Better Together: Forest and Open Habitat Support Bumble Bees in Late Summer

THESIS

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By

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Abstract

Bumble bees must forage continuously during their long flight periods. Floral resources change spatially and temporally, leading bumble bees to forage from different flower species. As the growing season progresses, flower availability shifts between habitats. These habitats may offer distinct floral resources for bumble bees to collect pollen and nectar. To properly conserve bumble bee habitat, we must determine where bumble bees find their food. Late summer is an especially important time in the bumble bee life cycle, as the colony shifts from producing non-reproducing workers to producing reproductives (i.e., gynes and males). Determining the habitats and plants bumble bees forage from in late summer is important for effective habitat management to support these essential pollinators.

I investigated the floral availability and wild bumble bee use of flowers in two habitats, forest and open habitat (fields and meadows). These habitats complement one another in the resources they offer to bumble bees. Through documenting flower visitation by bumble bees as well as floral abundance and richness, I determined the use and preference of flowers in these two habitats during late summer. Although bumble bees did not prefer one habitat over another, my data established that specific flower species were favored by bumble bees in both forest and open habitats.

Bumble bee forage can influence colony success. My research unveils insights into bumble bee foraging patterns and their impact on reproductive success. Through collecting the foraged pollen from sentinel bumble bee colonies, I determined where bumble bees collect pollen in late summer. Pollen is essential and a limiting resource to bumble bees, contributing to colony size and adult bee size. I found that both forest and open habitats contribute significantly to colony reproductive success in their own respective ways. Collecting open habitat pollen enhanced the number of gynes produced within a colony. Meanwhile, colonies that collected more forest pollen developed larger gynes.

Forest and open habitat complement each other by providing distinct forage for bumble bees in late summer. Both wild and sentinel bumble bees foraged in both habitats, collecting pollen to feed to their reproductives. Neglecting forest or open habitat resources in late summer jeopardizes the important floral species that sustain bumble bee colonies during this crucial period.

Dedication

To bumble bees everywhere

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Introduction:

There is considerable interest in wild bumble bee (*Bombus*) habitat conservation as they are incredibly efficient pollinators (Spivak et al. 2010) who play a key role in the pollination of wild plants (Garibaldi et al. 2013). There is increasing evidence of bumble bee population declines in some parts of the world (Cameron et al. 2010, Goulson et al. 2008). Broad scale habitat loss (Meehan et al. 2011) and the associated decline of their floral resources (Mola et al. 2021) continue to be stressors for wild bumble bee populations. These trends emphasize the need to conserve their habitats. To do so, we need to know what bumble bee habitat looks like, what resources they rely on during the different periods of their life cycle, and how local and landscape level factors influence their colonies. Bumble bees have a long flight season that extends from early spring to late fall, typically being one of the last bees active each year. Different habitat types may provide unique resources at distinct times of the year. Understanding how bumble bees use resources across habitats can help us to support bumble bee habitat. Specifically, determining how forested and open habitats affect bumble bee floral preference will shed light on the relationship between bumble bees and their landscape and resources.

Bumble bees are affected by the landscape context and flowering phenology within their habitats (Hines and Hendrix 2005, Mola et al. 2021). Bumble bee flight periods span several months, during which colonies need a constant supply of floral resources. Bumble bees may need to collect floral resources from several different habitat types over their colony lifetime because of their long foraging period (Mandelik et al. 2012). Some forests offer key floral resources in early spring, which is a critical time for colony success (Malfi et al. 2018). Forested land is associated with higher occupancy of some bumble bee species, higher worker abundance, and increased colony performance (Mola et al. 2021, Novotny et al. 2021). These observational studies suggest a key role of forested habitat in providing required resources for bumble bees. However, we need to determine which specific resources forests provide and which periods of a bumble bee colony's lifecycle rely most on these resources.

Deciduous, evergreen, and mixed forests may provide key resources at distinct times of the year, such as forage flowers (Mola et al. 2021). Floral distributions change over the growing season, which change where bumble bees may find their food. In early and mid-spring, before the canopy closes, forests can provide many floral resources for use by bumble bees (Mallinger et al. 2016, Wray et al. 2014, Kaemper et al. 2016). This timing coincides with emerging bumble bee queens that tend to forage in forested habitats for floral resources and search for nesting sites (Carvell et al. 2017, Novotny et al. 2021). The value of these early flowering forest flowers has been recognized (Mola et al. 2021). As the growing season progresses and the forest leaf canopy closes, most spring ephemeral flowers finish blooming and floral resources become scarce in the understory. Meanwhile, open habitats, which are not light-limited by a closed canopy, begin to offer greater densities of flowers (Wray and Elle 2015). Many bumble bee workers tend to forage in more open habitats with readily available mid-summer resources. The majority of pollinator habitat restoration projects plant species that bloom in mid to late summer (Lybbert et al. 2021) in open meadow habitats (Goulson, 2009). Near the end of summer and into autumn in midwestern USA, open areas continue to provide dense flowers, but forest flowers begin to bloom again. Some forest flowers reach peak abundance when bumble bee colonies move onto their next life stage, male and gyne reproduction. Bumble bees are known to use nesting resources provided by wooded areas in early spring (Liczner and Colla 2019) and spring queens may choose to nest near spring forage to optimize energy intake (Suzuki et al. 2007, 2009, O'Conner et al. 2017). Bumble bees are central place foragers and while they can disperse widely in search of resources (Rouslton and Goodell 2011), bumble bee colonies located within forest habitats may forage more efficiently on forest flowers than those located in further open habitats. Forest understories may also be less water stressed than open areas during late summer (Webb et al. 1978). Specifically, riparian forest habitats may offer higher resource levels for native bumble bees than other habitat types (Williams & Kremen, 2007). On the other hand, bumble bees tend to show fidelity for flower patches and flower constancy while foraging, which could cause a lag in their use of newly blossomed forest resources at the outset of their bloom in the fall (Thomsom et al. 1997). Research on the use of forest by bumble bees in the late summer and early fall will help illuminate whether this habitat may complement open habitats at the end of the colony life cycle.

To better maintain bumble bee habitat, we need to understand not only environmental influences on bumble bee floral use but also plant characteristics. Native to non-native plant composition can vary significantly in individual forests (Parscarella et al. 2000), as well as open habitats (Abella et al. 2023), especially in human-altered landscapes (Morandin & Kremen, 2012). There are many contrasting studies on bumble bee use of native vs. non-native plants. Non-native plants may provide less attractive flowers to native pollinators (Kearns et al. 1998) but may also offer important resources during phenologically distinct times (Stout & Morales, 2009). A meta-analysis of 40 studies showed an overall negative impact on native pollination in the presence of non-native plants (Morales and Traveset, 2009), while some studies have found that bumble bees simply use non-native plants in proportion to their abundance (Williams et al. 2011, Cameron et al. 2011, Hingston 2005). Even within studies results can vary, with different bumble bee species using native and non-native plants differently across the growing season (Lanterman et al. 2023). More data is needed to determine the best way to conserve bumble bee floral resources.

To study the mechanism by which local and landscape level factors impact wild bumble bee preference in late summer, I surveyed floral resource availability and documented where wild bumble bees foraged at 10 sites in Ohio, USA. These sites had a varying landscape composition from which I surveyed the available flower species' abundance and richness. I compared the proportion of bumble bee floral use to flower availability to quantify selection indices for 24 flower species. I address the following questions: 1) What floral resources are available in forest understories and open habitats in late summer and how do they compare in abundance and diversity? 2) which floral resources do bees use in forested and open habitats during late summer? 3) do bumble bee workers show a preference for flowers in forested or open habitats when both are available in late summer and which flowers? I also document preference for native vs. non-native flowers.

Methods:

Study sites:

This study was performed in Ohio, USA a region dominated by agricultural and urban land cover, but with many metropolitan parks consisting of greenspace. I chose 10 sites that varied in the amount of forest in the landscape within 1 km (Figure 1.1). This allowed for a gradient of percent forest ranging from 7.46% - 58.05% (Table 1.1). Forage availability of floral resources within 1km of bumble bee colonies has been found to be a good predictor of colony metrics (Knight et al. 2009), so I chose a 1km radius. I used ArcGIS ver. 10.8.2 to plot landscape composition and land cover data provided by the 2019 National Land Cover Database (NLCD 2019). The NLCD is a comprehensive database updated every 3 years and shows current land cover data on a raster map comprised of 30 x 30 meter pixels. I calculated forest by summing the areas of all forest categories (41- Deciduous, 42- Evergreen, and 43- Mixed). These forests are characterized by their trees generally greater than 5 meters tall that dominate the area, with vegetation cover greater than 20% (NLCD 2019). I calculated open habitat by summing shrub/scrub (52), herbaceous (71) and open space (21) (NLCD 2019).

Flower and bumble bee surveys:

I conducted weekly surveys for six contiguous weeks from August 8th, 2022 to September 19th, 2022 between the hours of 0900 and 1600 on clear weather days above 60˚F. There was an average of seven days between each survey conducted at each site. Based on the characterization of the land cover, surveys were conducted in habitats classified as forest or open habitat. Two 25 m x 1 m line transects (100 m^2 total area) were delineated in both forest and open habitats in different areas each week. For these surveys, forest surveys were conducted in patches with an area of at least 100 m^2 (with most being much larger). Some transects crossed narrow park walking paths or streams, so these transects stopped at the forest edge then started again on the other side. Open habitat surveys were performed in areas not enclosed by trees consisting of natural vegetation in open meadows, grass/prairie, or clear-cuts. The surveyed area within each habitat was randomly chosen each week and transects were haphazardly placed. Transects were walked slowly one-way to determine the number of flower species and the number of floral units of each species within the transect. Plants were identified in the field using a guide (Newcomb, 1977) and clippings were collected and pressed for later verification. Flower origin was determined by using data from the Biota of North

America Program (BONAP). Flower heads were counted in different units depending on flower type and were defined on a species-by-species basis (Williams and Kremen 2007). The genera *Actaea*, *Ageratina*, *Cirsium*, *Clethra*, *Daucus*, *Dipsacus*, *Laportea*, *Persicaria*, *Phytolacca*, *Prunella*, *Solidago*, and *Vernonia*, were quantified by counting each inflorescence. All other species were quantified by counting individual flowers. For *Helianthus* and *Solidago* species, flower abundance and bee foraging data were aggregated due to inconsistencies in species-level identification. At the end of each transect, surveyors walked the same transect in the opposite direction to count the number of bumble bees foraging from each flower species, from now on referred to as bumble bee "use". Wild bumble bees seen foraging were noted along with the floral species they foraged from. Bumble bees observed were most commonly *Bombus impatiens*, a generalist and common species at this time of year in Ohio (Novotny et al. 2021). Bumble bees were observed until they left the transect area to ensure they were only counted once. Bumble bees that were not foraging were not counted.

Data analysis:

To address the question of how floral resources in forest and open habitats compare in abundance and diversity, the total number of flower species and flower heads found in transects were summed across days for each site by habitat type and the overall totals among all sites were compared using a paired t-test.

To determine wild bumble bee selection of a flower species, use and availability were calculated following Novotny et al. (2022). I defined flower species “use” as the number of individual bumble bees seen foraging from flowers of a species i divided by the total number of bumble bee visits to all species and ranges from zero to one. I defined availability as the number of flower units of species i within transects over total flower abundance within habitat types. To test how use and availability differed between forest and open habitats, average use and availability were averaged across species at each site and compared between habitat types using a one-way ANOVA test with pairwise comparisons using the Tukey HSD post-hoc test. A Mantel test for correlation of Bray-Curtis dissimilarity was performed to determine if site-level use and availability matrices were correlated.

Following Cole et al. (2020), use was divided by availability to calculate selection indices. If $use_i/availability_i > 1$ then selection was considered positive and $selection_i = use_i/availability_i$. If $use_i/availability_i < 1$ selection was considered negative and $selection_i = -1 \times ([use_i/availability_i]^{-1})$. A selection index over 1 indicates a preference for that flower species, meaning it was used more than expected based on proportionate availability. I only calculated selection indices for flower species that had at least one documented foraging observation.

To explore the factors that best explain selection for each flower species, I examined the impact of local and landscape scale habitat type on site-level selection index. I constructed generalized linear models with both local and landscape scale variables using JMP®, Version 17 (SAS Institute Inc., Cary, NC, 1989–2023). I started with the model: *selection index ~ rank abundance + habitat + % forest + site + plant sp.,* in which *site* and *plant sp.* were included as random effects. Interacting terms were excluded. I tested the distribution of the variables and used the one that best fit the model assumptions. *Rank abundance* for each flower species at each site was determined by overall number of flower heads in descending order (Whittaker, 1965). *Site* was defined by the different area's surveys were performed, *plant sp.* indicated plant species, *habitat* indicated forest versus open habitat, and *% forest* indicated the amount of land cover within 1km occupied by forest. I then used the leave-one-out method to determine the model that minimized Akaike Information Criterion corrected (AICc) values. The model with the lowest AIC was considered the best fit to the data (Burnham and Anderson 1998). To explore whether plant species origin (native vs. non-native) affected the selection index, this variable was added as a fixed effect to a separate mixed model and the steps described previously were followed.

Results:

What floral resources are available in forest understories and in open habitats in late summer and how do they compare in abundance and diversity?

I recorded 1,706 foraging bumble bee observations and 35,359 flower heads from 45 plant taxa. Overall flower head abundance in forested and open habitats was similar (Forest = 16,411, Open = 18,291, $t = 0.74$, $df = 1$, $p = 0.24$). Open habitats had significantly more unique flower species than forest (t = 0.01, df = 1, p = 0.013). (Table 1.2). Furthermore, the composition of the flower community differed between forest and open habitats; they only shared two flower species (*Polymnia canadensis* and *Rudbeckia laciniata*). Forest had 18 unique flower species and open habitats had 25 unique flower species (Table 1.2).

Which floral resources do bees use in forested and open habitats during late summer?

Bumble bees used forest flowers significantly more than either open habitat flowers or flowers found in both habitats based on raw use data (ANOVA, $F_{2,20} = 11.93$, p = 0.0104). Open habitat flowers were also used significantly more than flowers found in both habitats. Average use, which is the proportional flower use, for each category was Forest = 0.08, Open = 0.04, and Both = 0.01. The three most used flower taxa were two species of Jewelweed, *Impatiens capensis* and *Impatiens pallida*, both of which are found in forest, and *Solidago* species commonly found in open habitats (Table 1.3). 21 flower species had no observations of bumble bee forage (Table 1.4).

Do bumble bee workers show a preference for flowers in forested or open habitats when both are available in late summer and which flowers?

Overall, average availability of flower heads was not significantly different between forest and open habitat. There were significantly less flower heads for species found in both habitats (ANOVA, $F_{2,20} = 3.49$, p = 0.032). Mean availability for each category was Forest = 0.06, Open = 0.05, and Both = 0.01. Similar to use, the three most available flower taxa included *Solidago* species, *Impatiens capensis*, and *Impatiens pallida* (Table 1.3), with *Solidago* and *Impatiens capensis* appearing at all 10 sites and *Impatiens pallida* at 9. By graphing the percent use against percent relative abundance, I can determine whether a flower species was used more or less by bumble bees than I would expect based on proportionate availability (Figure 1.2).

Taking availability of flowers into account changes the interpretation of bumble bee preference (Lanterman et al. 2023). There was an association between availability of flower heads and bumble bee use (Mantel test for correlation of Bray dissimilarity between site-level use vs. availability matrices, R = 0.55, p = 0.048) meaning that in general, flowers that were more abundant were used more, opposing the idea of an overall preference for one habitat or another. Although I couldn't distinguish against random use in a Mantel test, I can still calculate selection indices to analyze specific cases of preference or avoidance by bumble bees. I calculated 24 selection indices for different flower species (Table 1.5). There was no correlation between selection index and percent use of flower taxa by bumble bees (t = 0.07, p = 0.28). Of these 24 species, five were from forest, 17 from open habitat, and two from both habitats. Of the top five flower taxa based on use, only two were included in the top five for selection index (*Impatiens capensis* and *Verbesina alternifolia*). Mean site-level selection indices were calculated and graphed for visualization (Figure 1.3). The 21 species with zero observations could not have selection indices calculated as they had no use data (Table 1.4). Overall, 10 flower species were preferred (had a selection index higher than 1).

three forest flower species (*Impatiens capensis*, *Impatiens pallida*, and *Lobelia siphilitica*), six open habitat flower species (*Clethra alnifolia*, *Ratibida pinnata*, *Silphium perfoliatum*, *Verbesina alternifolia, Daucus carota, Vernonia gigantea*), and one flower species found in both habitats (*Rudbeckia laciniata*). The least preferred flower taxa included *Helianthus*, *Symphyotrichum pilosum*, and *Trifolium repens*, all species that commonly grow in open habitats. Comparing the ranks of flower species use vs. selection index, *Helianthus and Symphyotrichum lateriflorum* had the biggest drops in rank, dropping 10 or more ranks from use when their availability was accounted for using selection indices, showing that while the flower taxon had a high use value, it was also highly abundant. The largest increases were from *Ratibida pinnata*, *Rudbeckia laciniata*, and *Siphium perfoilatum*, increasing at least 12 ranks (Table 1.6).

The model that best explained selection index included only *rank abundance* as a fixed significant factor. There was no evidence that percent forest was an important factor, nor was habitat type. Comparison of AICc values for competing models showed that this model was significantly better than the next best model, which included *% forest* and *habitat* as fixed effects (AICc = 497 vs. AICc = 501). This shows that rank abundance was the best predictor for selection index for a flower species, aligning with the results of the mantel test. This correlation was positive, indicating that a higher rank abundance correlated with an increased selection index, meaning that there was no overall preference for forest or open habitat. Adding native vs. non-native as a variable to a secondary model showed that both rank and native vs. non-native were significant factors in explaining selection index. six flowers with at least one bumble bee forager visit were determined to be non-native, while 18 were native to Ohio. This indicates that native flowers correlated with a higher selection index, showing that native flowers were more likely to be selected for.

Discussion:

In this study, I investigated the abundance, use, and preference of forest and open habitat flowers in late summer, a critical time in the bumble bee colony lifecycle. Bumble bees have a flight period that lasts several months and need continuous access to floral resources during this time (Timberlake et al. 2019). Bumble bees are generalist foragers, meaning they can forage from a wide variety of flower species, but foraging bees visit some plant species disproportionately based on their abundance, selecting some flower species and avoiding others (Novotny et al. 2022). Selection may differ across habitats due to differences in flower community composition and or other characteristics. Bumble bees with access to multiple habitat types may selectively forage in one habitat over another due to these factors. Knowing bumble bee patterns for selection is critical for effective habitat maintenance. To determine how wild bumble bee flower selection may differ between habitats and their floral resources, I surveyed sites with varying habitat composition and documented observations of bumble bee forage and forage flower abundance. This shed light on which habitats and flower species bumble bees prefer and avoid. From this, I gain important insight on where bumble bees prefer to forage in late summer while colonies are producing reproductives, a time period that currently lacks comprehensive bumble bee research.

What floral resources are available in forest understories and in open habitats in late summer and how do they compare in abundance and diversity?

I found that forests offer substantial and different floral resources than open habitats to bumble bee foragers despite short distances involved and small forest patches. This contrasts with a prior study that documented late summer bumble bee gynes foraging from nine floral genera, with only two primarily found in forest (Williams et al. 2014). Of 45 documented flower species, 18 were found uniquely in forest and 2 were found in both habitats. These forest species may be unique in the amount of nectar vs. pollen they have available. *Impatiens capensis* and *Impatiens pallida*, both riparian flowers that I found to be highly abundant at my sites, may offer a large amount of nectar to bumble bee foragers (Rust 1977). Overall, after summing the flower heads in each habitat at all sites, around half of the flower heads available were forest flower heads, indicating that forest is offering a similar amount of flower forage to open habitat in late summer.

Which floral resources do bees use in forested and open habitats during late summer?

Bumble bees were observed foraging from 24 of the 45 flower species documented. Of the flowers used, 5 were from forest, 17 from open habitat, and 2 from both habitats. While most of the species used come from open habitats, forager use of forest flowers exceeded that of open habitat flowers and that of flowers found in both habitats. This contrasts prior studies that show the importance of early spring forest flowers, when few flowers are blooming in open habitats, by showing that in late summer bumble bees use forest flowers despite near equal abundance of flowers in the open. These observations confirm that bumble bees are not only using the forest flowers available to them but are using the few species so much that it is significantly more than open habitat flowers. The top 3 most used taxa included 2 forest flowers, *Impatiens capensis*, *Impatiens pallida*, and 1 open flower genus, *Solidago*. Goldenrods (*Solidago*) are another highly abundant plant taxa and are nutritionally rewarding for bumble bees (Carvell et al. 2006).

Do bumble bee workers show a preference for flowers in forested or open habitats when both are available in late summer and which flowers?

Considering flower abundance data greatly changes the interpretation of bumble foraging patterns and shows clear preference for some forest flower species. Selection indices for 10 flower species exceeded one, showing that bumble bees preferred these species (Figure 1.3) with 3 species from forest, 6 from open habitat, and 1 from both habitats. Bumble bees significantly selected against the remaining 14 taxa. These results are similar to a study that found bumble bee preference for only certain woody species (Rivers-Moore et al. 2020), but the mechanism behind this preference remains unclear. Phylogenetically conserved foraging preferences may drive bumble bees to select for certain plants (Wood et al. 2021). Comprehensive nutritional analysis between habitats is also needed to better understand pollen preferences (Vaudo et al. 2018). Species selected against included the heavily used *Ageratina altissima*. Proportional use of this species lagged behind its high availability, more than I should expect. Some less abundant species, such as the forest-dwelling flower *Lobelia siphilitica*, were visited more frequently than expected based on its abundance, giving it a positive selection index. These flower species were ranked based on both use and abundance (Table 1.6). A flower species with a high rank for use was used more than other species based on raw data use, but was then ranked again taking flower head abundance into account. This created rank changes, where flowers who were used a lot but were also highly abundant had a high use rank and low use/abundance rank, giving it a negative rank change. Conversely, species who were not used many times but had a very low abundance had a large positive rank change. These changes in rank (Table 1.6) can be useful in determining how flower use interpretations change with the addition of availability metrics. Similar to Novotny et al. (2022), *Trifolium repens* was found to be highly abundant yet rarely used, indicating a heavy avoidance by bumble bee foragers. However, it should be noted that an extremely high abundance can contribute to low selection ratios, as it’s more difficult to properly survey flowers with a large distribution that saturate the existing flower and pollinator community.

The results from the mixed model show that abundance was the most important factor in determining how much a plant was selected for. Flowers that had a higher rank abundance tended towards a higher selection index. This may be because foraging on a dominant flower type is more efficient, as search time is reduced (Pyke, 1980). Other studies have found that floral dominance is the most important factor in *Bombus impatiens* colony growth and reproduction, possibly showing that foraging efficiency is improved in locations with greater floral dominance (Spiesmen et al. 2017). Habitat was not an important factor in determining how much a plant was selected for, showing that overall, bumble bees did not select for forest or open habitat flowers. Although forest flower use was significantly more than open habitat use, this was due to a high preference for certain forest flowers, not a general preference for all forest flowers. When the variable native vs. non-native was added into the model, it was also found to be significant in explaining selection index. Native flowers tended to have a higher selection index, with all 10 preferred flowers being native in Ohio. Other studies have found similar results, showing wild bee preference for native hedgerows (Morandin and Kremen 2012) or native pollen (Harmon-Threatt and Kremen 2015). This research adds to the ever-growing list of studies investigating native plants and their connection to native bees, in support of the idea that native plants are preferred.

Conclusions:

With this study, I expanded our knowledge of bumble bee use and preference of forest and open habitat flower species in late summer. Providing key information on bumble bee flower selection is critical for habitat managers to effectively provide bumble bee forage. I show that having both forest and open habitat and their associated resources can be beneficial to bumble bees. Heterogeneous landscapes can support bumble bee species richness (Mola et al. 2021) with habitats that complement each other, each offering resources at distinct phenological times. Bumble bees are more likely to occur in landscapes with a continuous supply of flowering resources (Hemberger et al. 2023). Many bumble bee species have been shown to be positively associated with forest (Crowther et al. 2014, Richardson et al. 2019), while some only use them seasonally (Ushimaru et al. 2008), or only prefer open habitats (Gomez-Martinez et al. 2020). It has been shown that forest provides key flower forage in early spring to bumble bee queens (Carvell et al. 2017), and this study supports the idea that forest is again important in late summer at the end of the colony's lifecycle when they are producing gynes, the queens of next year. While I may not be able to conclude that there is an overall preference for forest flowers, I can still see that certain flower species are preferred by bumble bees. If we do not conserve forest resources in late summer, we risk losing its floral species that are supporting bumble bee colonies during this important time.

Table 1.1 Percent landcover within 1km of each site (NLCD 2019). *Spring Valley was excluded from Chapter 2 analysis.

Habitat Type	**Latitude**	**Longitude**	Forest	Open	Urban	Cultivated Crops	Other
Civic Park	**39.97523**	**-82.82196**	7.46%	27.05%	65.09%	0.00%	0.40%
Rocky Fork	**40.11469**	**-82.79887**	22.91%	30.26%	15.66%	30.83%	0.32%
Evans Bike Path	**40.05527**	**-82.48613**	32.98%	31.30%	33.21%	2.25%	0.22%
Dawes Arboretum	**39.97668**	**-82.41421**	37.19%	38.18%	5.51%	18.18%	0.95%
Battelle (Open)	**39.91287**	**-83.21676**	38.78%	33.58%	1.26%	22.88%	3.50%
Infirmary Mound Park	**40.02789**	**-82.51716**	41.20%	43.50%	3.57%	10.35%	1.38%
Spring Valley *	**40.05655**	**-82.52997**	45.87%	27.76%	20.31%	4.84%	1.19%
Highbanks Park	**40.14939**	**-83.04053**	50.39%	29.11%	18.29%	0.00%	2.21%
Battelle (Forest)	**39.89157**	**-83.21093**	55.99%	23.95%	7.19%	10.55%	2.33%
Lobdell Reserve	**40.10486**	**-82.60059**	58.05%	24.65%	1.44%	15.59%	0.26%

Table 1.2 Number of flower heads and plant species by habitat for each site.

Site	**Open habitat flower heads**	**Forest flower heads**	**Both habitat flower heads**	**Forest species**	**Open habitat species**	**Both habitat species**
Battelle (Forest)	714	1305	0	6	7	0
Battelle (Open)	3144	138	0	3	14	0
Civic Park	1939	1385	8	6	12	1
Dawes Arboretum	1694	1917	0	9	11	0
Evans Bike Path	1402	3722	0	11	12	0
Highbanks Park	1463	1657	511	6	9	1
Infirmary Mound Park	2540	2749	0	8	16	0
Lobdell Reserve	1386	1585	0	9	11	0
Rocky Fork	1661	884	13	10	11	1
Spring Valley	2230	1416	0	6	11	0
Grand Total	18173	16758	532	74	114	3

Table 1.3 Metrics for flower species with at least one bumble bee foraging observation.

Flower Species	**Common Name**	**Total # of Bombus found**	**Total # of flower heads**	**Floral Availability**	**Use**	**Use/ Availability**	**Habitat**	**Native?**
Trifolium repens	White Clover	12	1857	0.053	0.007	0.134	Open	N
Symphyotrichum pilosum	Hairy White Oldfield Aster	6	852	0.024	0.004	0.146	Open	Y
Daucus carota	Queen Annes Lace	14	1474	0.042	0.008	0.197	Open	N
Erigeron annuus	Fleabane	4	359	0.010	0.002	0.231	Forest	N
Rudbeckia triloba	Brown-eyed Susan	11	715	0.020	0.006	0.319	Open	Y
Oenothera elata	Evening Primrose	2	99	0.003	0.001	0.419	Open	Y
Vernonia gigantea	Tall Ironweed	12	499	0.014	0.007	0.498	Open	Y
Symphyotrichum lateriflorum	Calico aster	70	2710	0.077	0.041	0.535	Open	Y
Symphyotrichum novae-angliae	New England Aster	28	757	0.021	0.016	0.767	Open	Y

Polymnia canadensis	Leafcup	27	636	0.018	0.016	0.880	Both	Y
Ageratina altissima	White Snakeroot	130	3025	0.086	0.076	0.891	Forest	Y
Cirsium arvense	Thistle	43	999	0.028	0.025	0.892	Open	N
Rudbeckia laciniata	Cutleaf Coneflower	1	21	0.001	0.001	0.987	Both	Y
Taraxacum officinale	Dandelion	9	188	0.005	0.005	0.992	Open	N
Ratibida pinnata	Yellow Coneflower	3	59	0.002	0.002	1.054	Open	Y
Clethra alnifolia	Sweet Pepperbush	24	464	0.013	0.014	1.072	Open	Y
Solidago	Goldenrod	285	4864	0.138	0.167	1.214	Open	Y

Helianthus	Sunflowers	30	545	0.015	0.018	1.141	Open	Y
Impatiens pallida	Pale Jewelweed	502	6561	0.186	0.294	1.586	Forest	Y
Verbesina alternifolia	Wingstem	105	1340	0.038	0.062	1.624	Open	Y
Lobelia siphilitica	Great Blue Lobelia	13	165	0.005	0.008	1.633	Forest	Y
Impatiens capensis	Jewelweed	351	4387	0.124	0.206	1.658	Forest	Y
Silphium perfoliatum	Cup Plant	10	116	0.003	0.006	1.787	Open	N
Echinacea purpurea	Purple Coneflower	14	150	0.004	0.008	1.934	Open	Y

Table 1.4 Flowers with zero bumble bee foraging observations, whose selection indices could not be calculated.

Flower species	Common name	Flower heads available
Agrimonia parviflora	Swamp Agrimony	55
Ataea racemosa	Black Cohosh	6
Calystegia sepium	Hedge Bindweed	4
Calystegia spithamaea	False Bindweed	5
Campanula americana	Tall Bellflower	6
Cichorium intybus	Chicory	111
Dipsacus fullonum	Wild Teasel	72
Dipsacus laciniatus	Cutleaf Teasel	213
Hypericum perforatum	St. Johns Wort	32
Laportea canadensis	Wood Nettle	448
Oxalis stricta	Common Yellow Woodsorrel	694
Persicaria longiseta	Low Smartweed	364
Persicaria virginiana	American Jumpseed	11
Phlox paniculata	Blue Phlox	170
Phytoloacca americana	American Pokeweed	74
Prunella vulgaris	Common Selfheal	98
Rudbeckia hirta	Black-eyed Susan	61
Solanum carolinense	Carolina Horsenettle	2
Symphyotrichum cordifolium	Blue wood-aster	20
Trifolium hybridum	Alsike Clover	3
Trifolium pratense	Red Clover	68

Table 1.5 Overall selection index and mean site selection index for each flower with at least one bumble bee foraging observation.

Flower Species	**Common Name**	**Overall selection index**	**Mean site selection index**
Trifolium repens	White Clover	-7.466	-5.154
Symphyotrichum pilosum	Hairy White Oldfield Aster	-6.851	-3.477
Daucus carota	Queen Annes Lace	-5.080	1.117
Erigeron annuus	Fleabane	-4.330	-1.210
Rudbeckia triloba	Brown-eyed Susan	-3.136	-1.627
Oenothera elata	Evening Primrose	-2.388	-1.842
Vernonia gigantea	Tall Ironweed	-2.006	1.080
Symphyotrichum lateriflorum	Calico aster	-1.868	-1.705
Symphyotrichum novae-angliae	New England Aster	-1.304	0.568
Polymnia canadensis	Leafcup	-1.137	1.419
Ageratina altissima	White Snakeroot	-1.123	-0.236
Cirsium arvense	Thistle	-1.121	-0.399
Rudbeckia laciniata	Cutleaf Coneflower	-1.013	1.741
Taraxacum officinale	Dandelion	-1.008	0.845
Ratibida pinnata	Yellow Coneflower	1.062	1.208
Clethra alnifolia	Sweet Pepperbush	1.080	1.445
Solidago	Goldenrod	1.223	0.040
Helianthus	Sunflowers	1.298	-4.984
Impatiens pallida	Pale Jewelweed	1.597	-0.108
Verbesina alternifolia	Wingstem	1.636	1.015

Lobelia siphilitica	Great Blue Lobelia	1.645	1.979
Impatiens capensis	Jewelweed	1.670	1.240
Silphium perfoliatum	Cup Plant	1.800	2.363
Echinacea purpurea	Purple Coneflower	1.949	-0.340

Table 1.6 Ranks based on use, then ranks after availability is considered for selection indices, along with the differences in these ranks.

Flower Species	Common Name	Rank based on use	Rank based on SI	Rank change	# of sites present
Ageratina altissima	White Snakeroot	4	15	-11	10
Cirsium arvense	Thistle	7	17	-10	5
Clethra alnifolia	Sweet Pepperbush	11	4	7	1
Daucus carota	Queen Annes Lace	13	8	5	8
Echinacea purpurea	Purple Coneflower	12	16	-4	3
Erigeron annuus	Fleabane	21	18	3	6
Helianthus	Sunflowers	8	23	-15	3
Impatiens capensis	Jewelweed	2	6	-4	10
Impatiens pallida	Pale Jewelweed	1	5	-4	9
Lobelia siphilitica	Great Blue Lobelia	14	2	12	5
Oenothera elata	Evening Primrose	23	21	2	3
Polymnia canadensis	Leafcup	10	12	-2	2
Ratibida pinnata	Yellow Coneflower	22	7	15	2
Rudbeckia laciniata	Cutleaf Coneflower	24	3	21	2
Rudbeckia triloba	Brown-eyed Susan	17	19	-2	5
Silphium perfoliatum	Cup Plant	18	1	17	1
Solidago	Goldenrod	3	13	-10	10
Symphyotrichum lateriflorum	Calico aster	6	20	-14	10
Symphyotrichum novae-angliae	New England Aster	9	14	-5	4
Symphyotrichum pilosum	Hairy White Oldfield Aster	20	22	-2	6

Taraxacum officinale	Dandelion	19	11	8	7
Trifolium repens	White Clover	16	24	-8	10
Verbesina alternifolia	Wingstem	5	10	-5	7
Vernonia gigantea	Tall Ironweed	15	9	6	9

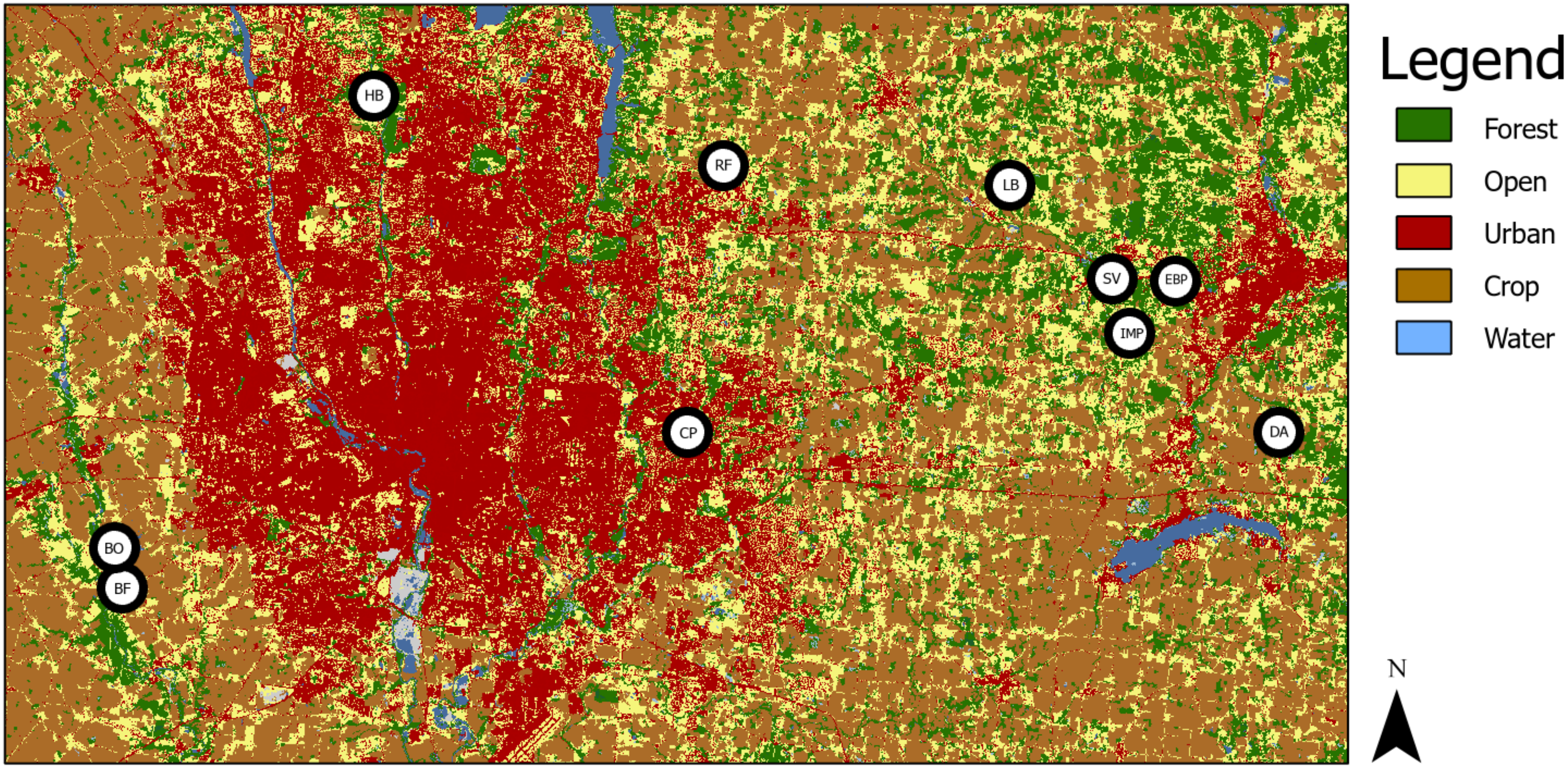

Figure 1.1 Map of sites where surveys took place. All located in Ohio, USA. Made using ArcGIS (Esri, NSAS, USGS, City of Gahanna, HERE, Garmin, SafeGraph, GAO, METI, EPA, NPS).

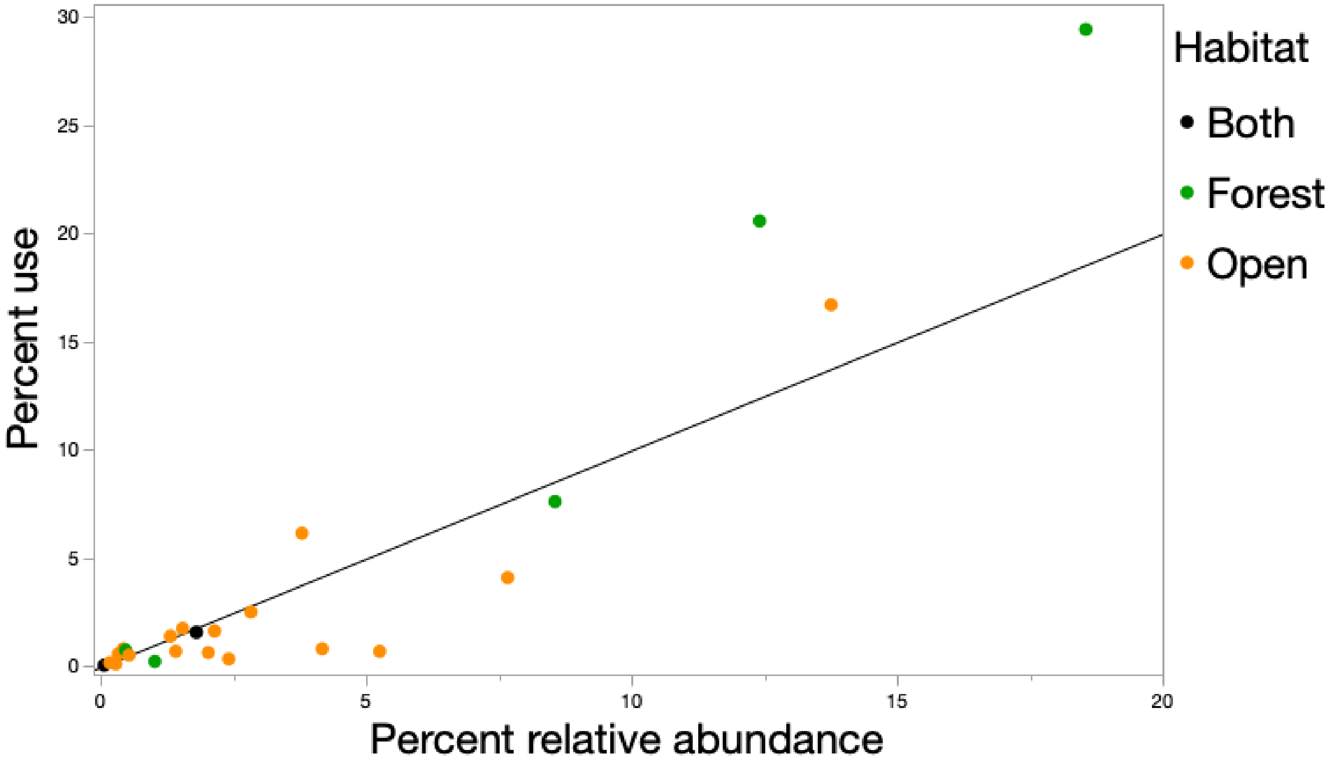

Figure 1.2 Percent use versus availability for each flower species that had at least one bumble bee foraging observation.

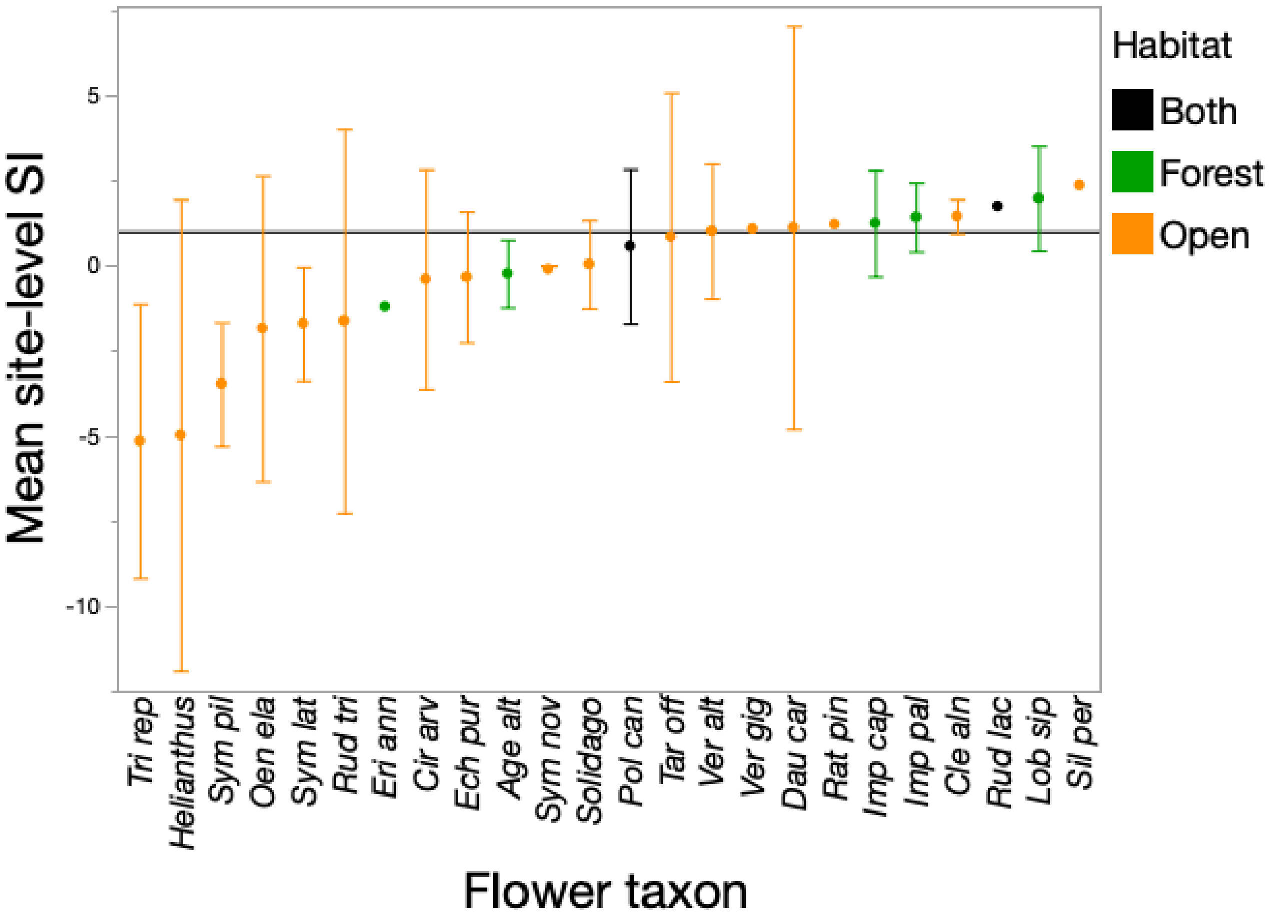

Figure 1.3 Mean site-level SI for each flower species with at least one bumble bee foraging observation. Points above the horizonal line at 1 represent preferred flower species. Error bars represent 95% confidence intervals.

Chapter 2: Forest and open habitat late summer pollen enhance bumble bee colonies reproductive success

Introduction:

Bumble bees (*Bombus* spp.) are critical pollinators that are essential to many wild plant communities (Memmott et al. 2004, Roubik 1995). There is evidence of declining populations globally for many bumble bee species (Cameron et al. 2011, Goulson et al. 2008). Several factors may be causing these declines, including the loss of floral resources (pollen and nectar) (Kearns et al. 1998, Potts et al. 2016), which can lead to declines in bumble bee abundance and reproduction (Crone and Williams 2016). To properly conserve populations, we need to determine where bumble bees are foraging and what factors affect colony reproductive success. By studying the relationship between foraging and reproductive patterns, we can learn to enhance conservation practices (Jha et al. 2013, Williams et al. 2012), which is especially true for late summer. Despite late summers significance in the bumble bee colony lifecycle (Alford, 1975), we currently lack comprehensive research on where bumble bees forage and how forage affects colony success during this period.

Bumble bees are central place foragers that need a constant supply of resources during their long flight periods because of the spatial and temporal turnover of flower species in temperate regions (Ogilvie and Forrest 2017, Caradonna et al. 2017). Temperate regions often have a mosaic of habitats, allowing bumble bees to use multiple types of habitat throughout their colony lifetime. Bumble bees typically use forest in early spring, when ephemeral flower communities in the forest understory offer abundant forage for new queens (Mola et al. 2021). Later in the spring, after the trees leaf out, forest understory flowers taper their blooms and less forage is available for bumble bees. At this time, and throughout the summer, bumble bees forage in open meadow habitats, roadsides, and other habitats that offer abundant resources and are not light-limited by forest canopy. In Ohio during late summer, some bumble bees species appear to forage from both forest and open habitats, coinciding with a guild of late summer-fall blooming plants in forest (Chapter 1). Forests are characterized by their trees generally greater than 5 meters tall that dominate the area, with vegetation cover greater than 20% (NLCD 2019). Open habitats are typically dominated by herbaceous or shrub vegetation. Having both forest and open habitat benefits bumble bees by providing a consistent supply of floral resources throughout their long flight period. Heterogeneous landscapes can be beneficial in this way, allowing bumble bees to access multiple habitat types throughout their colony lifetime. Bumble bee colonies may be more successful in heterogeneous environments (Rundlof et al. 2008, Pugesek and Crone 2021). Habitats that are adjacent to each other can complement one another by supplementing resources for species that can travel between them, such as bumble bees (Mola et al. 2021). These habitats are known as complementary habitats and are important for mobile species (Holt 1993). Complementary resources from these habitats may be especially beneficial during key stages of the bumble bee colony life cycle.

During late summer, many bumble bee species begin a critical stage in their colony life cycle, gyne and male production. Gynes are the new queens that will mate at the end of the summer, then overwinter in hibernacula to initiate the next generation of colonies the following spring (Goulson, 2003). Therefore, the number of colonies in the next generation directly depends on the number of gynes (Chapman and Bourke 2001), so gyne production and their survivorship over winter directly affects bumble bee population demography (Crone and Williams 2016). Therefore, it is crucial that we understand what factors affect the number and quality of gynes a bumble bee colony produces.

The number and size of reproductives (i.e., gynes and males) a colony produces may vary with the local and landscape scale factors that affect pollen availability (Williams 2015). Bumble bees are generalist foragers that can forage from many plant species. While bumble bees use flowers for both pollen and nectar foraging, they have been shown to be more selective of their pollen resources (Wcislo and Cane 1996, Cane and Sipes 2006). Bumble bees are ideal for studying pollen use because of their high floral resource demands (Jha et al. 2013) and sensitivity to floral resource changes (Williams et al. 2012). Pollen is an essential component of larval bee food (Jha et al. 2013) because of the proteins it provides. These proteins play a key role in determining adult bee size (Nooten and Rehan 2019). Larger adult bees may forage more efficiently (Pyke 1978, Ogilvie and Forrest 2017) or may be more robust (Heinrich 1983). Additionally, large size is associated with better overwinter survivorship in gynes (Owen

1988). The factors that affect pollen availability directly relate to the composition of pollen fed to larvae and may thus affect colony reproductive success.

An increase in the richness and diversity of floral resources can enhance colony growth (Jha and Kremen 2013). Bumble bees may be more likely to encounter high quality pollen sources in areas with more diverse flora. Pollen can vary in its nutritional quality (Roulston and Cane 2000, Tasei and Aupinel 2008) with pollen protein being limiting to bumble bees (Vaudo et al. 2015), so having more types of pollen may indicate a more nutritionally complete pollen diet (Tasei and Aupinel 2008). Nutritional diversity has been found to be important to bumble bees, stimulating colony growth (Goulson et al. 2002). Nutritional diversity may be higher in areas with multiple habitat types, because each habitat may offer distinct floral resources with different nutritional benefits. Different habitats may complement each other in this way, helping bumble bees fulfill their nutritional needs. These nutritional differences may lead bumble bees to forage in different habitats.

Having a more diverse pollen and nutritional diet may be beneficial, but it could also be a symptom of increased heterogeneity and thus landscape richness, defined as having more habitat types in an area. Landscape richness and diversity has been shown to be positively correlated with pollen diversity (Matthias et al. 2015). Having a richer and more diverse landscape may mean more flower species are available for bumble bees to forage from, as each habitat has a unique community of floral resources that shift spatially and temporally (Mallinger et al. 2016). Increased landscape richness may make collecting pollen easier for bumble bees, because workers may not have to travel as far to find forage, and that greater richness provides a more consistent source of forage throughout their long flight period (Speisman et al. 2016). Landscape composition may impact colony reproduction (Spiesmen et al. 2017) through its interactions with pollen diversity in this way.

Determining exactly which habitats and plants bumble bees collect pollen from and how pollen affects reproductive success can help us support bumble bee habitat (Jha et al. 2013). While we can determine which flower species bumble bees use for foraging from using visitation data, these data typically confound pollen and nectar foraging (Alarcon 2010). Visitation data is also limited to bees observed and therefore does not give complete information on habitat use (Novotny and Goodell 2022). Other methods must be utilized to deduce exactly in which habitat pollen is collected. Investigating how landscape diversity may affect pollen diversity can shed light on the relationship between landscape and the pollen available for bumble bee forage and how both may affect colony reproductive success.

To study the mechanism by which local factors and land cover affect bumble bee pollen use and colony success, I placed sentinel colonies at nine sites in Ohio, U.S.A. and documented their pollen use and reproductive success. These sites were located within landscapes of varying percentages of forest and open habitat in which colonies foraged. I collected foraged pollen and quantified colony reproductive output to understand relationships between habitat distribution, floral resources, and colony reproductive success. I address the following questions: 1) What habitats and plant species are bumble bee colonies collecting pollen from in late summer? 2) How is reproductive success affected by landscape and foraged pollen and does this change depending on habitat? 3) Are colonies more successful in areas that have more diverse landscape and pollen?

Methods:

Study sites:

This study was performed in Ohio, U.S.A. at metropolitan parks in the Greater Columbus area that were located within landscapes with differing land cover composition. I chose nine sites that had a gradient of forest land cover within 1km (Figure 1.1) as 1km has been shown to be a good predictor of colony metrics (Knight et al. 2009). Having a gradient of forest ensured that each site had a different habitat composition that could be analyzed in comparisons (Table 1.1). Land cover was determined using ArcGIS ver. 10.8.2. using data from the National Land Cover Database (NLCD). The NLCD provides a raster dataset of land cover on a 30m x 30m pixel map updated every 3 years (NLCD 2019). Land cover classes were lumped together based on habitat type. I classified land cover into four categories by combining NLCD classes: forest (deciduous, conifer, and mixed), open (shrub/scrub, herbaceous, open space), crop (cultivated crops), and other (all other classes).

Bumble bee colonies:

For the purpose of this study, colony success was measured by colony reproductive output. Quantifying the number of reproductive individuals, i.e., gynes and males, is a commonly used metric for colony success (Vaudo et al. 2018, Samuelson et al. 2018), as the number and size of gynes produced directly relates to colony fitness (Chapman and Bourke 2001). I used these characteristics to quantify success.

Bumble bees were purchased from Koppert Biological Systems ™ (Howell, MI). I chose *Bombus impatiens* as the study species because it is native to the study area, commercially available, and commonly used as a model species. Colonies arrived at the beginning of August. I kept colonies in a rearing room at Rothenbuhler Honey Bee Lab (Columbus, OH) at 28°C and 65% relative humidity for one week before placement into the field sites. Colonies were fed pesticide-free pollen patties made from local honey bee collected pollen and were allowed to drink 80% sugar solution *ad libitum* from the Koppert ™ nectar reservoirs. During this time, I counted the number of initial workers. I documented that each colony had one only queen at the time of placement in the field. All colonies were placed at their respective sites on August 8th, 2022.

I weighed and then placed bumble bee colonies in forested areas and away from public walking trails. Colonies were placed at least 30m away from the forest edge and in patches of at least 100m^2, though many were much larger. I placed colonies on small patches of bare ground. I kept all colonies within the Koppert™ box they arrived in, which consisted of a hard plastic inner shell with an outer cardboard box. However, nectar bags were removed before placement (Figure 2.1). I placed adhesive tiles on top of the cardboard to protect the colony from rain. To protect from predatory cursorial insects, such as ants, I set the colonies on top of bricks within a small plastic tub with holes cut into the bottom for water drainage. A band of sticky Tanglefoot ™ was spread around the outside of the plastic tub to deter ants. I surrounded the colony with 1" hardware cloth to deter small mammals. Colonies were weighed using a Dymo® Postal Scale and checked for damage weekly over six weeks. I started with ten colonies but lost the colony placed at Spring Valley that was destroyed by a large mammal; it was excluded from analyses.

To collect pollen from bumble bee colonies while they were in the field, I produced pollen traps using a 3D Printer and a pollen trap 3D model (Hudd et al. 2020). These traps modified the entrance to the colony, forcing workers through a narrow opening, thus brushing off collected pollen balls. These pollen balls fell into a catch basin that can be easily removed for collection (Figure 2.1). The pollen traps were placed once weekly on each colony for pollen collection. Pollen was not collected the first week to allow colonies to adjust to their surroundings. I completed five weeks of pollen collection. I removed pollen traps after 48 h to ensure that colonies had adequate nutrition to continue growing. Pollen traps were emptied weekly into tubes for later processing and identification. I was not able to collect pollen from every colony every week because of rain or small mammals knocking the pollen traps off.

After six weeks, the colony exits were closed for 48 h to prevent bumble bees from leaving, but one-way entrances left open to allow for the return of foragers. I then collected all colonies on September 16th, 2022 and transported them in a cooler to a –34°

C laboratory freezer to be euthanized. I photographed and measured the height, width, and length of the frozen colonies. After one final weighing, I opened the colonies to collect and count the number of gynes and workers within. I then dissected the colonies and documented the number of worker and gyne brood cells, which can be easily differentiated for *Bombus impatiens* based on size (Thomson 2004). These brood cells were measured by height, width, and volume. Brood cell size is correlated to bumble bee size (Chole et al. 2019). Volume was determined by weighing the amount of water that filled the cell, but since some cells were damaged volume could not be determined for every cell.

Pollen reference collection and identification:

To determine pollen use, I first collected a pollen reference collection of flowers local to the area following Jha (2013). Flowers were identified in the field using a guide (Newcomb, 1977) and plant specimens were collected and pressed for later verification. I then used sterile forceps to collect anthers and stored the anthers in microcentrifuge tubes until they dehisced. Once anthers dehisced, the tubes were filled with 70% ethanol and vortexed to suspend the pollen in the ethanol. Immediately after vortexing, two drops of this ethanol were placed on a microscope slide and stained with fuchsin gel (Kearns and Inoue 1993) then allowed to rest and rehydrate for 24 hours (Jha et al. 2013). These slides were used for morphological pollen identification via light microscopy. For samples collected from bumble bee colonies, each pollen sample was homogenized by crushing and mixing the pollen balls using a mortar and pestle that was sterilized between uses. Pollen samples for each site and week were kept separately. As described above, samples were then placed back the respective tube with ethanol, vortexted, and immediately had two drops placed on a microscope slide that were stained with fuchsin gel.

A single researcher identified all pollen grains for consistency using a compound microscope and brightfield microscopy to determine which plant species were used by each colony. After choosing a starting point on the slide, pollens were identified along a straight line transect until 100 pollen grains were identified (Punyasena et al. 2022). The height and width of each pollen grain was measured using an ocular grid that had been calibrated for each objective using a stage micrometer. Sample pollens were compared to the reference collection for identification. It was not possible to collect a reference of every pollen type available to the colonies because of the large foraging range of bumble bees, which can be up to 20 km (Mikkola, 1984), but is typically no more than 200 m (Osborne et al. 2001). As such, other methods of identification were used to identify pollen grains not included in the reference collection (e.g., Kapp et al. 2000, Jones et al. 1995). Some taxa are extremely hard to differentiate from related taxa by the methods used so some taxa such as Asteraceae were combined (Table 2.1). Pollens of Asteraceae species are morphologically very similar, especially those of closely related species, and difficult to distinguish from each other using light microscopy (Bahadur et al. 2022). Nevertheless, I was able to distinguish some groups of Asteraceae based on size, exine morphology, or apertures. I was particularly careful to pick out *Ageratina altissima*, an abundant plant and one of the few forest-dwelling Asteraceae in the study sites. *Ageratina altissima* typically has white or light grey pollen and was only available in the first two of weeks of pollen collection, which made it easier for me to differentiate it from other Asteraceae pollen. Thus, I could distinguish Asteraceae pollen by habitat, though it is important to note that Asteraceae is a large family and may include other forest dwelling species. However, the only exclusively forest flower in Asteraceae that I observed was *Ageratina altissima.* I placed unidentifiable pollen into an "Unknown" category that ultimately made up only 73 of 2800 of all pollen grains (2.6%) and never more than 7 % of any one sample. To determine the volume of the sample contributed by each pollen type, pollen grain volume was calculated using the height and width measurements, following the equation $V = (4/3)\pi a^2 b$, where height was a considering some pollens were oriented differently on the slide and were measured either from an equatorial view or polar view.

<u>Data analysis:</u>

To determine which habitat and plants bumble bee colonies used for collecting pollen, I categorized each plant taxa by its primary habitat based on my field observations and information from iNaturalist (https://www.inaturalist.org/). I summed pollen grain volumes of each plant taxon for each sample and divided the sum by the volume of the entire sample to get percent volume for each plant taxa. For example, if *Plantago lanceolata's* sum of volume made up 40% of a total sample volume, 40% was used in analyses. Percent volume was used as a dependent variable in analyses to account for different sample sizes for the colonies due to weather and other factors. The sum of the percent volume of pollen from each habitat and plant family was compared by site and week. To determine whether bees used pollens from different habitats across sites, I used a Kruskal Wallis test in JMP, Version 17 (SAS Institute Inc., Cary, NC, 1989–2023) using site as the independent variable and percent pollen from each habitat as the dependent variable. To determine whether bees used pollens from different habitats across weeks, I did the same test with week as the independent variable. Kruskal Wallis is good for non-parametric comparisons between pairs (Kruskal and Wallis, 1952). I used the same test to determine whether bees used pollens from different plant families across both sites and weeks, with site or week as the independent variable and percent pollen from each plant family as the dependent variable. I only used the top four plant families in this analysis as all other families made up less than 5% of the overall pollen volume from all samples. To determine whether the diversity of collected pollen varied between sites, I also calculated Shannon and Chao diversity indices for pollens collected in each site. The Shannon Index provides inference on community composition that places a greater weight on species richness (Kim et al. 2017), while the Chao Index gives more weight to low abundance species, so it is a good index for data skewed towards singletons and doubletons (Hughes et al. 2001). For all other analyses, plant taxa that did not make up at least 5% of the volume of any one sample were excluded.

To determine how colony reproductive success was affected by foraged pollen, the number of gyne cells produced (*total gyne cells*) and the size of those gyne cells (*average gyne cell size*) were compared to percent pollen volume using Generalized Linear Models (GLMs) in JMP ®. *Total gyne cells* were calculated for each site by summing the number of gyne cells found during colony dissections. The volume of each gyne cell was determined and averaged for each site, to determine *average gyne cell size*. *Total gyne cells* and *average gyne cell size* were not highly correlated. I used a reciprocal link function to account for the distribution of the *total gyne cells*. The distribution of *average gyne cell size* was not significantly different than normal so a link function was unnecessary. The percent volume of pollen from each species was grouped by habitat, flower taxa, and flower family. I ran a series of GLMs to explain either *total gyne cells* or *average gyne cell size* as a function of these explanatory variables: *volume of forest pollen*, *volume of open pollen*, *forest land cover*, and *open land cover*. Interaction terms were included. *Initial weight* was included as a covariate to account for the differences in colony weight at the outset of the study. *Volume of forest pollen* indicates the percent volume of pollen collected from forest while *volume of open pollen* indicates volume of pollen collected from open habitat. *Forest land cover* and *open land cover* represent the percentage of land cover with 1km made up by that land cover type. I started with these variables to model the combined effects of pollen and land cover, then pared them down to find the best fit models. I used Spearman's Rank to determine correlations between variables because Spearman's Rank is good for non-parametric data (Gauthier 2001). Explanatory variables were not highly correlated with each other, except for *volume of forest pollen* and *volume of open pollen* that were highly negatively correlated. I then used the leave-one-out method for each model to determine the model that minimized Akaike Information Criterion corrected (AICc) values. The model with the lowest AIC was considered the best fit to the data (Burnham and Anderson 1998). While there was some pollen collected from flowers that grow agricultural habitat or in both open and forest habitat, these amounts were small enough to exclude from GLMs.

I tested whether landscape heterogeneity improved pollen diversity or colony reproductive success. Landscape richness was measured by counting the number of land cover types within 1km. Landscape diversity was calculated using Simpson's Index, *D* (Simpson 1949). Simpson's Index can be used to examine variance in the proportion of area for each land cover type using the equation $D = 1/ \Sigma (p_i)^2$ (Gardiner et al. 2009). Pollen richness was the number of plant species represented in pollen samples. Shannon's Diversity Index, *H* was used to determine pollen diversity using the equation $H = -\sum[(pi) \times \log(pi)]$ (Shannon 1948). Chao1 Diversity Indices were also calculated using the equation $\text{Chao1} = S_{obs} + N_1(N_1-1)/(2*(N_2+1))$, where N_1 and N_2 are pollen species singletons and doubletons, respectively (Chao 1984). Using GLMs, landscape and pollen diversity were compared to determine associations with each other and the reproductive response variables (*total gyne cells* and *average gyne cell size*).

Results:

What plant species and habitats are bumble bee colonies collecting pollen from in late summer?

From the nine bumble bee colonies and five weeks of pollen samples, I identified 2800 pollen grains foraged by bumble bees representing 59 different plant taxa and the habitat occupied by that plant species (Table 2.1). Not all of the pollen grains could be identified to species; these I left at the family level. Plant species were combined by family for non-habitat analyses because of difficulties in identifying some families, like

Asteraceae, down to species. The most common pollen collected was Asteraceae 43%, Balsaminaceae (*Impatiens capensis* and *I. pallida*) 19%, Plantaginaceae (*Plantago lanceolata*) 12%*,* and Campanulaceae (*Lobelia siphilitica*) 9%. All other families made up less than 5% of the overall volume (Figure 2.2). Collected pollen differed by site (Figure 2.3). The family Asteraceae had significantly more pollen collected than any other family across sites (Figure 2.4). The percent volume of Balsaminaceae, Plantaginaceae, and Campanulaceae did not differ significantly from one another. Volume percent for each family by week (Figure 2.5) shows temporal changes in bumble bee plant family use. Asteraceae made up the largest volume for every week except for week 2, when the largest volume was made up of Balsaminaceae. *Impatiens capensis* had its peak bloom during this period (Chapter 1), which may explain this difference. However, the Kruskal Wallis test did not show any significant differences in the volume collected for the top four plant families across weeks stated above (Figure 2.6).

Collected pollen by habitat differed by site (Figure 2.7). The *volume of open pollen* and the *volume of forest pollen* were highly negatively correlated (Spearman rank correlation: $\rho = -0.81$, $p = 0.0072$). The Kruskal Wallis test comparing collected pollen by habitat by site showed that, overall, pollen was collected from open habitat significantly more than Crop and Both. (Kruskal-Wallis, $p = 0.0001, 0.0054$, $df = 3$). Bumble bees did not collect significantly more pollen from open habitat than forest (Kruskal-Wallis, $p = 0.1876$, $df = 3$). Forest was significantly higher than Crop, but not Both. Similarly, Crop and Both were not significantly different (Figure 2.8). Collected pollen by habitat also differed by week (Figure 2.9). Forest and open habitat pollen was collected significantly more than crop (Kruskal-Wallis, p = 0.0017, 0.0196). No other categories were significantly different from one another (Figure 2.10). Similar to the plant family by week graph (Figure 2.5), week two showed more forest pollen collected than any other week, with open habitat pollen making up the majority of all other weeks.

Sites differed in the diversity of pollen collected (Table 2.2). Differences in sample size, due to periodic difficulties in collecting pollen from each colony, should be taken into consideration when looking at these values. The site IMP had the largest Shannon Diversity Index and LB had the largest Chao Diversity Index. While IMP had the largest richness, LB had the most singletons which account for these differences. Shannon Diversity indices ranged from 1.9 to 2.85, while Chao Diversity indices ranged from 17.5 to 57.5 (Table 2.2).

Although foragers collected pollen from habitat in different amounts, bumble bees overall did not collect pollens from each habitat in proportion to the area of each habitat within a 1-km radius of the colony. *Forest land cover* and *volume of forest pollen*, along with *open land cover* and *volume of open pollen*, were not highly correlated (Spearman rank correlation: $\rho = -0.25, 0.33$ $p = 0.5165, 0.3807$).

How is reproductive success affected by landscape and foraged pollen and does this change depending on habitat?

The best fit GLM for *total gyne cells* showed a significant positive effect of *volume of open pollen* (Table 2.3). However, *average gyne cell size* (and presumably the gynes produced) were significantly negatively related to the *volume of open pollen*. In another model, *average gyne cell size* was best explained by the *volume of forest pollen*. *Volume of forest pollen* had a marginally significant negative effect on *total gyne cells* (Table 2.3).

Forest land cover and *open land cover* were not included in the best fit models and showed no significant relationship to *total gyne cells* or *average gyne cell size* (Table 2.3), indicating that the percent cover of forest or open habitat within a 1-km buffer did not significantly affect colony reproductive success. The covariate *initial weight* did not show a relationship with either response variable and was not included in the best model with the lowest AICc value for any model.

Are colonies more successful in areas that have more diverse landscape and pollen?

The diversity of land cover types was not related to the reproductive output of bumble bee colonies (Table 2.3). Neither the Shannon nor the Chao diversity indices, which measure the diversity of pollen in a collected sample, significantly explained total gyne cells or average gyne cell size. This non-significant result shows that more pollen diversity did not benefit colony reproductive success. Land cover diversity, which measures the land cover richness at a site using Simpson's Diversity Index, also was not significant (Table 2.3). Simpson's Diversity Index was not significantly related to Shannon or Chao diversity indices, indicating that a more diverse landscape did not mean bumble bees were collecting pollen from more flower species. In the models that looked at both pollen and land cover diversity indices, along with their interactions, no variables showed as significant.

Discussion:

Late summer coincides with the end of bumble bee colony life cycle and a key stage for producing gynes. Therefore, foraging success of bumble bees during this stage of colony development could directly impact reproductive output. Bumble bees are mobile species that can move between habitats as the growing season progresses (Mola and Williams, 2019). The contribution of forested habitats in early spring and open habitats in summer has been documented for multiple bumble bee species in temperate regions (Novotny et al. 2021). Few studies have focused on bumble bee foraging in late summer and fall.

Bumble bees feed pollen to their larvae as the main protein source that supports larval development (Jha et al. 2013). Larval pollen diet is positively associated with adult size (Nooten and Rehan 2009). Adult bumble bees eat pollen as well, especially spring queens, and it is thought to influence egg development (Moerman et al. 2016). Gyne and male production is key to colony fitness, but also has demographic implications. Producing more gynes that are larger and have better survivorship will contribute to population growth (Crone and Williams 2016). Knowing what pollen bumble bees are using during this time and the relationship between collected pollen and reproductive success can help us shed light on what makes a bumble bee colony more successful. In a study by Vaudo et al. 2018, it was found that protein, lipid, and carbohydrate values of collected pollen was not significantly different between habitats. My investigation into bumble bee reproductive success as related to pollen foraging indicates a high degree of integration across habitats, with forested and open habitats contributing similarly to bumble bee pollen diets. More research is needed to determine the nutritional quality of the collected pollen and whether it played a role in the differences found across colonies.

What plant species and habitats are bumble bee colonies collecting pollen from in late summer?

Bumble bees collected Asteraceae more than any other plant taxa or family. The Asteraceae pollen in this study was comprised of several taxa, including *Solidago* spp., *Helianthus* spp., and *Cirsium* spp. Other studies such as Novotny et al. (2023) have found several Asteraceae species to be highly preferred by bumble bees, though most of the data was collecting during mid-summer. The next most used family was Balsalminaceae, represented by *Impatiens pallida* and *I. capensis* (Figure 2.2). This genus is known to offer large amounts of nectar to bumble bees (Rust 1977), but the high volume of this pollen collected by bees in my study suggests that it was not just incidentally collected by nectar foraging bees, but actively collected, though I cannot rule out nectar being the primary target for bees visiting *Impatiens.* In Chapter 1, I found that wild bumble bees preferentially foraged from *Impatiens capensis*, *I. pallida* and *Lobelia siphilitica* (Figure 2.11). These forest flowers were also highly used in sentinel colonies. Wilson and Tomson, (1996) found that *Impatiens* spp. flowers are clearly adapted to bumble bee pollination and that bumble bees brush against the anthers as they drink its nectar. The third largest family used was Plantaginaceae, represented solely by *Plantago lanceolata* in pollen samples. This flower is a weedy, non-native flower that does not provide nectar

(Lovell 1926), which means bumble bees must forage elsewhere for nectar. Through the span of the study, bumble bees collected pollen from plant families in different amounts each week (Figure 2.5). However, foragers collected pollen from the top four plant families, Asteraceae, Balsaminaceae, Plantaginaceae, and Campanulaceae in similar amounts each week (Figure 2.6).

Although Asteraceae, a family largely made up of open habitat plants in this region (Henn, 1998), made up most of the pollen volume collected, bumble bees did not overall collect significantly more open habitat pollen than forest pollen. One reason for this discrepancy is that *Ageratina altissima*, a forest blooming Asteraceae, added substantially to the forest collected pollen (Table 2.1). This result shows that bumble bees are foraging for pollen in both forest and open habitat during late summer. Forest and open habitat did have significantly more pollen collected than crop habitat. Bumble bees also collected significantly less crop pollen than forest and open pollen each week (Figure 2.10). Pollen was rarely collected from crop habitat, even though crop sometimes made up to 30% of the land cover at one site (Table 1.1). Increasing cropland extent is associated with declines in bumble bee species in Midwest, U.S.A. (Hemberger et al. 2020). While bumble bees can use crop pollen, the colonies in this study seemed to avoid it. This avoidance could be because crop flowers were not as attractive or were not at peak bloom during late summer.

Interestingly, I found that the volume of forest or open habitat pollen was not highly positively correlated with the amount of its respective land cover within the 1-km radius used. Independence of pollen collection from land cover patterns may reflect the distribution of flower resources within each habitat, which can be patchy and in forests concentrated along edges (Van Halder et al. 2011). Forest edge can have a higher intensity and less variable light environment than forest (Galloway 2005). This greater access to light may contribute to greater plant diversity (Eldegard et al. 2015) which may thus lead to greater insect diversity (Riwidiharso et al. 2020, Widhiono et al. 2017). Forest edges have been shown to have a higher species richness and stem density (Davies-Colley et al. 2000) of which bumble bees can forage from (Kammerer et al. 2016). These resources may be particularly valuable for bee species (Ammann et al. 2023) that prefer foraging along linear habitat features (Bertrand et al. 2019). For example, forest edge flowers received more pollinator visits than forest interior flowers of the same species (Goodell et al. 2010). Because of the floral resource density of forest edge, the amount of forest perimeter may play a role in how much pollen is collected from forest by bumble bees.

How is reproductive success affected by landscape and foraged pollen and does this change depending on habitat?

The total number of gynes cells was significantly positively related to the volume of open habitat pollen collected. Yet gyne cells were larger in colonies that collected more pollen from forest habitat. The latter may reflect shorter foraging distances. This result could also suggest that either the quantity or the nutritional quality of the pollen collected from forests was better. Forest and open habitat may provide different plant secondary compounds important to different aspects of colony success. For example, consuming sunflower pollen (*Helianthus annuus*, Asteraceae family) has been shown to reduce the intensity of infections in *Bombus impatiens* (LoCasico et al. 2019, Adler et al. 2020). Sunflower pollen can also enhance bumble bee colony production (Giacomini et al. 2018), which may corroborate the finding of enhanced reproduction from open pollen in this study as Sunflower pollen may be included in the Asteraceae pollen found. Additionally, it has been found that colonies placed in closer proximity to forest outperformed colonies placed in open habitat based on gyne and male production (Vaudo et al. 2018), but the size of the gyne cells were not compared. There may be other factors causing these differences in size. Late-season nectar availability has been shown to play an important role in colony growth (Timberlake et al. 2021), with forest flowers such as *Impatiens* offering large amounts of nectar (Rust 1977), so it is possible that the forest flowers are supplementing bee size in this way. Another factor to consider is the potential trade-off between number of brood cells and the size of the bumble bees. Colonies may put more energy into increasing the colony size which may result in smaller gynes, workers, and males (Castillo et al. 2015). In the same study, Castillo et al. (2015) found that environmental factors such as temperature and precipitation affect both colony and body size, suggesting that these factors may influence the size-number trade-offs in bumble bee colonies. Ultimately, my results show that both forest and open habitat contribute to colony success but in different ways.

Are colonies more successful in areas that have more diverse landscape and pollen?

Land cover diversity did not have a significant effect on pollen diversity, in contrast to results by Matthias et al. (2015) and Pasquale et al. (2013). However, Danner et al. (2017) found that landscape diversity did not influence honey bee collected pollen abundance or diversity, later suggesting that this may have been due to bees compensating for lower resource availability by increasing their foraging range. Having a less rich landscape may result in bees foraging longer distances (Steffan-Dewenter and Kuhn 2003, Danner et al. 2016).

Land cover diversity also did not have an effect on the number or size of gynes produced. This result could be because bumble bees were mostly using forest and open habitat no matter the site, despite having different land cover richness and composition at each. For example, crop habitat nearby would increase the land cover diversity in the area, but would not necessarily mean that bumble bees would collect pollen from the habitat, as found in this study. Looking at only land cover diversity without taking floral resources into account typically does not capture a well-rounded landscape perspective (Bartual et al. 2019, Hellwig et al. 2022), floral resource maps typically predict wild bee communities better than land cover maps (Ammann et al. 2023). Interestingly, pollen diversity also did not have a significant effect on the number or size of gynes produced. My study shows that using more open or forest pollen enhanced colonies in certain ways, but that using more pollen sources overall did not necessarily mean colonies were more or less successful. While bumble bees in my study were collecting from both forest and open habitat, they may collected most of the pollen from just a few species. For example, Balsamaniceae was made entirely of *Impatiens capensis* and *I. pallida*, Plantaginaceae of

Plantago lanceolata, and Campanulaceae of *Lobelia siphilitica*. These four families made up most of the total volume collected. Identifying Asteraceae down to species to determine whether some species dominated the pollen samples would shed light on whether bumble bees were mostly foraging from only a few species. Additionally, high diversity of collected pollen might reflect overall scarcity of floral resources, which necessitates foraging on suboptimal floral resources (Ogilvie and Forrest, 2017). In Chapter 1, I found that rank abundance of flowers was overall the most important factor in determining bumble bee preference towards a flower species and its selection index. Floral dominance may allow foragers to focus on a smaller number of rewarding species (Spiesman et al. 2016). This result from chapter 1 may explain why reproductive success did not benefit from increased pollen diversity, but it is important to note that it was not negatively affected either.

Conclusions:

I provide insight on bumble bee foraging patterns and their impact on reproductive success. Bumble bee populations are declining globally (Ghisbane et al. 2023, Goulson et al. 2008), emphasizing the need to act. Information on the plants and habitats used by bumble bees is crucial for maintaining their forage habitat. Floral resource diversity offered by forest and open habitat have recently been identified as key drivers for different wild bees, some of which are rare or of conservation concern (Ammann et al. 2023). My research reveals that bumble bees gather pollen from both forest and open habitats, each contributing uniquely to colony success. Gathering more open habitat pollen increases the production of gynes, boosting bumble bee populations (Crone and Williams et al. 2016). Meanwhile, collecting more forest pollen results in larger and more resilient adult gynes, improving their chances of surviving winter (Owen 1988). Forest and open habitat complement each other in this way, both providing beneficial forage that bumble bees use in late summer. These complementary habitats work together to enhance bumble bee colony reproductive success.

Table 2.1 Plant taxa identified in pollen along with associated habitat and plant family.

Plant taxon:	**Habitat:**	**Family:**
Actaea racemosa	Forest	Ranunculaceae
Ageratina altissima	Forest	Asteraceae
Agrimonia parviflora	Both	Rosaceae
Arundinaria gigantea	Forest	Poaceae
Asteraceae	Both	Asteraceae
Calystegia Sepium	Both	Convolvulaceae
Campsis radicans	Forest	Bignoniaceae
Castanea dentata	Forest	Fagaceae
Cephalanthus occidentalis	Forest	Rubiaceae
Cichorium intybus	Open	Asteraceae
Cirsium arvense	Open	Asteraceae
Cirsium vulgare	Open	Asteraceae
Clethra alnifolia	Forest	Clethraceae
Corylus americana	Forest	Betulaceae
Cryptotaenia canadensis	Forest	Apiaceae
Dactylis glomerata	Open	Poaceae
Daucus carota	Open	Apiaceae
Desmodium canadense	Both	Fabaceae
Dipsacus species	Both	Caprifoliaceae
Echinacea purpurea	Open	Asteraceae
Erigeron species	Both	Asteraceae
Eupatorium coelestinum	Forest	Asteraceae
Gaillardia pulchella	Open	Asteraceae
Galium aparine	Open	Rubiaceae
Hedera helix	Both	Araliaceae
Impatiens capensis	Forest	Balsaminaceae
Impatiens pallida	Forest	Balsaminaceae
Laportea canadensis	Forest	Urticaceae
Lobelia siphilitica	Forest	Campanulaceae
Lotus corniculatus	Open	Fabaceae

Medicago lupulina	Open	Fabaceae
Menispermum canadensis	Forest	Menispermaceae
Mirabilis nyctaginea	Both	Nyctaginaceae
Oenothera biennis	Open	Onagraceae
Oxalis stricta	Both	Oxalidaceae
Persicaria species	Forest	Polygonaceae
Phytolacca americana	Both	Phytolaccaceae
Plantago lanceolata	Open	Plantaginaceae
Polymnia canadensis	Forest	Asteraceae
Prunella vulgaris	Both	Lamiaceae
Frangula alnus	Both	Rhamnaceae
Rudbeckia species	Both	Asteraceae
Silphium perfoliatum	Both	Asteraceae
Solanum carolinense	Open	Solanaceae
Solanum lycopericum	Crop	Solanaceae
Taraxacum offcinale	Open	Asteraceae
Trifolium spp.	Open	Fabaceae
Unknown	Unknown	Unknown
Urtica procera	Both	Urticaeae
Vernonia gigantea	Open	Asteraceae
Zea mays	Crop	Poaceae

Table 2.2 Shannon and Chao1 Diversity indices for pollen at each site.

Site:	Overall # of taxa (Richness):	Chao Diversity Index:	Shannon Diversity Index:	Evenness:	Total Count:	Avg pop size:
BF	17	17.5	2.23	0.787	200	11.8
BO	23	24	2.35	0.751	498	21.7
CP	22	22	2.51	0.812	300	13.6
D	30	22.75	2.66	0.781	400	13.3
EBP	16	35	1.9	0.686	200	12.5
HB	24	19	2.66	0.838	200	8.33
IMP	41	29.25	2.85	0.773	500	12.5
LB	31	57.5	2.56	0.747	300	9.68
RF	22	50.5	2.32	0.752	200	9.09

Table 2.3 Reported values for Generalized Linear Models.

Response Variable:	**Model:**	**Source of variation**	**DF**	**Estimate**	**Std Error**	**L-R ChiSquare**	**Prob>ChiSq**	**AICc**
Total Gyne Cells	~ open pollen vol	Open vol	1	0.43	0.23	4.47	**0.0344**	68.27
	~ forest pollen vol	Forest vol	1	-0.26	0.14	2.86	0.0905	69.88
	~ open %	Open %	1	0.25	0.42	0.38	0.5355	72.36
	~ forest %	Forest %	1	0.12	0.12	0.93	0.3327	71.8
	~ Shannon	Shannon	1	-0.04	0.09	0.25	0.6136	72.48
	~ Chao	Chao	1	0	0	0.22	0.6316	72.51
	~ Simpsons	Simpson	1	0	0.02	0.03	0.8596	72.71
Gyne Cell Size	~ open pollen vol	Open vol	1	-2.38	0.56	8.85	**0.0029**	11.3
	~ forest pollen vol	Forest vol	1	2.71	0.88	5.93	**0.0148**	14.28
	~ open %	Open %	1	1.01	2.08	0.23	0.6294	19.99
	~ forest %	Forest %	1	-1.26	0.72	2.52	0.1122	17.69
	~ Shannon	Shannon	1	1.07	0.58	2.75	0.0971	17.47
	~ Chao	Chao	1	0	0.01	0.01	0.924	20.21
	~ Simpsons	Simpson	1	0.03	0.15	0.04	0.8351	20.18

Figure 2.1 (Left) Sentinel colony set-up. (Right) Catch basin containing pollen collected from foraging bumble bees.

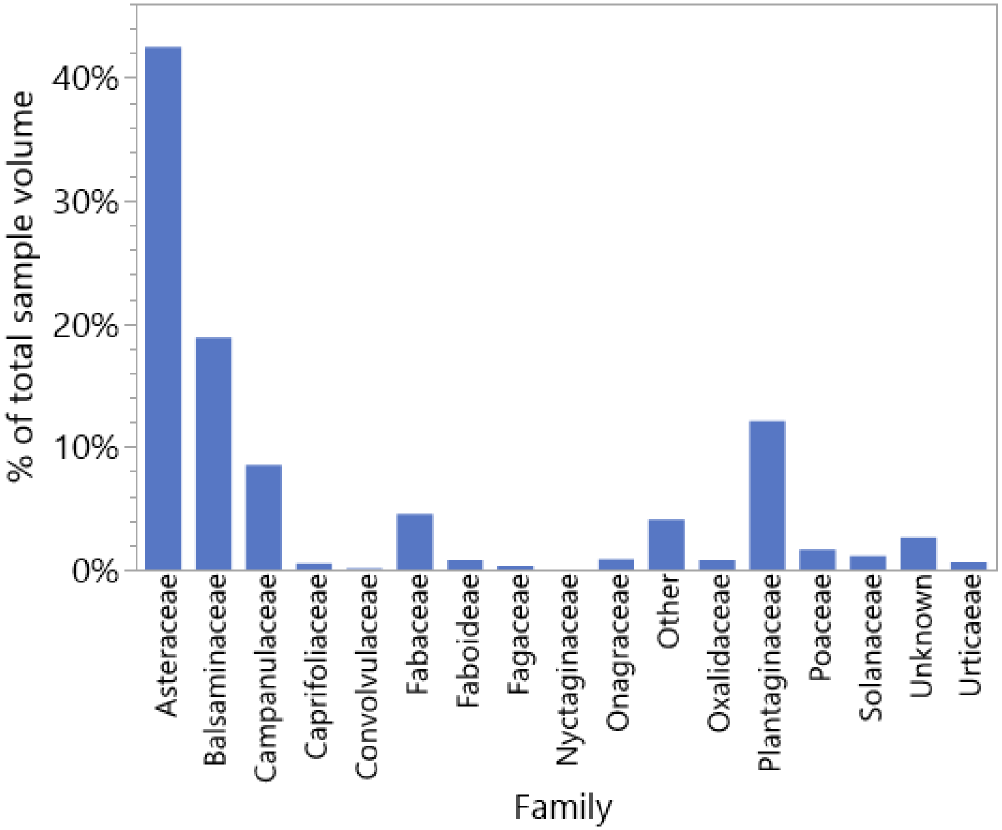

Figure 2.2 Percentage of overall volume of each plant family.

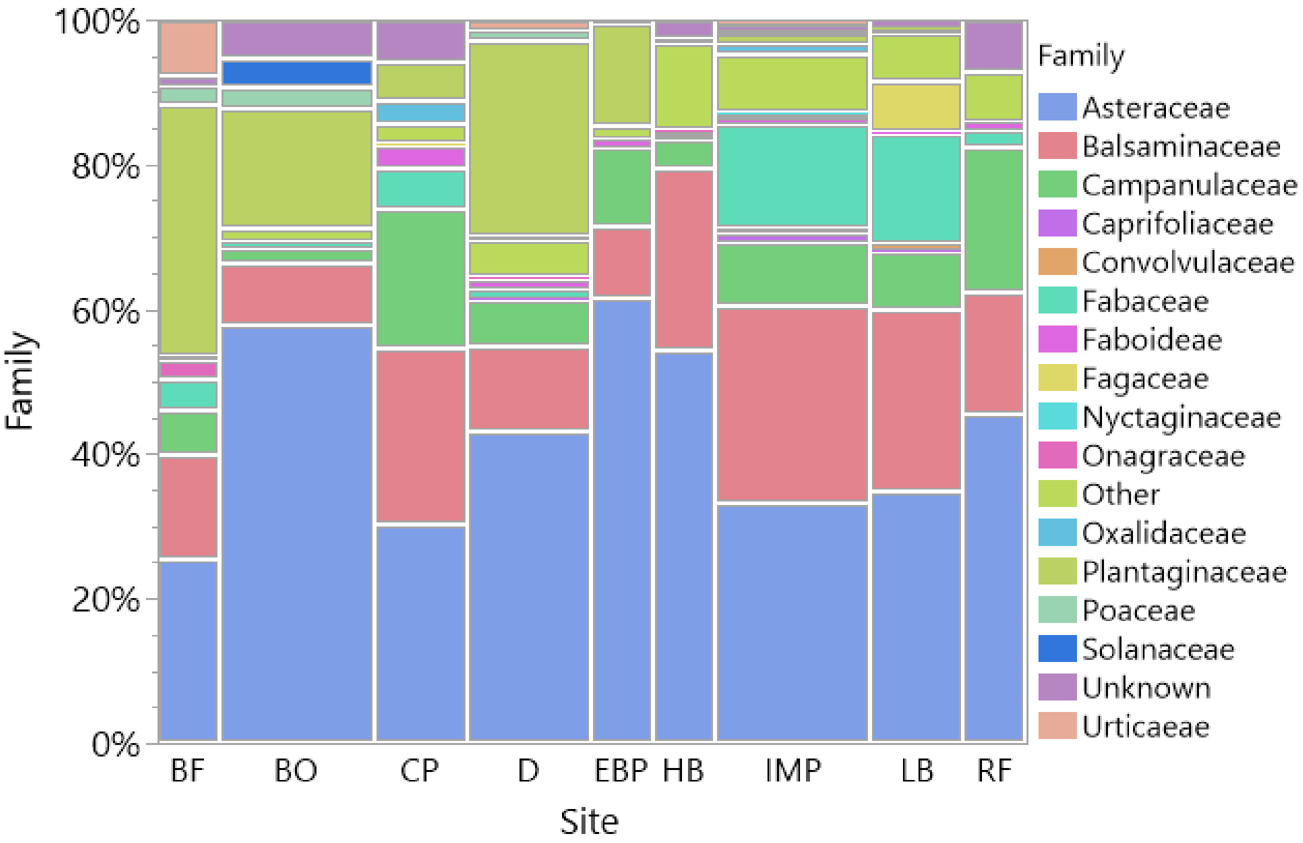

Figure 2.3 Percentage of total volume of pollen sample from each family by site.

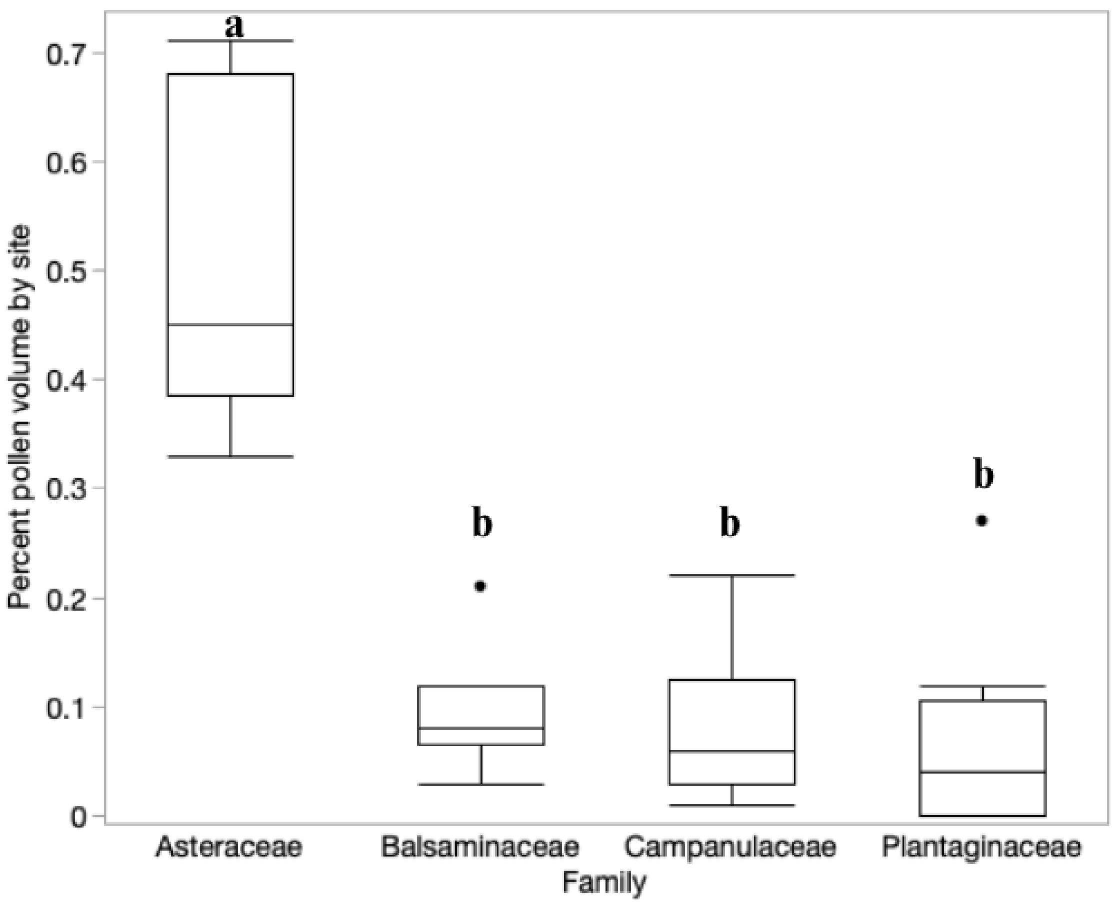

Figure 2.4 Differences between the amount of pollen collected from each plant family across sites averaged over weeks. The line at the center indicates the median, the top of the black box indicates the upper quartile, and the bottom represents the lower quartile. Whiskers represent 95% confidence interval. Points represent outliers. Box plots with the same letters above them are not significantly different from one another.

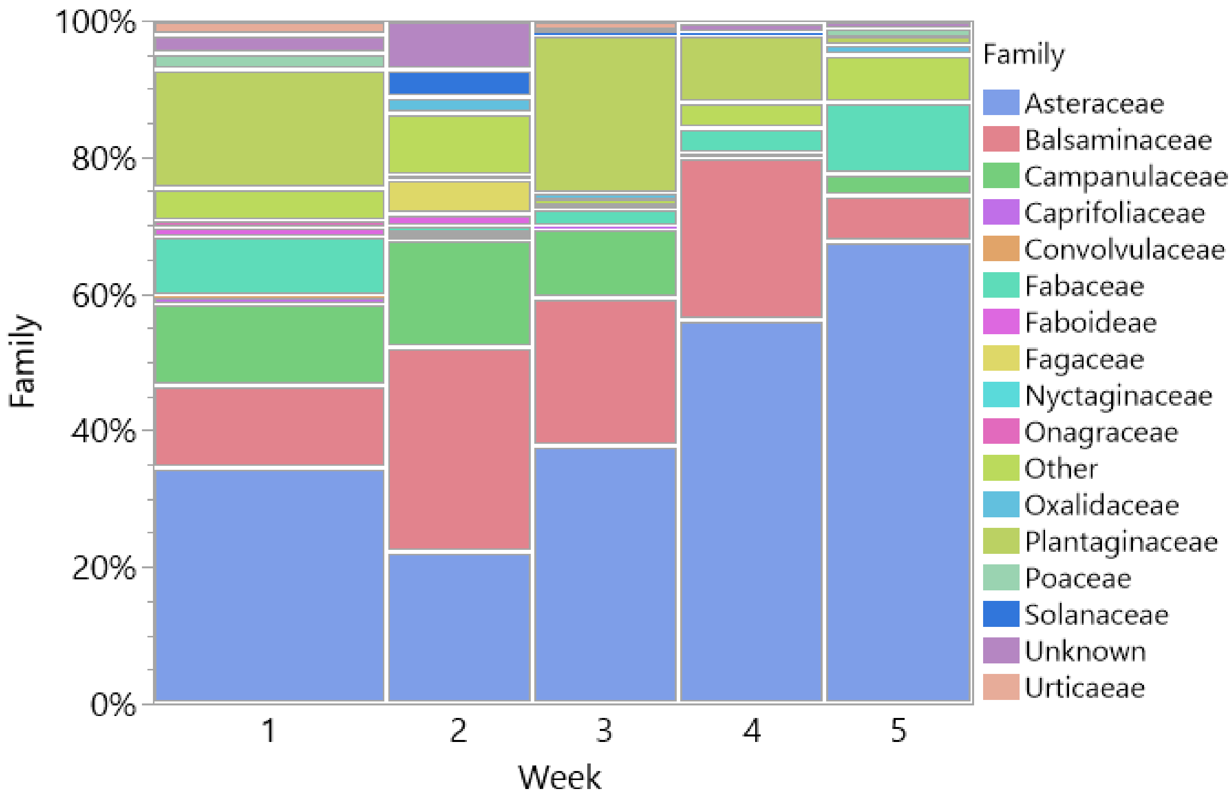

Figure 2.5 Percentage of total volume of pollen sample from each plant family by week.

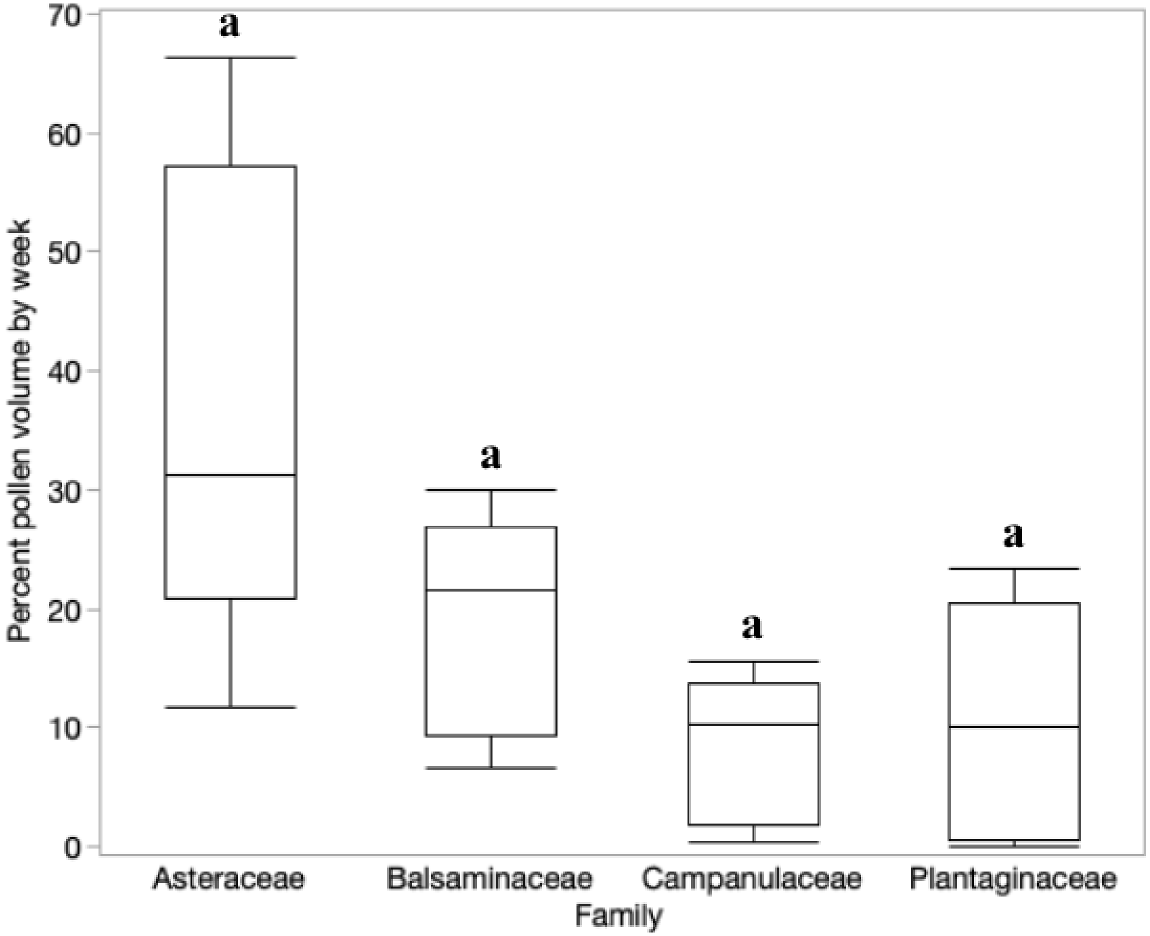

Figure 2.6 Differences between the amount of pollen collected from each plant family across weeks averaged over sites.

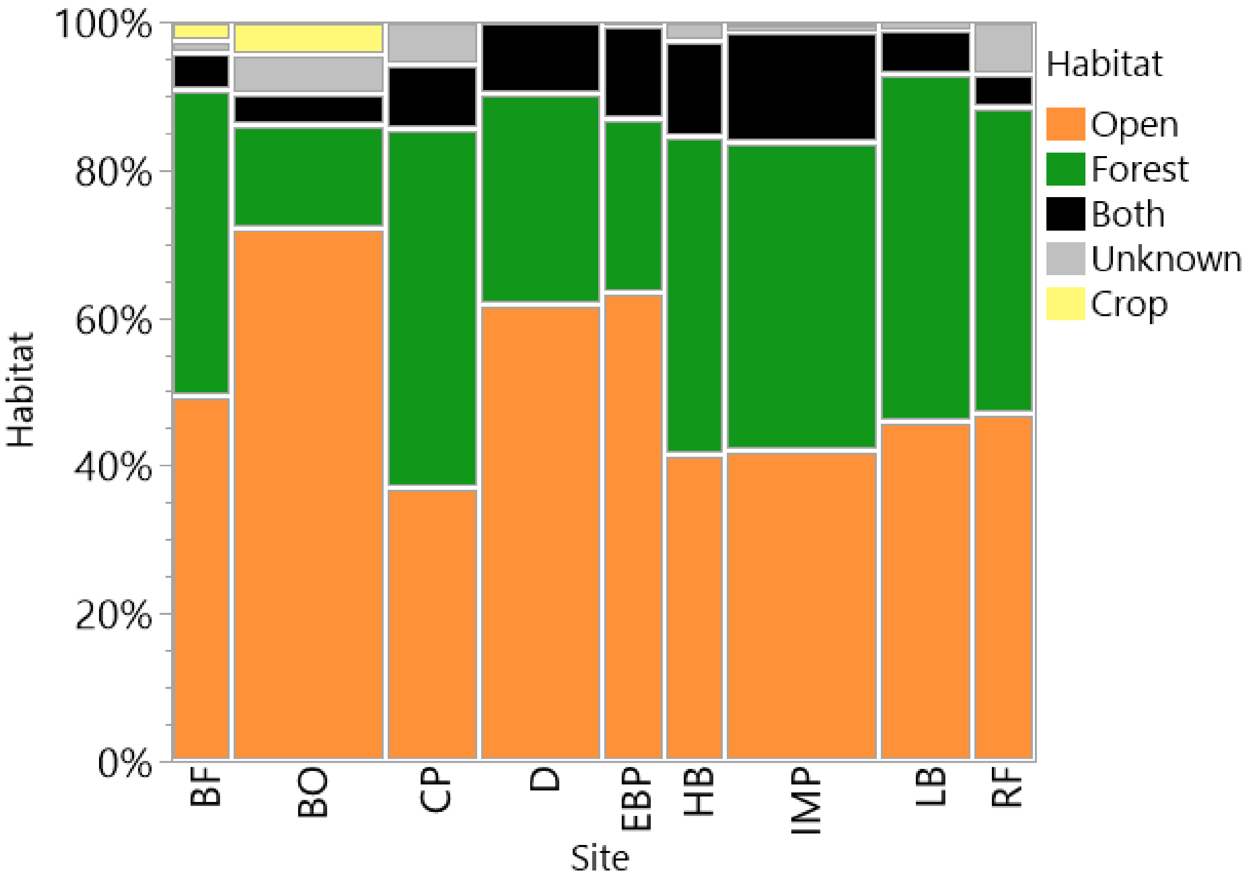

Figure 2.7 Percentage of total volume of pollen sample from each habitat by site.

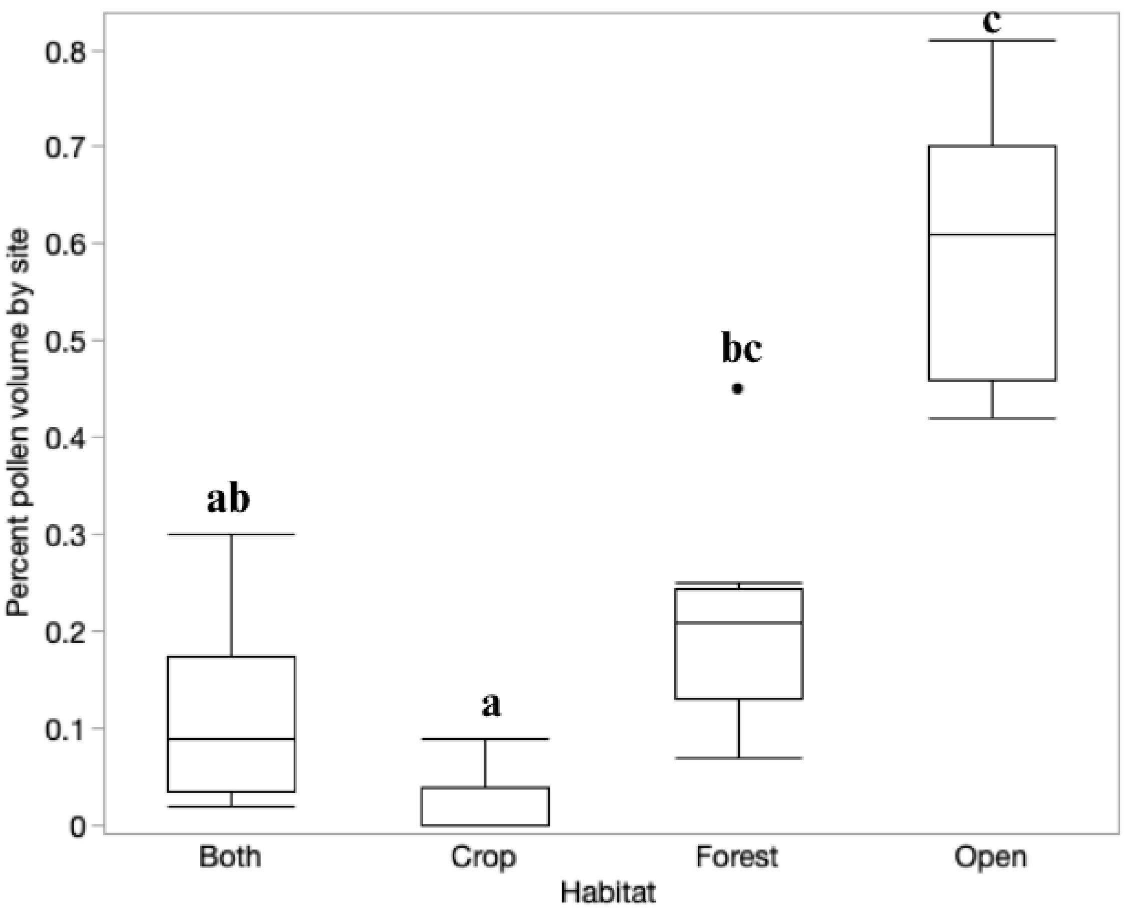

Figure 2.8 Differences between the amount of pollen collected from each habitat across sites averaged over weeks.

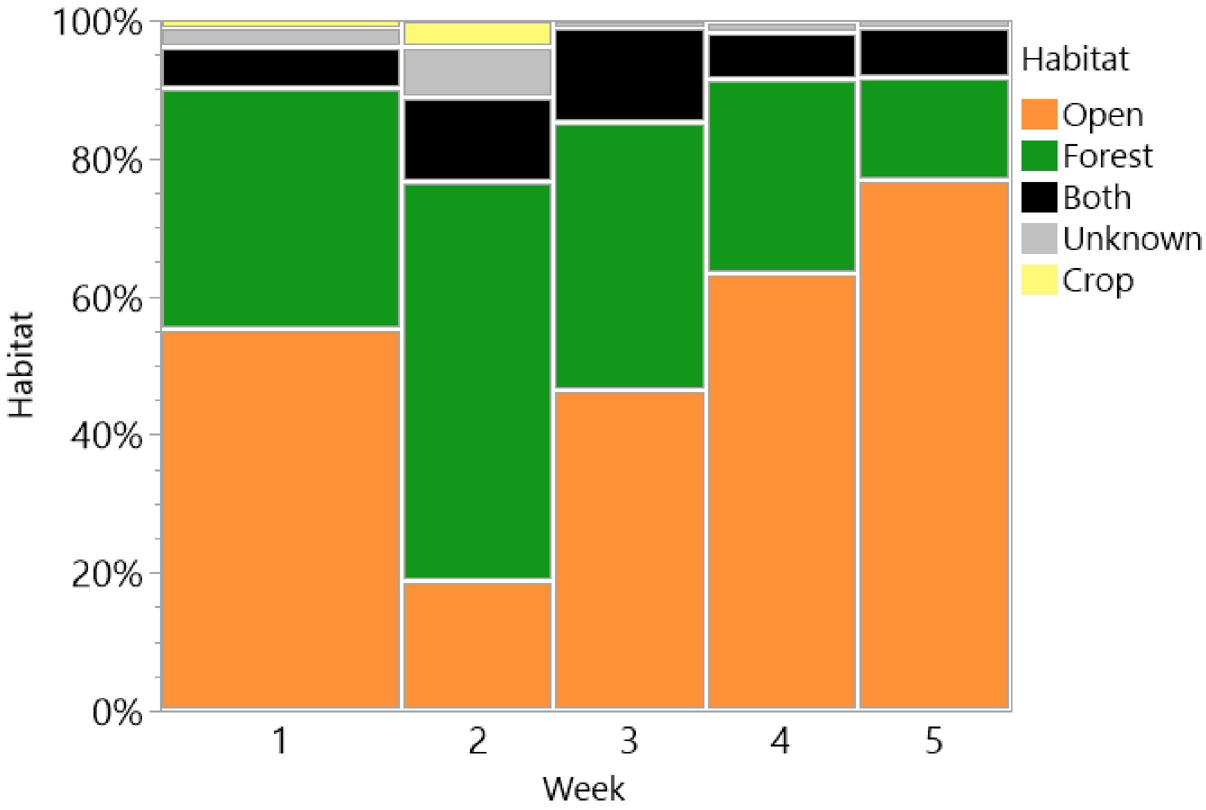

Figure 2.9 Percentage of total volume of pollen sample from each habitat by week.

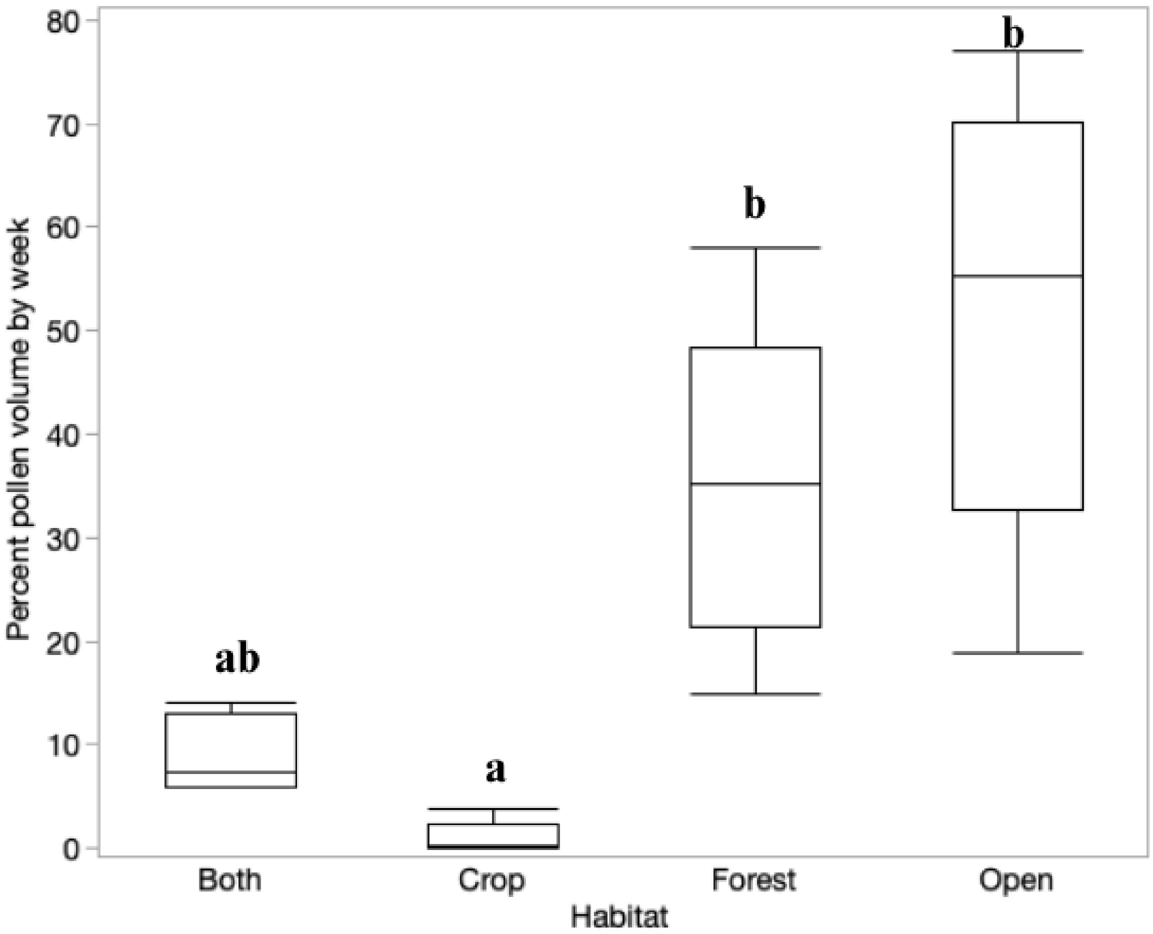

Figure 2.10 Differences between the amount of pollen collected from each habitat across weeks averaged over sites.

Figure 2.11 (Left) *Bombus impatiens* gyne foraging from *Lobelia siphilitica*. (Right) Forest edge filled with *Impatiens capensis*, *I. Pallida*, and *Ageratina altissima*.

Appendix A: Percent volume of plant taxa from samples.

Table A.1 Percent volume each plant taxa made up for each site.

ID:	**BF**	**BO**	**CP**	**D**	**EBP**	**HB**	**IMP**	**LB**	**RF**
Actaea racemosa	0.00%	0.00%	0.00%	0.15%	1.48%	0.00%	0.00%	0.00%	0.00%
Ageratina altissima	6.06%	0.42%	1.66%	2.37%	0.20%	1.91%	1.32%	1.66%	0.41%
Agrimonia parviflora	0.00%	0.00%	0.00%	0.13%	0.00%	0.00%	0.00%	0.00%	0.00%
Arundinaria gigantea	0.00%	2.95%	0.00%	2.52%	0.00%	0.00%	0.92%	0.00%	0.00%
Asteraceae	1.56%	16.29%	8.25%	8.86%	37.52%	13.06%	6.76%	13.26%	9.09%
Calystegia Sepium	0.00%	0.00%	0.00%	1.59%	0.00%	0.00%	5.53%	11.12%	0.00%
Campsis radicans	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.31%
Castanea dentata	0.00%	0.00%	0.13%	0.00%	0.00%	0.02%	0.05%	2.45%	0.00%
Cephalanthus occidentalis	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.80%	0.00%
Cichorium intybus	0.00%	10.82%	0.00%	4.13%	1.31%	0.35%	1.16%	0.00%	0.00%
Cirsium arvense	19.70%	32.98%	16.64%	14.66%	18.61%	31.82%	26.52%	12.56%	35.64%
Cirsium vulgare	3.07%	5.55%	0.00%	0.00%	0.00%	1.47%	0.00%	0.00%	0.00%
Clethra Alnifolia	0.00%	0.00%	0.35%	0.00%	0.13%	0.00%	0.00%	0.19%	0.00%
Coprinus cordisporus	0.00%	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Corylus americana	0.00%	0.00%	0.00%	0.00%	0.00%	1.79%	0.53%	0.00%	0.00%

Cryptotaenia canadensis	0.00%	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Dactylis glomerata	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.23%	0.00%
Daucus carota	0.00%	0.00%	0.19%	0.00%	0.00%	0.00%	0.06%	0.00%	0.15%
Desmodium canadense	0.72%	0.00%	3.72%	2.91%	1.34%	1.10%	0.64%	0.00%	0.70%
Dipsacus species	0.00%	0.00%	0.00%	2.55%	0.00%	5.33%	9.45%	5.56%	0.00%
Echinacea purpurea	0.79%	0.00%	0.73%	0.15%	0.00%	3.79%	0.80%	4.65%	0.90%
Erigeron	0.20%	0.00%	0.00%	0.62%	0.48%	0.00%	2.00%	0.00%	0.00%
Eupatorium coelestinum	0.00%	0.00%	0.00%	1.54%	0.00%	0.00%	0.00%	0.00%	0.00%
Frangula alnus	0.00%	0.09%	0.00%	0.69%	0.00%	0.00%	0.00%	0.00%	0.00%
Gaillardia pulchella	0.00%	0.00%	0.00%	0.26%	0.00%	0.00%	0.10%	0.00%	0.00%
Galium aparine	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.17%	0.00%	0.00%
Hedera helix	0.13%	0.00%	0.00%	0.00%	0.00%	0.00%	0.11%	0.23%	0.00%
Helianthus species	0.00%	0.77%	0.80%	0.22%	0.00%	3.20%	0.19%	0.74%	0.85%
Impatiens capensis	6.02%	1.79%	18.04%	3.85%	4.11%	2.47%	7.11%	4.66%	6.91%
Impatiens pallida	2.67%	0.89%	3.89%	2.79%	1.80%	4.73%	5.58%	6.83%	1.70%
Laportea canadensis	4.33%	0.00%	0.00%	0.14%	0.00%	0.00%	0.09%	0.00%	0.00%
Lobelia siphilitica	5.09%	0.78%	22.30%	4.51%	8.53%	1.87%	6.14%	8.12%	16.71%
Lotus corniculatus	0.00%	0.06%	0.00%	0.00%	0.00%	0.00%	0.05%	0.00%	0.40%
Medicago lupulina	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.16%	0.00%	0.00%
Menispermum canadensis	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.03%	0.00%
Mirabilis nyctaginea	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.07%	0.00%	0.00%

Oenothera biennis	21.92%	6.00%	0.00%	8.21%	0.00%	5.33%	0.00%	0.00%	0.00%
Oxalis stricta	0.00%	0.00%	4.13%	0.36%	0.00%	0.11%	1.71%	0.23%	0.00%
Persicaria species	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.46%	0.00%
Phytolacca americana	0.00%	0.00%	0.70%	0.00%	0.00%	0.60%	0.00%	0.00%	1.62%
Plantago lanceolata	8.95%	6.92%	3.61%	25.88%	12.02%	0.00%	0.95%	0.48%	0.60%
Polymnia canadensis	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.27%
Prunella vulgaris	0.00%	0.11%	0.00%	0.00%	0.00%	2.52%	0.11%	0.37%	0.89%
Rudbeckia laciniata	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.62%
Rudbeckia species	0.00%	1.35%	1.59%	1.05%	7.67%	0.52%	1.48%	1.63%	0.81%
Silphium perfoliatum	1.57%	0.21%	0.42%	0.00%	0.00%	4.15%	0.91%	1.66%	0.00%
Solanum carolinense	0.00%	0.00%	0.78%	0.00%	0.00%	0.35%	1.23%	0.74%	0.00%
Solanum lycopericum	0.00%	1.22%	0.00%	0.00%	0.00%	0.00%	0.19%	0.00%	0.00%
Solidago species	0.00%	0.80%	1.64%	0.77%	0.00%	5.60%	0.29%	2.53%	0.83%
Symphyotrichum cordifolium	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.15%	0.00%	0.00%
Symphyotrichum ericoides	0.00%	0.00%	0.00%	0.20%	0.00%	0.00%	0.83%	0.00%	0.00%
Symphyotrichum lateriflorum	0.00%	0.00%	0.00%	3.45%	0.00%	0.00%	0.46%	0.00%	0.00%
Symphyotrichum novae-angliae	0.00%	0.00%	0.00%	1.38%	0.00%	0.00%	1.95%	0.00%	0.13%
Taraxacum offcinale	0.00%	0.00%	2.94%	0.00%	1.16%	0.00%	0.54%	0.43%	10.31%
Trifolium species	4.10%	0.52%	5.15%	0.68%	0.00%	0.00%	5.44%	14.46%	1.19%
Unknown	0.30%	5.02%	2.07%	0.00%	1.31%	5.37%	0.66%	0.97%	7.98%

Urtica procera	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.08%	0.00%
Verbesina species	3.19%	0.00%	0.27%	1.22%	2.33%	0.34%	1.62%	1.10%	0.00%
Vernonia gigantea	0.00%	0.00%	0.00%	0.00%	0.00%	2.21%	0.00%	0.77%	0.00%
Zea mays	9.63%	4.42%	0.00%	2.15%	0.00%	0.00%	0.00%	0.00%	0.00%

Table A.2 Percent volume each plant taxa made up for each week.

ID:	**1**	**2**	**3**	**4**	**5**
Actaea racemosa	0.00%	0.00%	0.00%	0.12%	0.60%
Ageratina altissima	2.15%	0.53%	2.73%	2.38%	0.00%
Agrimonia parviflora	0.07%	0.00%	0.00%	0.00%	0.00%
Arundinaria gigantea	2.07%	0.00%	0.00%	0.00%	2.65%
Asteraceae	5.25%	3.35%	2.84%	20.98%	32.34%
Calystegia Sepium	1.66%	6.47%	4.04%	0.00%	0.00%
Campsis radicans	0.00%	0.00%	0.00%	0.13%	0.00%
Castanea dentata	0.00%	1.56%	0.00%	0.00%	0.00%
Cephalanthus occidentalis	0.30%	0.00%	0.00%	0.00%	0.00%
Cichorium intybus	0.09%	0.00%	5.13%	0.62%	10.11%
Cirsium arvense	24.59%	16.25%	31.51%	36.25%	8.45%
Cirsium vulgare	0.00%	6.12%	1.20%	0.00%	0.00%
Clethra Alnifolia	0.07%	0.20%	0.00%	0.00%	0.05%
Coprinus cordisporus	0.02%	0.00%	0.00%	0.00%	0.00%
Corylus americana	0.00%	1.23%	0.00%	0.00%	0.00%
Cryptotaenia canadensis	0.00%	0.02%	0.00%	0.00%	0.00%
Dactylis glomerata	0.00%	0.13%	0.00%	0.00%	0.00%
Daucus carota	0.11%	0.00%	0.05%	0.00%	0.00%
Desmodium canadense	2.27%	2.57%	0.55%	0.00%	0.00%
Dipsacus species	4.23%	7.83%	2.10%	0.00%	0.00%

Echinacea purpurea	1.98%	1.73%	1.08%	0.37%	0.00%
Erigeron	0.00%	0.00%	2.11%	0.62%	0.00%
Eupatorium coelestinum	0.00%	0.00%	0.00%	0.00%	1.25%
Frangula alnus	0.00%	0.00%	0.09%	0.58%	0.00%
Gaillardia pulchella	0.00%	0.00%	0.00%	0.00%	0.31%
Galium aparine	0.10%	0.00%	0.00%	0.00%	0.00%
Hedera helix	0.03%	0.24%	0.00%	0.00%	0.00%
Helianthus species	1.52%	0.00%	0.17%	0.80%	0.36%
Impatiens capensis	4.48%	0.61%	7.31%	15.72%	2.45%
Impatiens pallida	2.68%	6.96%	2.83%	1.48%	3.69%
Laportea canadensis	1.07%	0.00%	0.15%	0.03%	0.03%
Lobelia siphilitica	9.74%	14.01%	8.09%	0.39%	3.06%
Lotus corniculatus	0.13%	0.06%	0.00%	0.00%	0.00%
Medicago lupulina	0.00%	0.00%	0.00%	0.00%	0.15%
Menispermum canadensis	0.00%	0.02%	0.00%	0.00%	0.00%
Mirabilis nyctaginea	0.00%	5.76%	0.00%	0.00%	0.00%
Oenothera biennis	5.41%	8.13%	0.00%	0.00%	6.66%
Oxalis stricta	0.00%	2.54%	0.45%	0.00%	1.48%
Persicaria species	0.00%	0.00%	0.00%	0.00%	0.85%
Phytolacca americana	0.57%	0.00%	0.00%	0.41%	0.00%
Plantago lanceolata	5.86%	0.00%	17.95%	12.16%	1.13%
Polymnia canadensis	0.00%	0.00%	0.00%	0.11%	0.00%

Prunella vulgaris	0.86%	0.46%	0.00%	0.00%	0.00%
Rudbeckia laciniata	0.41%	0.00%	0.00%	0.00%	0.00%
Rudbeckia species	0.95%	0.68%	0.00%	1.28%	5.71%
Silphium perfoliatum	0.94%	2.52%	0.61%	0.00%	0.00%
Solanum carolinense	0.39%	0.45%	0.00%	0.19%	0.96%
Solanum lycopericum	0.00%	1.15%	0.07%	0.19%	0.00%
Solidago species	3.65%	0.31%	0.77%	0.00%	0.00%
Symphyotrichum cordifolium	0.00%	0.00%	0.00%	0.00%	0.14%
Symphyotrichum ericoides	0.00%	0.00%	0.00%	0.00%	0.94%
Symphyotrichum lateriflorum	0.00%	0.00%	0.00%	0.00%	3.24%
Symphyotrichum novae-angliae	0.00%	0.00%	0.00%	0.05%	2.98%
Taraxacum offcinale	3.57%	0.24%	0.48%	0.53%	0.25%
Trifolium species	3.17%	0.43%	2.13%	3.20%	9.13%
Unknown	2.63%	6.70%	0.99%	1.10%	0.52%
Urtica procera	0.03%	0.00%	0.00%	0.00%	0.00%
Verbesina species	0.00%	0.31%	4.57%	0.32%	0.51%
Vernonia gigantea	0.58%	0.45%	0.00%	0.00%	0.00%
Zea mays	6.35%	0.00%	0.00%	0.00%	0.00%

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