Evaluating Light-Dependent Olfactory Responses in Adult Black Soldier Flies for Improved Rearing Practices

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ABSTRACT

Black soldier flies (BSF; *Hermetia illucens*) are insects known for their ability to upcycle food waste into useful agricultural products such as fertilizers, soil amendments, proteins, fats, and chitin. These qualities make BSF larvae pivotal in the food waste recycling industry.

Consequently, significant research has been dedicated to optimizing larval growth conditions, while adult BSF behavior remains largely unexplored. This gap may impede further improvements in rearing operations and overall production. To address this, we tested BSF adults using a Y-tube olfactometer—a robust assay for olfaction—to evaluate the olfactory preferences of adult BSF. We compared the response rate of adult BSF to a known attractant and a negative control. Our experimental setup involved varied light conditions: no light, fluorescent lamps, and a custom-made UV full spectrum light to simulate outdoor conditions.

Our preliminary results suggest that BSF adults exhibit a higher response to the known attractant under full spectrum light, suggesting that specific lighting conditions enhance responsiveness to olfactory cues. These insights imply that light may play a crucial role in key behaviors such as oviposition, mating, and attraction. By better understanding these processes, we can refine BSF rearing techniques, which could lead to advancements in the waste recycling industry.

KEYWORDS: black soldier fly, light stimulus, UV full spectrum light, waste recycling

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Kerry Mauck is an associate professor in the Department of Entomology. She received her Ph.D. from The Pennsylvania State University in 2012. In her current research, she works to discover and implement sustainable methods for controlling pests and pathogens in agriculture. A key part of this research is the study of black soldier fly (BSF) products as agricultural amendments that prime plant defenses against attackers.

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Wesley is a third-year biology student at the University of California, Riverside, with a minor in data science. In the Mauck Lab, he works with *Hermetia illucens*, also known as black soldier flies. His research focuses on their food decomposition properties and behavior given certain olfactory stimuli.

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Ricky Le is a 3rd year biology student studying the behavioral tendencies and chemical preferences of black soldier flies in the Mauck Laboratory. In his free time, he enjoys playing badminton, trying new cooking recipes, and doing arts and crafts. In the future, he hopes to become a pharmacist.

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INTRODUCTION

Black soldier flies (BSF; *Hermetia illucens*) are key components of the food waste recycling industry, due to their ability to convert discarded food waste into several different useful agricultural products, such as insect-based fertilizers and soil amendments. Compared to chemical fertilizers, BSF products avoid the toxic poisoning of water bodies, soil, and plants often associated with fertilizers (Bisht and Chauhan, 2020). The digestate that the BSF creates from breaking down food waste is called frass, and it is rich in macronutrients, chitin, and minerals (Manan et al., 2024). These qualities make it an effective soil amendment for plants. Due to this, considerable research has been focused on optimizing BSF larvae growth, since larvae create the frass. However, much less research has focused on optimizing behavior of the adult flies, which may limit improvements to BSF rearing operations since they have an important role in egg-laying. To address this knowledge gap, our study investigates some of the olfactory and visual cues that the adult BSF may respond to, specifically focusing on BSF response to odor cues in the context of different light cues.

Like humans, insects rely on their multiple sensory systems to make behavioral decisions, including vision, olfaction, thermosensation, and hygrosensation (Drury, Whitesell, & Wade, 2016; Enjin, 2017; Piersanti et al., 2024). Environmental factors such as lighting can impact insect behavior because many species of flies use natural light sources to regulate flight, mating, and oviposition behaviors (Fabian et al., 2024). Multiple studies have found that natural sunlight is the gold standard for fly interactions, with artificial lighting having negative effects such as disorientation (Zhang et al., 2010; Fabian et al., 2024). However, the usage of artificial light that has been tailored to match the UV spectrum of natural sunlight may promote typical behavioral responses.

In this study, we tested adult BSF olfactory responsiveness using a Y-tube olfactometer under varying light conditions: no light, fluorescent light, and a custom UV full-spectrum light setup. We used the Gainesville diet, a standard positive control attractant in the industry that is a mixture of wheat bran, alfalfa, corn meal, and water. This was to ensure that the experimental design works. Because females are responsible for egg laying, understanding how these cues specifically affect female behavior is of particular interest. Therefore, we also explored whether there were sex-specific differences in response rates. Understanding the lighting conditions that encourage female adults to visit and lay eggs can lead to improved BSF rearing strategies, such as facilitating egg harvesting.

METHODOLOGY

Insect rearing. Adult black soldier flies were obtained from a controlled greenhouse environment. The colony operates under a steady state system, meaning that all life stages of the black soldier fly are continuously present to ensure a consistent supply of adults. The larvae were reared on homogenized food waste that was routinely checked and distributed in larval rearing bins. As the larvae reached maturity, they self-harvested by actively crawling out of the rearing substrate toward drier areas, which was facilitated by slopes in the bins. These larvae would then exit into collection containers. Following collection, pupation and eclosion occurred under the controlled conditions of the greenhouse. Every three to four days, metrics were recorded to ensure consistency in rearing conditions. These included quantities of pupae harvested, food waste applied, bulking agent added, and water supplementation. Internal bin temperatures and ambient greenhouse temperatures were measured, alongside pH levels of the bin mixture.

Experimental setup. To evaluate the olfactory preferences of adult black soldier flies, we used a Y-tube olfactometer. Each arm contained a known treatment source: either Gainesville diet, or an empty control (no attractant). They were initially contained in plastic cups connected to the Y arms by tubing, and then were succeeded by small metal tea strainers placed directly in the ends of the arms of the Y-tube. Experiments were conducted under three different lighting conditions: no light, fluorescent light, and a custom-built UV full spectrum light simulating outdoor conditions. Fluorescent lighting was provided by standard overhead ceiling fixtures commonly used in laboratory spaces.

The Y-tube olfactometer setup (Figure 1) consisted of a central release chamber connected to two odor arms. Air was pushed through each odor arm via a compressed air source

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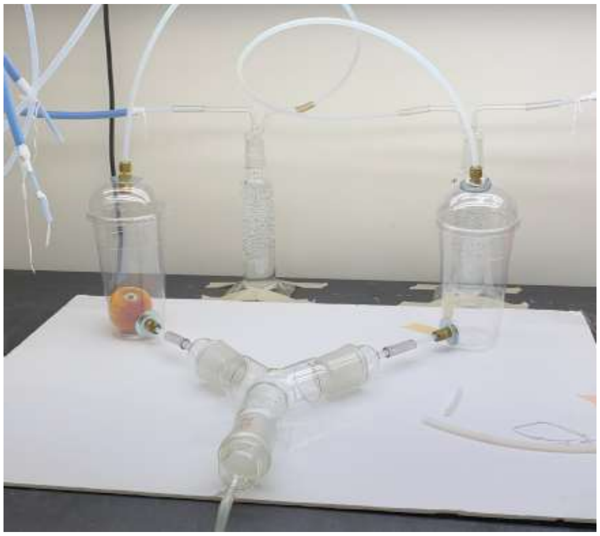

Figure 1: Y-tube olfactometer under fluorescent lighting, with odorant stimuli presented in open cups at each arm.

Figure 2: Y-tube olfactometer under UV lighting, with odorant stimuli held in tea strainers to allow scent diffusion.

regulated to 1.5 L/min, passed through a bubbler-style humidifier to ensure consistent humidity, and then pushed into the odor chambers. A vacuum system at the base of the Y-tube maintained constant airflow through the system at 1.5 L/min. Treatments were alternated between arms every three flies to avoid side bias.

Procedure. Individual flies were introduced to the base of the Y-tube and were given up to three minutes to make a choice. A choice was defined as the fly fully entering one of the two arms and reaching the terminal end of the arm. If a fly did not enter either arm within the three-minute window or did not travel all the way to the end of an arm, the trial was recorded as a no choice outcome. The sex of the fly, the choice of arm (left or right), and the time taken to make the decision were documented. Data collection and environmental conditions were kept consistent as much as possible. A total of 145 flies were tested across the three lighting conditions: 28 flies under no light, 39 flies under fluorescent light, and 78 flies under UV full-spectrum lighting. 21 flies (not included in the previous total) were tested with no treatment in either arm, under fluorescent lights. This was to evaluate side bias in the Y-tube setup, and these were excluded from the main analysis.

Analysis. Only experiments that included both a treatment and a control were considered. Control-only trials are described in the procedure section above. The analysis consisted of evaluating different response rates under different lighting conditions; preference for Gainesville diet over a control; and sex-based behavioral differences. We performed a chi-square test of independence using a contingency table comparing response categories across the different lighting treatments. This test evaluates whether the distribution of behavioral responses was significantly different between lighting treatments. Sex-based differences in behavior were also recorded and are available for exploratory analysis.

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RESULTS

The following table and bar charts summarizes our findings:

Lighting	Sex	Total Flies	Chose Gainesville		Chose Control		No Choice	
			n	%	n	%	n	%
No Light	Female	15	4	26.7	3	20.0	8	53.3
No Light	Male	13	2	15.4	4	30.8	7	53.8
Fluorescent	Female	23	8	34.8	7	30.4	8	34.8
Fluorescent	Male	16	9	56.3	4	25.0	3	18.8
UV	Female	38	21	55.3	12	31.6	5	13.2
UV	Male	40	19	47.5	13	32.5	8	20.0

Table 1: Table of BSF responses given lighting conditions and sex.

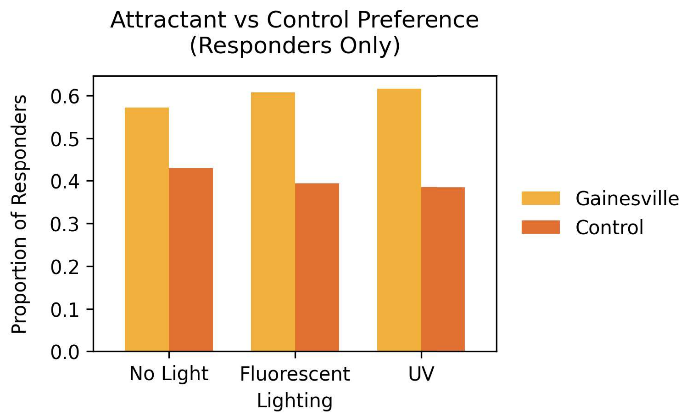

Figure 4: Choice preference among responsive adult BSF for Gainesville diet versus negative control under different lighting conditions. Only flies that made a choice in the Y-tube olfactometer are shown (No-light n = 13, Fluorescent n = 28, UV n = 65).

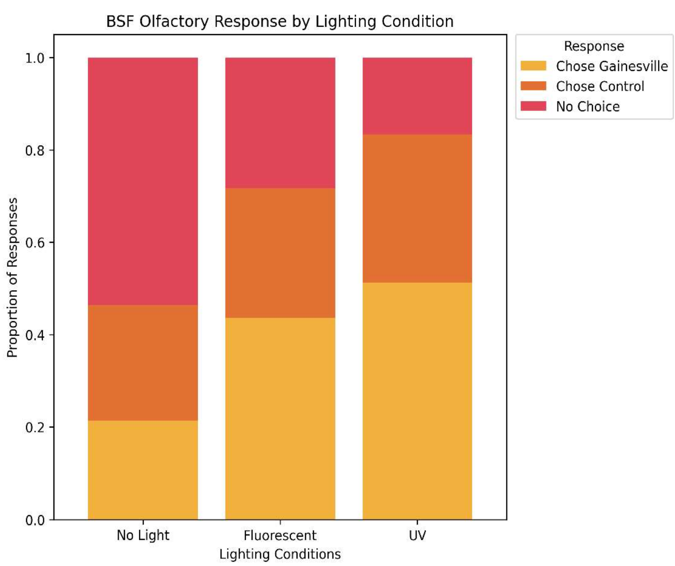

Figure 3: Proportion bar chart showing the distribution of responses across lighting conditions, irrespective of sex (matplotlib).

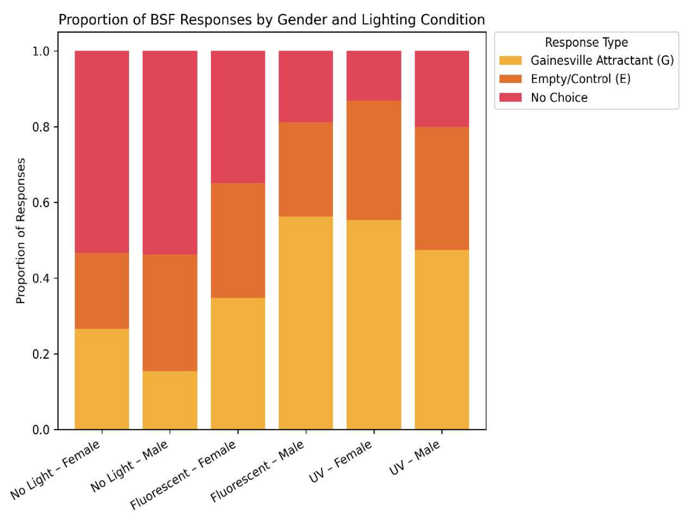

Figure 5: Proportion bar chart showing distribution of responses given sex and lighting conditions (matplotlib).

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RESULTS

In no-light conditions, few flies (21%) responded to the attractant, an equal percentage selected the control, and a majority (54%) made no choice. Under fluorescent lighting conditions, 44% of adult flies chose the attractant arm, compared to 28% choosing the negative control, with the remaining 28% showing no choice. Under UV full-spectrum lighting, we observed a higher attraction rate of 51% attractant preference, with only 17% showing no choice.

Statistical analysis and interpretation. The global chi-square analysis indicated a significant association between lighting conditions and fly choices ($\chi^2 = 15.08$, df = 4, $p = 0.0045$), demonstrating that BSF responses vary significantly with changes in lighting conditions. Follow- up analyses showed that the proportion of flies making a choice (versus no choice) differed significantly under UV ($\chi^2 = 34.7$, $p < 0.001$) and fluorescent ($\chi^2 = 7.41$, $p = 0.006$) conditions, but not under no light ($p = 1.0$). However, among flies that made a choice, the preference between the Gainesville attractant and the control was not statistically significant under any lighting condition.

The statistically significant result for the global chi-square analysis provides evidence that variations in BSF behavior observed under different lighting conditions are unlikely to be due to chance alone. Examination of the observed minus expected frequencies highlighted particularly strong deviations under the UV lighting condition, as flies exhibited higher-than-expected responsiveness to the Gainesville diet and a lower-than-expected incidence of no choice behavior.

DISCUSSION

UV full-spectrum lighting. Our findings demonstrate that UV full-spectrum lighting significantly increases responsiveness to olfactory cues compared to fluorescent or no-light conditions. Under UV conditions, a significantly greater proportion of flies made a behavioral choice rather than remaining inactive ($\chi^2 = 34.7$, $p < 0.001$). Among responsive flies, UV conditions produced a 61% preference for the Gainesville attractant. Although this preference was not statistically significant ($\chi^2 = 1.32$, $p = 0.251$), the numerical trend aligns with previous research that suggests UV radiation influences insect behavioral ecology (Shimoda & Honda, 2013). Previous studies also have noted that insects often exhibit improved visual and olfactory processing under natural lighting conditions, particularly involving UV spectra (Land, 1997). Since BSF are naturally active in daylight, the observed enhanced responsiveness under UV lighting is likely evolutionary adaptation for oviposition site selection and food resource localization.

No lighting conditions. Under no lighting conditions, adult BSF demonstrated a reduction in overall responsiveness. Over half of the tested individuals did not choose any arm in the Y-tube olfactometer. Statistical analysis confirmed no significant difference between the proportion of flies that responded versus remained inactive ($\chi^2 = 0.00$, $p = 1.000$). This reduced responsiveness has implications for indoor rearing facilities that may operate under reduced or artificial lighting conditions, potentially limiting productivity.

Fluorescent lighting. Fluorescent conditions yielded intermediate results, with moderate responsiveness (44%) to the attractant, which is better than dark conditions but inferior to UV lighting. This may reflect incomplete spectral emission provided by fluorescent lights. Statistical tests indicated that significantly more flies made a behavioral choice under fluorescent lighting compared to no choice ($\chi^2 = 7.41$, $p = 0.006$). However, among responsive flies, there was no statistically significant preference for the Gainesville attractant versus the empty control ($\chi^2 = 0.29$, $p = 0.591$). Given that fluorescent lamps emit limited UV radiation that differ from the spectral characteristics of natural daylight, BSF visual systems that are sensitive to UV wavelengths may not be fully activated, reducing the effectiveness of behavioral responses compared to full-spectrum UV illumination (Briscoe & Chittka, 2001).

Limitations. There are a few potential limitations that should be acknowledged. First, variations in testing conditions such as slight differences in airflow rates, temperature, or humidity between testing days may have influenced fly behavior. Consistency across these parameters is crucial as adult insect responsiveness is known to vary with minor environmental fluctuations (Tomberlin & Sheppard, 2002). Future experiments should also apply standardization of fly handling to minimize external stressors.

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Future directions. Since the positive control, Gainesville diet, has shown to be preferentially chosen under UV lighting, further research can substitute with other chemicals of interest such as indole, or a combination of chemicals—which would involve substituting the negative control with another compound—to determine which one is more preferred.

Conclusion. The statistical support for lighting effects has important implications for the optimization of industrial-scale BSF rearing systems. Facilities aiming to maximize adult activity, mating, and oviposition rates should consider adopting full-spectrum or UV-inclusive lighting setups. This result supports previous findings about the relevance of lighting spectra in insect and waste-recycling management contexts. Flies responded with a significant preference for the Gainesville attractant under UV; however, there were no significant differences under fluorescent and no light conditions, which may be tested with further replication and larger sample sizes. Further research can investigate detailed spectral analyses to identify which specific wavelengths within the UV spectrum maximize responsiveness, along with investigations into behavioral differences between sexes under varied lighting, given potential differences in sensory or behavioral priorities between male and female BSF. Long-term assessments of BSF behavior under varying light conditions can also be explored to see the effects on health and fecundity in rearing and waste-upcycling operations.

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