Determining Hymenoptera Preference Pairing to Color and Odor in College Station, TX

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Abstract: One third of all food production is reliant on pollination, the transfer of pollen to allow for fertilization. For this process to occur, plants use visual and olfactory cues to attract a wide variety of pollinators. The persistent decline of pollinating insects has captured the attention of the public and has catalyzed the need for research focused on the affects that the overuse of pesticides, insecticides, and climate change has on pollinators. The interconnectedness of pollinators and food production has risen in prevalence, along with the increasing risks that threaten the agricultural and ecological sectors. The attraction to the combination of colors (blue and white) and essential oils (lavender and eucalyptus) were tested to determine which combined variables resulted in a higher capture of hymenopterans, as the order is the third most diverse pollinator and is familiar to the public. The experiment occurred at an urban site to reflect the decreasing numbers of pollinators in cityscapes. In the isolation of color and essential oil, and the summation of all captured data, there was no significant difference in the attraction of hymenopterans. With further research, this information has the potential to open the door for examining how hymenopterans react to artificial scents and common colors, in both isolated and paired treatments.

Keywords: *Hymenoptera, pollination, essential oils, color, pollinator attraction*

Pollination, the process of transferring pollen from a male anther to a female stigma, is crucial in ensuring the reproductive success of plants (Lord and Russell 2002). The production and diversity of agriculture is highly dependent on biotic pollination, specifically those within the order Hymenoptera (Aizen et al. 2009). The hymenopteran order, with the acknowledgement that many insect species have yet to be discovered, is the third most biologically diverse group of pollinators. However, the effectiveness of pollination is not reliant on theoretical capability, but rather the overall abundance of a specific animal species, the likelihood for an animal to encounter pollen, and the probability that the animal will transfer the pollen to a plant of the same species (Ollerton 2017). With this consideration, pollinators play a critical role in the reproductive success of plants and global biodiversity (Patricio-Roberto and Campos 2014).

To ensure their reproductive success, plants use unique blends of isoprenoids, benzenoids, and aminoid compounds to produce volatiles that are designed to attract a wide array of pollinators (Dobson 1993). Along with the chemical compatibility between plants and pollinators, the morphology of plants has evolved to compete for the attention of specialized and generalist species (Varassin et al. 2001). Despite a great variety of visual and olfactory cues, pollination systems are more generalized, implying resilience to linked extinctions and potential displacement of native species (Waser et al. 1996).

A wide variety of essential oils have been utilized for the application of biopesticides, due to their attractive and repellent activities against many insect pests (Mossa 2016). However, recent studies have suggested that the use of marketed "safe" essential oils has a negative effect on non-target species, specifically beneficial pollinators (Gostin and Popescu 2023). One study found that eucalyptus oil, garlic extract, neem oil, and rotenone repelled and decreased the walking activity in adult *Apis mellifera* (Hymenoptera: Apidae) (Linneas) workers (Xavier 2015). With the increase of organic agriculture, these supposedly safe methods of biocontrol are marked to the common man, despite the underlying danger that threatens the very livelihood of the pollinators that campaigns like "Save the Bees" are trying to protect.

With the public developing an increased awareness of how biotic and abiotic factors negatively affect the population of pollinators, the need and desire for research relating to the overuse of pesticides, insecticides, and climate change has increased (Cedillo 2016). To increase knowledge of pollinator attraction, one study tested the preference of *Apis mellifera caucasica* (Hymenoptera: Apidae) (Linneas) for odor and color, using essential oils and colored dishes (Erdogan and Yavus 2022). The results indicate a strong preference towards the blue dish and the control (sucrose) was the preferred odor. These preferences were isolated and did not allow for the analysis of the combined effect of color and odor.

With the acknowledgement that the population of hymenopterans, along with all other pollinators, is declining, the goal of the experiment is to determine the optimal combination of color and scent regarding the attraction of hymenopterans. The experiment will focus on the introduction of combined variables to a natural population, specializing in color and odor. Due to the results of the study, it can be assumed that the combination of the blue dish and the lavender essential oil will result in the greatest number of hymenopterans collected. With this research, methods used for conservation efforts can be addresses and applied. The experiment is conducted with the hopes of raising awareness for the attractants of pollinators, to encourage a growing population and the stability of the ecosystem.

Materials and Methods

Time and Location. The experiment took place at the Texas A&M University Gardens in College Station, TX. This site was chosen due to its proximity to impervious structures including concrete, buildings, and roads (McDonnell and Hahs 2008). The experiment took place over the course of one weekend in April 2024. Both trials occurred during partly cloudy days, in the same urban site over the course of 48 hours.

Set-Up. To act as visual volatiles, nine 12-ounce bowls (Anderson's, White Bear Lake, Minnesota) in the colors glistening gold, navy, and white were used for this experiment. For the sake of simplification, the colors are referred to as yellow, blue, and control. Three bowls of each color were paired with olfactory volatiles: eucalyptus essential oil, (Guru Nanda, Buena Park, California), lavender essential oil (Guru Nanda, Buena Park, California), and no applied scent (Fig. 1). The white bowl acted as the control for the visual stimuli and the plain distilled water (ULINE, Pleasant Prairie, Wisconsin) acted as the control for the olfactory stimuli. The combination of scents and colors is as follows:

	Blue	Yellow	Control
Eucalyptus	B_E	Y_E	C_E
Lavender	B_L	Y_L	C_L
Control	B_C	Y_C	C_C

Fig. 1. Color and scent combinations

All nine bowls were placed 15.25 meters from one another in a grid formation, with a total surface area of 930.25 m^2 (Fig. 2). The placement of the bowls was randomly assigned for both trials to prevent attraction due to overall proximity and familiarity. 2 mL Autosampler Vials (Premium Vials, Tullytown, Pennsylvania) were filled with 2mL of each essential oil. A single 100mm glass capillary tube for the essential oil. To dispense the essential oils at a steady rate, the dispensing rate was set to 0.5 ng/h with a concentration of 0.1 g/mL. Once the vials were filled and the dispensing rate was set, the vials were attached to the inside of the bowls using 2.54 cm Industrial Masing Tape (ULINE, Pleasant Prairie, Wisconsin). The bowls were then placed in their randomized location:

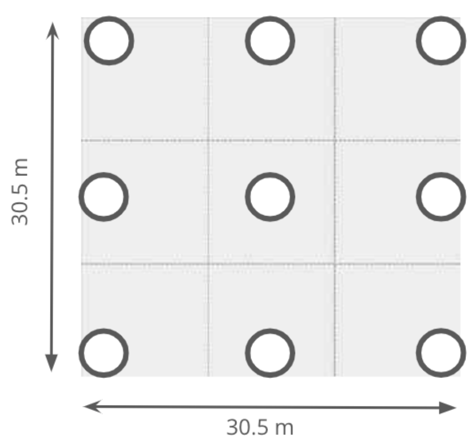

Fig. 2. Attractant grid formation spacing

First Trial. The bowls and dispensers were set up at 1800 on a Thursday. 150mL of powdered laundry soap (OxiClean, Princeton, New Jersey) was added to each bowl to lower the surface tension of the water. This was done to ensure that any arthropod interacting with the bowls would be trapped. The soap was then mixed with the water until suds appeared. A single rock was placed in each of the bowls to weigh them down. The rocks used were found at the site and were approximately an inch in diameter. The randomized placement of the bowls for the first trial is as follows:

B_C	C_C	B_L
Y_C	C_C	C_L
Y_E	B_E	Y_L

Fig. 3. Trial one color and scent formation

The first day of collection occurred after 24 hours had passed, at 1800 on Friday. The contents of the bowls were drained into Ziploc snack bags (Ziploc, San Diego, California), and the insects were allowed to remain in the water mixture until the entirety of the collection was identified to the order. The bags were labeled with the combination using a fine-point permanent marker (Sharpie, Atlanta, Georgia).

Second Trial. The second trial followed the same methods as the first. After collecting the samples, the bowls were randomized, refilled with water, soap, and rocks. The vials of the essential oils were also refilled to 2mL. The randomized placement of the bowls for the second trial is as follows:

C_L	B_C	B_E
B_L	C_C	Y_E
Y_L	W_E	Y_C

Fig. 4. Trial two color and scent formation

The second day of collection occurred at 1800 on Saturday. The contents were drained into labeled Ziploc snack bags and were kept separate from the previous trial's collection. The collections were then analyzed to determine the order of the captured organisms. Only insects identified as Hymenoptera were recorded, and the remaining orders were discarded.

Results

The mean of each combination of the two trials was calculated and is represented in Figure 5. A *p*-value, along with an overall trend of the data, was not able to be accurately determined due to the limitations of only two trials.

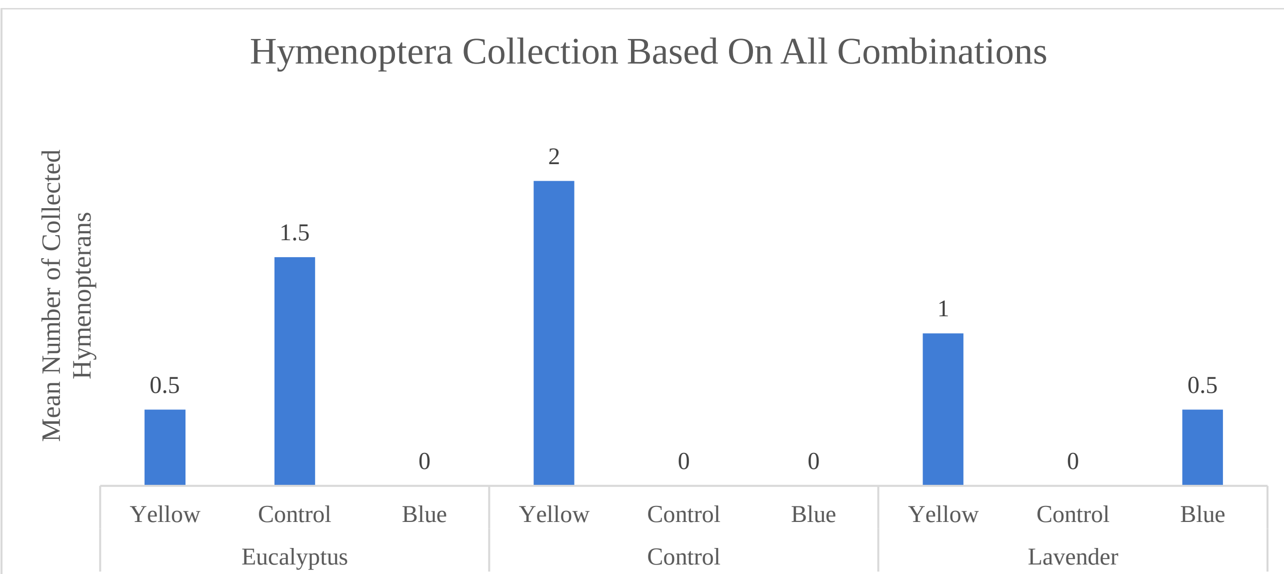

Fig. 5. The mean number of collected Hymenopterans based on all color combinations: Yellow + Eucalyptus, Control + Eucalyptus, Blue + Eucalyptus, Yellow + Control, Control + Control, Blue + Control, Yellow + Lavender, Control + Lavender, and Blue + Lavender.

Figure 6 is representative of the mean collection of Hymenoptera based on the isolation of olfactory volatiles. Visual volatiles were not accounted for in the distribution of data to determine if there was a significant difference in the attraction of hymenopterans based solely on olfactory stimuli. A one-way ANOVA was used to determine the level of significant difference. With a P=0.20, there was no significant difference in the essential oils' effectiveness in attracting hymenopterans (Figure 6).

Figure 7 is representative of the mean collection of hymenopterans based on the color of the bowls. The pairing of the essential oils was not considered to determine if there was a distinct attraction to the colors of the bowls alone. A one-way ANOVA was used to determine if there was a significant difference in the attraction of hymenopterans based solely on visual stimuli. With *P*=0.94, there was no significant difference in the colors' effectiveness in attracting hymenopterans.

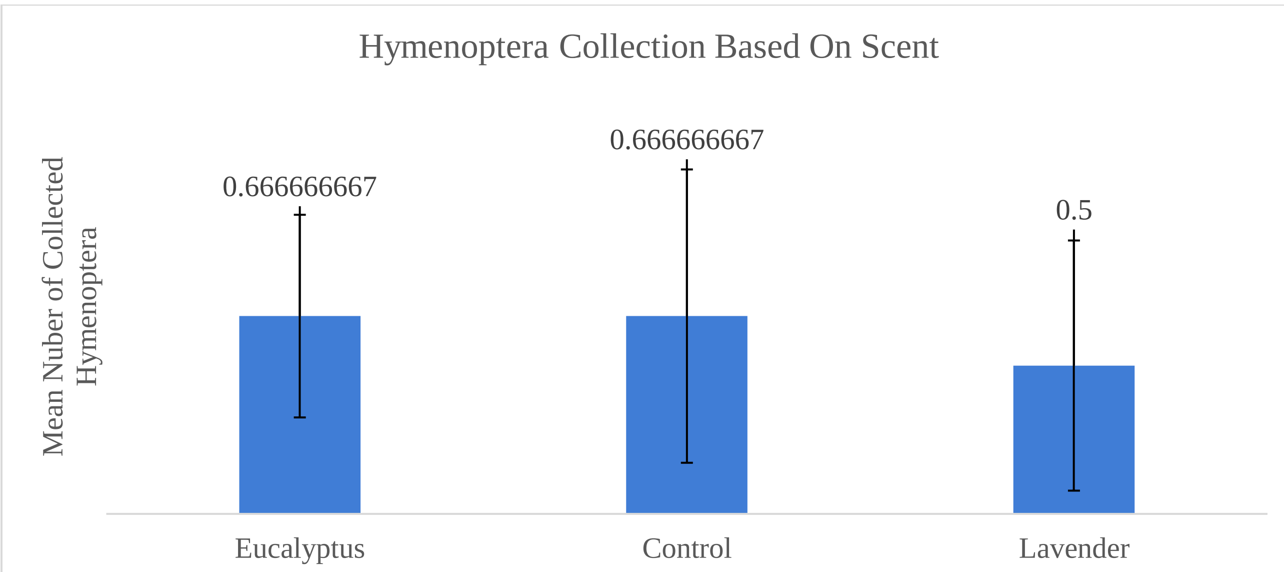

Fig. 6. The mean number of collected hymenopterans based on the scent of essential oils, regardless of colored bowl pairing. (*P*=0.20)

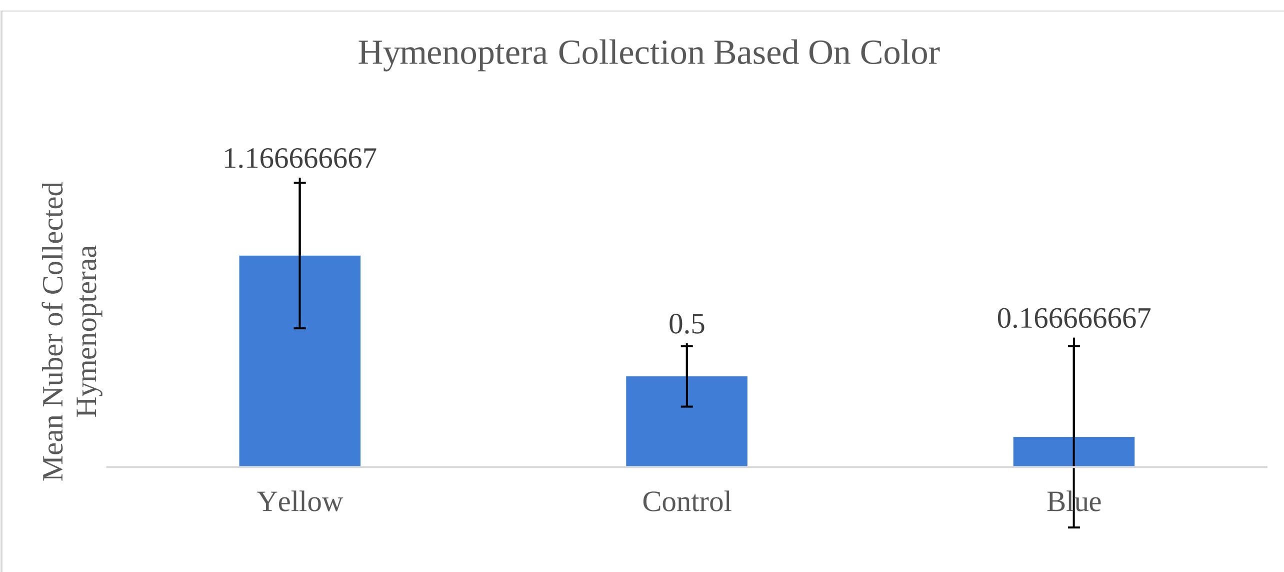

Fig. 7. The mean number of collected hymenopterans based on the colors of the bowls, regardless of essential oil pairing. (*P*=0.94)

Discussion

Pollinators serve an important role within the ecological and agricultural sector. Because of their interconnectedness with worldwide food production, the global trend of declining pollinator populations has caught public attention. Increased awareness for the importance of pollinators has encouraged more research, diving into the factors that repel and attract these crucial organisms. In this experiment, we strive to determine what combination of olfactory and visual stimuli attracted the most hymenopterans.

The collected data concluded that there is no significant difference of the levels of attraction based on color and scent, combined or isolated. Despite the *P*-values not allowing us to definitively determine the causation, an observational conclusion can be made. Regarding the olfactory stimuli, the lavender essential oil did not attract as many hymenopterans as the control nor the eucalyptus essential oil. These findings are reminiscent to a similar study, in which another floral essential oil, rose, did not attract as many hymenopterans as the control group, sucrose. (Erdogan and Yavus 2022) These results could have been influenced by the overall dispersion rate of the essential oils. Like perfume and cologne, there is a fine line in using the proper amount to attract instead of repelling an individual. (Teixeira et al. 2009) The potency of the essential oils, though using the same concentration, can differ from one another and may need to be altered accordingly. Additionally, the essential oils used may be reflective of the natural volatiles that plants use to attract pollinators and repel herbivores. (Pichersky and Gershenson 2002)

In future replications of this experiment, it would be advised to perform more trials. This would allow for the ability to determine p-values for the overall data and a potential Tukey test. Additionally, the experiment should take place over a longer period, ensuring little variation in weather and human interaction. The bowls should also be placed further apart from one another to reduce the overlapping scents of the essential oils. To allow for more dimension in this experiment, an urbanization gradient can be applied, with the experiment occurring in rural, suburban, and urban sites. The use of an urbanization gradient can determine the negative effects of urbanization on hymenopteran biodiversity and abundance. (Liang et al. 2023) This application can also provide classification thresholds, which can be used for selecting study areas in the future and aid in conservation efforts. The use of this gradient can emphasize the importance of using different landscape types for the application of conservation methods (Udy, et al. 2020)

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