Laboratory evaluation of trap color and vinegar, yeast and fruit juice lure combinations for monitoring of *Zaprionus indianus* (Diptera: Drosophilidae)

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**ABSTRACT**

*Zaprionus indianus* Gupta (Diptera: Drosophilidae) is a successful invasive drosophilid that is currently impacting fig production in Mexico and Brazil. Very few studies have examined the improvement of trapping strategies for this pest. Here, we compared visual responses of *Z. indianus* to different colors and olfactory cues. Orange and brown colored traps were among the most attractive in choice and no-choice tests, with violet and white being the least attractive colors. Orange traps with brown circles around the access holes were more attractive than uniformly orange traps. Apple cider vinegar was significantly more attractive to adults than sugar cane vinegar or grape juice but was not significantly more attractive than white wine and red wine vinegars. Captures of *Z. indianus* in apple cider vinegar-baited traps were not improved by the addition of grape, pineapple or apple juices, or when compared against a sucrose solution or grape juice fermented with *Saccharomyces cerevisiae*, or grape juice fermented with *Candida tropicalis*. Pairwise comparisons of *Z. indianus* attraction to *S. cerevisiae* and *C. tropicalis* indicated a high dependence on the growth media used. Orange traps with brown circles baited with apple cider vinegar may prove useful for monitoring this pest under field conditions.

**Introduction**

The fig fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), is an invasive species that occupies a broad ecological niche and has the capacity to adapt to diverse food and climatic conditions (Parkash and Yadav 1993-2004). Originally native to Africa, this insect has spread rapidly through the Americas since its introduction to Brazil at the end of the 1990’s (Vilela 1999), and has now invaded Mexico, USA and Canada (van der Linde et al. 2006; Castrezana 2007; Renkema et al. 2013). The fly became a pest in Brazil in the production of figs, *Ficus carica* L. Females lay eggs on the bracts of the ostiole in intact fruits at the start of maturation and larvae enter the fruit through small natural wounds of the fruit. The fig variety “Roxo de Valinhos” was highly susceptible and losses in production often reached 50% (Tidón et al. 2003). In Mexico, the fig variety “Netzahualcoyo” is currently attacked by this pest so that *Z. indianus* now represents a new phytosanitary problem for the production of figs destined for export to Canada (Bautista et al. 2017). Although most varieties of figs seem not to be affected, in susceptible varieties, the open ostiole and small cracks in the surface of developing fruit appear to influence susceptibility (Franco and Penteado 1986; Bautista et al. 2017). It is unclear whether the pest status of *Z. indianus* is restricted to figs or whether it attacks additional species in the Malpighiaceae and Rosaceae families. This drosophilid has been reared from ripe fruits of acerola cherry (Malpighia emarginata de Candolle), longan fruit (*Dimocarpus longan* de Loureiro) (Steck 2005) and from ripe blackberry fruits (*Rubus fruticosus* L.) (R. Lasa, unpublished observations).

Few studies have addressed the issue of managing trapping technologies for *Z. indianus* (Raga and Souza-Filho 2003; Aluja and Rull 2009; Pasini and Link 2011; Pasini et al. 2011). The selection of specific trap features with visual stimuli and the use of effective attractants determine the efficacy of traps for the capture of drosophilid pests in monitoring surveys. High capture rates can contribute to the development of alternative control strategies such as mass trapping and bait stations.

As pests can discriminate between colors, shapes and sizes, visual stimuli have been investigated to improve the efficiency of traps for tephritids (Cytrynowicz et al. 1982; Sivinski 1990; Aluja and Rull 2009) and some drosophilids. Previous studies
on trap color have identified red as highly attractive to *Drosophila melanogaster* Meigen (Wave 1964), whereas red, purple, orange and combinations of black and white were attractive to *Drosophila repleta* (Patterson) (Hottel et al. 2015), and red traps with a black horizontal stripe were effective for trapping *Drosophila suzukii* (Matsumura) (Basoalto et al. 2013; Rice et al. 2016; Lasa et al. 2017). However, to our knowledge no information exists on the colors that attract *Z. indianus*.

Visual stimuli play a role in attraction at short distances, whereas lures are required for attraction over long distances and determine the efficacy of each trap-lure combination. Fermentation products such as apple vinegar and cider vinegar plus wine (Landolt et al. 2012), or fermented sugars inoculated with fruit-associated yeasts (Hampton et al. 2014; Iglesias et al. 2014; Marcus 2014; Lasa et al. 2017), have been tested for their capacity to attract *D. suzukii*, although attraction of *Z. indianus* has been reported on occasions (Iglesias et al. 2014; Lasa et al. 2017). In the case of *Z. indianus*, studies have focused on responses to fermented fruit juices (Pasini et al. 2011; Epskay and Gill, 2017).

The present study examined the attraction of *Z. indianus* adults to different colors and color combinations. Lures, such as vinegars, commercial fruit juices and fermenting lures inoculated with specific yeasts were also evaluated. These studies serve as the basis for the development of effective trap-lure combinations for testing under field conditions.

### Material and methods

**Insect colony.** A laboratory colony of *Z. indianus* was started in an insectary at the Instituto de Ecología AC, Xalapa, Veracruz, Mexico, using adults that emerged from naturally infested sapodilla (*Manilkara zapota* L. van Royen) collected in June 2016 in Apazapan, Veracruz (19°19′14″N; 96°43′08″W, 292 m altitude). Adults were allowed to oviposit in a cornmeal-based artificial diet (Dalton et al. 2011) that was dispensed into 30 ml plastic cups and covered with a fine nylon gauze. The colony was maintained at 24 ± 1°C, 60 ± 10% relative humidity (RH) and 12:12 h (L:D) photoperiod with a light intensity of 3500 - 4500 lux, measured using a light meter (YK-10LX, LT Lutron, Taipei, Taiwan). The sex ratio of adults in cages was approximately 50% males and 50% females. Flies used in tests were 1 week old and had been kept together since emergence (males and females) in 31 cages with water supplied in a moist cotton pad and a 3:1 sugar-yeast mixture.

### Visual attraction of different colors under cage conditions

Three independent experiments were performed to evaluate the visual attraction of *Z. indianus* adults to different colors:

**Experiment 1.** A preliminary no-choice experiment involved eight different-colored traps. Traps were constructed from 120-ml plastic cups (35 mm diameter, 87 mm high) that were drilled with three equidistant lateral holes through which translucent conical tubes (9 mm external diameter, 6 mm internal diameter, 20 mm deep) were inserted to decrease the frequency of fly escape once inside the trap. Holes were placed at 45 mm above the base (Figure 1a). The traps were covered with paper (Irasa Industrial SA de CV, Mexico) of one of the following colors: green, yellow, white, black, violet, brown, orange and red. Colors were characterized by their RGB values, determined using the Paint Software (Microsoft Inc.) from a photograph of all traps together (Supplemental material, Table S1). Traps had a white screw-cap with a central 10 mm hole in which a 1.5 ml polyethylene centrifuge tube was inserted (Figure 1a (i)). Two rectangular openings, each of 1 cm², were perforated 2 mm below the lid of the centrifuge tube and covered with 0.2 mm nylon mesh. This allowed volatiles to escape into the trap headspace and prevented flies entering the internal vial device (Figure 1a (i)). The centrifuge tube was baited with 0.5 ml of grape juice (Jumex, Saborex SA de CV, Mexico) before inserting into the screw-cap. Grape juice was used as an attractant because it has been reported to be an effective lure for this pest (Epskay and Gill 2017). A volume of 20 ml of water with 10 μl of Tween 80 was used as the drowning solution.

Each trap was placed in the center of an acrylic cage (30 × 30 × 30 cm) with 0.1-mm nylon mesh sides under the laboratory conditions described above. A 50-ml cup of water with a moist cotton wick was placed in the cage during each experiment. A group of 40 non-starved 7-day old flies (both sexes) were released at the front of each cage at 10:00 am. After 23 h, traps were removed from cages and the number of captured flies was recorded. The remaining flies inside the cage were discarded. Eight cages, one per trap color, were prepared simultaneously. A total of eight replicates per color trap were performed.

**Experiment 2.** Multiple-choice tests were performed to compare trap colors that captured higher numbers of insects during experiment 1 (orange, brown, green and yellow). Traps covered with colored paper re-used from experiment 1 were placed at the corners of acrylic and nylon mesh cages (30 × 30 × 30 cm), containing a moist cotton wick.
as a water source. Trap colors, with grape juice attractant as described above, were initially assigned at random and subsequently rotated clockwise for each new replicate. A group of 40 non-starved 7-day old flies (both sexes) were released at the front of each cage at 10:00 am. After 23 h, traps were removed from the cages and captured flies recorded. Four independent cages were prepared simultaneously. A total of four replicates were performed for each cage by rotating the position of each trap at each replicate (1 per position, n = 16 replicates in total).

Experiment 3. Multiple-choice tests were conducted to compare fly responses to an orange-colored trap with other orange traps with brown-colored stimuli. Four color designs were compared (Figure 1b): i) orange, ii) orange with a 20 mm wide brown horizontal stripe at the height of trap holes, iii) orange with a 26 mm diameter brown circle around each of the access holes, and iv) orange with a 20 mm wide brown vertical stripe at the trap access hole. To increase the surface area involved in the release of volatile compounds, attraction to traps

Figure 1. Traps used in experiments: (a) components of the two trap devices: (i) trap with a perforated screw cap and ventilated top vial baited with grape juice and (ii) trap with a simple screw cap, (b) orange-brown color combination traps used in choice tests, (i) simple orange trap, (ii) orange trap with a brown horizontal stripe, (iii) orange trap with a brown circle around the entrance hole and (iv) orange trap with three vertical stripes.
and capture was achieved using 20 ml of grape juice with 10 μl of Tween 80 as the drowning solution and the small tube device used in experiments 1 and 2 was eliminated from the trap (Figure 1a [ii]). Traps were placed at the corners of acrylic and mesh cages, as described in experiment 2. Traps were initially assigned to random positions and were subsequently rotated clockwise for each new replicate. A moist cotton wool wick was present in each cage. A group of 40 non-starved 7-day old flies (both sexes) were released at the front of each cage and were collected and counted 23 h later, as described in experiments 1 and 2. A total of four replicates were performed for each cage by rotating the position of each trap at each replicate (1 per position, n = 16 replicates in total).

**Attraction to odors under cage conditions**

**Experiment 4.** Multiple-choice tests were performed to compare attraction of *Z. indianus* to a 20 ml volume of the following four types of commercial vinegars: i) apple cider vinegar (La Costeña, 5% acidity, Ecatepec, Mexico), ii) sugar cane vinegar (La Costeña, 5% acidity, Ecatepec, Mexico), iii) red wine vinegar (Carbonell, 6% acidity, Córdoba, Spain), and iv) white wine vinegar (Carbonell, 6% acidity, Córdoba, Spain). All vinegar lures contained 10 μl of Tween 80 to reduce surface tension. The trap with the highest capture of adult flies identified in experiment 3 (orange with a brown circle around the access hole) was used for all treatments. Traps were randomly assigned to the corners of cages and subsequently rotated for each new replicate. As responses to traps placed within cages were clear and differed markedly, we assumed that odors did not interfere with one another. The experimental procedure was identical to that of experiment 3.

**Experiment 5.** Multiple-choice tests were performed to compare attraction of *Z. indianus* to apple cider vinegar or mixtures of apple cider vinegar and 20% different fruit juices. Experimental treatments were: i) 20 ml of apple cider vinegar as control, ii) 16 ml of apple cider vinegar + 4 ml apple juice, iii) 16 ml of apple cider vinegar + 4 ml pineapple juice and iv) 16 ml of apple cider vinegar + 4 ml of grape juice. All juices were produced by Jumex (Saborex SA de CV, Mexico). All lures contained 10 μl of Tween 80 to reduce surface tension. The highest capture of adult flies identified in experiment 3 (orange with a brown circle around the access holes) was used for all treatments. Traps were prepared 1 h before the start of each experiment, following the procedure used to test *D. suzukii* (Lasa et al. 2017). The procedure, using the orange trap with a brown circle around the access hole, was identical to that of experiment 3.

**Experiment 7.** *Candida tropicalis* is a yeast that has been isolated from figs infested by *Z. indianus* and is closely associated with this pest (Gomes et al. 2003). Independent pairwise comparisons of attraction were conducted for *C. tropicalis* and *S. cerevisiae*, the latter of which is commonly used in lures designed to monitor drosophilid pests. As the growth medium can influence the composition of volatile compounds produced during fermentation (Dzialo et al. 2017), three different media were used to culture each species of yeast. The trials were designed to compare attraction to different species of yeasts rather than to evaluate the attraction of flies to yeast per se.

Both yeasts were previously grown in 250 ml Erlenmeyer flasks with 50 ml YPD broth (10 g yeast extract, 20 g peptone and 20 g dextrose per liter; Dibico, Cuatitlán, México) inoculated with 1x10⁶ cells and incubated for 48 h at 25 °C in an orbital agitation shaker at 120 rpm. Lures were prepared by inoculating 1.5 x 10⁸ cells of *C. tropicalis* or *S. cerevisiae* in 20 ml volumes of one of three different growth media: (i) 5.5% (wt/vol) sucrose, (ii) 5.5% corn syrup (Karo bebe®, 72.6 g total carbohydrates, ACH-Foods Mexico, Santa Fé, Mexico) and (iii) YPD broth. Lures were prepared 24 h prior to experiments, incubated at 24 °C under laboratory conditions and added to traps with 10 μl of Tween 80. The traps used and the experimental procedures were identical to those described in experiment 3, except that *C. tropicalis* and *S. cerevisiae* inoculated in each type of medium were compared in pairwise tests involving two traps per cage and rotated once for position (a total of eight replicates per treatment).

**Experiment 8.** Based on the results of the previous experiment an additional experiment was performed to compare the attraction of *Z. indianus* to apple cider vinegar or grape juice fermented with *C. tropicalis*. Two attractants were compared in choice tests involving the orange trap with brown circles: i) 20 ml of grape juice inoculated with 1.5x10⁶ cells of *C. tropicalis*, and ii) 20 ml of apple cider vinegar. A 10 μl volume of Tween 80 was included in both
treatments. Yeast was added to grape juice 1 h prior to conducting the experiments. The experimental procedures were identical to that of experiment 7 with four cages and two traps from each treatment per cage (1 trap in each position, eight replicates per treatment in total).

Statistical analyses. Mean numbers of captured flies were initially compared by a two-way analysis of variance (ANOVA) with cage and treatment as factors. Cage effects were not significant in tests (P > 0.05), therefore numbers of captured flies were compared by a one-way ANOVA with treatment as main factor. Mean separation was performed using Tukey HSD test. Only in experiment 5, mean numbers of captured flies were root transformed (\(\sqrt{x}\)) to homogenize variances. Comparisons of yeast species and fermented grape juice and apple cider vinegar were performed by t-test. All analyses were performed using the R-based program Jamovi v.0.9.1.12 (Jamovi 2018).

Results

Visual attraction to colored traps under cage conditions (experiments 1 - 3)

No-choice experiments revealed significant differences in fly captures among different uniformly-colored traps (F = 3.75; df = 7.56; P = 0.002). Orange traps had a significantly higher capture of flies than violet or white traps. Brown traps also captured significantly more flies than white traps, whereas the remaining colors had intermediate numbers of captured flies (Figure 2a). A choice experiment using the colors that elicited the highest captures in experiment 1, revealed that orange and brown were significantly more attractive than yellow or green (F = 16.47; df = 3.60; P < 0.001) (Figure 2b). In this test, yellow was selected over black as it has been used for commercial traps of other pestiferous flies, such as tephritids and drosophilids.

The inclusion of brown circles around the access holes resulted in a significant increase in the capture of flies compared to uniformly orange traps or orange traps with a brown horizontal stripe at the access holes (F = 4.78; df = 3.60; P = 0.004), whereas orange traps with vertical brown stripes had an intermediate number of captured flies (Figure 2c).

Attraction to odors under cage conditions (experiments 4–8)

The attraction to traps differed significantly among types of vinegar (F = 2.80; df = 3.60; P = 0.047). Traps with apple cider vinegar captured significantly more flies than traps with sugar cane vinegar, whereas traps with red wine and white wine vinegars did not differ significantly from the other two lures (Figure 3a). Captures in traps baited with apple cider vinegar were not significantly affected when this vinegar was mixed with 20% of grape, pineapple or apple juice (F = 0.81; df = 3.60; P = 0.495) (Figure 3b).

Apple cider vinegar baited traps captured significantly more Z. indianus than traps baited with grape juice without S. cerevisiae, whereas traps containing grape juice with S. cerevisiae or S. cerevisiae in 5.5% sucrose solution had intermediate captures (F = 34.10; df = 3.60; P < 0.001) (Figure 3c).

The attraction of Z. indianus to yeasts was dependent of the growth media used. When yeasts were grown in YPD broth, Z. indianus was significantly more attracted to traps containing C. tropicallis than to S. cerevisiae (t = 3.68; df = 14; P = 0.002) (Figure 4a). When grown in corn syrup (Karo
captures in traps baited with *C. tropicalis* or *S. cerevisiae* did not differ significantly ($t = 1.52; \text{df} = 14; P = 0.151$) (Figure 4b). In contrast, the use of a 5.5% sucrose solution as a growth medium resulted in higher captures in traps baited with *S. cerevisiae* than *C. tropicalis*, although this effect was borderline significant ($t = 2.14; \text{df} = 14; P = 0.051$) (Figure 4c).

Finally, the capture of *Z. indianus* in traps baited with apple cider vinegar alone was significantly higher than in traps baited with grape juice and *C. tropicalis* ($t = 3.30; \text{df} = 14; P = 0.005$) (Fig 5).

**Discussion**

Based on our results *Z. indianus* was most attracted to orange colored traps with brown rings around the access holes. Among the lures evaluated, the most effective one was apple cider vinegar that was more attractive than combinations of yeasts, fruit juices and other vinegars in different experiments.

The shape, size and color of a trap influences fruit fly attraction, especially when traps resemble the host fruits in which females oviposit (Prokopy...
Vinegars have been shown to attract drosophilid pests, although vinegars have not been widely tested against *Z. indiana*. Our laboratory results suggest that vinegars, particularly apple cider vinegar, are effective lures for *Z. indiana*. Apple cider vinegar was significantly more attractive than sugarcane vinegar whereas wine vinegars elicited intermediate levels of attraction. Traps baited with apple cider vinegar to monitor *D. suzukii* populations trapped considerable numbers of *Z. indiana* adults in cherry and guava crops in Pennsylvania (Joshi et al. 2014) and Mexico (Lasa et al. 2017). Apple cider vinegar was significantly more attractive than grape juice, which was previously considered to be an effective lure for *Z. indiana* (Epsky and Gill 2017).

In our study, apple cider vinegar baited traps also captured higher numbers of flies than grape juice fermented with *C. tropicalis*. The period of fermentation of juice-based lures could influence attraction, but for *D. suzukii*, fermentation of sucrose solution with *S. cerevisiae* over a 24 h period was more attractive than apple cider vinegar (Lasa et al. 2017), whereas 2–4 days of ambient fermentation was considered optimal for attraction of *Z. indiana* to grape juice (Epsky and Gill 2017). However, the differences of attraction between an induced or natural fermentation will likely vary, not only in the duration of the fermentation process but also in the microorganisms responsible for the fermentation.

Insects display species-specific responses to different blends of fermentation products (Landolt and Alfaro 2001), and specific ecological interactions between insects and microbes can be of great value in the development of targeted pest monitoring or control techniques (Hamby and Becher 2016). However, the volatile profile of a fermentation depends on the yeast species and strain and the type of growth media, among several other factors (Dzialo et al. 2017). Our tests, designed to compare attraction to two different yeasts in different growth media, revealed both yeast and media-based differences in attraction. The yeast *C. tropicalis* grown in YPD captured nearly twice as many flies as *S. cerevisiae* grown in YPD, whereas the opposite pattern was observed when these yeasts were inoculated in sucrose solution. However, adding yeasts to grape juice or sucrose solution did not improve the capture rates of traps over that of traps baited with apple cider vinegar.

In a previous study in Florida mainly targeted at *D. suzukii*, captures of *Z. indiana* were similar in traps baited with rice vinegar + wine or yeast in sucrose solution whereas the highest captures were observed in traps containing a mixture of apple cider vinegar, sucrose, wheat flour and *S. cerevisiae* (Iglesias et al. 2014).

In Mexico, trials targeted at *D. suzukii* using the 2C trap containing ACV and a *S. cerevisiae* + sucrose solution in the upper tube device, captured similar numbers of *Z. indiana* as a wheat flour + sucrose + *S. cerevisiae* mixture, and higher numbers of *Z. indiana* flies than ACV alone (Lasa et al. 2017).

A 50% dilution of fig juice in water is recommended for controlling and monitoring *Z. indiana* populations in Brazil (Pasini et al. 2011). That said, the rarity of fig fruits and fig juice in supermarkets in Mexico prevented comparison of these products with apple cider vinegar in the present study.
Nonetheless, given its marked preference for figs, attractants based on natural or synthetic fig fruit volatiles might prove useful for the development of effective and selective lures targeted at this pest. 

Interest in managing this pest will probably increase in the future as it continues to spread across the USA (Joshi et al. 2014), Europe (Carles-Tolrá 2009; Kremmer et al. 2017) and the Middle East (Al Jaboory and Katheh-Bader 2012). The present study demonstrated that trap color is important in the attraction of this pest. An orange trap with brown circles around the access hole, baited with apple cider vinegar, may be a useful trap-lure combination for field testing in Z. indianus-infested crops.

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Disclosure statement

We have no conflict of interest with respect to any of the products mentioned in this study.

References


