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ATTRACTING DARK-EYED FRUIT FLIES, *DROSOPHILA REPLETA* (DIPTERA:  
DROSOPHILIDAE), IN SWINE FACILITIES USING COLOR AND ODOR

BY

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THESIS

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## ABSTRACT

Color and volatile preferences of *Drosophila repleta* were tested using sticky card and bottle traps. Species identity of *D. repleta* was confirmed using morphological characters; DNA COI sequence comparisons were inconclusive. Attraction to primary and secondary colors was tested with sticky cards in the laboratory; only primary colors were tested in the field. Laboratory trials were performed in clear plastic chambers. Other sticky card combinations included white, black, and grey. A white card with a black border was tested to examine contrast effects. Field trials were conducted at the University of Illinois Imported Swine Facility. Pinot Noir red wine, apple cider vinegar, and wet swine feed (the on-site material fed on by the flies) were used in volatile preference field trials. While there appeared to be some color preference in laboratory trials, field color trials failed to replicate laboratory results. Red wine was extremely attractive to *D. repleta*, but there were no differences in response to colors when tested in combination with a red wine volatile lure ( $P>0.05$ ). It appears that odor plays a dominant role in attracting *D. repleta*, whereas color is less important.

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## CHAPTER 1: INTRODUCTION

### 1.1 BIOLOGY AND PEST STATUS

*Drosophila repleta* (Diptera: Drosophilidae), commonly referred to as dark-eyed fruit flies or dark-eyed vinegar flies, are a synanthropic species of Nearctic-Neotropical origin (Ashburner et al. 1981). This species is slightly larger and more darkly pigmented than the common fruit fly, *Drosophila melanogaster* (Fig. 1). The reproductive potential and development time also differ between the two species. While ovariole number isn't a sole indicator of reproductive potential, it does show how many eggs can potentially be produced (Markow and O'Grady 2006). *D. melanogaster* have a total of 43 ovarioles which is slightly more than the 36 ovarioles in *D. repleta* (Robertson 1957; Kambysellis and Heed 1968). From an egg, *D. melanogaster* develops to adult hood in 13 days while it takes *D. repleta* 3 more days to reach adulthood (Markow and O'Grady 2006). The larvae of *D. repleta* are commonly found in drains or cracks and crevices feeding on yeast in decaying and fermenting organic matter (Wegner 1997). These flies can become nuisance pests in restaurants and bars, and the flies are viewed as contaminants in food and beverage processing facilities (Wegner 1997). *D. repleta* have also been found to be nuisance pests in poultry and swine facilities (Harrington and Axtell 1994). In addition, *Drosophila* spp. are potential carriers of disease (Ewing 1962). The ability to mechanically transmit disease may be particularly concerning in hospital or other medical facility infestations (Wegner 1997).

### 1.2 RAISON D'ÊTRE

Given that the larvae of *Drosophila* feed in decaying food and other organic matter, cleaning up and getting rid of this food source will reduce or even eliminate fly populations (Potter 1994).

Sanitation will only get rid of larvae, but adults will still be present. Since the adults are perceived as the direct pest problem, it is important to also develop a strategy to eliminate the adult stage. While insecticides could be used to kill adult flies, insecticides may not be permitted in areas where the flies are located. Various trap designs using color, odor, or a combination of both to attract adult flies into a container or sticky surface are one non-chemical option of dealing with these flies. The trap must be able to compete with odors and visual signals in the flies' environment. Finding the right combination of stimuli to illicit a high attractive response is therefore imperative in the overall effectiveness of the trap.

## CHAPTER 2: REVIEW OF LITERATURE

### 2.1 ODOR, COLOR, AND SHAPE: HOW THESE VARIABLES AFFECT HERBIVOROUS DIPTERAN ATTRACTION

While *D. repleta* is certainly not considered a herbivore, studies performed on diptera that are agricultural pests display how important color, shape, and odor can be in attracting flies. In search of a shape and color that was attractive to apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae); Prokopy (1968) found small spheres colored red, blue, violet, dark orange, and black to be more attractive than other colors tested. The blueberry maggot, *Rhagoletis mendax* (Diptera: Tephritidae) also is attracted to spheres and was found to be most attracted to green, red, and blue in combination with an ammonia lure (Liburd et al. 1998). Color alone was tested on the oriental fruit fly, *Bactocera dorsalis* (Diptera: Tephritidae), and green and ultraviolet were found to be the most attractive (Wu et al. 2007). Harris and Miller (1988) found color, shape, and odor to all be important in host recognition by female onion flies, *Delia antiqua* (Diptera: Anthomyiidae). The study also found that chemicals were no more important than visual stimuli from shape and color in attracting these flies. The right color, shape, and odor can attract these herbivorous flies, who perceive these combinations of stimuli as a potential host plant. Since *Drosophila* feed on fermenting and decaying organic matter, visual and volatile stimuli may play a slightly different role in attraction.

### 2.2 VOLATILE ATTRACTION IN DROSOPHILA

Color preference and volatiles that attract *D. repleta* are unknown; however, there are many attractant studies on other drosophilids. Studies examining volatile attraction report *D. melanogaster* to be attracted to various volatiles produced from fermenting fruit or vinegar



(Barrows 1907; Reed 1938; West 1961; Zhu et al. 2003). Barrows (1907) found ethyl alcohol, acetic acid, and acetic ether to be attractive to *D. melanogaster* (referred to as *D. ampelophila* in the study). Ethyl alcohol, acetic acid, and acetic ether are all compounds common in fermenting fruit (Barrows 1907). Barrows also found that a ratio of ethyl alcohol to acetic acid that proved to be most attractive was similar to the ratio of ethyl alcohol to acetic acid in apple cider vinegar. Reed (1938) expanded upon Barrows study and found further evidence that ethyl alcohol and acetic acid alone were attractive at varying concentrations. These compounds, however, were less attractive than an actual fermenting banana. West (1961) found that a 25% malt extract solution was the most attractive to *D. melanogaster*, *D. virilis* and *D. pseudoobscura*. West also discovered that a house fly, *Musca domestica* (Diptera: Muscidae), bait consisting of 5% liquid malt, 0.5% ethyl alcohol, and 0.02% skatole with the addition of 1% acetal was the most attractive combination to *D. melanogaster* in field trials. Interestingly acetic acid was not as attractive as other compounds tested with *D. melanogaster*, *D. virilis*, and *D. pseudoobscura*. Zhu et al.(2003) found an aqueous solution of ethanol, acetic acid, and 2-phenylethanol in a 1:22:5 ratio to be more attractive than non-blended chemical volatiles. The blend itself was based on overripe mangos which were found to be more attractive than other fruits such as bananas, strawberries and grapes. While the blend fared well in the laboratory, it was less attractive in field trials.

Flowers with volatiles that mimic fermenting fruit are also attractive to drosophilids. The flower, *Arum palaestinum* (Alismatales: Araceae), that releases chemicals associated with yeast found in fermenting fruit to attract deceived pollinating drosophilids. These drosophilids include:

*Drosophila simulans*, *D. melanogaster*, *D. subobscura*, *D. hydei*, *D. immigrans*, *D. busckii*, *Zaprionus tuberculatus*, and *Z. indianus* (Stökl et al. 2010). The blend used by *A. palaestinum*

consists of 2,3-butanediol acetate, acetoin acetate, ethyl hexanoate, hexyl acetate, 2-phenylethyl alcohol and 2-phenethyl acetate. In addition to these studies, Wheeler (1971) noted that *D. hydei* is attracted to the odors of beer and wine.

### 2.3 LURED AND NON LURED COLOR ATTRACTION IN *DROSOPHILA*

Few studies have examined color attraction in combination with some type of volatile attractant. Most *Drosophila* vision studies are focused on physiological aspects of vision, such as wavelength discrimination showing true color vision or the utility of various photoreceptors (Hernández de Salomon and Spatz 1982; Yamaguchi et al. 2010). A color-baited trap study done by Wave (1964) found red to be the most attractive color for catching *D. melanogaster* when using a bait mixture described by Mason et al. (1963) of 10% granulated sugar, 1% apple cider vinegar, 4% Fleischmann's® active dry yeast, 0.5% lindane wettable powder, and water. In *Drosophila suzukii* color attraction was tested with color cups filled with soap water (Lee 2010). Red and black were found to be more attractive than all other colors tested except for orange.

Taking into account these past experiments performed on other *Drosophila*, this study will examine color preference, volatile attraction, and lured color preference in a wild population of *D. repleta* at the University of Illinois Urbana-Champaign Imported Swine Research Facility (UI Swine Facility). Molecular and morphological species identification methods were also performed to confirm species identification of field collected flies.

## CHAPTER 3: METHODS

### 3. 1 TEST INSECTS AND SPECIES IDENTIFICATION

Wild *D. repleta* were collected from the UI Swine Facility using an electric aspirator and frozen. Flies captured at this field site were compared genetically to a laboratory strain of *D. repleta* (Yucatan, Mexico stock #15084-1611.02; San Diego Species Stock Center) using the cytochrome c oxidase I (CO1) sequence. The wild *D. repleta* CO1 sequence was also compared to other CO1 sequences in GenBank using BLAST. DNA was extracted using a DNeasy blood and tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The PCR protocol of Mardulyn and Whitfield (1999) was followed. PCR primers used were Ben (5'-GCWACWACRTAATAKGTATCATG-3') and LCO (5'-GGTCAACAAATCATAAAGATATTGG-3'). Sequences were edited using BioEdit software (Hall 2007). Geneious Pro was used to align sequences with MUSCLE and create a neighbor-joining tree (Edgar 2004; Drummond et al. 2010). The tree was edited using FigTree (Andrew Rambaut 2009) and Inkscape (Inkscape Team 2011). Both laboratory and field strains were also keyed out morphologically by David Grimaldi at the American Museum of Natural History. 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; 557,618).

### 3. 2 EXPERIMENTAL CHAMBER

Wild caught *D. repleta* from the UI Swine Facility were tested for color preference in a clear plastic 5.08cm x 5.08cm x 2.54cm five sided chamber (Fig. 2). The chamber was placed on top of a white poster board covered in a white plastic table cover. A 10.15cm diameter hole was

present on top of the chamber to allow operator access to the arena. During testing the hole was covered with a clear piece of transparency film. A white cotton sheet was placed over the chamber to diffuse light and block external visual interference. The lighting consisted of 32 watt Philips high performance fluorescent lights.

### *3.3 COLOR CARDS*

Red, yellow, blue, green, orange, purple, black, gray, and white card stock (Hobby Lobby, Champaign, Illinois, 61820) were used in both laboratory and field trials. The reflectance from each color was analyzed using a spectrophotometer (Fig. 3). Color cards were cut to 12.7cm x 12.7cm squares for the laboratory experiment and 26.67cm x 20.32cm for the field trials. The cards were glued, using a hot glue gun, to 16.51cm x 13.97cm 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, MI 49504) was evenly applied on top of the transparency film or the plastic-laminated card prior to mounting on the side of the chamber or on the walls of the swine facility.

### *3.4 EXPERIMENTAL LABORATORY PROTOCOL COLOR PREFERENCE*

Colors were grouped into three different experimental sets. Red, yellow, blue, and a white control were tested in the primary color trials. Orange, purple, green, and a white control were tested in a second set. A third set of trials evaluated contrast by comparing flies caught on black, gray, a 10.16cm x 10.16cm white card on top of a 12.7cm x 12.7cm black card (a contrast treatment), and a white control. Color cards in each set were shuffled to randomize their order before placement in the center of the walls of the chamber in a clock-wise fashion. The cards were attached to the walls using drops of hot glue. Two vials holding a total of twenty-five flies

were released into the chamber. Neither  $\text{CO}_2$  nor ether were not used to anesthetize the flies at any point during the experiments. The vials were opened and placed in the center of each chamber. Food was prepared in fly-rearing bottles (Genesee Scientific, San Diego, CA, 92126). The food consisted of 20ml of instant fly diet (Carolina Biological, Burlington, NC, 27215), 25ml of water, yeast, and opuntia powder. One vial holding 20ml of water with a flug attached to the end was added as a water source. The food and water vials were placed next to fly vials in the chamber. Temperature was maintained at  $22.15 \pm 0.75$  °C and relative humidity ranged between 62-75% during the trials. A light cycle was not used for these experiments. The facility where the flies were caught did not have a light cycle. The flies were released from their vials into the chambers and the number of flies caught on the cards was observed after 24h. Six replications were performed for primary color, secondary color, and contrast experiments.

### *3.5 FIELD SITE*

Field experiments were conducted in the grower room of the UI Swine Facility (Fig. 3). The grower room houses swine aged 9 to 16 weeks old. The room is enclosed and temperature is regulated using an automated fan and water cooling system. Temperature and humidity were recorded throughout all experiments. Temperature varied between 71.6°C and 82.0°C. Relative humidity ranged between <15% and 64%. No pesticides were applied in this room.

Experimental design and protocols were limited by various animal care constraints in the UI Swine Facility. Placement of traps was highly dependent on where swine were located in the room. Sticky traps had to be high enough or separated by a barrier so that the swine could not access the traps. Jar trap placement was limited to pipes on the ceiling from which traps could

be hung in the grower room. Air turbulence from fan ventilation system also limited the placement of many traps.

### *3.6 EXPERIMENTAL FIELD PROTOCOL COLOR PREFERENCE*

Red, yellow, blue, and white 21.59cm x 27.94cm sticky traps were placed on a wall 91.44cm above the floor of an empty swine stall between two occupied stalls (Fig. 4). The horizontal placement of traps across the wall was randomized (Fig. 5). After 24h, the traps were retrieved and the number of *D. repleta* were counted.

### *3.7 EXPERIMENTAL FIELD PROTOCOL ODOR PREFERENCE*

Four jar traps (Starbar® CAPTIVATOR® fly traps, Wellmark International, Schamburg, IL, 60173) were hung at different locations in the room (Fig. 5; Fig. 6). One jar in all trials was left empty as a negative control. In the first set of trials, jars were filled either with 100ml of apple cider vinegar, 100ml of a Pinot noir red wine (Alice White, Madera and Woodbridge, CA), or a mixture of 100ml water and 100ml of swine feed. 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, IL). Flies were counted in each jar trap after 24h. Experiments were performed from late August to mid September in 2010. Four replications were performed.

### *3.8 EXPERIMENTAL FIELD PROTOCOL COLOR PREFERENCE WITH VOLATILE ATTRACTANT*

Eight polypropylene fly rearing bottles (Genesee Scientific, San Diego, CA, 92126) were each filled with 25ml of Pinot noir red wine. Color cards were rolled into cones and inserted into the openings of the bottles.. Red, yellow, blue, green, orange, purple, white, and white on black cards (17.5cm x 7cm ) were used. In empty swine stalls, bottles were placed randomly in two

rows 22cm apart (Fig. 7). Bottles were collected after 24h and the number of flies caught in the bottles was recorded. Temperature ranged from 70.2°C to 75.6°C. Relative humidity stayed under 15%. Experiments were performed in mid November 2010. Seven replications were performed.

### 3.9 STATISTICAL ANALYSIS

Data from all experiments were analyzed using a single factor ANOVA at  $\alpha=0.05$ . If the ANOVA was significant, a Tukey test was performed to make pairwise comparisons between treatment means. Data from the laboratory color bioassays were square root transformed ( $\sqrt{X + 0.5}$ ) before analysis. Data from both the field volatile, and color combined with a volatile attractant trials were log transformed ( $\log_{10}(X + 1)$ ) before analysis. All data were tested for normality using the Shapiro-Wilk test and equal variance with the Levene test ( $P<0.05$ ). Data were analyzed using R statistical software *stats* package (R Development Core Team 2011) and graphs were generated using the *sciplot* package (Morales et al. 2010), *ggplot2* package (Wickham 2009), and Microsoft Paint. Images were edited using Adobe InDesign CS5.5 (Adobe Systems Incorporated 2011).

## CHAPTER 4: RESULTS

### 4.1 SPECIES IDENTIFICATION

Genetic distance analysis revealed a 6% variation in the COI sequences between field-collected *D. repleta* and laboratory strains and a 0.1% variation within field or within laboratory strain flies. Furthermore, N-J analysis clustered all field specimens consistently in a separate clade to laboratory strains (Fig. 8). *D. repleta* from the Tokyo Metropolitan University (Wang et al. 2006) were the closest match using BLASTn at 94% in the field population and 99% similarity in the laboratory strain. Despite the large variation found in the COI sequence between field and laboratory strains, both were keyed to *D. repleta* based on morphological characteristics (Vilela 1983; D. Grimaldi, pers. comm.)

### 4.2 COLOR PREFERENCE IN THE LABORATORY

In the primary color trials (Fig. 9), red caught significantly more flies than yellow and white ( $P_{red-yellow}=0.00787$ ,  $P_{red-white}=0.00099$ ). Red was not different from blue ( $P_{red-blue}=0.36189$ ). In the secondary color assays (Fig. 10), purple and orange caught significantly more flies than the control but not more than green ( $P_{purple-white}=0.03131$ ,  $P_{orange-white}=0.04704$ ,  $P_{purple-green}=0.93537$ ,  $P_{orange-green}=0.97927$ ). A significant difference was not found between black, white, or grey and the contrast experiment at  $P<0.05$  (Fig. 11). However, the white on black contrast treatment did catch significantly more flies than the black, grey, and white cards ( $P_{wb-black}=0.03656$ ,  $P_{wb-grey}=0.02971$ ,  $P_{wb-white}=0.00137$ ).



#### 4.3 COLOR PREFERENCE IN THE FIELD

No significant difference was found between colors in the field trials. A total of only 11 flies were caught in all of the color preference field trials.

#### 4.4 ODOR PREFERENCE IN THE FIELD

Pinot noir red wine caught significantly more flies than the swine feed, apple cider vinegar and the control ( $P_{\text{wine-feed}}=0.00001$ ,  $P_{\text{wine-vinegar}}<0.00001$ ,  $P_{\text{wine-control}}<0.00001$ ) (Fig. 12).

Swine feed, where flies normally feed and oviposit, was more attractive than the control ( $P=0.00549$ ).

#### 4.5 COLOR PREFERENCE WITH A VOLATILE ATTRACTANT

No difference was found between any color tested with volatiles ( $F=0.4568$ ,  $P=0.8606$ ) (Fig. 13). Variability in fly captures in the white on black bottle traps was large and unexplained. Alternative parametric and non-parametric analyses also failed to detect statistical significance (i.e. Welch ANOVA and the Kruskal-Wallis rank sum test ( $F=0.5643$ ,  $P=0.776$ ;  $\chi^2=3.5198$ ,  $P=0.8331$ )).

## CHAPTER 5: DISCUSSION

While there are no previous studies examining color and volatile preference in *D. repleta*, studies performed on other *Drosophila* found red and volatile compounds associated with fermenting fruit or vinegar to be the most attractive (Barrows 1907; Reed 1938; West 1961; Wave 1964; Zhu et al. 2003; Lee 2010; Stökl et al 2010). In this study, laboratory color trials also found red to be the most attractive primary color, although not statistically distinguishable from blue. A preference between secondary colors could not be found.

Other flies such as *M. domestica* and *Rhagoletis pomonella* (Diptera: Tephritidae) are attracted to red and also usually black (Waterhouse 1948; Pospisil 1962; Prokopy 1968). However, some of this attraction was not based on color, but on the contrast of the “colored” object with its environment (Prokopy 1968; Howard and Wall 1998). This study also examined the effects of contrast, by testing white, black, and grey sticky cards on a white background. A fourth white sticky card with a black border was also used. Interestingly, while this study failed to show a preference among contrasts between white, black, and grey; there was an attraction to sticky cards that were white with a black border. This border attraction phenomenon was also reported in *M. domestica*; why both these fly species are attracted to high contrast border is uncertain (Howard and Wall 1998). The bordered cards could be perceived as an exit from the enclosure. Color preferences discovered in the laboratory could not be replicated in the field with or without a lure. Based on color preference field trials, color may not provide any significant attraction for *D. repleta*. The lack of color preference may be due to the vast range of possible visible features associated with decaying and fermenting organic matter that *D. repleta* normally seeks out in the wild. Volatiles released by these feeding and oviposition substrates, may have more consistency.

Volatiles used in the experiment were selected both because of their past history as good attractants to drosophilids, but also because they were safe to use around livestock. Since the flies in the UI Swine Facility use the swine feed as an oviposition and feeding substrate, the swine feed was used as a benchmark to see if any of the other volatiles were more attractive. These data show that *D. repleta* were highly attracted to red wine and it was preferred over all other tested volatiles. Many other *Drosophila* have been found to be attracted to wine, beer, and whiskey production facilities, so it is not surprising that these flies are also attracted to wine (Kaneko et al. 1966).

The commonality that many *Drosophila*, including *D. repleta*, are attracted to alcoholic beverages makes controlling these flies much easier. The importance of knowing what specific *Drosophila* species you are dealing with may not be important when using red wine as a lure. This generality of red wine attraction in *Drosophila* is especially important in this study because of the present uncertainty of the identity of the *Drosophila* at the UI Swine Facility. Given the high COI variation found between the *D. repleta* from the UI Swine Facility and the laboratory *D. repleta* strain from the San Diego Species Stock Center, there is a possibility these two fly strains are separate species. The issue arises in the current species description of *D. repleta* (as revised by Vilela 1983). *D. austrorepleta*, *D. betari*, *D. brunneipalpa* and *D. melanopalpa* were synonymized under *D. repleta* by Vilela (1983) due to similar male genital morphology. In contrast, Wharton (1943) found differences in metaphase chromosomes between *D. repleta* and *D. melanopalpa*. It is also difficult to produce successful hybrids between *D. repleta* and *D. melanopalpa* (Ward and Stone 1952). A single fertile female hybrid was created between a *D. repleta* female and *D. melanopalpa* male. All other crosses were sterile or intersexes. Some or

all of these synonymized species may in fact be separate species. The field or laboratory strain flies from this study may be undescribed or one of the species synonymized.

There could be other explanations for the high COI variation between the field and laboratory strains in this study. Smith et al. (2008) found a high COI intraspecific variation within a few species of parasitoid wasp. They proposed that this variation could be caused by “the recent mixing of formerly separated incipient species, naturally large intraspecific variation, or immigration from a different population.” Ultimately, additional *D. repleta* populations need to be sampled from other localities before any conclusions can be made regarding species identification. Due to the general attractiveness of red wine to *Drosophila*, these findings may not be critical in the overall role of red wine in luring *Drosophila* to traps for control purposes.

Results from this study could be used in combination with sanitation to reduce fly populations. Mass trapping is an IPM strategy that uses attractants to attract and trap target pest species as a means of controlling the pest population (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, mass trapping could be implemented to control these flies. A study done on the Mediterranean flour moth, *Ephestia kuehniella* (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 reliance on insecticide fumigations (Trematerra and Gentile 2009). Similar measures could be implemented at the UI Swine Facility to help control *D. repleta* populations. Increased sanitation including power spraying floors to clean off spilled feed regularly and distributing red wine lured bottle traps throughout the 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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## GRAPHICS



Figure 1. Visual comparison of the larger and darker *Drosophila repleta* (bottom) with the smaller *Drosophila melanogaster* (top) at 2x magnification. Photo by Joseph Spencer.

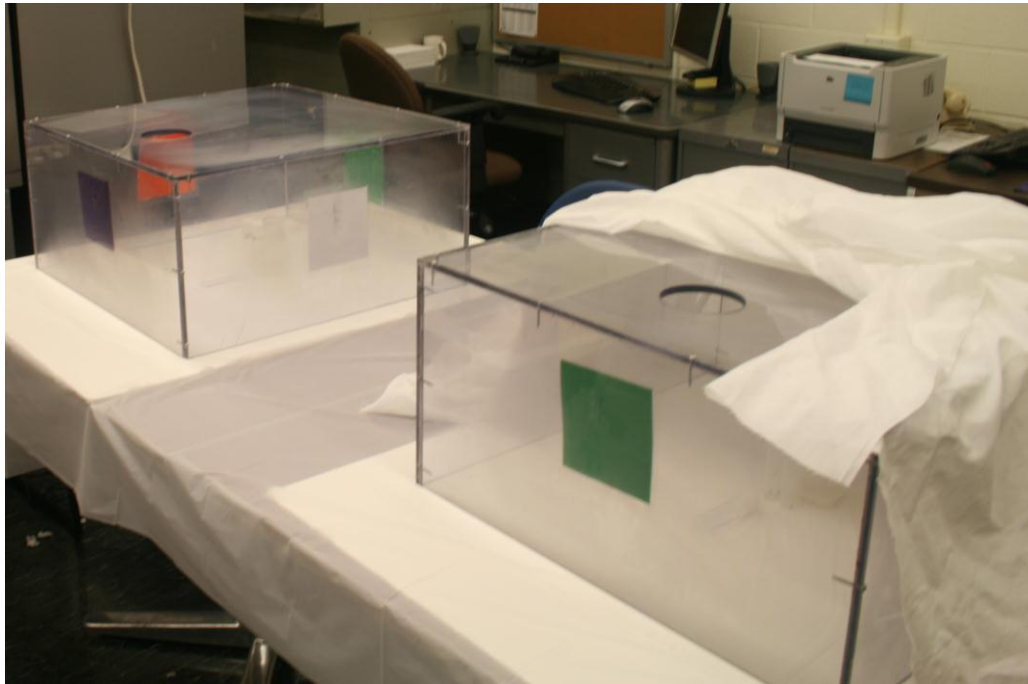


Figure 2. Plastic chambers used for the laboratory color preference tests. Flies were caught on colored sticky cards coated in Tanglefoot®. The white sheet in the photograph was draped over the chambers to provide a homogenous background.

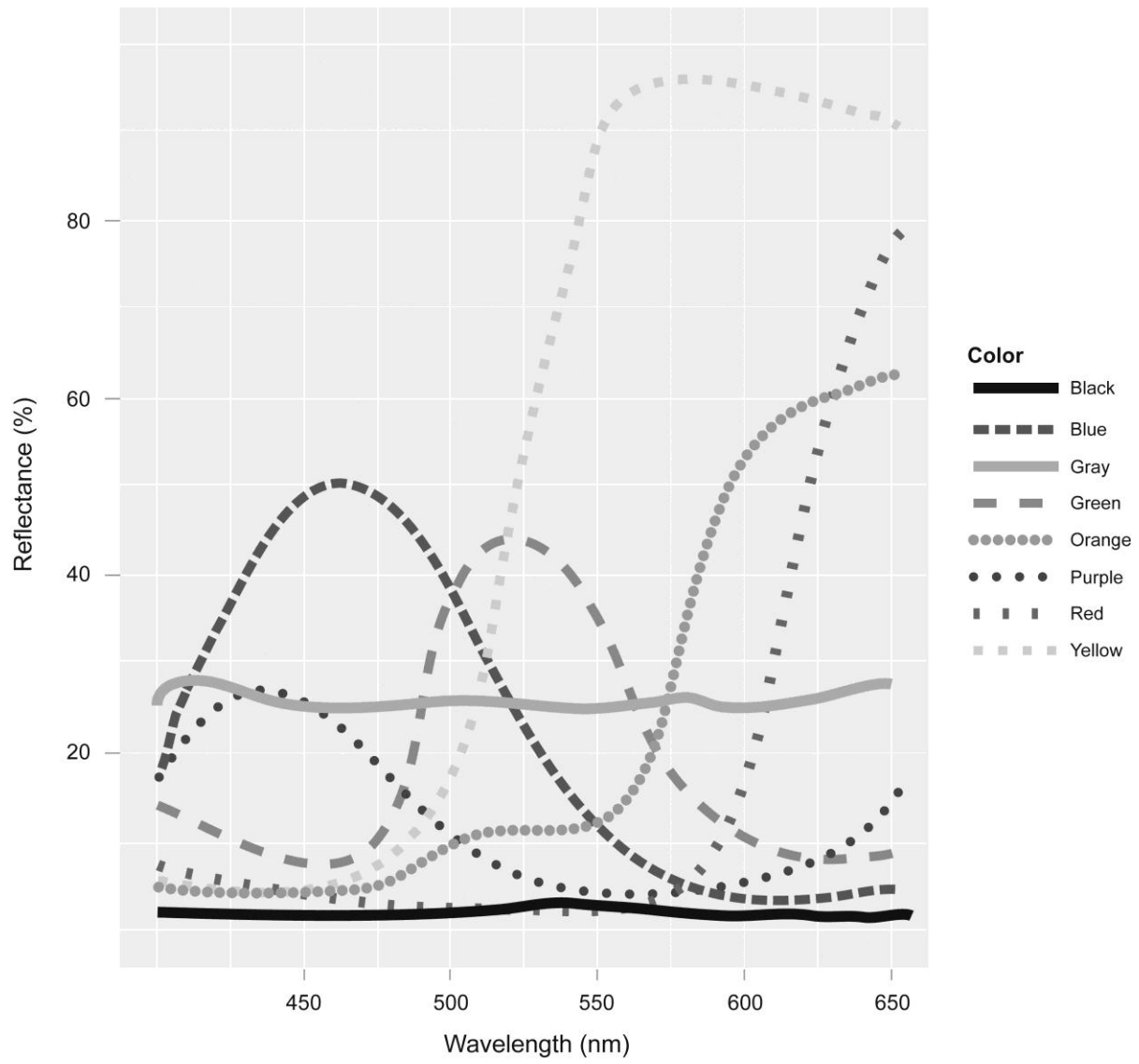
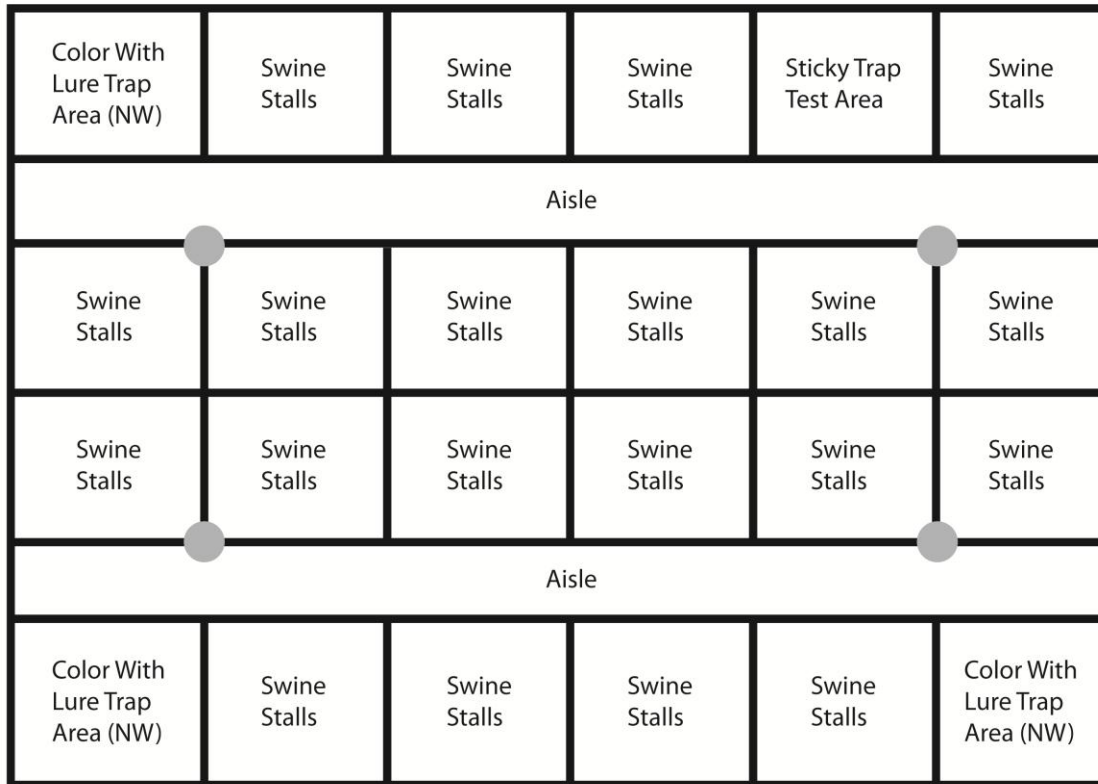


Figure 3. Wavelength and reflectance of color standards used in all color preference experiments. White was also tested and remained at 100% reflectance across the wavelength spectrum.



Figure 4. Field color preference set-up at the University of Illinois Imported Swine Research Facility. Flies were caught on laminated colored cards coated in Tanglefoot®.



● Volatile Trap Experiment

Figure 5. Swine facility growing room layout showing areas and placements of various experiments performed. Sticky traps and color traps baited with a lure were deployed in empty swine stalls. Traps in the volatile experiment were attached to water pipes on the ceiling.



Figure 6. Setup of volatile preference experiment. Starbar® CAPTIVATOR® fly traps were attached to pipes on the ceiling and filled with Pinot Noir red wine, a swine feed and water mixture, apple cider vinegar, or left empty as a control.



Figure 7. Field color test with red wine lures placed in an empty swine stall at the grower room of the University of Illinois Imported Swine Facility.

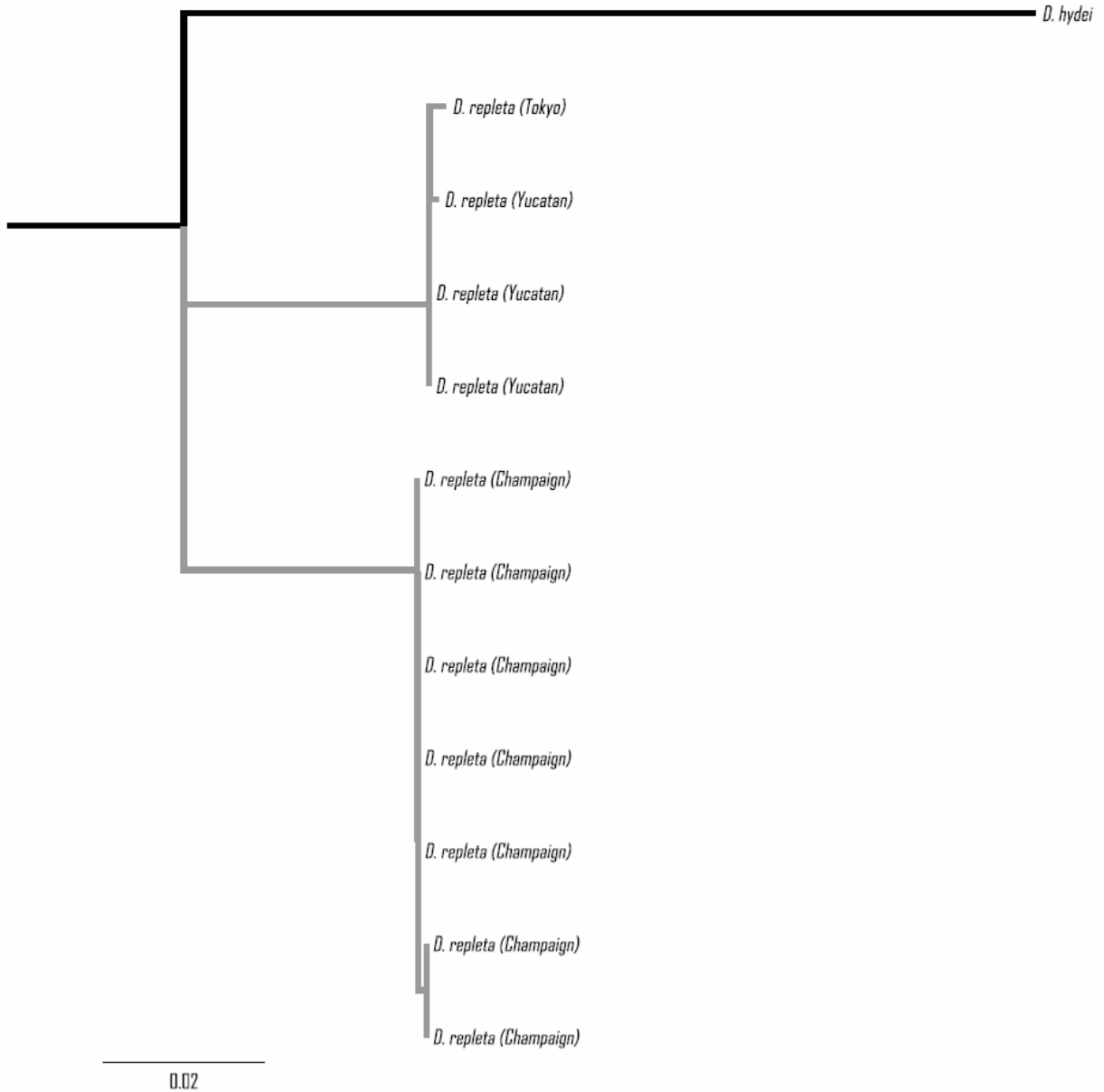


Figure 8. Neighbor joining tree showing a 6% variation in the CO1 sequence between the laboratory strain of *Drosophila repleta* (San Diego Species Stock Center Yucatan Strain) and *D. repleta* found at the University of Illinois Imported Swine Facility in Champaign, IL. Another *D. repleta* strain found on GenBank from the Tokyo Metropolitan University (Wang et al. 2006) was also compared. A closely related species, *Drosophila hydei* (Spicer and Pitnick 1996), was added as an outgroup.



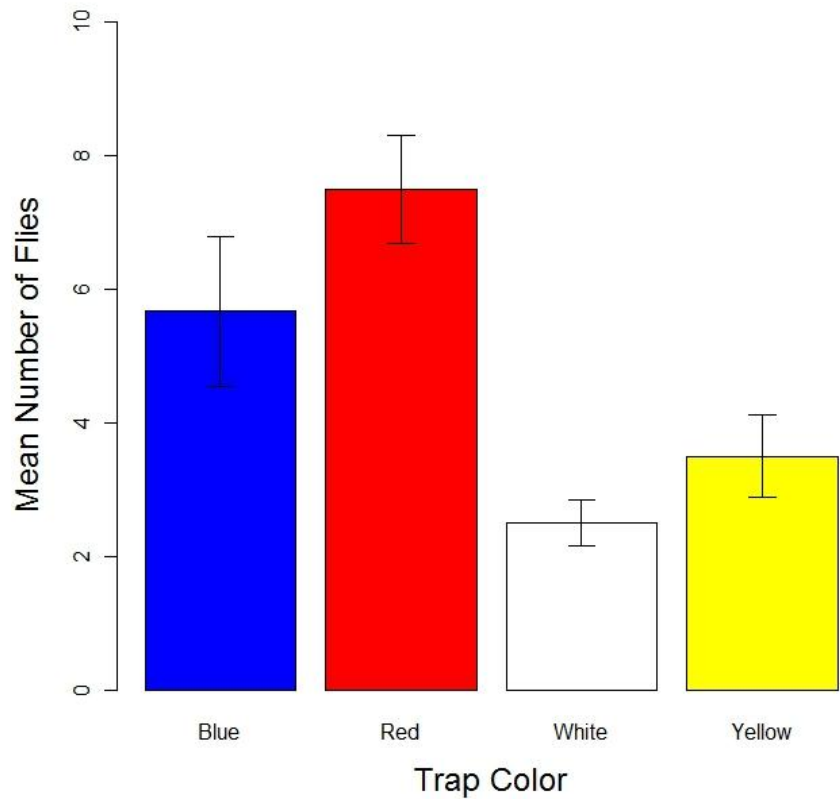


Figure 9. Mean number of *Drosophila repleta* ( $\pm$  SE) caught on primary color sticky traps in the laboratory. The white sticky trap served as the control. Means bearing same letter are not statistically significant at  $P < 0.05$  using the Tukey Test. There were 6 replications and 307 total flies tested.

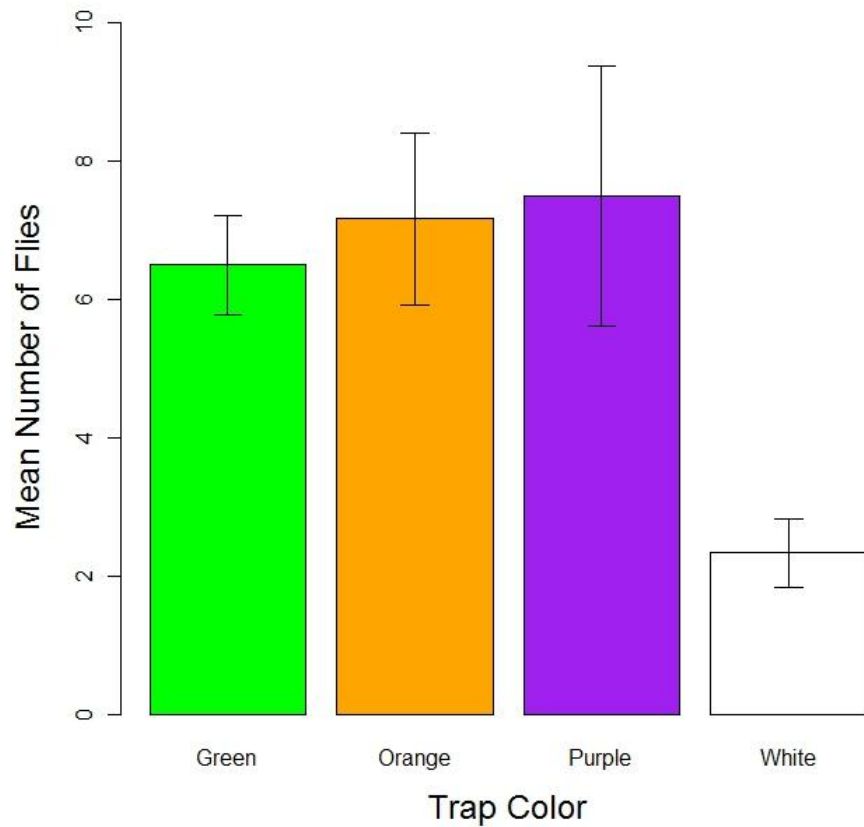


Figure 10. Mean number of *Drosophila repleta* ( $\pm$  SE) caught on secondary color sticky traps in the laboratory. The white sticky trap served as the control. Means bearing same letter are not statistically significant at  $P < 0.05$  using the Tukey Test. There were 6 replications and 307 total flies tested.

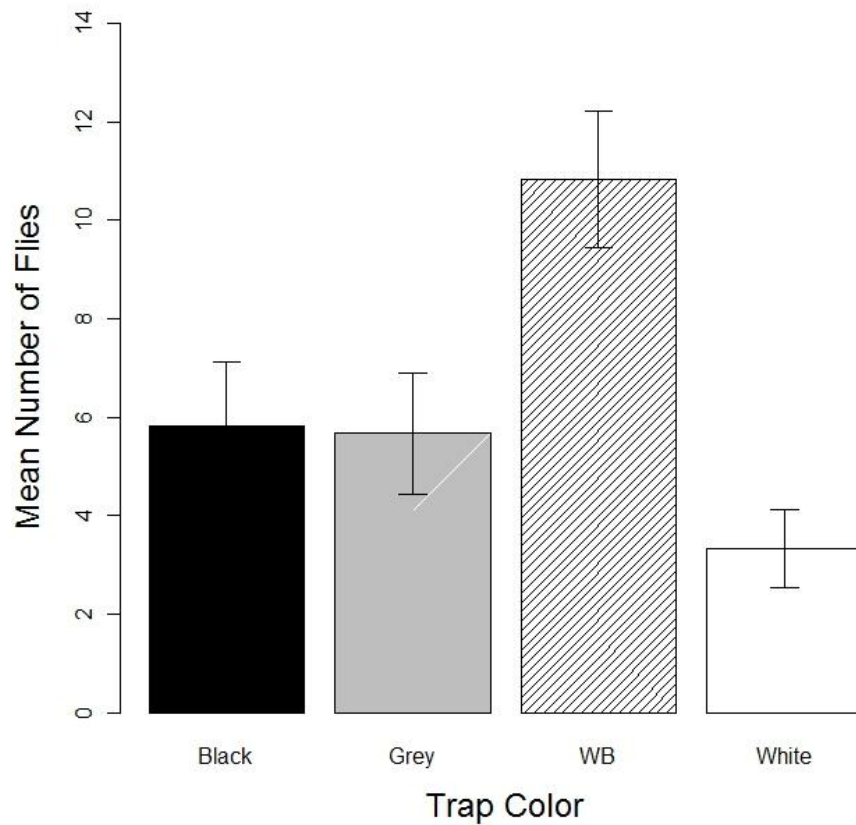


Figure 11. Mean number of *Drosophila repleta* ( $\pm$  SE) caught on sticky cards when testing for contrast in the laboratory. White on black sticky cards consisted of a smaller white card attached on top of a larger black card. A white card was used as a control in this experiment. Means bearing same letter are not statistically significant at  $P < 0.05$  using the Tukey Test. There were 6 replications and 314 total flies tested. White on black traps are abbreviated WB.

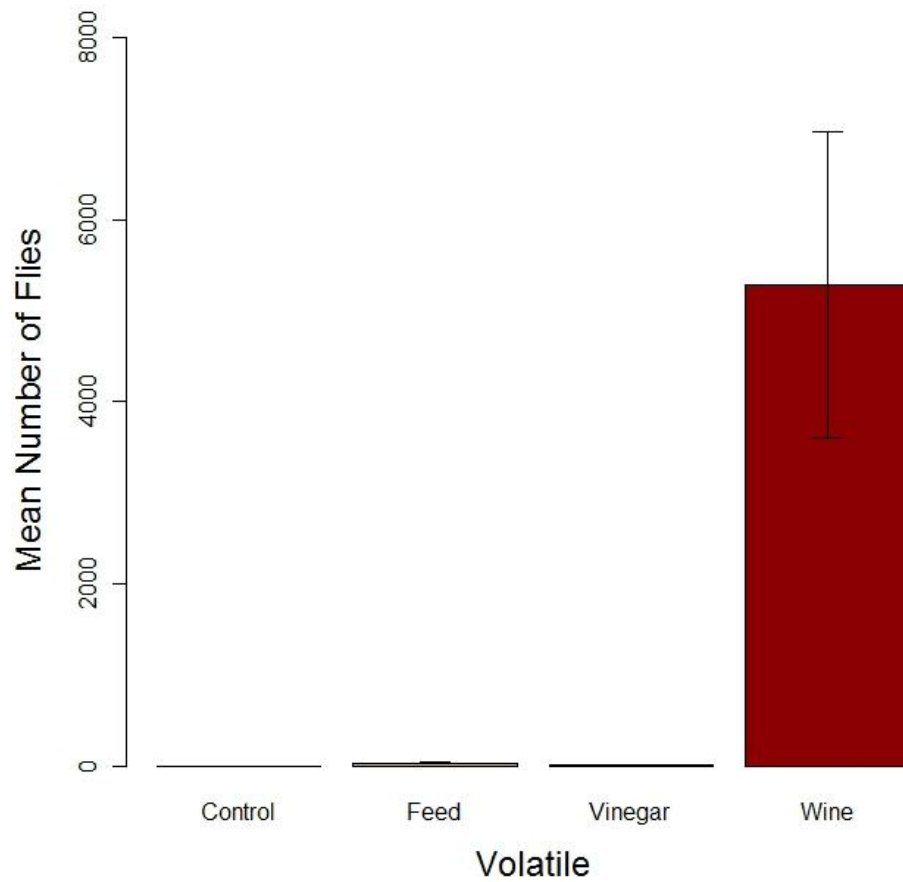


Figure 12. Mean number of *Drosophila repleta* ( $\pm$  SE) caught in *Musca domestica* bottle traps filled with different volatile attractants. An empty bottle was used as a control. Means bearing same letter are not statistically significant at  $P < 0.05$  using the Tukey Test. There were four replications and 21,300 total flies caught.

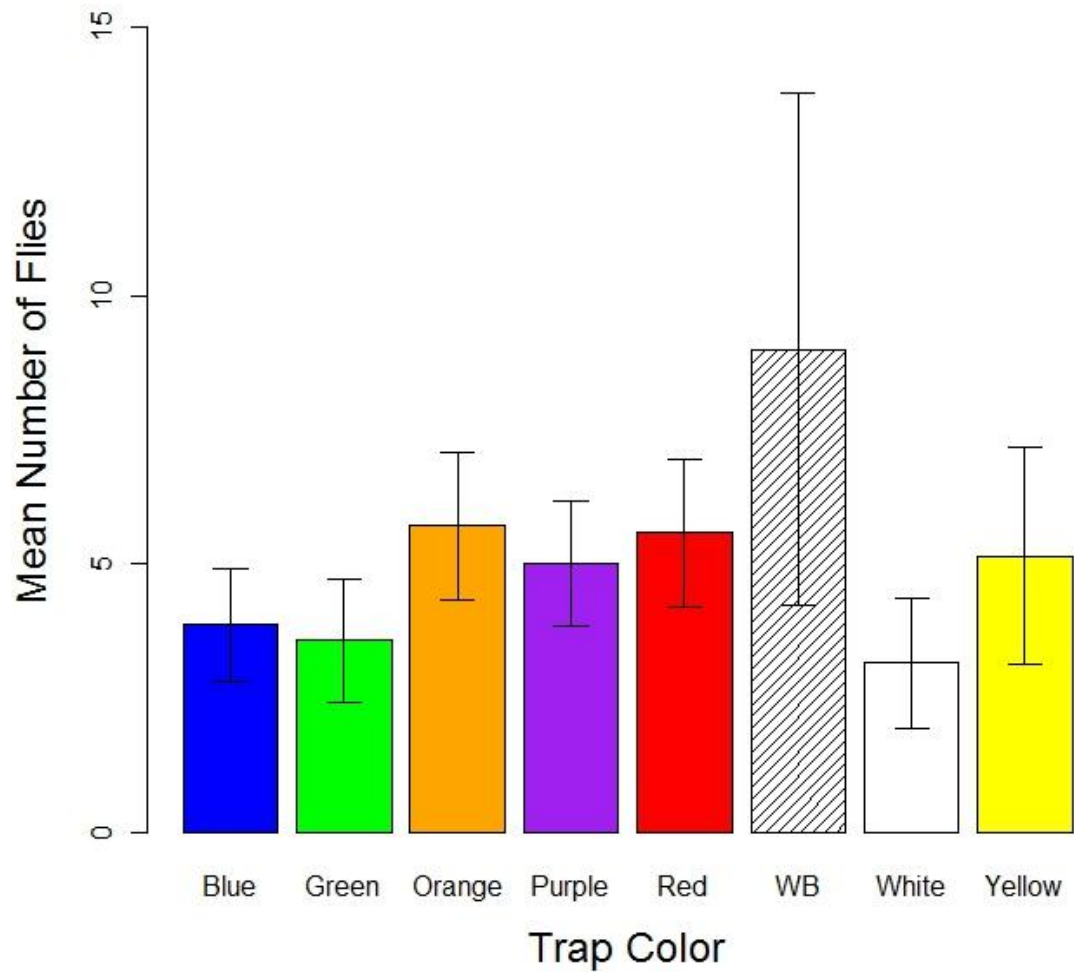


Figure 13. Mean number of *Drosophila repleta* ( $\pm$  SE) caught in different colored cones placed on fly rearing bottles filled with red wine. A white cone was used as a control. The white on black cone consisted of a smaller white strip placed on top of a larger black strip so that when formed into a cone, white was present around the cone's tip. There were seven replications and 287 total flies caught. White on black traps are abbreviated WB.