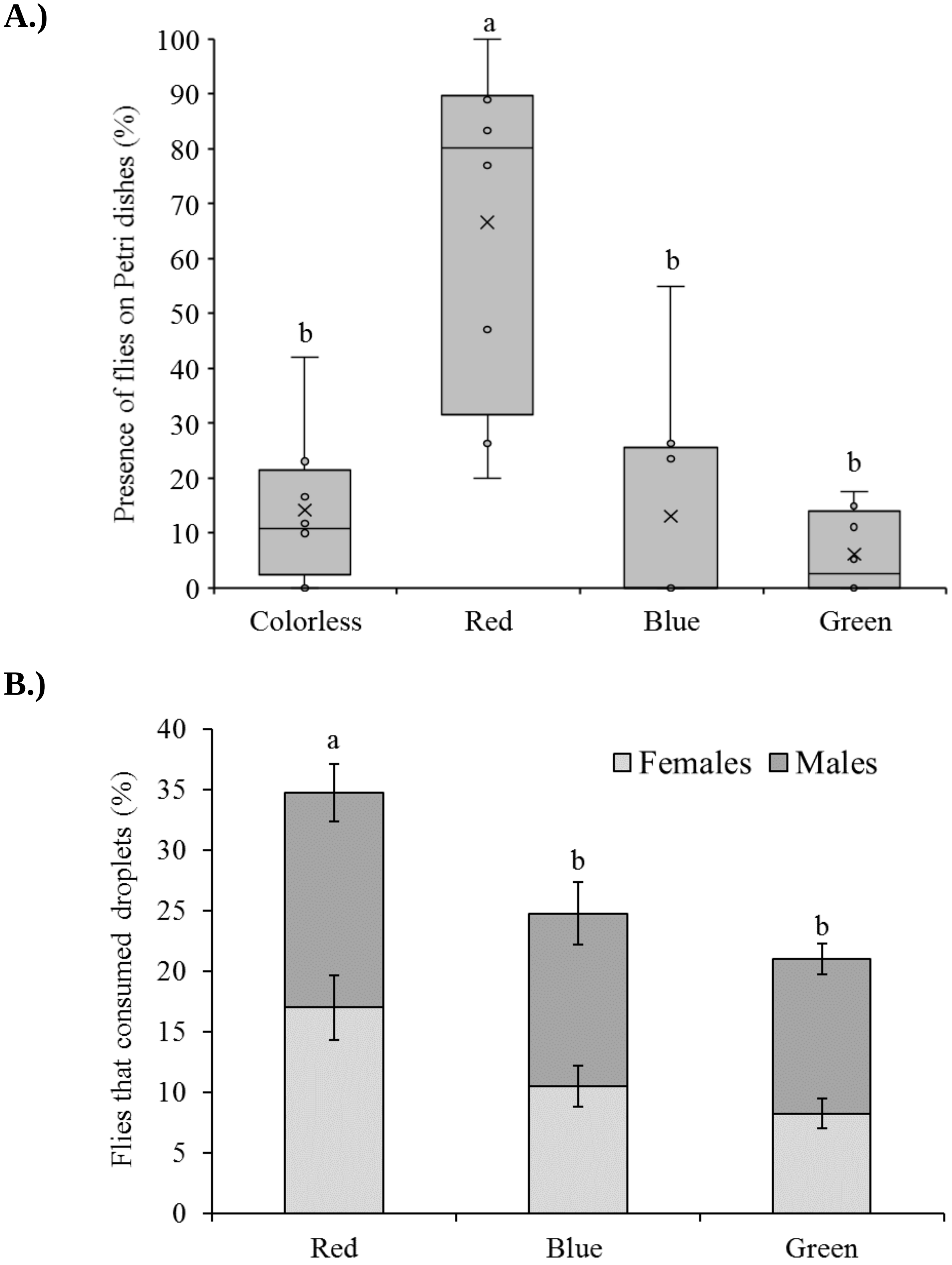

A.)
Presence of flies on Petri dishes (%)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
b
Colorless
Red
Blue
Green
B.)
Flies that consumed droplets (%)
40
35
30
25
20
15
10
5
0
a
b
b
Females
Males
Red
Blue
Green

Fig 2

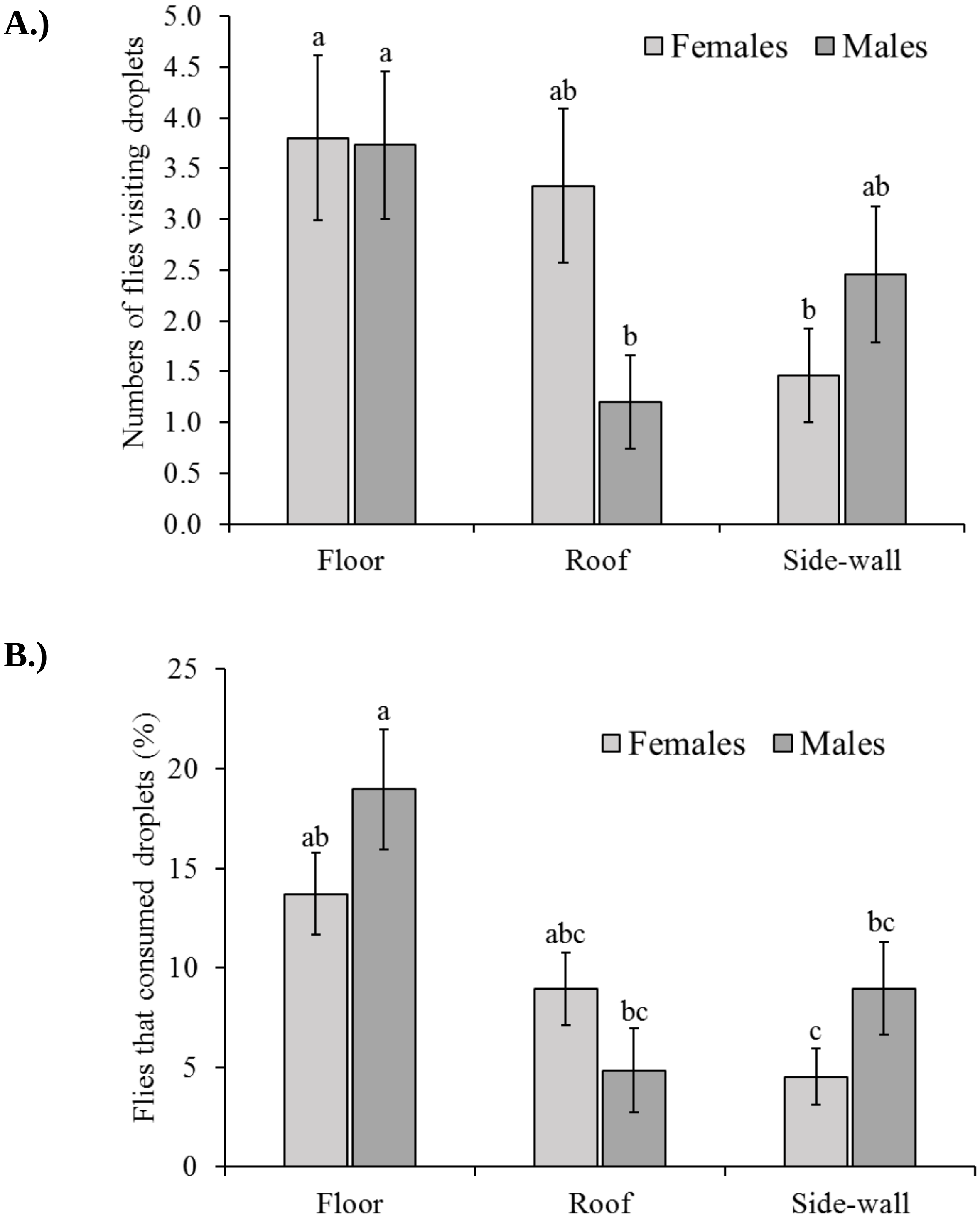
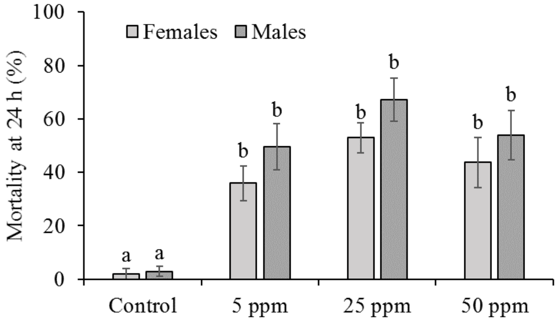
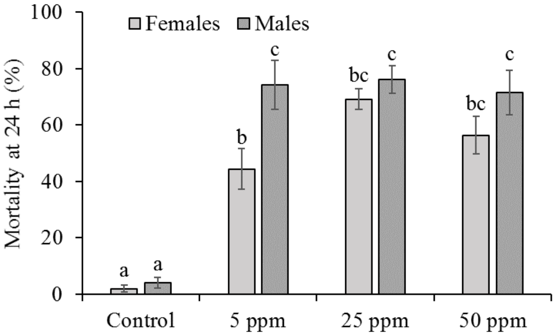

A.)
Numbers of flies visiting droplets
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Females
Males
a
a
ab
b
b
ab
Floor
Roof
Side-wall
B.)
Flies that consumed droplets (%)
25
20
15
10
5
0
Females
Males
ab
a
abc
bc
c
bc
Floor
Roof
Side-wall

Fig. 3

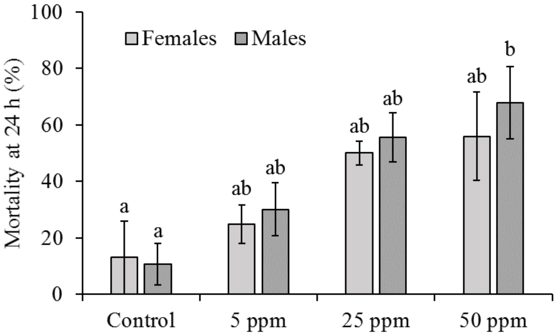
A.) Deltamethrin



B.) Spinosad



C.) Spinetoram



D.) Abamectin

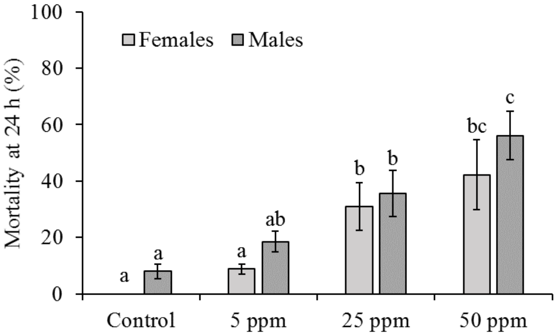


Fig. 4

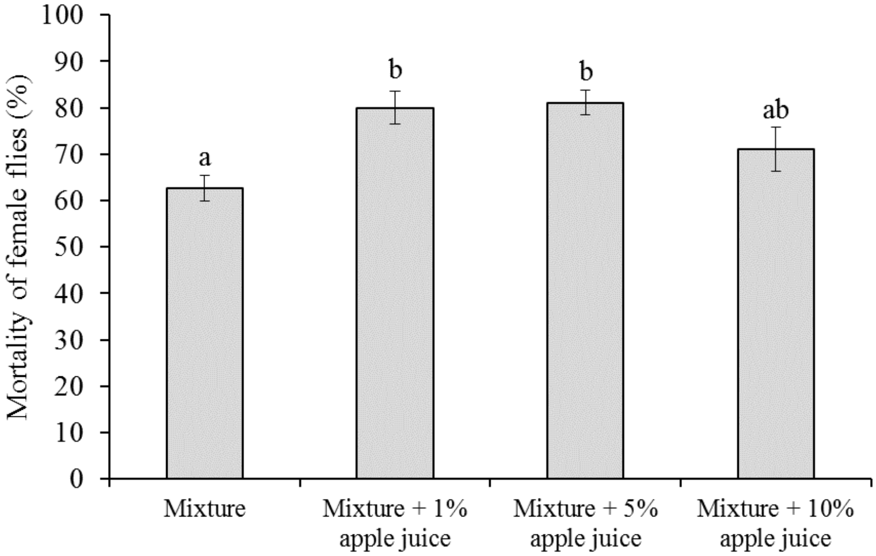

100
90
80
70
60
50
40
30
20
10
0
Mortality of female flies (%)
a
b
b
ab
Mixture
Mixture + 1% apple juice
Mixture + 5% apple juice
Mixture + 10% apple juice

Fig. 5


Mortality of female flies (%)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
b
b
0.3% sucrose
Mixture + apple juice
Mixture + apple juice + S. cerevisiae
Mixture + apple juice + H. uvarum
Mixture + apple juice + S. cerevisiae + H. uvarum