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Buzzing Havens: Plant-Insect Interactions in Pollinator Gardens

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Abstract

Pollinating insects promote plant diversity, increase the stability of ecosystems, and maintain the reproductive success of most flowering plants; therefore, monitoring plant-pollinator interactions is necessary for conservation efforts. Little research is available on pollinator communities at the local scale in the Southeastern United States. Our investigation assessed plant-insect interactions in pollinator gardens at UNC-Asheville by evaluating diversity, abundance, and species assemblages through weekly visual surveys and pan trap collections in two gardens, Garden 1 and Garden 2, located on-campus in front of university buildings. The primary focus of this research study was preference and relationships between color, plant, and insect species. Plant assemblages and insect diversity varied significantly between sites. Garden 2 had greater insect diversity and abundance than Garden 1. Yellow flowers attracted a higher diversity of insects than purple or white flowers. Green and yellow pan traps had the highest insect diversity in both gardens and among pan trap colors, specifically for Hemiptera, Diptera, and one Coleopteran family. Andrenidae and Apidae had the highest abundance in blue traps, but this trend was not evident within Hymenoptera. These findings can inform conservation decisions around the impacts of cultivating and maintaining plant assemblages to increase pollinator diversity.

Introduction

Plant-insect interactions are a field of growing interest, and although much research has given insights into this phenomenon, questions remain regarding the ecological mechanisms behind their complexity. Plant-insect interactions include: herbivory, recruitment, oviposition host selection, and pollination. When under stress due to herbivory, plants can activate several metabolic pathways that either directly deter the attacking insect from continued consumption or recruit natural predators, typically other insects, of the herbivorous insect (Wu et al. 2007, Arimura et al. 2000, Paré and Tumlinson 1997, Godfray et al. 1995, Zhu et al. 2015, Hilker et al. 2002). Host selection during oviposition is dependent upon pheromone and phytochemical release from the target plant. Female insects then utilize the cues to orient themselves and choose a plant with the highest probability of maximizing offspring fitness (Honda 1995, Ferguson and Williams 1991, Videla et al. 2012, Heisswolf et al. 2005). Pollination involves a combination of olfactory, sensory, and visual cues given by the host plant that insect pollinators receive and interpret (Riffel et al. 2014, Kevan and Lane 1985, Wright and Schiestl 2009).

Insect pollinators orient themselves toward the target flower with an array of olfactory signals detected by their antennae, microtextural tactile cues after physical contact, and floral visual cues exhibited by the plant (Riffell et al. 2014, Kevan and Lane 1985, Wright and Schiestl 2009). Insects can distinguish the released plant volatiles from surrounding odors, including anthropogenic pollutants, and utilize floral scent to select flowers that offer pollen or nectar rewards after visiting the plant's reproductive organs (non-deceptive flowers) (Riffel et al. 2014, Wright and Schiestl 2009). Olfactory signals may be a driver in the establishment of pollinator specialist relationships with certain flowering plants (Wright and Schiestl 2009). Epidermal microstructure

differences of flower petals are detectable by sensilla on the antennae of pollinating insects and act as nectar-guides for foraging insects. Pollinators exploit this sensory cue as an additional discriminatory pathway when selecting a flowering plant (Kevan and Lane 1985, Deora et al. 2021).

Floral visual cues, including flower shape, size, and color, have developed a dynamic relationship between plant and insect pollinators (Weiss and Lamont 1997). Flower visual cues inform pollinators of reward quality and direct insects to locate the flower (Barragán-Fonseca et al. 2019). The co-occurrence of plant species and aggregation of floral traits may lead to increased visitation rates and pollinator sharing in communities with similar vegetation composition (De Jager et al. 2010). However, despite color similarity, the majority of pollinators are flower constant, consistently visiting the same plant species and relying on secondary cues or signals for discrimination (De Jager et al. 2010). Evidence for pollination syndromes, which drive floral trait evolution and selection for certain floral features, has implicated the importance of insect pollinator specificity for certain plant species (Reynolds et al. 2009). Plant senescence induces color change in different floral structures which results in a spectrum of colors that corresponds with age, this generates distinct color phases insects can recognize. Insect pollinators perceive the color phases and respond by preferentially visiting non-pollinated flowers or young flowers (Weiss and Lamont 1997, Barragán-Fonseca et al. 2019). Selection of flower color includes both innate preferences and learned behaviors by insects (Weiss and Lamont 1997). Some pollinating insects require a combination of olfactory and visual cues, instead of a single stimulus, for plant selection and participation in pollination (Raguso and Willis 2005), while other species preferentially orient themselves towards plants that exhibit visual cues over olfactory cues when given a choice between the two (Barragán-Fonseca et al. 2019).

Insects are effective pollinators because of their morphological capacity to transfer larger loads of pollen between flowers and increase fruit set (Ajerrar et al. 2020). The majority of angiosperm species and terrestrial habitats significantly depend on pollination services by insects for seed dispersal and promoting heterogeneity of species (Ollerton et al. 2011). Pollinating insects help increase the resilience and stability of ecosystems since the reproductive success of up to 94% of flowering plants rely on pollination (Portman et al. 2020). Thus, the evolution of angiosperms and plant mating systems are heavily influenced by insect visitor behavior and preferences (Wright and Schiestl 2009). Pollinator communities need proper monitoring procedures and conservation practices to ensure stable population growth and protection of the plant communities that rely on them.

Although there are studies on pollinating communities based on regional and temporal scales in the Southeastern United States, more localized research in urbanized, human disturbed areas is needed (Galletto et al. 2023). The garden spaces at the University of North Carolina–Asheville were established to provide essential habitat for native flora and insect communities, promote biodiversity, and maintain stable pollinator populations, which offer an opportunity to observe and learn about insect interactions with their environment. The purpose of this study was to elucidate the relationship between plants and insects, specifically the effects of environmental and biological factors on plant selection. This investigation aimed to understand the

difference in plant composition between gardens, the effect of garden type on insect diversity, whether climatic conditions affect visitation rates, the effect of trap color on diversity and abundance of visitors, and whether insect pollinators preferred specific flower colors. These plant-insect interactions were analyzed to understand the pollinating networks on a local scale in Western North Carolina.

Methods

2.1. Plant Diversity

Two gardens on the UNC-Asheville campus, denoted Garden 1 and Garden 2, were established in 2015 in collaboration with Burt's Bees Foundation to provide native flora habitat for local pollinators. Plant species for each garden were identified before and after all field data were collected.

2.2. Pan Traps

Pan traps elevated on a platform assessed insect richness and visitor color choice. The platform consisted of a wooden block (25.4 x 16.5 x 3.8 cm) drilled into a wooden post (5 x 5 x 127 cm). The pan traps were 325 mL plastic bowls colored blue, green, yellow, and white. One bowl of each color was placed in random order on the block and adhered by strips of Velcro (Fig. 1). Six platforms per garden were installed haphazardly approximately 2 m from other platforms and the garden perimeter (Table 1). Every week, pan traps were filled with 220 mL of soap solution consisting of 10 mL of soap per 1.00 L of water and left uncovered for 24 h. Pan traps were checked after each collection period, and specimens were stored in 70% ethanol at -17.7 °C. Specimens were then identified to the family. Data and specimens were collected at the two gardens for six weeks, from July 11 to August 18, 2023.

2.3. Visual Surveys

Visitation rates and flower color preference were determined via weekly visual surveys from July 11 to August 18, 2023. Individual observations were performed between 9:00-11:00 AM and again between 12:00-2:00 PM. Six flowering plants were chosen per garden, and the same plants were observed in both morning and afternoon. Each flowering plant was observed for 10 min, and any insect interactions that lasted at least five seconds were recorded. Insects were identified by order, and only adults were considered. Interactions were classified by behaviors including: hovering, direct contact with reproductive and non-reproductive structures, resting, climbing, and consumption of plants. Flower color, sun exposure (full sun, partial sun, partial shade, or shade), temperature, humidity, precipitation, wind speed, and wind direction were recorded for each survey period.

2.4. Data Analysis

Data were imported to R V4.3.1 using RStudio. Packages tidyverse, car, emmeans, vegan, multcomp, lme4, and ggplot2 were used in the analysis, plotting, and model interpretation. Shannon-Wiener Indices were calculated for both visual survey and pan trap data based on garden type, flower color, pan trap color, and insect count. Linear regression was used to assess the effects of (1) climatic conditions on insect visitation and diversity, (2) garden type and flower color in insect diversity, and (3) garden type and pan trap color on insect diversity and abundance. Best fit models were chosen and post-hoc comparisons were made with the Estimated Marginal Means test.

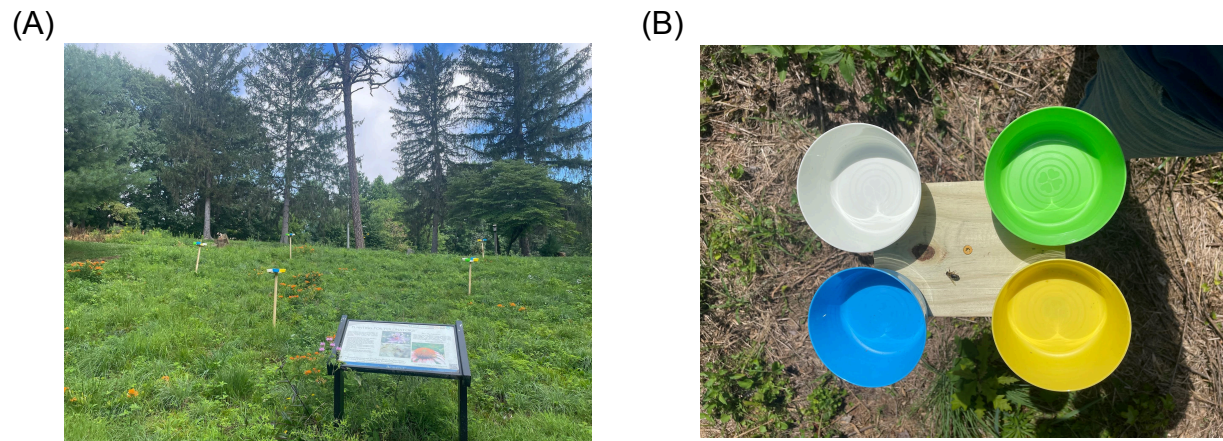


Figure 1. (A) Pan trap platforms were installed in each garden with 2 m spacing between each platform. (B) Example of colored pan traps placed in random order on a wooden platform.

Table 1. GPS Coordinates of Pan Traps in Gardens 1 and 2

Garden	Pan Trap #	Coordinates
1	1	35.61715°N, 82.56346°W
1	2	35.61717°N, 82.56336°W
1	3	35.61704°N, 82.56351°W
1	4	35.61715°N, 82.56342°W
1	5	35.61702°N, 82.56331°W
1	6	35.61744°N, 82.56217°W
2	1	35.61708°N, 82.56321°W
2	2	35.61704°N, 82.56329°W

2	3	35.61721°N, 82.56337°W
2	4	35.61705°N, 82.56318°W
2	5	35.61691°N, 82.56323°W
2	6	35.61631°N, 82.56243°W

Results

3.1. Plant Diversity

Plant assemblages differed greatly between gardens (Fig. 2). Garden 1 had 21 unique species and Garden 2 had 24 species, with only one species, *Solanum carolinense*, identified in both. The majority of plants in Garden 1 produced yellow (33.3%) or white (38.1%) flowers. Garden 2 contained mostly white flowers (41.7%) or purple flowers (25%).

Garden 1	Both	Garden 2
<ul style="list-style-type: none"> ● <i>Asclepias tuberosa</i> ● <i>Rudbeckia hirta</i> ● <i>Lactuca serriola</i> ● <i>Acalypha virginica</i> ● <i>Lactuca canadensis</i> ● <i>Chaecrista nictitans</i> ● <i>Coreopsis major</i> ● <i>Coreopsis verticillata</i> ○ <i>Geum canadense</i> ○ <i>Trifolium repens</i> ○ <i>Penstemon digitalis</i> ○ <i>Gamochaeta pensylvanica</i> ○ <i>Cerastium fontanum</i> ○ <i>Sherardia arvensis</i> ○ <i>Cerastium glomeratum</i> ○ <i>Pycnanthemum incanum</i> ● <i>Veronica persica</i> ● <i>Salvia lyrata</i> ● <i>Monarda fistulosa</i> ● <i>Geranium dissectum</i> ● <i>Viola odorata</i> 	<ul style="list-style-type: none"> ○ <i>Solanum carolinense</i> 	<ul style="list-style-type: none"> ● <i>Liquidambar styraciflua</i> ● <i>Solidago canadensis</i> ● <i>Bidens aristosa</i> ● <i>Helianthus angustifolius</i> ● <i>Oenothera biennis</i> ● <i>Sonchus asper</i> ○ <i>Symphyotrichum pilosum</i> ○ <i>Erigeron annuus</i> ○ <i>Doellingeria umbellata</i> ○ <i>Cicuta maculata</i> ○ <i>Hydrangea arborescens</i> ○ <i>Erigeron philadelphicus</i> ○ <i>Solanum americanum</i> ○ <i>Croton glandulosus</i> ○ <i>Lepidium virginicum</i> ○ <i>Sambucus canadensis</i> ● <i>Eutrochium fistulosum</i> ● <i>Asclepias syriaca</i> ● <i>Vernonia missurica</i> ● <i>Symphyotrichum novae-angliae</i> ● <i>Geranium maculatum</i> ● <i>Symphyotrichum cordifolium</i> ● <i>Triodanis perfoliata</i> ● <i>Vicia sativa</i>

Figure 2. List of plant species identified in each garden. *Solanum carolinense* was the only species in common between both gardens. Colored circles represent the dominant flower color for each plant species.

3.2. Pan Traps

Pan trap collections captured a total of 3,647 insects within the six-week collection period. Diptera had the highest count of specimens ($n=2,112$) followed by Hemiptera ($n=683$), Hymenoptera ($n=568$), and Coleoptera ($n=244$). The orders with the fewest number of collected insects were Lepidoptera ($n=18$), Thysanoptera ($n=18$), Neuroptera ($n=1$), Orthoptera ($n=1$), and Psocoptera ($n=1$). Across pan trap colors and garden type, insect diversity was higher in Garden 2 compared to Garden 1 ($F=24.86$, $df=1$, $P<0.0001$). Between gardens, yellow and green pan traps had higher diversity in Garden 2 than traps of the same colors in Garden 1 ($t=-3.765$, $df=35$, $P=0.0006$; $t=-4.387$, $df=35$, $P=0.0001$). Within Garden 1, only blue and white pan traps had significantly more insect diversity than green pan traps ($t=3.117$, $df=35$, $P=0.0182$; $t=-3.241$, $P=0.0133$).

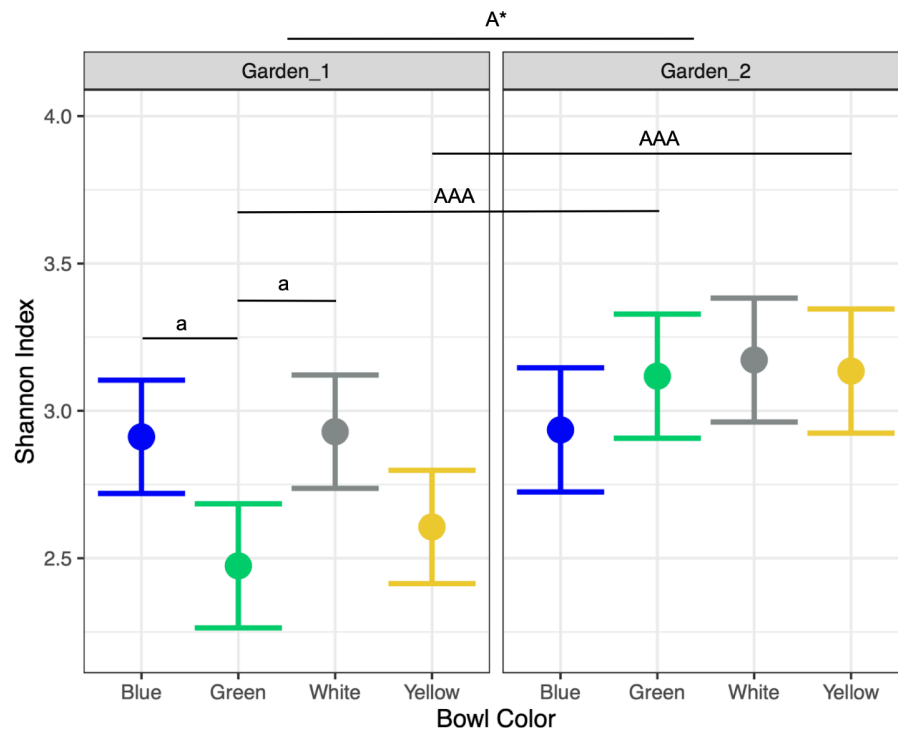


Figure 3. Insect diversity was significantly different depending on garden and pan trap color. (A*) denotes higher diversity in Garden 2. (AAA) refers to higher diversity for green and yellow traps between gardens. (a) demonstrates greater diversity for blue and white traps within Garden 1.

Additionally, traps were assessed for effect on insect abundance and revealed certain insect orders and families were more attracted to specific colors (Fig 4). Blue pan traps captured a higher amount of Andrenidae than white and yellow traps (Fig 4a; $t=2.575$, $df=105$, $P=0.0304$; $t=3.154$, $df=105$, $P=0.0059$) and Apidae than green traps (Fig. 4b; $t=2.782$, $df=105$, $P=0.0320$). No significant difference in abundance was evident for the order Hymenoptera.

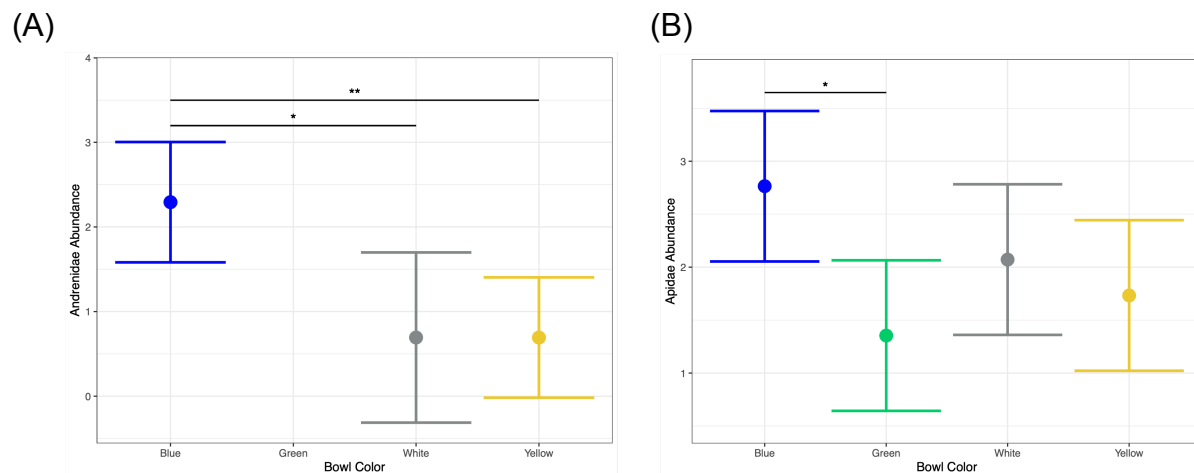


Figure 4. (A) Differences in abundance for Andrenidae are shown. Higher abundance was found in blue traps over white ($P=0.0304$) and yellow traps ($P=0.0059$). (B) Greater abundance was associated with blue traps over green traps ($P=0.0320$) for Apidae. * = $P < 0.05$ and ** = $P < 0.001$

Green pan traps had a higher abundance of Hemiptera than blue and white traps (Fig. 5a; $t=-3.383$, $df=20$, $P=0.0144$; $t=2.782$, $df=20$, $P=0.0518$). Only one Hemipteran family, Cicadellidae, demonstrated a similar pattern with green traps capturing more than blue traps (Fig 5b; $t=-4.967$, $df=105$, $P=<0.0001$). Green also contained significantly more Mordellidae (Coleoptera) compared to blue traps (Fig. 5c; $t=-2.602$, $df=105$, $P=0.0511$). Dolichopodidae (Diptera) was significantly more attracted for green traps than blue traps and had a higher abundance for this color (Fig. 5d; $t=-4.824$, $df=105$, $P=<0.0001$).

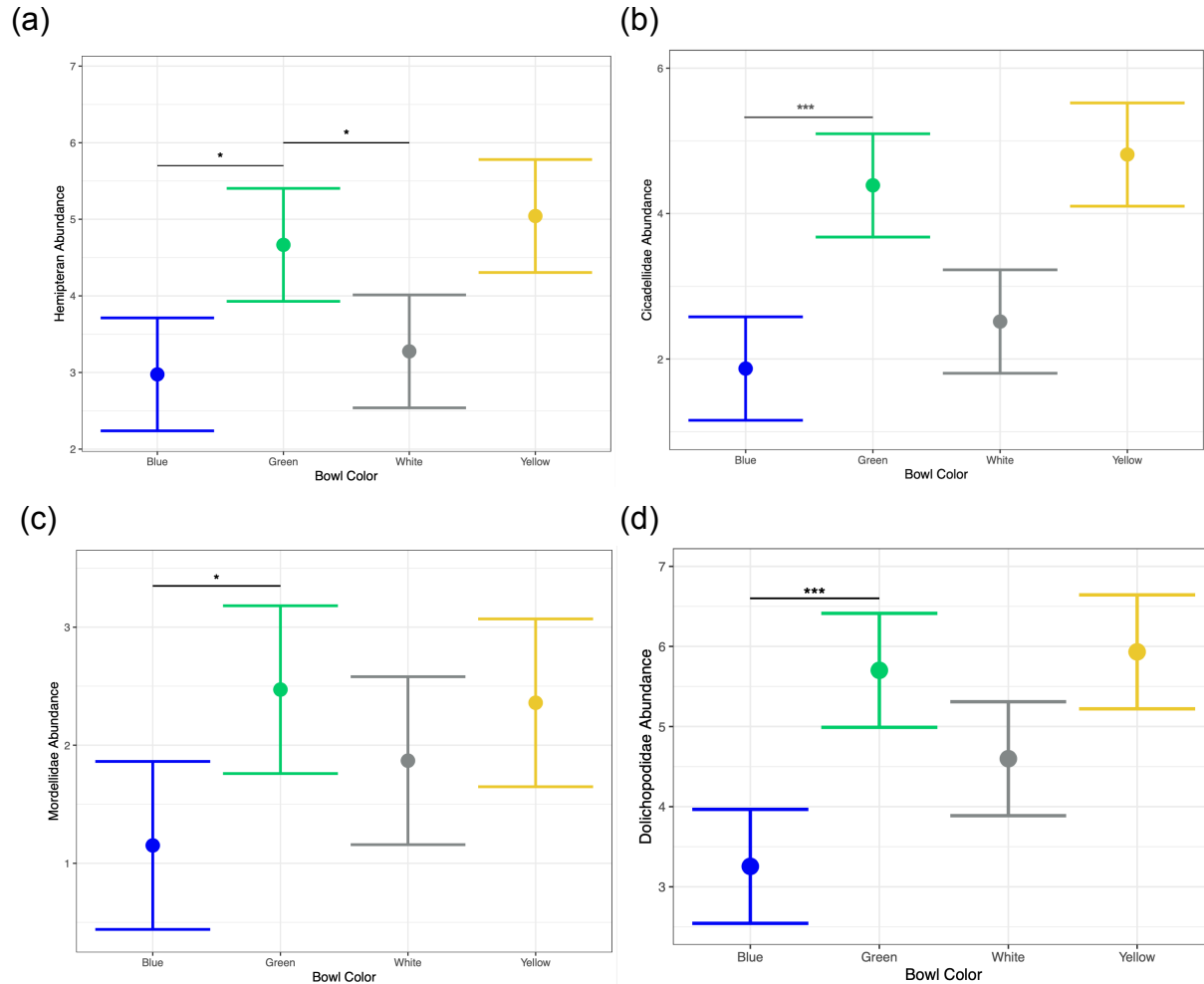


Figure 5. Significant differences in abundance correlated with green pan traps for insect order Hemiptera and insect families Cicadellidae and Mordellidae.

Lastly, yellow traps had more Hemiptera than blue or white traps (Fig. 6a; $t=-4.136$, $df=20$, $P=0.0027$; $t=-3.535$, $df=20$, $P=0.0103$). The family Cicadellidae followed this trend, with yellow traps differing significantly in abundance compared to blue or white pan traps (Fig. 6b. $t=-5.804$, $df=105$, $P=<0.0001$; $t=-4.529$, $df=105$, $P=0.0001$). Diptera also had a higher abundance in yellow traps than blue traps (Fig. 6c; $t=-4.095$, $df=20$, $P=0.0029$). Similarly, the family Dolichopodidae had higher abundance in yellow traps compared to blue traps and white traps (Fig. 6d; $t=-5.278$, $df=105$, $P=<0.0001$; $t=-2.629$, $df=105$, $P=0.0477$).

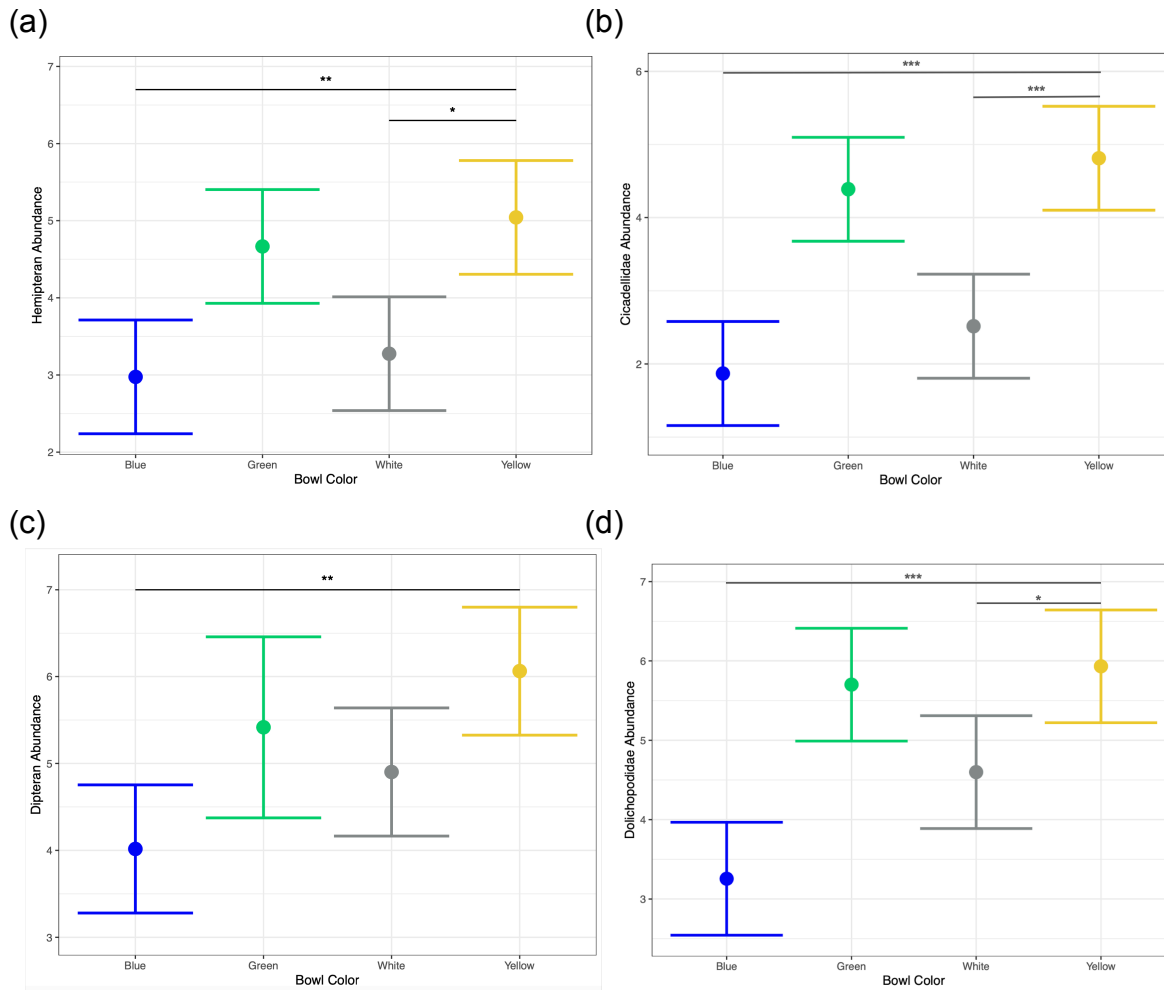


Figure 6. Abundances were higher in yellow pan traps over all other colors (blue, green, and white) for two insect orders (Hemiptera and Diptera) and two families (Cicadellidae and Dolichopodidae).

3.3. Visual Surveys

In total, 2,110 adult insects were recorded over 60 hours of observation. Insect visitation rates and insect diversity were not significantly affected by time of survey, sun exposure, or temperature ($F=0.0667$, $df=1$, $P=0.7966$; $F=0.1840$, $df=1$, $P=0.9071$; $F=0.1768$, $df=1$, $P=0.6748$). Humidity, precipitation, wind direction, and wind speed also did not significantly impact insect visits or diversity during visual surveys ($F=0.0$, $df=1$, $P=0.9968$; $F=2e^{-4}$, $df=1$, $P=0.9897$; $F=0.39$, $df=2$, $P=0.6778$; $F=0.2328$, $df=1$, $P=0.6302$). However, Garden 2 had more diverse insect visits compared to Garden 1 (Fig. 7; $t=2.102$, $df=112$, $P=0.0378$).

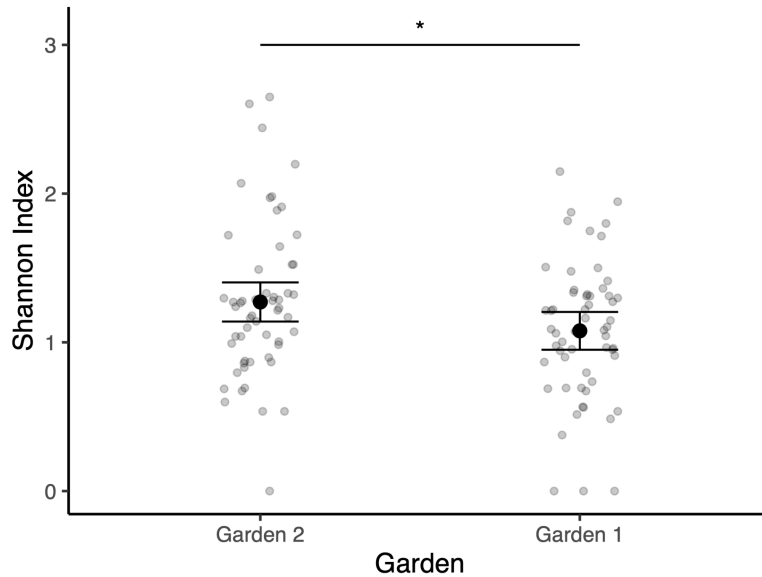


Figure 7. Shannon Index revealed greater insect diversity in Garden 2 during visual surveys.

Overall, yellow flowers had more diverse insect activity than white or purple flowers between the two gardens (Fig. 8; $t=-3.809$, $df=109$, $P=0.0022$; $t=-3.490$, $df=109$, $P=0.0062$).

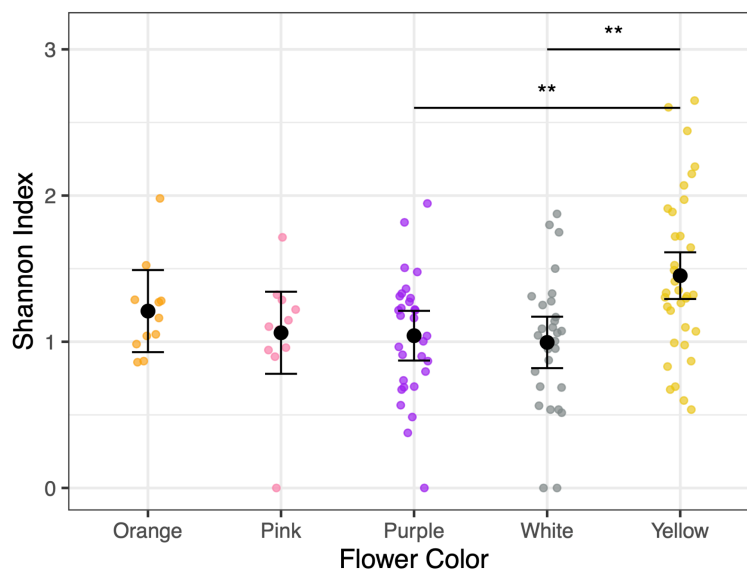


Figure 8. Yellow flowers had higher insect diversity than two other colors. No significant difference was found for pink or orange flowers.

Discussion

This study sought to assess insect diversity, examine whether climatic conditions impact visitation rates, investigate the influence of trap color on visitor diversity and abundance, and determine if insect pollinators show a preference for particular flower colors with visual surveys and pan trap collections. Plant assemblages differed between gardens in terms of numbers and types of plant species. Garden 2 had the highest insect diversity which could be attributed to the diversity of plant species growing. The dominant floral color was white (41.7%), which has been documented to primarily attract generalist pollinators (McCall et al. 2012), meaning they collect nectar and pollen from a variety of plant species often belonging to different families (Thompson 2001, Fontaine et al. 2008). Frequent visitors of white blooms are moths, flies, and beetles (Goyret et al. 2008, Johnson and Midgley 2001), in addition to primary pollinator species such as bees and wasps. Oftentimes, plants exhibit an olfactory cue concentrated around white blooms since the majority of dipterans, lepidopterans, and coleopterans have dichromatic vision and require additional cues to orient themselves toward a food source (Van Der Kooi et al. 2021). Insects identified during visual surveys were likely generalist pollinators and were attracted by additional structural and color cues. Secondary dominant flower color in Garden 2 was purple (25%) which typically attracts bees and wasps. The majority of Hymenopterans have trichromatic sensitivity; bees and wasps are better adapted to perceiving wavelengths within 200-300 nm and consequently steer themselves towards blue and purple colored objects (Van Der Kooi et al. 2021, Giurfa et al. 1995, Ostroverkhova et al. 2018). Interestingly, out of the five colors observed during visual surveys (orange, pink, purple, white, and yellow), yellow flowers had higher diversity compared to white and purple flowers. Some pollinating insects have been recorded to prefer yellow floral cues and arrangements over other flower colors even at the expense of longer foraging expeditions (Campbell et al. 2010, Kevan 1972). Furthermore, yellow flower abundance was significantly higher than that of other floral colors, which may have influenced insect-pollinator preference. Greater abundance would increase the frequency of yellow flowers available, and therefore, pollinators may have been more likely to select a yellow blossom due to spatial positioning throughout the garden.

Insect order association with pan trap color further corroborates existing literature on effective insect trapping methods (Gumbert 2000). Hymenopterans were most frequently captured in white, yellow, and blue traps (Hall 2016). Substantial data has established an innate preference for shorter wavelength emissions (blue/purple) for the family Andrenidae both in natural environments and laboratory settings. Other Hymenoptera families (Apidae and Vespidae) often select blue/purple over other chromatic options but will periodically choose white or yellow flowers in field conditions (Csanády et al. 2021, Reverté et al. 2016, Buffington et al. 2020). Hemiptera has innate

color preferences for green and yellow pigmented objects that correspond to pigments reflected by the plant structures Hemiptera most interacts with (Fernier et al. 2014, Döring and Chittka 2007, Rodriguez-Saona et al. 2012). Insects with phytophagous diets rely primarily on visual cues, shape, and color for selection of host plant and will direct themselves towards plants most similar in the absence of favored species (Bullas-Appleton et al. 2004, Fernier et al. 2014). Leafhoppers (Cicadellidae) particularly displayed this behavioral pattern by having a high abundance in green and yellow pan traps, which complements other studies on predatory and beneficial Cicadellids (Bullas-Appleton et al. 2004, Döring and Chittka 2007). Coleopteran pests are best captured by traps with dark chromaticity (dark green and black) while flower-visiting beetle species tend to have similar attraction patterns as other pollinators (Cavaletto et al. 2020). Diptera typically associates with white and green plant structures, but certain species are also attracted to yellow traps despite their dichromatic vision (Van Der Kooi et al. 2021, Campbell et al. 2010).

Providing adequate food and nesting resources is important not only for promoting insect diversity but also for maintaining the ecological mechanisms provided by insects (Majewska and Altizer 2019, Ebeling et al. 2011). Incorporating a diversity of plant species in personal and communal gardens is beneficial to native plants and insects (Zhang et al. 2016, Haddad et al. 2001, Janz et al. 2006). Efforts to increase public support and interest in 'pollinator gardens' would benefit from including information on the best plant species based on temporal and geographical location. Generalist plants should be included since a diverse array of insects depend on these plants for resources, but specialist species should also be included to support more vulnerable insect species with more specific preferences. Farmers wanting to implement non-abrasive and non-toxic methods of pest control should select traps that correspond to color attraction trends evidenced in this study and previous literature (Vrdoljak and Samways 2011, Haddad et al. 2001, Janz et al. 2006).

This study only observed insect visitation and did not investigate pollination success. Continuing research on plant-insect interactions presents an opportunity to determine the efficacy of specific insect guilds in aiding plant reproduction and their exact contribution to pollination and plant fitness. Our understanding of true pollinator species is based on indirect evidence, such as the presence of pollen on an insect's anatomy (Bischoff 2008). Therefore, species-specific studies on plants and insects would improve current perspectives on these interactions. Additionally, future research should also focus on gardens with different plant assemblages or with more diverse plant compositions to assess if similar patterns of insect-flower color attraction are identified or if plant composition significantly alters visitation trends. UNC-Asheville's Bee Hotel, located directly in front of Garden 2, could influence pollinator communities as it provides shelter and nesting for solitary bees and wasps. Further investigations are

needed to address the effects, if any, the Bee Hotel may have on the diversity, abundance, and richness of local insect communities.

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