

Trapping *Drosophila repleta* (Diptera: Drosophilidae) using color and volatiles

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Abstract

Color and volatile stimulus preferences of *Drosophila repleta* (Patterson) (Diptera: Drosophilidae), a nuisance pest of swine and poultry facilities, were tested using sticky card and bottle traps. Attractions to red, yellow, blue, orange, green, purple, black, grey and a white-on-black contrast treatment were tested in the laboratory. *Drosophila repleta* preferred red over yellow and white but not over blue. Other than showing preferences over the white control, *D. repleta* was not observed to have preferences between other colors and shade combinations. Pinot Noir red wine, apple cider vinegar, and wet swine feed were used in volatile preference field trials. Red wine was more attractive to *D. repleta* than the other volatiles tested, but there were no differences in response to combinations of a red wine volatile lure and various colors. Odor was found to play the primary role in attracting *D. repleta*.

Key Words: *Drosophila repleta*; color preference; volatile preference; trapping

Resumen

Se evaluaron las preferencias de estímulo de volátiles y color de *Drosophila repleta* (Patterson) (Diptera: Drosophilidae), una plaga molesta en las instalaciones porcinas y avícolas, utilizando trampas de tarjetas pegajosas y de botella. Su atracción a los tratamientos de color rojo, amarillo, azul, anaranjado, verde, morado, negro, gris y un contraste de blanco sobre negro fue probado en el laboratorio. *Drosophila repleta* prefirió el rojo más que el amarillo y el blanco, pero no sobre el azul. Aparte de mostrar una preferencia por el control de color blanco, no se observó que *D. repleta* tiene alguna preferencia entre los otros colores y combinaciones de tonos. Se utilizaron el vino tinto Pinot Noir, vinagre de sidra de manzana, y alimento para cerdos húmedo en los ensayos de campo de preferencias volátiles. El vino tinto fue el más atractivo para *D. repleta* de los otros volátiles probados, pero no hubo diferencias en la respuesta a la combinación de un señuelo volátil de vino tinto y los colores. Se encontró que el olor juega un papel principal en atraer *D. repleta*.

Palabras Clave: *Drosophila repleta*; preferencia de color; preferencia volátil; atraqueo

Drosophila repleta (Patterson) (Diptera: Drosophilidae), commonly referred to as dark-eyed fruit fly or dark-eyed vinegar fly, is a synanthropic species of Nearctic-Neotropical origin (Ashburner et al. 1981). The larvae of these flies feed on yeast in decaying and fermenting organic matter (Wegner 2007). Given this ecological niche, it is no surprise that these flies have become pests in various settings where food debris is present. In the agricultural setting, *D. repleta* can be a nuisance pest in poultry and swine facilities where they feed on spilled animal feed (Harrington & Axtell 1994). *Drosophila repleta* has been shown to disperse from pit privies into nearby houses from about 1,000 feet (305 m) away (Pimentel & Fay 1955). Dispersal of flies from swine facilities to local residences may be of some concern because *Drosophila* species can carry pathogens (Ewing 1962). Excessive fly populations could lead to litigation from homeowners (Hayes 1993). In addition to animal facility infestations, *D. repleta* is a nuisance pest in restaurants, bars, food and beverage processing facilities, and hospitals (Wegner 2007).

There has been little research to examine methods to monitor and manage *D. repleta*. While attractiveness of volatiles has been examined in *D. repleta*, direct comparisons between stimuli are lacking. Baits consisting of overripe bananas, fermenting applesauce vinegar, stale beer, and a freeze-dried banana tea bag lure were all attractive to *D. repleta*

(Pimentel & Fay 1955; Birmingham et al. 2011). Studies on *Drosophila* in general, primarily *D. melanogaster* Meigen and *D. sukii* (Matsumura), have found volatiles produced from fermenting fruit and vinegar to be attractive (Barrows 1907; Reed 1938; West 1961; Zhu et al. 2003; Stökl et al. 2010; Cha et al. 2012; Landolt et al. 2012; Cha et al. 2014). *Drosophila hydei* Sturtevant is not only attracted to fermented fruit, such as wine, but also fermented grains such as beer (Wheeler 1971).

No color preference studies have been performed on *D. repleta*, but other *Drosophila* species have been examined. A color-baited (10% granulated sugar, 1% apple cider vinegar, 4% yeast, 0.5% lindane wettable powder, and water) trap study by Wave (1964) found red to be the color that was most attractive to *D. melanogaster*. An apple cider vinegar jar trap study on *D. sukii* observed that jars with red and black caps caught more flies than white capped jars (Basoalto et al. 2013). Another study done on *D. sukii*, however, did not find any statistical evidence for color preference with baited traps (Oregon State University 2012).

Color, odor, or both color and odor may be useful to attract adult flies into a container or onto a sticky surface. Trapping adults with these attractants may be a viable non-insecticidal option for managing *D. repleta*. Based on previous experiments performed with other

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Drosophila species, this study examined color preference, volatile attractiveness, and a combination of both in a feral population of *D. repleta* at the University of Illinois Urbana-Champaign Imported Swine Research Facility (UI Swine Facility).

Materials and Methods

TEST INSECTS

Drosophila repleta used for laboratory experiments were caught using an electric aspirator (Hausherr's Machine Works, Toms River, New Jersey) at the University of Illinois Swine Facility and placed in holding vials before each experiment. To confirm the species identity of the field population, David Grimaldi at the American Museum of Natural History keyed flies out morphologically. A molecular marker, cytochrome c oxidase I (CO1) (Hottel 2011), was also used for species identification. Voucher specimens have been deposited in the Insect Collection of the Illinois Natural History Survey at the University of Illinois, Urbana-Champaign (INHS Insect Collection #557,616; #557,617; and #557,618).

COLOR CARDS

Red, yellow, blue, green, orange, purple, black, grey, and white card stock (Hobby Lobby, Champaign, Illinois) was used in both lab and field trials. Wavelength and reflectance of these colors were quantified using an Ocean Optics USB 2000 spectrophotometer (Ocean Optics, Dunedin, Florida) (Fig. 1). Color cards were cut to 12.7cm × 12.7cm squares for the lab experiments and 20.3cm × 26.7cm for the field trials. Cards were hot glued to 16.5 cm × 14.0 cm pieces of transparency film for laboratory experiments; the pieces were laminated for use in field trials. Tanglefoot Tangle-trap® insect trap coating (The Tanglefoot Company, Grand Rapids, Michigan) was evenly applied on top of the transparency film or the plastic-laminated card before mounting on the side of the laboratory color preference chamber or on the walls of the swine facility. Response to color was measured as the number of flies caught on sticky cards.

LABORATORY COLOR PREFERENCE

Preference tests with various colors were grouped into 3 different experiments. Red, yellow, blue, and white were tested in experiment 1. Orange, purple, green, and white were tested in experiment 2. Experiment 3 evaluated attraction to contrast by presenting black, grey, and white cards along with a white card (10.2 cm × 10.2 cm) placed in the center of a black card (12.7 cm × 12.7 cm). Color cards were randomly assigned to a position on one of the 4 walls of a 61.0 cm × 61.0 cm × 30.5 cm clear plastic chamber. Positions were re-randomized for each

replication. Two fly-rearing vials (Genesee Scientific, San Diego, California) were placed in the chamber before starting the experiments to supply the flies with food and water. One vial was filled with 25 mL of instant fly diet (Carolina Biological, Burlington, North Carolina) mixed with 20 mL of water, and the other vial was filled with 20 mL of water and plugged with a cotton ball. A white sheet was placed over the chamber to homogenize the external visual background. Temperature was maintained at 22.2 ± 0.8 °C and relative humidity ranged between 62-75% during the trials. Lights were left on for the entire duration of the experiment (24 h) because the room at the UI Swine Facility did not have a light cycle. A total of 50 swine-facility *D. repleta* of mixed age and sex were caught and placed in a rearing vial. The flies were sight identified to species using a key to common domestic species of *Drosophila* (Wheeler 1971). The rearing vials were placed into the chambers and the flies released shortly after the flies were captured. This was repeated 6 times for each color/contrast combination. The number of flies caught on the cards was counted after 24 h.

FIELD VOLATILE PREFERENCES

Four clear 3.5 L jar traps (Starbar® CAPTIVATOR® fly traps, Wellmark International, Schamburg, Illinois) were suspended from ceiling pipes in the center of the grower room. Flies entered the trap through a 5.1 cm diameter opening and were unable to relocate the opening once they were inside the jar trap. Each trap was spaced at least 3.7 m apart and was placed at least 1.2 m from the nearest wall. Each jar trap was randomly assigned either 100 mL of apple cider vinegar (Schnucks, St. Louis, Missouri), or 100 mL of a 2008 Pinot noir red wine (Alice White, Madera and Woodbridge, California), or a mixture of 100 mL water and 100 mL of swine feed, or was left empty without any contents. The swine feed was a soybean and corn based diet (soybean meal, dical, lime, swine TM, Vitmix ADEK tylan-40, lysine, corn, and qualfat) made by the University of Illinois Feed Mill (Champaign, Illinois). Treatments were re-randomized for each replication. *Drosophila repleta* were sight identified as described in the laboratory color experiment and counted in each jar trap after 24 h. The experiment was repeated 4 times. Experiments were performed from late Aug to mid Sep in 2010.

FIELD COLOR PREFERENCE WITH RED WINE LURE

Paper funnel traps were designed in a similar manner to previous trapping studies performed on *Drosophila* (Hunter et al. 1937; Mason et al. 1963). Eight polypropylene fly rearing bottles (Genesee Scientific, San Diego, California) were each filled with 25 mL of Pinot noir red wine. Color cards were rolled into cones and the tip of the cone was inserted into the openings of the bottles. Red, yellow, blue, green, orange, purple, white, and white on black cards (17.5 cm × 7 cm) were used. Bottles were randomly assigned a position in a 2 by 4 grid with each bottle spaced 22 cm apart and placed on the floor of 1 of 3 empty swine stalls. Positions were re-randomized for each group of bottles tested. Bottles were collected after 24 h and *D. repleta* were sight identified and recorded as performed in the previous 2 experiments. Two to 3 of the empty swine stalls were used simultaneously over the 24 h time period. Temperature ranged from 21.2 °C to 24.2 °C. Relative humidity was < 15%. Seven groups of bottle traps were tested in total in mid Nov 2010.

STATISTICAL ANALYSIS

Both the laboratory color and field volatile experiments were analyzed using a single-factor randomized complete block design ANOVA model at $\alpha = 0.05$. Blocks were designated as random factors. The field color preference with a red wine lure experiment was analyzed using a single-factor ANOVA with the empty stall location and day tested set as random factors. If the ANOVA was significant, a least-squares means pairwise comparison was performed with a Bonferroni correction. To meet ANOVA assump-

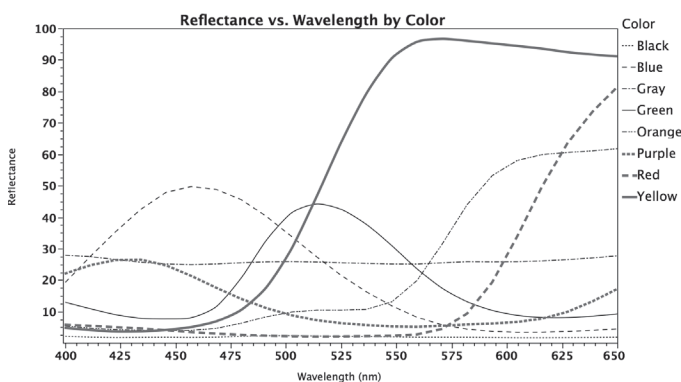


Fig. 1. Spectrophotometer analysis of colors used in laboratory and field experiments.

tions, laboratory data were square root transformed before analysis, while data from both the field volatile and color/volatile attractant trials were log transformed ($\log_{10}[x+1]$). Data were analyzed using R statistical software stats, lme4, and lsmeans packages (R Development Core Team 2014).

Results

LABORATORY COLOR PREFERENCE

Color (red, yellow, blue, and white) was observed to have a significant effect on the number of *D. repleta* caught in experiment 1 ($F = 7.50$; $df = 3, 20$; $P = 0.001$). Red caught more flies than both white and yellow but not significantly more than blue color cards (Table 1). Color (orange, green, purple, and white) also has a significant effect on the number of *D. repleta* caught in experiment 2 ($F = 4.76$; $df = 3, 20$; $P = 0.012$). More flies were caught on orange, purple, and green cards than on white sticky cards (Table 1). A significant preference among the contrasting sticky cards was found in laboratory experiment 3 ($F = 5.74$; $df = 3, 20$; $P = 0.005$). The white on black sticky cards caught more flies than the white colored sticky cards (Table 1). There were no significant differences among the number of flies caught on black, grey, and white on black cards.

FIELD EXPERIMENT 1: VOLATILE PREFERENCE IN THE FIELD

More than 21,000 total flies were caught in field experiment 1. In addition to the *D. repleta* caught, 10 flies of various species were captured. Bait treatment was found to have a significant effect on the number of *D. repleta* caught ($F = 101.0$; $df = 2, 6$; $P < 0.0001$) (Table 2). Pinot noir red wine caught more *D. repleta* than the swine feed, apple cider vinegar or the control ($P < 0.001$ for all 3 factors). Swine feed, where *D. repleta* normally feed and oviposit, was more attractive than both the control and vinegar ($P = 0.002$ and $P = 0.034$, respectively). Vinegar traps did not catch more *D. repleta* than the control traps ($P = 0.244$).

FIELD EXPERIMENT 2: COLOR PREFERENCE WITH A VOLATILE ATTRACTANT

Almost all of the insects caught in field experiment 2 were identified as *D. repleta*. One wasp and a muscid fly were also caught during the experiment. Color was not found to have a significant effect on the number of *D. repleta* caught when used in combination with the red wine lure. ($F = 0.502$, $df = 7, 42$; $P = 0.828$) (Table 2). The use of any color elicited a higher number of flies caught than the white treatment, with the numerically highest number caught in the white on black treatment.

Table 1. Mean number of *Drosophila repleta* caught on colored sticky cards in plastic laboratory chambers ($n = 6$).

Experiment	Stimulus treatments	Flies captured \pm SE*
Lab Experiment 1	Red	7.50 \pm 0.81 a
	Blue	5.67 \pm 1.12 ab
	Yellow	3.50 \pm 0.62 b
	White	2.50 \pm 0.34 b
Lab Experiment 2	Purple	7.50 \pm 1.88 a
	Orange	7.17 \pm 1.25 a
	Green	6.50 \pm 0.72 ab
	White	2.33 \pm 0.49 b
Lab Experiment 3	White on Black	10.8 \pm 1.38 a
	Black	5.83 \pm 1.30 ab
	Grey	5.67 \pm 1.23 ab
	White	3.33 \pm 0.80 b

*Means within an experiment with the same letter are not significantly different, least-squares means pairwise comparison, with Bonferroni correction at $P \leq 0.05$. Data were square root transformed before being analyzed.

Table 2. Mean numbers of *Drosophila repleta* captured in bottle traps containing volatile lures (field experiment 1, $n = 4$) and in colored bottle traps filled with red wine (field experiment 2, $n = 7$).

Experiment	Stimulus treatments	Flies captured \pm SE*
Field Experiment 1	Wine	5289 \pm 3371.4 a
	Swine Feed	32.3 \pm 29.1 b
	Vinegar	3.50 \pm 3.51 c
	Empty Control	0.25 \pm 0.50 c
Field Experiment 2	White on Black	9.00 \pm 4.79 a
	Orange	5.71 \pm 1.38 a
	Red	5.57 \pm 1.38 a
	Yellow	5.14 \pm 2.02 a
	Purple	5.00 \pm 1.18 a
	Blue	3.86 \pm 1.06 a
	Green	3.57 \pm 1.15 a
	White	0.25 \pm 0.50 a

*Means within an experiment with the same letter are not significantly different, least-squares means pairwise comparison, with Bonferroni correction at $P \leq 0.05$. Data were log transformed before being analyzed.

Discussion

Flies other than *D. repleta* such as *Musca domestica* L. (Diptera: Muscidae) and *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) are attracted to red and often to black (Waterhouse 1948; Pospisil 1962; Prokopy 1968). However, some of these attractions were not based on color, but on the contrast of the "colored" object with its surrounding environment (Prokopy 1968; Howard & Wall 1998). This study examined the effects of contrast, by testing white, black and grey versus white on a black background contrast treatment. Interestingly, more *D. repleta* were attracted to the white with black border contrast treatment than by the white. This attraction phenomenon was also reported in *M. domestica*. Why both these fly species are attracted to high contrast borders is uncertain (Howard & Wall 1998).

Previous research on other *Drosophila* species found red color and volatile compounds associated with fermenting fruit or vinegar to be highly attractive (Barrows 1907; Reed 1938; West 1961; Wave 1964; Zhu et al. 2003; Stökl et al 2010; Cha et al. 2012; Landolt et al. 2012). Color preferences of red over either yellow or white were also observed in *D. repleta* but these results could not be replicated in preliminary field trials (BAH, unpublished data). Red, yellow, blue, and white sticky cards placed in the UI Swine Facility caught very few flies despite there being thousands of flies in the nearby vicinity. Given the success of the field experiments with volatiles in capturing flies, more effort was dedicated to discovering whether or not a color preference existed in the presence of a successful volatile lure. When used with a lure, a color preference was not observed. These findings are similar to the lack of color preference found in one study on *D. suzukii*, but they conflict with studies that observed color preferences in *D. suzukii* (Oregon State University 2012; Basoalto et al. 2013). The higher trap catch observed in our field volatile preference experiment versus the experiment that examined both color and volatiles reflects the lower population of flies available at the UI Swine Facility in Nov when the color and volatile field experiments were taking place. The change in trap types in the 2 field experiments may have also contributed to much lower trap catches in the Nov field trial.

Volatiles used in the experiment were selected both for their past history as suitable drosophilid attractants, and also because they are safe to use around livestock. Because the flies in the UI Swine Facility use the swine feed as both an oviposition and feeding substrate, the swine feed was used as a benchmark for comparison with other treatments.

Drosophila repleta were observed to have a strong attraction to red wine over all other volatiles tested. Many other *Drosophila* have been found to be attracted to wine, beer, and whiskey production facilities, so it was not surprising that these flies are also attracted to wine (Kaneko et al. 1966). Despite recent experiments on other *Drosophila* species showing attraction to vinegar (Becher et al. 2010; Cha et al. 2012; Landolt et al. 2012). *D. repleta* showed little response to vinegar volatiles. Even more perplexing is the fact that wine and vinegar release several common volatile compounds. In a study done on the gas chromatography electroannographic detection (GC-EAD) responses of *D. suzukii* to Merlot wine and rice vinegar volatile compounds, all vinegar compounds that elicited GC-EAD responses were also found in wine (Cha et al. 2012). These compounds included: acetic acid, ethanol, ethyl acetate, acetoin, ethyl lactate, isoamyl acetate, 2-methylbutyl acetate, grape butyrate, and 2-phenylethanol. Compounds that were unique to wine included: ethyl butyrate, 1-hexanol, methionol, isoamyl lactate, ethyl sorbate, and diethyl succinate. An examination into how these various red wine compounds affect *D. repleta* behavior could lead to a successful synthetic attractant as was accomplished with *D. suzukii* (Cha et al. 2014).

These findings suggest there is potential for lures to be used in combination with sanitation to reduce *D. repleta* populations. Mass trapping is an IPM strategy that uses lures to attract and trap target pest species as a means of managing pest populations (El-Sayed et al. 2006). Attractants may include synthetic pheromones, food volatiles, or host attractants. Due to the high affinity of *D. repleta* to red wine, it could be used as an attractant in a mass trapping effort to manage these flies. A study done on the Mediterranean flour moth, *Ephestia kuehniella* Zellar (Lepidoptera: Pyralidae), in a flour mill found that a combination of sanitation measures, localized insecticide applications, and mass trapping not only helped reduce pest populations but decreased the need for insecticide fumigations (Trematerra & Gentile 2009). Similar measures could be implemented at wine facilities to help control *D. repleta* populations. Increased sanitation including regular removal of spilled feed, and distribution of red wine-baited bottle traps throughout a facility could decrease both larval and adult populations. Additional field trials will be required to assess mass trapping and increased sanitation on management of *D. repleta* in livestock facilities.

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