SFU SURJ SIMON FRASER UNIVERSITY SCIENCE UNDERGRADUATE RESEARCH JOURNAL
Vol. 3 (2017-18)

Phototactic Responses of *Drosophila Melanogaster* to UVA, UVB, White Light, and Dark Environments

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Abstract

Ultraviolet A (UVA) radiation comprises a large portion of the solar radiation found on Earth. UVA exposure has been shown to induce both harmful and beneficial biological effects on insects. The objective of this study was to observe if *Drosophila melanogaster*, a relative of the North American common fruit fly, are attracted to or repelled by UV light when alternative light conditions are present. We performed three experiments that gave the flies a choice between (1) UVA or UVB light areas, (2) UVA light or a dark space, (3) a white light only area or a UVA in combination with white light space. In all three cases, we found significantly more flies in the UVA exposed areas ($\geq$ 80%). This suggests that fruit flies exhibit positive phototaxis toward UVA light. Our data provide insight into the behavioral preferences of fruit flies, and show potential for a UVA component to be involved in a successful pest-trapping device.

Keywords — Phototaxis, Ultraviolet, Fruit Fly, Two-Choice Bioassay, Pest Management, Light Trap

1. Introduction

Ultraviolet (UV) radiation is visible to insects [1]. Three types of UV are emitted from the sun: UVA (400-315 nm), UVB (315-240 nm), and UVC (240-100 nm). UVC and most UVB radiation are largely absorbed by the ozone layer, which is beneficial as they are both harmful to living organisms [2]. This leaves UVA as the primary component ($>$ 90%) of UV that reaches the Earth's surface, causing it to be the main type of radiation interaction with ecosystems and organisms [3].

The fly genus *Drosophila* are common pests that are present on almost every continent on Earth [4]. *Drosophila* possess photoreceptive cells which have specific spectral sensitivities, allowing them to detect differences between light [1]. One type of response that comes from these cells is phototaxis, which can be either positive or negative [1]. A positive phototactic response provides potential for pest trapping uses, however it has been found that effective intensities and wavelengths vary greatly between insects

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[1]. A negative phototactic response can be used to protect cultivation areas by displaying light at an intensity or wavelength that repels a particular species [1]. This study explores how such innate reactions to light can be better implemented into pest management.

Our ultimate goal with this research was to observe if UVA light serves as a repellant or attractant for *Drosophila melanogaster*, a relative of the North American common fruit fly, when alternative light options are present [1]. For *D. melanogaster*, UVA exposure is disadvantageous to the F1 generation's fitness, and causes an increase in the duration of time from egg to hatching of the first filial generation *5*. There seems to be a gap in existing research involving more long-term studies. Although UVA has been found to be primarily damaging, it has also been noted that UVA radiation can stimulate beneficial photorepair in some organisms [2]. Developing ways to attract these flies to UVA can be utilized in traps to help manage infestations.

The purpose of our first experiment was to determine if different wavelengths of UV light were more attractive to the flies than others. Our second experiment's goal was to confirm the previous finding that the flies would be found in a UVA light environment as opposed to a dark environment [1]. Our final experiment explored fruit flies' phototactic response to UVA light when white light was also present, as a way to mimic an indoor setting.

We hypothesized that, because it is often evolutionarily beneficial to avoid harmful conditions, *D. melanogaster* would (1) be found more often in the UVA light environment than the UVB environment, (2) be found less often in the UVA light environment than the dark environment, and (3) be found less often in the UVA light environment than a white light only environment.

2. Materials and Methods

2.1. Experimental Insects

The *D. melanogaster* used in this experiment are of the Oregon-Roseburg wild-type strain, sourced from Simon Fraser University's Department of Biological Sciences. The flies received a standard fruit fly diet composed of distilled water, yeast, agar, cornmeal, molasses, tetracyclin and tegosept [5].

2.2. General Experimental Setup: Two Choice Bioassay

We used two-choice bioassays to compare which light environment captured more flies, modelling a previous study [6]. For our experiment, we used a T-tube adapted from black construction paper to connect two test tubes (Ultident Scientific Borosilicate Disposable GlassCulture Tubes) together (Figure 1). This setup allowed the fruit flies to move towards one test tube or the other. Any fly that did not move toward either of the environments, and instead remained in the T-tube, was identified as a "non-responder". The T-tube served as a way to insert fruit flies into the bioassay apparatus and expose them to the two environments at the same time.

To control for external variables, we used cardboard boxes as chambers to surround the test tubes and enclose the different lighting conditions. We hot glued heavyweight black construction paper to the inside of both boxes. We inserted one of the test tubes 3.0 cm up from the bottom of the first box, so that approximately 0.7 cm of the open end of the test tube remained outside the box. This was repeated for the second test tube, which was set up identically in the adjacent box. Each cardboard box was exposed to one of two lighting conditions for each experiment. In a preliminary test (not shown), we randomly alternated the UV bulbs and the sides of the T-maze, concluding that these caused no significant impact on our results.

A vial of fruit flies contained 25-137 flies. For each trial, we inserted fruit flies from a vial into the T-tube using a funnel which we then closed off using a cotton ball. When the flies were being inserted, the open end of the T-tube was facing upward. As soon as the flies were inserted, we rotated the T-tube apparatus 90 degrees to prevent the flies from being encouraged to crawl back upwards and to avoid a third-choice environment. We started a timer for two minutes, giving the fruit flies enough time to explore both lighting options and move towards one test tube or the other, without temperature acting as a confounding variable [7]. We then separated the test tubes from the T-tube using slips of paper, capturing the flies in their respective environments. The flies still in the T-tube (non-responders) were then transferred to a third test tube. All test tubes for each trial were placed into a freezer to sacrifice the flies, after which we counted and identified them by sex.

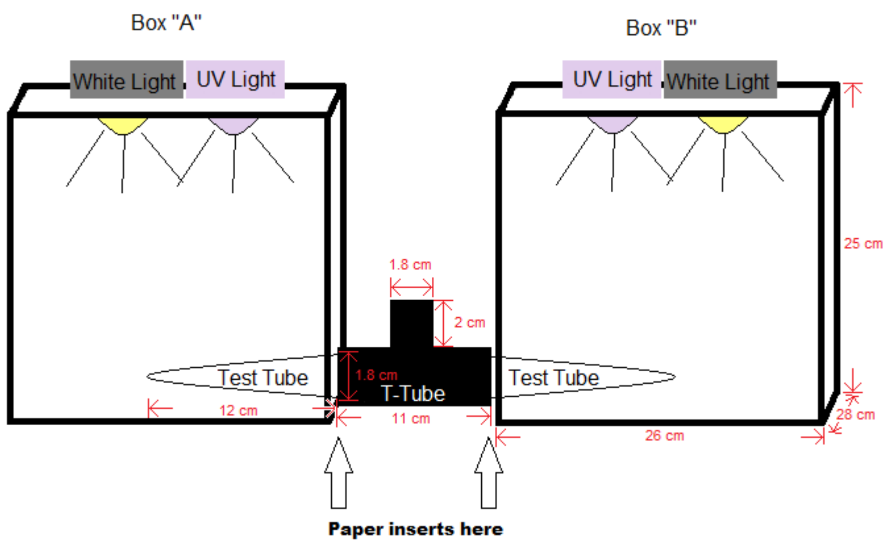

Figure 1: ***General Setup and Dimensions for Two-Choice Bioassay*** *Flies were deposited into the top of the T-Tube. At the end of the experiment, paper slips were inserted to trap the flies present in each condition. Any flies remaining in the T-Tube after the experiment ended were classified as "non-responders". The specific lighting conditions varied for each experiment.*

For each experiment, the bioassays were replicated 10 times. For every replication, new T-tubes and test tubes were used to avoid contaminating equipment with *D. melanogaster* stress hormones or other signals [8].

2.3. Experimental Light Conditions

The three experiments conducted had specific light conditions (Table 1). A Mineralight® lamp (Model UVGL - 58, 115 V) and a Voltax® LED Work Light (160 lm) were used as the sources for UV and white light, respectively. The Mineralight® lamp had two wavelength settings: long (365 nm, 1200 $\mu W\,cm^{-2}$) and short (254 nm, 1350 $\mu W\,cm^{-2}$). In Experiments 2 and 3, a UV light was turned on but covered by black construction paper to control for any noise, temperature, vibration, or other confounding factors that might have arisen from the UV lamp itself.

Table 1: *Light Conditions for Experiments.*

Experiment	Box A		Box B	
	Mineralight	Voltax Flashlight	Mineralight	Voltax Flashlight
1	UVA (365 nm)	-	UVB (254 nm)	-
2	UVA	-	UVA, covered	-
3	UVA	On	UVA, covered	On

2.4. Statistical Analysis

We converted the fly-count data to percentages of the total number of flies in each vial. We then graphed the mean percentages (with 95% confidence intervals) of the total number of flies, including non-responders. We performed chi-squared tests of goodness-of-fit ($\alpha = 0.05$) on the two responding groups of flies for all 3 experiments, to determine if the proportions differed significantly from 50%, our null hypothesis. This would indicate that our observed values were significantly different between the two environments. A Two Way ANOVA ($\alpha = 0.05$) was used to examine if the phototactic responses of female and male flies were statistically different for each experiment.

3. Results

3.1. Experiment 1: UVA vs. UVB Light

The UVA light environment captured significantly more flies that the UVB light environment (Figure 2; $\chi^2(2, N = 10) = 188.16, p < 0.0001$).

3.2. Experiment 2: UVA Light vs. Darkness

The UVA light environment captured significantly more flies than the dark environment (Figure 3; $\chi^2(2, N = 10) = 360.08, p < 0.0001$).

3.3. Experiment 3: UVA And White vs. Only White Light

The UVA and white light environment captured significantly more flies than the "white light only" environment (Figure 4; $\chi^2(2, N = 10) = 206.35, p < 0.0001$).

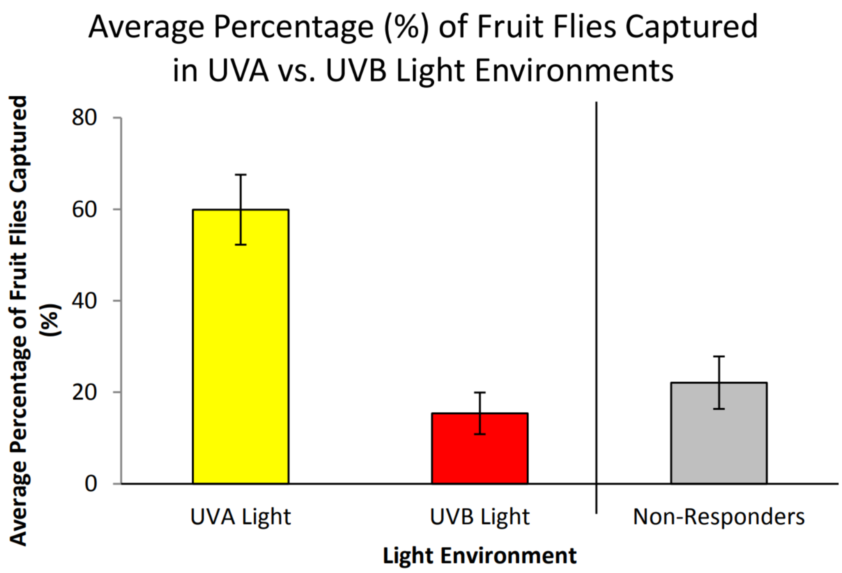

Figure 2: ***Average Percentage (%) of Fruit Flies Captured in UVA vs. UVB Light Conditions*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, UVB light, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

We found that there was no significant difference in the average percentage of male and female flies captured in the UVA environment in experiments 1 through 3 (graph not shown; $F(59) = 2.17, p = 0.124$).

4. Discussion

The results from the first experiment suggest that more fruit flies would be captured by the UVA light environment than UVB (Figure 2). While UVB radiation is exclusively harmful for organisms, UVA wavelengths display a variety of detrimental and beneficial effects [2]. Some beneficial effects of UVA involve the substantial inhibition of growth or survival in bacteria that are harmful to the flies [9]. Both UVA and UVB have been found to cause mortality and pyrimidine dimers, however, UVA causes damage to a much lesser degree [2, 10]. The flies may have avoided the UVB due to the damage associated to its higher intensity, or they may simply be more attracted to the specific wavelength of UVA.

More fruit flies were found in the UVA light environment when given the alternative of the dark environment (Figure 3). The observed phototaxic behaviour of the adults is opposite than that seen in larvae [11].To promote survival, larvae prefer to reside and feed in dark environments, such as rotting fruit, to simultaneously avoid predators while obtaining a source of food [12]. Therefore, from an evolutionary view, it would be beneficial for the larvae to seek dark conditions. Once the flies reach maturity and leave these dark spaces, their perception of a safe and opportunistic environment may change. A possible explanation of our results, and adult insect attraction to UVA light,

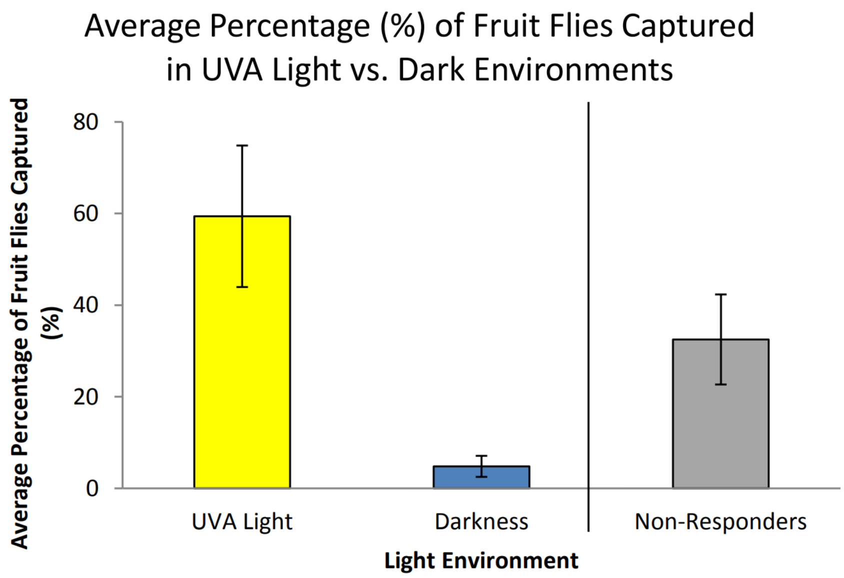

Figure 3: ***Average Percentage (%) of Fruit Flies Captured in UVA Light vs. Dark Environments*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, Darkness, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

is that UVA light is associated with the recognition of open sky, in which food and other essentials may be found [13].

Our final experiment suggests that UVA is still a superior attractant even when a white LED light is present (Figure 4). Williamson et al. [2] discovered that UVA radiation, in combination with visible light, stimulates photoenzymatic repair - a light-mediated process to repair UVB-damaged DNA. This could be a potential underlying reason for positive phototaxis to UVA light. The results from these experiments point toward the conclusion that a UVA trap may be effective for adults in both dark and light conditions during the day.

One aspect that may have added variability to the data was the possibility of social interactions influencing the observed response. Due to the large variation in sample size per trial, in some cases flies may have attempted to avoid crowded conditions in some trials, which may have impacted their location. This large variation, as well as the brief time limit given, may be responsible for the number of flies classified as "non-responders".

Regarding the investigation of the differences in behaviour between sexes, we used a one-way ANOVA and found that there was no statistically significant difference in the movement of male and female flies. The absence of a sex-dependent phototactic response suggests that a UVA light trap could be equally effective against male and female flies. This adds to the potential effectiveness of an indoor trap because UVA light has also been shown to lower fecundity in fruit flies [14]. Thus, overtime, it may reduce population growth as well.

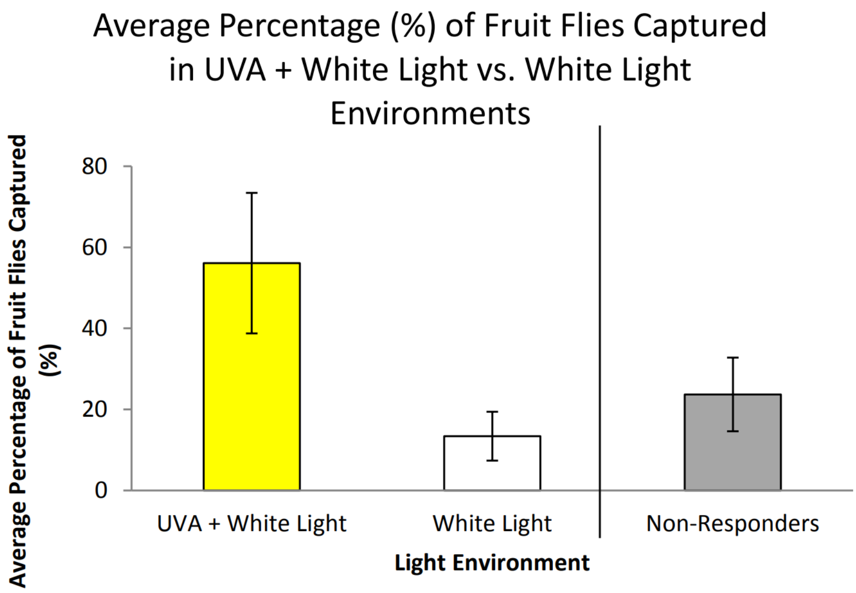

Figure 4: ***Average percentages (%) of Responding Flies Captured in UVA + White Light vs. Only White Light Environments*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, white light, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

5. Conclusion

Our results suggest that *Drosophila* are more attracted to UVA light than UVB. The results also suggest that fruit fly photoreceptors are sensitive to different wavelengths of light, and that certain wavelengths may be more attractive than others. A trap utilizing UVA radiation could be combined with olfactory stimuli to increase its effectiveness in attracting the flies [15]. A follow up experiment could be to determine which olfactory stimuli, when combined with UVA, is the most effective in attracting and trapping fruit flies.

A challenge in research with adult *Drosophila* is that their phototactic responses may be modulated by their circadian clock[16]. Due to the diurnal nature of fruit flies, it is possible that their phototactic response to UVA light can oscillate throughout the day [14, 17]. An additional future experiment could be to conduct similar bioassay trials at night and examine if there are any observable changes in the flies' phototactic behaviour.

A major practical application of our study's results is to use the attractive properties of UVA as bait to capture the disruptive populations of these flies in various environments. Our data offers potential for UVA radiation to be applied as part of a pest-control mechanism. This type of mechanism, which would involve light rather than scent, would be beneficial in terms of avoiding an unpleasant odor. The effectiveness of such a trap can be further explored by studying the behavioral and physiological effects of UVA exposure on *D. melanogaster*, particularly on their reproductive processes. These studies are needed to ascertain if a sanitation technique would be needed in a trap that includes UVA, or if UVA exposure alone is enough to impede population growth.

In conclusion, *Drosophila melanogaster* appear to be able to distinguish these different types of light from one another, and demonstrate positive phototactic responses to UVA light in both light and dark conditions. The difference in phototactic response between male and female fruit flies toward UVA light does not appear to be significantly different, which indicates that a UVA light trap could be effective for both sexes.

6. Acknowledgments

Facilities, equipment, and materials were provided by the Department of Biological Sciences at Simon Fraser University (SFU), as part of BISC 272: a Special Topics Biological Research course. Initial development of this course was funded by the Dean of Science Office at SFU, through the INSPIRE program. Technical support and expertise were provided by Cheryl Leonard and Fan Sozzi-Gu. Physics and statistical analysis consulting provided by Erik Andersen. Additional editing and proofreading assistance was provided by peer tutors.

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