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The Interactive Effects of Colour and Odour on *Drosophila melanogaster* Trap Design for Pest Control Applications

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Abstract

Drosophila species cause widespread agricultural damage, and designing effective traps for pest control requires an understanding of multimodal cues. Prior studies have looked at the effects of colour and odour on trap efficacy separately, but few have looked at the combined effects of these two types of stimuli. We tested the effect of multimodal cues on *Drosophila melanogaster* trap efficacy by setting up colour versus white two-choice tests for both banana-baited traps and odourless traps. While colour had no significant impact on banana-baited trap efficacy, red odourless traps were significantly more effective than white odourless traps at capturing *D. melanogaster* females. This suggests that colour only affects choice of female *D. melanogaster* in the absence of an attractive odorant. Additionally, both odorous and odourless traps were more effective at capturing females than males, which indicates that these traps do not necessarily target males and females equally, and that pest control strategies need to be designed accordingly.

Keywords — Drosophila Melanogaster, Trap Design, Two-Choice Test, Multimodal Cues, Sex Differences

1. Introduction

Drosophila species, commonly known as vinegar flies, are common domestic and agricultural pests that infest fermenting fruit [1]. *Drosophila melanogaster* is a particular problem in China, where cherry cultivars, which represent a growing agricultural sector, suffer 35%-80% crop loss due to the species [2]. Furthermore, *D. melanogaster* acts as a vector for *E. coli*, transmitting it from infected to uninfected fruits [3]. Additionally, the invasive species *Drosophila suzukii* has presented a new problem, as it has the unique ability to oviposit in fresh soft-skinned fruit [4, 5], causing crop losses of up to 80% in Europe. [6] Given the high economic damage that *Drosophila* species cause, it is valuable to study effective trapping mechanisms that offer non-insecticidal approaches for controlling these pests [7].

Previous studies have looked at colour and odour separately when exploring trap design. Two-choice tests with a white control found red, purple, and checkered disks to be most effective at trapping *D. suzukii* [8] Traps baited with overripe mango [9] and

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banana [1] have also shown to be effective attractants for *Drosophila* species. However, few studies have tested odour and colour in combination. One study of *Drosophila repleta* showed that red traps were the most effective in colour-only tests, while there was no differential response to different colours when colour was combined with an attractive red wine lure [7]. Many insects recognize and select host fruits for feeding and ovipositing based on the integration of olfactory and visual cues [10], so it is important to examine how these multimodal cues interact and how they can be combined to optimize trap designs.

Our study examined the effect of trap colour on the effectiveness of odourous and odourless *D. melanogaster* traps. We used banana-baited traps as our odourous traps, based on previous studies that indicate it is an effective attractant [1, 11]. We set up colour versus white two-choice tests, and hypothesized that traps with colours that resemble oviposition sites, such as red, yellow, and green, would be more effective than white traps [12]. Conversely, we hypothesized that blue, an ecologically unimportant colour for many insects [13], would decrease trap efficacy compared to white traps. We also hypothesized that there would be a larger difference in effectiveness between coloured and white traps in the absence of an odour [7].

2. Materials and Methods

2.1. Test Insects

We used lab-reared adult *D. melanogaster* fruit flies of wildtype strain Oregon-R, from a colony maintained at Simon Fraser University. The colony was raised on a solid food diet of agar, yeast, and cornmeal. The food was checked daily and replaced when low, and the flies had the opportunity to feed until they were put in the cages (i.e., there was no period of starvation prior to the test). Males and females were inserted into two-choice cages simultaneously.

2.2. Coloured Cardstock

We made coloured cones and disks out of non-fluorescent cardstock (Michael's Craft Store, Recollections ®; grey : # 267824; white: # 267815; green: # 470525; red: # 26776; yellow: # 267780; blue: # 470530). For Experiment 1 and the control experiment, we made each cone out of an 8cm × 8cm square of cardstock, rolled and glued using a glue gun (Mastercraft General Purpose Glue Sticks). Each cone had a ∼ 3cm top and a ∼ 0.4cm hole cut out of the bottom through which the flies could pass. For Experiment 2, we made 2.7cm diameter flat circular disks.

2.3. General Two-Choice Cage Setup

We used Oak Stump Farms©Yellow Jacket Traps (12.5 cm high, 11.0 cm diameter; white with a black lid) as cages, plugging the holes in the sides of the cages with cotton balls. We taped two traps to the bottom of each cage (Figure 1). We added one vial of fruit flies into each cage by lightly anesthetizing the fruit flies with CO_2 for ∼30 seconds, then transferring them to the bottom center of the cage, ensuring that no flies fell into either trap. The number of flies per cage varied (means are provided in 3). We screwed the lids on the cages tightly and stored them in a room with both artificial light from above and natural light from a window; both traps in each cage were equidistant from the window (Figure 1). For all tests, cages received ∼8 hours of natural lighting. The duration of time in which artificial light was available varied between the tests, but for all tests the cages received at least 4 hours of artificial light. After ∼24 hours, we transferred the cages to a freezer for at least 24 hours in order to kill all fruit flies. We counted the number of flies in each trap and number of flies in neither trap for each cage, sorting flies by sex according to the Carolina Drosophila manual [14].

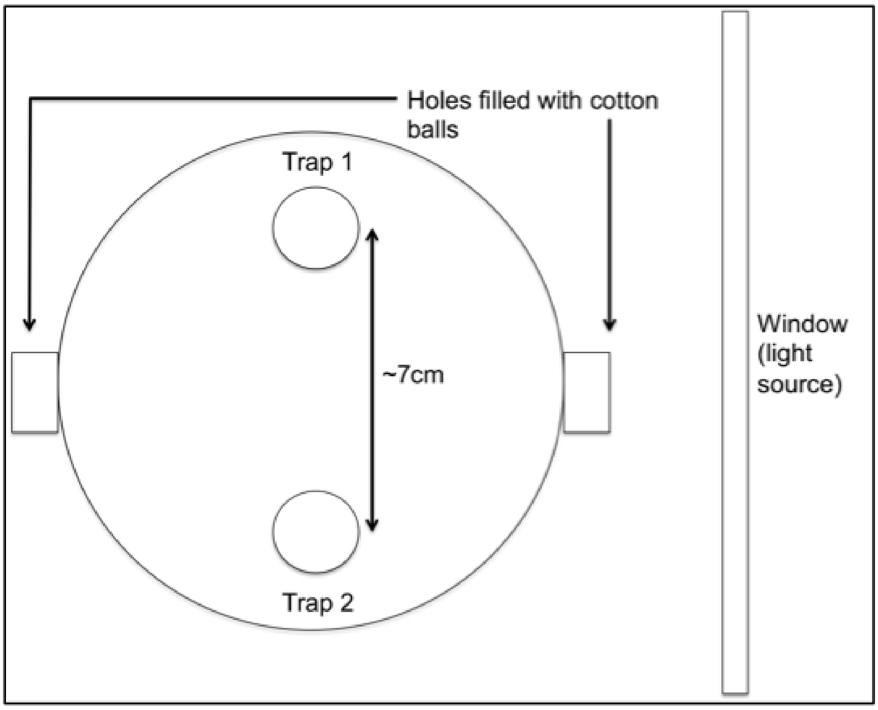

Figure 1: *Cage Setup for two-choise tests. In Experiment 1 and the control experiment, each trap consisted of a vial filled with mashed banana, and a cardstock cone inserted into the vial. In Experiment 2, each trap consisted of a petri dish with a cardstock circle taped to its bottom outer side, and filled with soapy solution (Table 1).*

All two-choice tests compared a coloured trap to a white trap, and were conducted as listed in Table 1.

2.4. Control Experiment: Grey vs. White Two-Choice Test

We conducted a control experiment, adapted from Arnold et al. [13], to ensure that white cardstock cones were appropriate to use as controls. To ensure that the response by *D. melanogaster* to the coloured cones was a response to the chromaticity of the cardstock rather than the fact that the coloured cardstock was darker in comparison to white cardstock, we set up a grey vs. white two-choice experiment. Any differences in response observed would be due to difference in the darkness of the cardstock, since there is no distinguishable difference in chromaticity between grey and white cardstock [13].

Table 1: *Summary of Two-Choice Experiments*

Experiment	Two-Choice Test Colour	Two-Choice Test Control	Trap Type (Odour/NoOdour)	Replicates
Control	Grey	White	Odour (Mashed Banana)	10
Experiment 1	Green Red Yellow Blue	White	Odour (Mashed Banana)	10 10 10 10
Experiment 2	Red Blue	White	No Odour (Soapy Water Mixture)	10 10

2.5. Experiment 1: Two-Choice Tests with Green, Red, Yellow, and Blue-Coned Traps

We used 20 mL glass vials, with coloured cardstock cones inserted into them, as traps, in a similar design to Hottel et al. [7]. We added $\sim$ 7g mashed ripe bananas (Chiquita®organic bananas, mashed to a paste with a mortar and pestle) to each glass vial, which filled about one third of the vial, and inserted the tip of a cone into the opening of each vial (Figure 2).

2.6. Experiment 2: Two-Choice Experiment with Traps of Various Colours Without an Attractive Odour

We set up an experiment analogous to Experiment 1 but removed the attractive banana odour. We used STARSTEDT petri dishes (35 mm diameter $\times$ 10 mm height) as traps. We taped a 27 mm circle to the bottom outer side of each petri dish (Figure 3). We then created a soapy water solution (170 mL distilled water and 0.85 g Sparkleen Fisherbrand detergent) and added 3.5 mL of the solution to each trap. The purpose of this soapy water mixture was to break surface tension and drown trapped flies [15].

We chose to change the trap design for Experiment 2 to better assess attraction to colour. The cone traps were designed such that the flies would be lured down the cone towards the attractive odour. These traps were appropriate in two-choice tests where both traps had the same attractive odour. In the absence of such attractive odour, the flies may have been attracted to the colour of the cones and may have approached them, but likely would not have gone down the cones and gotten trapped. We used petri dishes with soapy water because flies that were attracted to the colour and approached it would drown in the solution.

Due to time constraints, for Experiment 2, we were only able to set up two-choice tests for two colours of interest (red vs. white and blue vs. white).

2.7. Statistical Analyses

For all of our two-choice tests, we converted the counts to percentages for each cage (number of flies caught by coloured trap/number of flies caught in total). We plotted

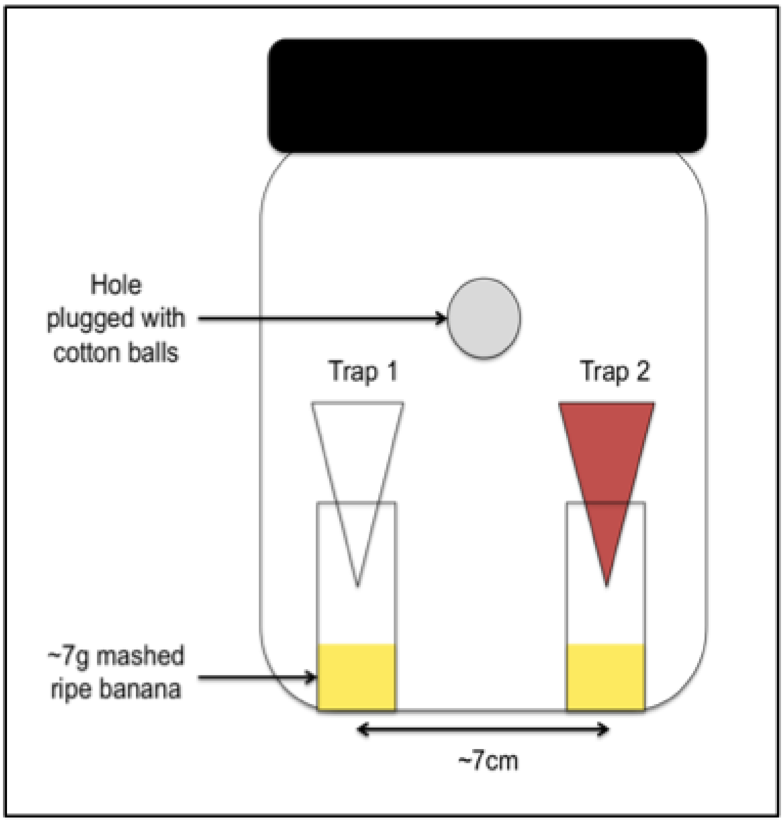

Figure 2: *Set up for Control Experiment and Experiment 1. Trap 1 had a white cone. For the Control Experiment, Trap 2 had a grey cone. For Experiment 1, Trap 2 had either a green, red, yellow, or blue cone (Table 1).*

mean percentages with 95% confidence intervals and used a one-sample t test ($\alpha = 0.05$) with a null hypothetical mean of 50% to test for significance. This test determined whether the percentage of flies captured by the coloured differed significantly from 50% , assuming that if the coloured and white traps were equally effective, then 50% of captured flies would be caught by each. We excluded non-responders from the above-mentioned analysis and analyzed them separately: we converted counts to percentages (# of non-responders/total # of flies in cage) for each cage and then calculated the mean percentages with 95% confidence intervals.

We used an unpaired two-sample t-test ($\alpha = 0.05$) to look for differences between male and female non-responders. For all statistical tests, values of $p < 0.1$ were considered marginally significant.

Both paired and unpaired t-tests were conducted using the GraphPad t-test calculator [16].

We chose to use a t-test rather than a chi-squared test because a t-test allowed each replicate cage to be treated as an individual experimental unit ($n = 10$ as there were 10 replicates for each two-choice assay). A chi-squared test would require combining the data of all 10 replicates into a single percentage for each assay, which would result in lost information.

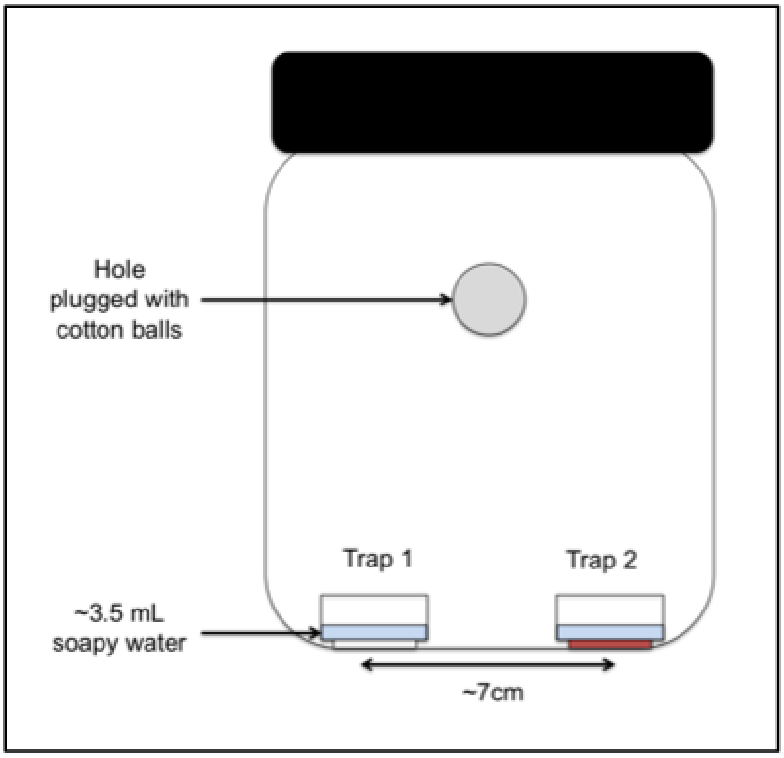

Figure 3: *Set up for Experiment 2. Trap 1 had a white circle stuck to the bottom outer side of the trap, while Trap 2 had either a red or blue circle.*

3. Results

3.1. Control Experiment: Grey vs. White Two-Choice Test

Percentage of captured flies caught by grey traps was not significantly different from 50% for males ($t(9) = 0.3556, p = 0.7303$) or females ($t(9) = 0.1017, p = 0.9212$) (Figure 1). There was a mean of 29.9 males and 25.7 females in each cage.

3.2. Experiment 1: Two-Choice Tests with Green, Red, Yellow, and Blue-Coned Traps

In the red vs. white two-choice test, the percentage of captured female flies caught by the red trap differed marginally significantly from 50% ($M = 60.43\% \pm 10.26\%; t(9) = 1.9923, p = 0.0775$). For all other two-choice tests, the percentage of captured flies caught by the coloured trap was not significantly different from 50% . There was a mean of 30.9 males and 33.6 females in each cage.

3.3. Experiment 2: Two-Choice Experiment with Traps of Various Colours Without an Attractive Odour

In the red vs. white two-choice test, the percentage of captured female flies caught by the red trap differed significantly from 50% ($M = 70.23\% \pm 7.72\%; t(9) = 5.136, p = 0.0006$). In the blue vs. white two-choice test, the percentage of captured male flies caught by

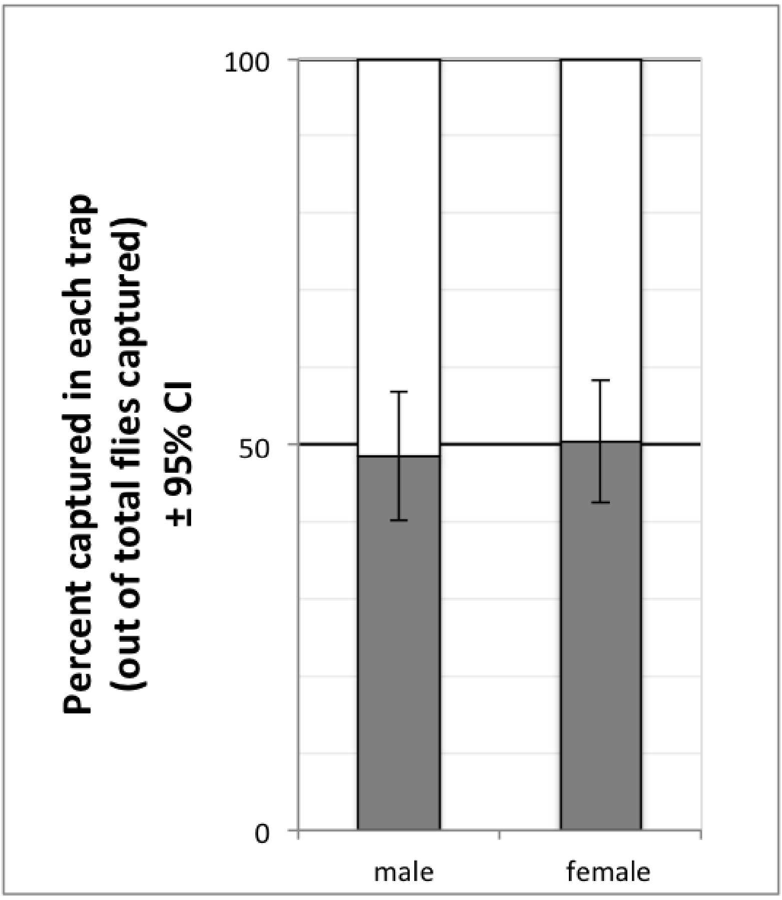

Figure 4: *Control Test Examining the Effect of Cone Darkness on Banana-Baited Trap Capture. 100% stacked bar chart with the grey segment representing mean percentage of captured flies caught by grey traps, and the white segment representing mean percentage of captured flies caught by white traps. Non-responders are excluded from this data.*

the blue trap differed marginally significantly from 50% ($M = 70.64\% \pm 20.64\%; t(9) = 1.9605, p = 0.0816$). For all other two-choice tests, the percentage of captured flies caught by the coloured trap was not significantly different from 50%. There was a mean of 6.0 males and 13.3 females in each cage, which was fairly low compared to the previous two experiments.

3.4. Non-Responder Data

In all two-choice tests across all 3 experiments, there was a significantly larger percentage of male non-responders than female non-responders (Table 2; Figure 7). In Experiment 1 (Figure 7b), there was also a significantly larger percentage of male non-responders in the red vs. white two-choice test compared to the blue vs. white two-choice test ($t(9) = 2.5806, p = 0.0189$). There were very low percentages of female non-responders in Experiment 2.

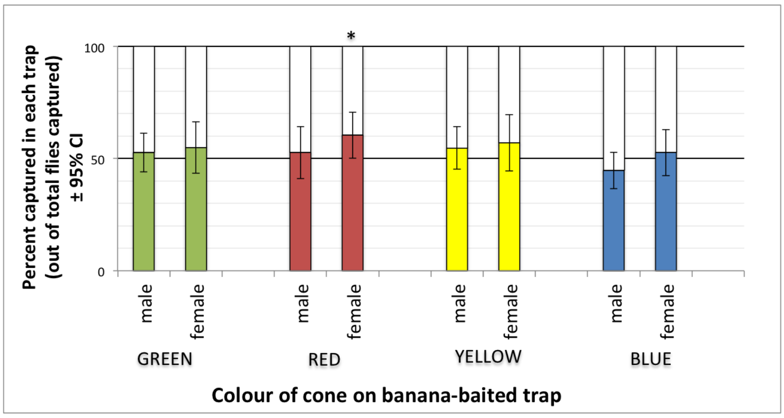

Figure 5: *Effect of Colour on Banana-Baited Trap Capture. 100% stacked bar chart with the coloured segment representing mean percentage of captured flies caught by coloured traps, and the white segment representing mean percentage of captured flies caught by white traps. Marginal significance (∗) is shown ($p < 0.1$). Non-responders are excluded from this data.*

Table 2: *Non-responders: Male vs. female unpaired two-sample t-test.*

	Two-Choice Test (Colour vs. White)	p-Value	t-Value	df
(a) Control Experiment	Grey	0.0075	3.0133	18
(b) Experiment 1	Green	0.0088	2.9368	18
	Red	0.0053	3.169	18
	Yellow	0.0119	2.7963	18
	Blue	0.0117	2.8036	18
(c) Experiment 2	Red	0.001	3.919	18
	Blue	0.0025	3.5137	18

4. Discussion

The results from the control study suggest that white and grey banana-baited traps are equally effective (Figure 4) and that *D. melanogaster* are not differentially attracted to darker or lighter colours, such that any significant results observed in Experiment 1 should be due to chromaticity rather than colour shade. However, we observed no significant differences in capture between coloured and white traps for any colour in Experiment 1 (Figure 5), though these banana-baited traps captured a large percentage of total flies (Figure 7b), confirming that banana is an attractive odour and an effective lure.

Results from the colour-only Experiment 2 tests (Figure 6) suggest that there is a preferential attraction to red by *D. melanogaster* females. A field study on *D. repleta* also showed a preferential attraction to red, though a sex difference was not examined [7]. Interestingly, this preference for red is not evident in the presence of an attractive odour. In red vs. white two-choice tests, the percentage of captured flies caught by red traps was both larger (roughly 70% vs. 60%) and more significant in the absence of an attractive banana odour (Figure 6) than in the presence of the odour (Figure 5). Similar

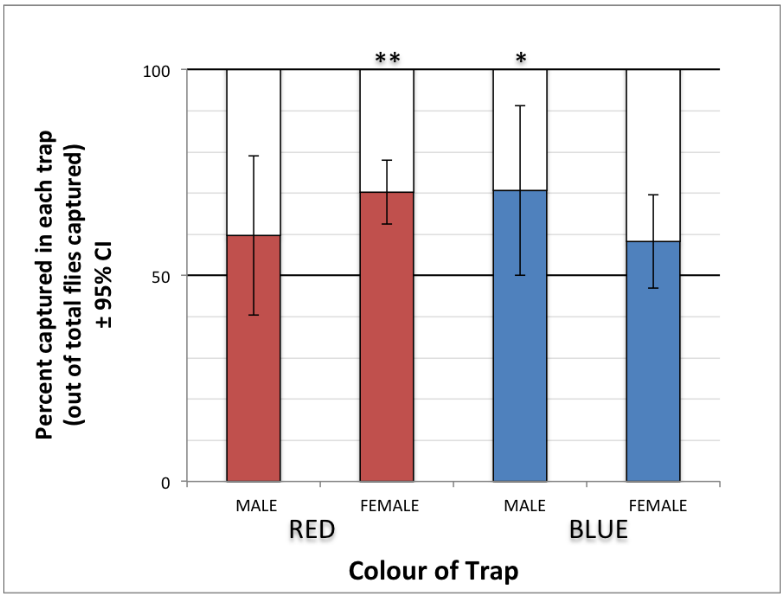

Figure 6: *Effect of Colour on Odourless Trap Capture. 100% stacked bar chart with the coloured segment representing mean percentage of captured flies caught by coloured traps, and the white segment representing mean percentage of captured flies caught by white traps. Significance* ($**, p < 0.05$) *and marginal significance* ($*, p < 0.1$) *are shown. Non-responders are excluded.*

effects were seen with *D. repleta*; a colour preference for red was seen when testing odourless coloured traps, but no colour preference was observed when colour was combined with an attractive red wine lure [7]. These results, therefore, suggest that in the presence of an attractive odour, colour is less important in *Drosophila* choice-making. *D. melanogaster* are highly sensitive to olfactory cues, able to navigate by detecting differences in odour concentration [17], and can use plant-specific olfactory cues to identify suitable hosts from a distance [10]; thus odour is evidently a primary stimulus in *D. melanogaster* navigation.

Although these results suggest that olfactory cues are more important than chromatic cues in this navigation process, it is possible that, comparing Experiment 1 and 2, the difference in type of trap (petri dish vs. cone) contributed to the differences in trap capture observed.

Both odourous and odourless traps seem to be more effective at trapping females than males (Figure 7). A similar female bias in baited trap catch was observed in blowflies, which was explained by the postulation that lures represent both feeding and egg-laying sites for females [18]. Likewise, our results support the hypothesis that colours may be preferentially attractive to *Drosophila* females because they resemble oviposition host sites [12]. A study on *D. suzukii* showed that males and females respond similarly to various colours of odourless traps, and thus traps can be designed to target them simultaneously [8]. This does not seem to be the case for *D. melanogaster*, and this differential response to colour must be considered when designing traps.

Notably, the design of Experiment 1 may have affected non-respondent results, as the cones were not tightly fixed to vials, so flies may have been able to escape

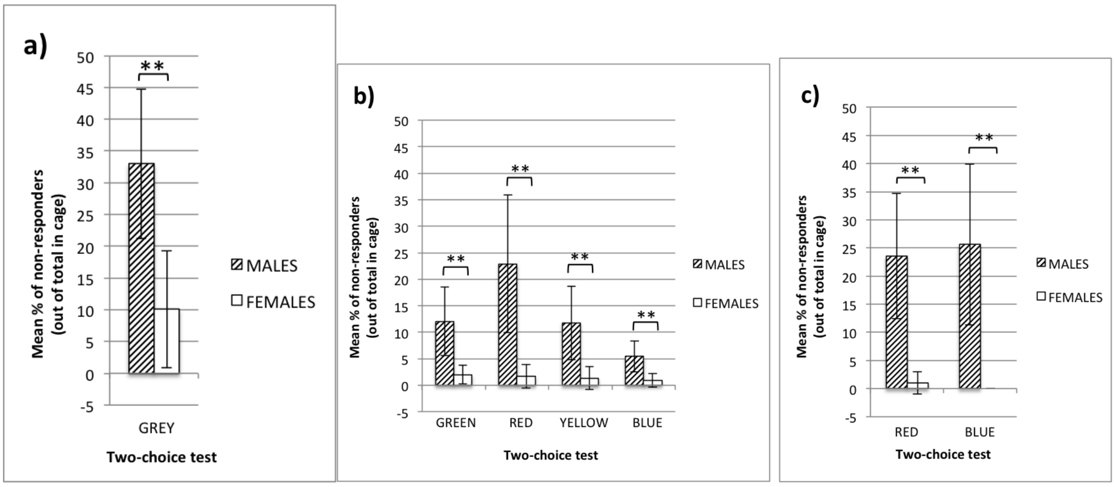

Figure 7: *Non-responders (flies caught by neither trap) compared by sex for each a) the control experiment; b) Experiment 1; c) Experiment 2. Significant differences between males and females are shown (∗∗).*

from the sides. As males are smaller and may escape more easily than females, this design flaw could potentially account for the significantly high proportion of male non-responders in Experiment 1 (Figure 7b). However, since a similar sex difference in non-responders was seen in Experiment 2 (Figure 7c), in which the trap design does not favour the escape of one sex over the other, we can infer that the sex difference seen in non-responders is truly reflective of the differential attractiveness of the traps to males and females.

Yeast-inoculated banana-baits are usually used for *Drosophila* traps [1], and previous studies comparing yeast-inoculated and non-inoculated banana baits showed that non-inoculated baits attracted very few flies [19]. However, our non-inoculated banana-baited traps had fairly low non-response rates for females, generally $< 10\%$ (Figure 7a, 7b). When yeast metabolizes fruit, it produces the phenolic compounds that are believed to be the primary attractants for *D. melanogaster* [20]. However, no yeast was added to our traps, representing a novel result.

The possibility that the traps were naturally exposed to yeast spores could explain this result. Assuming that any yeast spores in the lab are similar to those that would occur in natural settings, this result would show that active yeast-inoculation of banana baits is not necessary to produce effective lures. However, experimental evidence of this assumption is lacking, limiting this conclusion.

One limitation of this study is that we did not focus on insects of a particular age. We used the colonies as they were renewed. It is possible that the age of the flies may affect how attractive they find potential nutrition sources and how actively they are looking for oviposition sites. Additionally, in Experiment 2, a lower number of *D. melanogaster* was present in the cages (mean of 6.0 males and 13.3 females in each cage). It is possible this change may have affected the differences seen between Experiments 1 and 2.

These results offer insight into multimodal trap design, and should be further applied to field studies to test for variation in results [7]. Red colour-only water-based traps that were effective at trapping females could offer a simple, non-insecticidal option for pest control of *D. melanogaster*, and a possible solution to the agricultural damage that they cause. Females are the main threat to agricultural crops because they look for a suitable location with food resources to lay their eggs. By capturing and eliminating female *Drosophila*, the population would be controlled and less damage to crops would be caused.

5. Acknowledgments

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References

[1] Anna L. Birmingham, Ervin Kovacs, Jean Pierre Lafontaine, Norman Avelino, John H. Borden, Isak S. Andreller, and Gerhard Gries. A new trap and lure for drosophila melanogaster (diptera: Drosophilidae). *Journal of Economic Entomology*, 104(3):1018–1023, 2011. doi:10.1603/EC10432.

[2] Qing-Cai Lin, Yi-Fan Zhai, Cheng-Gang Zhou, Li-Li Li, Qian-Ying Zhuang, Xiao-Yan Zhang, Frank G. Zalom, and Yi Yu. Behavioral rhythms of drosophila suzukii and drosophila melanogaster. *Florida Entomologist*, 97(4):1424–1433, Dec 2014. ISSN 0015-4040. doi:10.1653/024.097.0417.

[3] W. J. Janisiewicz, W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. Fate of escherichia coli o157:h7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl Environ Microbiol*, 65(1):1–5, Jan 1999. ISSN 0099-2240. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC90974/`. 0671[PII].

[4] Santosh Revadi, Silvia Vitagliano, Marco V. Rossi Stacconi, Sukanya Ramasamy, Suzan Mansourian, Silvia Carlin, Urska Vrhovsek, Paul G. Becher, Valerio Mazzoni, Omar Rota-Stabelli, Sergio Angeli, Teun Dekker, and Gianfranco Anfora. Olfactory responses of drosophila suzukii females to host plant volatiles. *Physiological Entomology*, 40(1):54–64. doi:10.1111/phen.12088.

[5] Omar Rota-Stabelli, Mark Blaxter, and Gianfranco Anfora. Drosophila suzukii. *Current Biology*, 23(1):R8–R9, 2013. doi:10.1016/j.cub.2012.11.021.

[6] K Steffen, F Grousset, G Schrader, F Petter, and M Suffert. Identification of pests and pathogens recorded in europe with relation to fruit imports. *EPPO Bulletin*, 45(2):223–239, 2015. doi:10.1111/epp.12215.

[7] B. A. Hottel, J. L. Spencer, and S. T. Ratcliffe. Trapping drosophila repleta (diptera: Drosophilidae) using color and volatiles. *Florida Entomologist*, 98(1):272–275, Mar 2015. ISSN 0015-4040. doi:10.1653/024.098.0144.

[8] D. M. Kirkpatrick, P. S. McGhee, S. L. Hermann, L. J. Gut, and J. R. Miller. Alightment of spotted wing drosophila (diptera: Drosophilidae) on odorless disks varying in color. *Environmental Entomology*, 45(1):185–191, 2016. doi:10.1093/ee/nvv155.

[9] Junwei Zhu, Kye-Chung Park, and Thomas C. Baker. Identification of odors from overripe mango that attract vinegar flies, drosophila melanogaster. *Journal of Chemical Ecology*, 29(4):899–909, Apr 2003. ISSN 1573-1561. doi:10.1023/A:1022931816351.

[10] Toby J. A. Bruce, Lester J. Wadhams, and Christine M. Woodcock. Insect host location: a volatile situation. *Trends in Plant Science*, 10(6):269–274, Jun 2005. ISSN 1360-1385. doi:10.1016/j.tplants.2005.04.003.

[11] Melia R. Reed. The olfactory reactions of drosophila melanogaster meigen to the products of fermenting banana. *Physiological Zoology*, 11(3):317–325, 1938. doi:10.1086/physzool.11.3.30151465.

[12] P Ravikumar and Shashidhar Viraktamath. Attraction of fruit flies to different colours of methyl eugenol traps in guava and mango orchards. *Karnataka Journal of Agricultural Sciences*, 20(4):749, 2007.

[13] S.E.J. Arnold, P.C. Stevenson, and S.R. Belmain. Responses to colour and host odour cues in three cereal pest species, in the context of ecology and control. *Bulletin of Entomological Research*, 105(4):417–425, 2015. doi:10.1017/S0007485315000346.

[14] Raymond O. Flagg. *Carolina drosophila manual*. Carolina Biological Supply Co., Burlington, NC, 1981.

[15] Karla M. Addesso, Jason B. Oliver, and Paul A. O'Neal. Survey for spotted-wing drosophila (diptera: Drosophilidae) in the five-county nursery production region of middle tennessee, usa. *Florida Entomologist*, 98(4):1050–1055, Dec 2015. ISSN 0015-4040. doi:10.1653/024.098.0407.

[16] Graphpas quickcalcs: t test calculator. URL `https://www.graphpad.com/quickcalcs/ttest1.cfm`.

[17] Quentin Gaudry, Katherine I Nagel, and Rachel I Wilson. Smelling on the fly: sensory cues and strategies for olfactory navigation in drosophila. *Current Opinion in Neurobiology*, 22(2):216 – 222, 2012. ISSN 0959-4388. doi:10.1016/j.conb.2011.12.010.

[18] Anders Aak and Geir K. Knudsen. Sex differences in olfaction-mediated visual acuity in blowflies and its consequences for gender-specific trapping. *Entomologia Experimentalis et Applicata*, 139(1):25–34. doi:10.1111/j.1570-7458.2011.01103.x.

[19] Marcos R.D. Batista, Fabiana Uno, Rafael D. Chaves, Rosana Tidon, Carlos A. Rosa, and Louis B. Klaczko. Differential attraction of drosophilids to banana baits inoculated with *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* within a neotropical forest remnant. *PeerJ*, 5:e3063, March 2017. ISSN 2167-8359. doi:10.7717/peerj.3063.

[20] Geraldine A Wright. Olfaction: Smells like fly food. *Current Biology*, 25(4):R144–R146, Feb 2015. ISSN 0960-9822. doi:10.1016/j.cub.2014.12.052.