How do Human-Induced Environmental Changes and Parasitism Influence Plant Traits Governing Bumblebee Foraging?

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Abstract

This thesis examines how environmental changes shape interactions foraging behaviour of bumblebees (*Bombus*), important ecologically and economically pollinators. A literature review identified floral nutritional and morphological traits as being key for bumblebee attraction. Herbivory generally reduced attractiveness to bumblebees through their effects on these traits, while other environmental factors such as soil moisture or fertilizer use had context-dependent impacts. In an experimental study, I then tested how diet quality and infection with the parasite *Crithidia bombi* interactively influenced foraging behaviour and sensitivity to herbivore damage. Contrary to predictions, herbivory positively influenced bumblebee visitation, while having no effect on measured floral traits. Infection did not influence foraging decisions, but reduced overall foraging activity in a dose-dependent manner, while diet shaped visitation patterns without having an impact on infection intensity. Colony identity, plant height and floral display size were also key predictors of foraging behaviour. These findings highlight the hierarchical nature of interacting biotic and environmental factors in shaping pollinator foraging ecology.

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Chapter 1: Bumblebees, Flowers and Environmental Stressors: A Comprehensive Literature Review

Introduction

Human activity has had profound, and often detrimental impacts on the planet's environment (Vitousek, P. M 1992). In many cases, plants in particular are known to suffer severe effects resulting from human activities. Studies show that human-induced factors such as drought resulting from climate change, urbanization and agricultural development impact the quantity and productivity of vegetation across a landscape (Wang et al., 2015). In addition to this, other human activities such as fossil fuel combustion and use of fertilizers have been shown to alter soil quality, impacting the health of plants, (Penuelas & Sardans, 2023), with fertilizer use further being linked to increased damage to plants by insect pests (Sánchez-Bayo, F. 2021). This prompts a growing concern regarding the health of vegetation which makes up the primary producers of most ecosystems on earth.

Variation in plant traits associated with environmental stressors may represent the impact of physiological constraints (Georgieva & Vassileva, 2023), or they may stem from phenotypic plasticity, a genetically regulated capacity of plants to change their traits, or phenotype, in response to environmental changes (Schlichting, 1986). This mechanism is of particular importance to plants due to them being sessile organisms (Bradshaw, 1965). Phenotypic plasticity allows plants to allot different amounts of resources to different body structures including leaves, roots and reproductive organs in order to ensure that the plant is able to maximize its access to limiting resources (Sultan, 2003). For example, some plants may alter the size of their leaves in response to differing levels of sunlight in their environment (Schlichting, 1986; Sultan, 2003), while in other cases, plants can increase their root diameter to maximize water uptake in low-moisture environments (Sultan, 2003).

Phenotypic plasticity is widespread among plants (Schlichting, 1986), but the extent of plasticity of a certain trait can vary drastically, even in closely related species where the mean trait value is similar, suggesting that the evolution of a trait and that of its plasticity are not necessarily related (Schlichting, 1986). Furthermore, the extent of phenotypic plasticity has been demonstrated to vary among populations of the same species. Phenotypic plasticity is also sensitive to climatic variations, with the extent of phenotypic plasticity being correlated with the level of climatic variation in an environment (Stotz et al., 2021).

Aside from direct impacts to plants, human activity can also impact interactions between plants and the pollinators which rely on them. For example, experimental exposure of plants to extreme heat stress, akin to conditions experienced due to extreme drought, was proven to reduce fertility in pollinators relying on these plants for nutrition (Walters et al., 2024). Alterations to plants derived from environmental changes can further impact plants' ability to attract pollinators, as these alterations often involve traits related to pollination (Gambel & Holway, 2024).

Bumblebees (*Bombus* spp.) are insect pollinators of particular scientific and ecological interest, whose pollination habits are known to be influenced by environmentally induced phenotypic plasticity in plants from which they forage from (e.g., Höfer et al., 2021; Ludewig et al., 2023). These bees play an important role in pollinating commercially valuable crops and wild plants, and have many advantages over honeybees in this regard. These include longer flying seasons, less aggressive colonies that make bumblebees better suited to pollination of crops grown in greenhouses, longer tongues which can reach nectar from plants with longer flower tubes and the ability to perform 'buzz pollination', wherein the bees create vibrations which allow for more efficient extraction of pollen from the flowers of certain crops such as tomatoes (Wahengbam et al., 2019). Bumblebees are also capable of pollinating in cooler temperatures and lower light levels than honeybees (Karbassioon et al., 2023), and their 'buzz pollination' ability, which honeybees are incapable of performing, benefits numerous commercially important crops including tomato, eggplant, kiwi, and blueberry plants (Cooley & Vallejo-Marín., 2021). Unfortunately, many bumblebee species are threatened with extinction, with as many as 43% of all global bumblebee species facing some level of extinction risk (Arbetman et al., 2017). As a result, it is becoming increasingly important to understand bumblebee ecology, including their foraging behaviour, in order to ensure effective conservation policy aimed at protecting these key pollinators.

Environmental variables known to influence phenotypic plasticity in plants which bumblebees forage from include insect herbivory (Gustafson, 2022), soil quality (Vaudo, 2022), water availability (Gallagher & Campbell, 2021), and air temperature (Shrestha et al., 2018). Preliminary findings suggest that variation in each of these environmental variables is associated with variation in plant traits relevant to pollinator choice in bumblebees, which ultimately impacts bumblebee foraging behaviour (Gustafson, 2022; Vaudo, 2022; Gallagher & Campbell, 2021; Shrestha et al., 2018). These traits include nectar composition (ie. ratio of glucose and fructose to sucrose) (Gustafson et al., 2022), pollen count (abundance of pollen), number of flowers and nectar abundance (Vaudo et al., 2022).

Here, I conduct a comprehensive literature review in order to understand the scope of current scientific literature regarding the roles of different floral traits in influencing bumblebee foraging behaviour as well as those of environmental variables in driving variation in these floral traits,

ultimately impacting flower choice in bumblebees. In addition to this, I also seek to understand the similarities and differences in the relationships between floral traits and pollinator preference in bumblebees when compared to other bee species. In doing so, I further hope to understand whether the foraging behaviour and decisions made by bumblebees are unique to the genus, or if these behaviours and underlying drivers are conserved across the broader taxonomic group of bees. In particular, my study aims to address two broad research questions:

Q1: Which floral traits have been identified in scientific literature as having an influence on bumblebee foraging behaviour, and how do variations in these traits influence foraging in bumblebees? Are these patterns conserved across bees, or specific to bumblebees?
Q2: How do ambient temperature, water availability, soil quality and insect herbivory influence these, and how does this impact flower choice in bumblebees? How does this compare to other bees?

Relationship between bumblebee foraging and bumblebee traits

Bumblebee traits

While interactions between floral traits and bumblebees play an important role in governing foraging behaviour, and the topic is outside the scope of this literature review, it is important to note that the properties of bumblebees themselves can also often influence plant-pollinator interactions. Many notable examples of this are evident in scientific literature. In one such study, co-evolution between bumblebees and plants appeared to have an impact on corolla length, with this feature co-evolving with proboscis length in different bumblebee and host plant species (Suzui et al., 2007). In particular, *Isodon umbrosus* and *Clematis stans*, which have long corolla tubes and are pollinated by a wide variety of bumblebee species, exhibit considerably variation within and between populations in order to accommodate specialist bumblebee pollinators of varying proboscis lengths (Suzui et al., 2007).

Another study looked at *I.sodon umbrosus* and *I.sodon effusus*, two other Isodon species, where the primary, morphologically matched pollinator of *I. umbrosus* was *B. honshuensis* while the *B. diversus* was matched with *I. effusus. ,* subsequentlyIt found that *I. umbrosus* was had a higher level of deposition by *B. honshuensis* than by *B. diversis*, its' secondary pollinator, while *I. effusis* instead relied on frequent pollen deposition by *B. diversus* for reproduction (Suzuki & Akazome, 2000). This would suggest that many species of bumblebee- pollinated plants, particularly those with longer floral tubes, co-evolve appropriate floral tube lengths in order to accommodate specialist bumblebee pollinators. However, bumblebee-pollinated plant species with shorter corolla tubes are more accessible to bumblebees as a whole, attract a wider variety of bumblebee pollinators, and subsequently have less variation in corolla tube length (Suzui et al., 2007). This therefore indicates that floral- tube- length- based bumblebee pollinator syndromes are more so prevalent in long-tubed

plants. As a result, this suggests that multiple types of pollination systems exist in bumblebee-pollinated plants, involving generalist and specialist systems, where proboscis length is only important for specialist plant-bumblebee systems rather than all bumblebee-pollinated plants in a region.

Of further interest is the existence of bumblebee-plant systems where generalist systems are present despite one pollinator species being the most efficient. This is evident in research on *Melampyrum roseum* in a study system involving *Bombus consobrinu*s, *Bombus diversus*, and *Bombus honshuensis* pollinators (Hiei, K & Suzuki 2001). In the study, while *B. honshuensis* was considered to be the most efficient pollinator, the plant lacked any form of specialist pollinator system, with pollination intensity being similar for all three species (Hiei, K & Suzuki 2001). This would further suggest that one bumblebee pollinator being better morphologically suited for a given plant species does not guarantee the existence of a bumblebee pollinator syndrome. This therefore indicates that bumblebee-pollinator syndromes form under strict circumstances, perhaps only in plants with long floral tubes, which can only be reliably accessed by a single bumblebee pollinator in a given region. As a result, the understanding of the circumstances of when pollinator syndromes do or do not form may present an area of interest for future scientific investigation.

These examples help show that features of bumblebees themselves and their interaction with plants can influence their foraging decision-making. This may be a topic for discussion in future literature reviews pertaining to bumblebee-plant interactions.

Methods

Evaluating literature

In order to conduct an effective literature review, I sought to critically look at literature addressing each of the above research questions, in order to identify common themes and knowledge gaps in current literature. In answering question 1, my aim was to understand the extent of current scientific knowledge regarding bee-pollinator interactions, and how these interactions are shaped by floral traits. In addressing question 1, I focused on several key themes, namely evolution of floral traits driven by bee pollinators (both bumblebees and other bee species), mechanisms that are speculated to or have been empirically demonstrated to drive this co-evolution and similarities versus differences in the relationship of between floral traits and bumblebees, when compared to those observed between floral traits and other species of bee pollinators. I further sought to identify and comment on the extent and subsequent limitations of scientific knowledge regarding the topic.

In addressing question 2, I sought to explore the impact of each of the four environmental variables: water availability, insect herbivory of host plants, soil nutrition and ambient temperature alter the foraging decisions made by bumblebee and other bee (apoidea) pollinators. My aim was to

understand how plants growing under varying water levels, soil quality levels and air temperatures as well as those exposed to the presence or different levels of insect herbivory can result in alterations to the plants' desirability to potential bee pollinators. Similarly to question 1, I sought to address the underlying mechanisms for the relationship between the environment, plants and bees, while also investigating whether these environmental drivers impact bumblebees versus other bees differently. Furthermore, as with question 1, I also wanted to assess the scope of and comment on available research and knowledge gaps relating to the topic.

Conducting literature review

In order to identify desired literature, I used the Omni, Google Scholar and Web of Science databases. For Question 1, I used the keywords: Flower Trait AND bee pollin*, Flower traits AND bumblebee pollin*. In using the wildcard (*) symbol after the root word 'pollin', which allowed me to search for all papers containing keywords with this root word, including 'pollinators' 'pollinating' etc., I aimed to maximise the number of potential papers that I can find (table 1.1).

For question 2, I used the keywords: Soil Quality, Water availability, Insect Herbivory or Air temperature with each search term connected by the word AND followed by bee or bumblebee pollin* order to find studies related to either bumblebees or other bees respectively (table 1.1).

In addition to finding papers directly from Omni, Google Scholar and Web of Science, I also identified some literature relevant to my study by looking at the list of citations from papers which I have previously screened, for additional studies on the topic. This was especially true for topics which had relatively few relevant papers.

Criteria for selecting literature

In answering question 1, I only included literature focusing on the relationship between bumblebees or other bee pollinators and floral traits into my synthesis. This included bee choice studies, which empirically demonstrated a relationship between specific floral traits, and bee preference. In addition to this, I also included studies which hint at a correlation between certain floral traits and pollination by bumblebees or other bees, even if these studies did not directly involve bee traits. Such studies often involved coevolution studies, whose findings indicated selective pressures acting on certain floral traits to make them more desirable to bumblebees or other bees. In filtering literature by pollinator species in my literature review to date, I found and incorporated studies which included bumblebees and/or other bee species alongside other species of pollinators (birds, other insects etc.). However, I omitted any studies which did not incorporate bumblebees or other bees. Lastly, I also sought to find and incorporate studies which compared the effects of flower traits choice on bumblebees and other bees (ie. honeybees and other wild bee species).

In answering question 2, I focused on literature directly related to the influence of the four aforementioned environmental variables: insect herbivory, water availability, soil quality and ambient temperature on flower choice in pollinating bees through their impact on flowers and their relevant properties. In conducting my literature review, I primarily focused on studies involving flower choice experiments, but additionally looked at literature involving the impacts of the environmental variables on the plants, where bee pollination was involved, and the findings could suggest some sort of relationship between plants, the environment and bee pollination. As with question 1, I only included studies which look at either bumblebees or other bees as pollinators in my literature review.

Table 1.1: **Numbers of results found using different search terms from Web of Science, Omni and Google Scholar Databases.** Date each Search was initially entered, alongside the search term and total results found for each search are indicated below.

Search Engine	Date	Search term	# of results
Web of Science	8/26/2024	floral traits bumblebee pollination	196
	8/26/2024	Floral traits and bee pollination	942
	8/26/2024	Soil quality and bee pollination	78
	8/26/2024	Soil N:P ration and bee pollination	0
	8/26/2024	Insect herbivory and bee pollination	79
	8/26/2024	Air temperature and bee pollination	69
	8/26/2024	Insect herbivory and bumblebee pollination	9
	8/26/2024	Air temperature and bumblebee pollination	11
	9/9/2024	Insect herbivory and bumblebee pollin*	16
	9/9/2024	Insect herbivory and bee pollin*	98
	9/9/2024	Soil fertility and bumblebee pollin*	8
	9/9/2024	Soil moisture and bumblebee pollination	5
	9/9/2024	Soil moisture and bee pollination	22
	9/9/2024	drought and bee pollin*	122
	9/9/2024	drought and bee pollination	86
	9/9/2024	drought and bumblebee pollination	15
	9/9/2024	drought and bumblebee pollin*	21
	9/9/2024	Insect herbivory and bee pollin*	98

	9/9/2024	Insect herbivory and bumblebee pollin*	16
	9/11/2024	Air temperature and bee pollin*	105
	9/16/2024	Floral traits and bumblebee pollin*	284
	9/17/2024	Floral traits and bee pollin*	1194
Omni	10/2/2024	Insect herbivory and bumblebee pollin*	38
Google scholar	12/30/2024	Floral traits and bumblebee pollin*	~19,000
	1/9/2025	effects of soil moisture on plant reproductive fitness	~72,400
	1/10/2025	Soil moisture and bumblebee pollin*	~15,100
	1/14/2025	floral traits and bee pollin*	567
	1/14/2025	Soil nutrients and bee polllin*	19,400
	1/14/2025	Soil phosphorous and bee pollin*	24,200
	1/14/2025	Soil Nitrogen and bee pollin*	17,300
	1/18/2025	Soil Nitrogen and bumblebee pollin*	17,400
	1/21/2025	air temperature and bumblebee pollin*	193

Underlying reasons for Bumblebee foraging preference

Associations between floral traits and bumblebee attraction

Across screened research, multiple underlying mechanisms for bumblebee foraging preferences could be observed. First, and most commonly, some floral traits were associated with increased levels of floral rewards; bumblebees use these traits as an indicator of reward availability and/or quality (e.g., Kariyat et al., 2021; Corbet & Huang, 2014; Bauer et al., 2017). Second, certain floral traits were associated with detection by bumblebees, making bumblebees more likely to find and forage from these flowers (e.g., Spaethe et al., 2001). Third, there seemed to be an inherent preference for certain floral traits in foraging bumblebees regardless of whether or not these traits were associated with increased rewards, suggesting an evolved preference in bumblebees (e.g., Gomez et al., 2008; Somme et al., 2014). Examples of the latter mechanism include bumblebees favouring floral traits which are often associated with increased rewards, even when those traits are uncoupled from floral rewards (e.g., Carr et al., 2015), as well as instances of co-evolution between bumblebees and host plants (e.g., Suzuki et al., 2000; Gomez et al., 2008).

Which floral traits have been identified in scientific literature as having an influence on bumblebee foraging behaviour, and how do variations in these traits influence foraging in bumblebees? Are these patterns conserved across bees, or specific to bumblebees?

When considering floral traits relevant to pollinator attraction themselves, three broad categories can be seen: 1) the availability and composition of floral rewards (i.e., nectar and pollen); 2) visual and olfactory cues, which consist of physical plant traits that pollinators can identify by sight and smell such as flower area or organic volatile compounds, and it turn, are often directly associated with floral reward abundance; 3) traits which impact accessibility to the flower, such as floral tube length and depth (Michelot-Antalik et al., 2025). Within these broad categories, my literature review identified a number of floral traits relevant to pollination by bumblebees along with one or more of the above mechanisms explaining these trends. Among traits relevant to bumblebee attraction, both abundance of floral resources and traits associated with bumblebee attraction were important drivers determining bumblebee foraging choice. Furthermore, among floral attraction traits, properties of the corolla and relevant reproductive structures were most commonly found in literature.

Floral reward abundance

Pollen and Nectar abundance

Unsurprisingly, abundance of pollen and nectar rewards are major driving factors behind bumblebee foraging preference, with bumblebees preferentially foraging from both flower species which produce more pollen and nectar (Somme et al., 2014) and, within a species, preferring individual flowers with greater quantities of these resources (Symington & Glover, 2024; Kariyat et al., 2021; Brunet el al., 2015).

Pollen protein content

In addition to the quantity of nectar and pollen, pollen composition is also important. Notably, high pollen protein content is important to bumblebees. One study found that bumblebees prefer pollen from flower species with higher ratios of proteins to lipids, with optimal protein to lipid ratios being 10:1, and adjust their diet accordingly (Vaudo et al., 2016). This may be attributed to the fact that increased mortality in bumblebees was found to be associated with reduced protein concentrations of 5:1 P:L or lower, suggesting that inadequate dietary protein is fatal to bumblebees (Vaudo et al., 2016). Research further found that bumblebee visitation rates are positively associated with pollen amino acid concentrations (Somme et al., 2014; Leach et al., 2023). This may be because adequate dietary amino acid content is important for healthy development of bumblebee larvae, where amino acid deficiencies are associated with increased larval mortality (Génissel et al., 2002). As a result, bumblebees likely preferentially visit plants with higher abundances of amino acids in order to ensure that their larvae receive adequate dietary amino acids. These findings therefore highlight the importance of dietary proteins as being important to bumblebees, with amino acids being particularly important. This may help to further explain their foraging preferences in relation to nutritional rewards.

Sugar composition

In addition to the abundance and composition of proteins, sugar composition was also found to be important to bumblebee foragers. Possible evidence of this could be seen in a study on *Helleborus foetidus* flowers, where bumblebees, alongside anthophorid bees, were the main pollinators, and most of the variation in fructose, glucose and sucrose ratios was present among individual nectarines on the same flower and individual flowers on the same plant as opposed to different plants within the population, most of this variation occurred between individual nectarines on a given flower (Herrera et al., 2006). This pattern of variation in floral resources resulted in high levels of unpredictability in nectar rewards to foraging bumblebees, with the study suggesting that plants evolved such nectar composition variations in order to make it more difficult for pollinators such as bumblebees to select individual flowers with a desired nectar composition, and instead visit, and subsequently pollinate, all open flowers on a plant. This could indicate that bumblebees, among other pollinators, have an inherent preference for certain chemical compositions in the nectar they consume, as suggested by older literature (Baker 1983b), whereas plants evolve random patterns of these compositions in their nectarines to ensure that the bees visit as many flowers as possible, ultimately helping to improve pollination in the host flower. Ultimately, these findings suggest that the abundance of nectar and pollen, as well as primary metabolites such as proteins and sugar play a key role in bumblebee foraging decision-making.

Floral Chemistry

Chemical cues in plants were also key factors in shaping bumblebee-plant interactions. In addition to sugar nectar composition, other components of nectar chemistry can shape bumblebee foraging. In one example, inbred horsenettle (*Solanum carolinense*) plants produced considerably lower levels octadecanal and (Z)-9-Octadecenal, two key scent chemicals in *S. carolinense* which are associated with pollinator attraction (Kariyat et al., 2021). Despite the importance of these chemical cues to bumblebees specifically not being tested, the study found that their production was correlated with pollen production, with previous research finding these chemicals to elicit physiological responses in other pollinators; moreover, bumblebees showed a strong preference for outbred as opposed to inbred *S. carolinense* plants (Kariyat et al., 2021). These findings suggest that bumblebees may demonstrate a positive response to the presence of these chemicals.

Another example of floral chemistry in bumblebee foraging behaviour can be seen in wild bergamot, *Monarda fistulosa*, a plant species which has multiple chemical phenotypes or 'chemotypes'. In a study involving different *M. fistulosa* chemotypes, bumblebee pollinators of the plant infected with the parasite *Crithidia bombi* were shown to preferentially forage from chemotypes associated with medicinal compounds associated with anti-parasitic 'medicinal' compounds (Fitch et al., 2025). This example demonstrates that bumblebees are capable of making foraging decisions in

relation to host plant chemistry that are not only associated with a desirable nutritional reward, but also with the health of the bee itself. Indeed, this would suggest that the plant, bumblebee pollinator and bumblebee pathogen interact to shape plant-pollinator interactions, which may point to a novel mechanism through which bumblebees make foraging decisions, which may prompt additional research on the topic. Overall, however, these examples show that nectar chemistry can play a vital role in bumblebee foraging, and can shape both bumblebees foraging as well as the evolution of host plants.

While some secondary metabolites in nectar and pollen attract bumblebee pollinators, others can instead repel them. In particular, anti-herbivore compounds are well known across literature for their capacity to deter bumblebee pollinators (Aguirre et al., 2020; Agrawal et al., 2003; Arnold et al., 2014a; Gegear et al., 2007), with adequately high concentrations of these compounds being associated with toxicity to pollinators (Thomson et al., 2015). One notable example of this is that of gelsemine, a nectar alkaloid associated with anti-herbivory defence, and is produced by the perennial vine (*Gelsemium sempervirens*). In a study involving *G. sempervirens* and captive bumblebees, bees demonstrated a preference for flowers with no nectar gelsemine, or low levels of the alkaloid versus those with higher concentrations (Gegear et al., 2007). However, when sucrose rewards were higher in gelsemine-containing plants, bees generally showed a preference for the more nutritionally rich flowers regardless of gelsemine presence. This would suggest that while defensive chemicals like gelsemine can deter bumblebee pollinators, this effect is dependent on the energetic content of the nectar. This suggests that bumblebees can make fitness trade-offs associated with defensive chemicals when foraging. Evidence of these fitness trade-offs is further evident in research looking at the effects of concentrations of caffeine, an alkaloid found in floral rewards of some species, on bumblebee visitation (Thomson et al., 2015). A study involving bumblebees and artificial flowers found that the bees preferred to forage from flowers with low concentrations of caffeine in nectar solutions when presented with options with either no, low or high caffeine content (Thomson et al., 2015). The authors suggest that this is due to some inherent preference in bumblebees for nectar with small amounts of caffeine, a phenomenon reported in previous studies (Singaravelan et al., 2005; Wright et al., 2013). This suggests that low levels of caffeine in nectar may incur some form of fitness advantage to bumblebees, despite the toxic effects of the metabolite in higher concentrations, highlighting the dose-dependent effects of phytochemicals in floral rewards.

Visual and olfactory cues

Corolla length and diameter

In addition to floral reward composition, morphological traits of flowers, which are often associated with these rewards, are also key to bumblebee attraction. Among these traits, properties of the corolla, especially corolla tube length (Basnett et al., 2019; Toji et al., 2021; Corbet et al., 2021; Corbet & Huang, 2014) and corolla diameter (Kariyat et al., 2021; Galen & Newport, 1987; Spaethe

et al., 2001) are particularly important, with longer and wider flowers attracting more bumble bees. For instance, in one study involving *Polemonium viscosum* and *Bombus kirbyellus*, bumblebees ignored 60% of plants with a corolla diameter ≤12 mm, while only doing so for 12% of plants where the diameter was ≥16 mm (Galen & Newport, 1987). This is likely due at least in part to the positive correlation between these morphological traits and floral rewards (e.g., Kariyat et al., 2021; Corbet & Huang, 2014). For example, Gomez et al. (2008) found that corolla length has a strong, positive correlation with increased nectar volume, while corolla diameter has a strong, positive correlation with pollen production, with evidence of larger pollinators, which would include many bumblebee species, being able to use these morphological cues to identify plants with greater floral reward quantities (Gomez et al., 2008). Several explanations are proposed for the relationship between corolla length and nectar production, including deeper corollas being associated with larger nectarines, and subsequently more nectar, or the fact that deeper corollas can help reduce nectar evaporation (Gomez et al., 2008). However, the study failed to provide an explanation for the relationship between pollen production and flower area, which may present a knowledge gap for future research to explore.

However, evidence exists in literature that factors other than reward availability influence the relationship between flower size and reward composition. For instance, at least one study (Spaethe et al., 2001) suggests that larger flowers may be easier for bumblebees to detect and navigate to. For example, *Bombus terrestris* workers took far longer to find smaller artificial flowers over larger ones, taking 10.4 s ± 8.5 to find 28 mm flowers, but as much as 124.3 s ± 86.0 s to find 5-mm flowers (Spaethe et al., 2001). This suggests that the physical size of the flower can make it easier for bees to identify, potentially resulting in bumblebees favouring these flowers regardless of actual reward availability.

Other literature seems to suggest a relationship between flower size, reward composition and flower genetics, where it is less clear which factors and relationships between them influence foraging behaviour in bumblebees. Notably, in *Solanum carolinense*, outbred plants had considerably wider corolla diameters in comparison to inbred plants; outbred plants also produced more pollen and higher levels of volatile compounds associated with pollinator attraction (Kariyat et al., 2021). As a result, pollinators, which were mostly *Bombus impatiens*, demonstrated a strong preference for these outbred plants (Kariyat et al., 2021). However, it was not clear whether the bees were responding to cues associated with plant genetic condition, floral rewards, flower size, or some combination of these; a limited amount of literature suggests that bumblebees have an innate preference for outbred plants regardless of floral rewards (Carr et al., 2015). As a result, it becomes less clear whether morphological traits, such as corolla diameter are indeed indicators of floral rewards, or if these traits are associated with genetic condition in plants, which influences bees regardless of floral rewards,

suggesting a possible knowledge gap in current literature. Studies that are able to isolate the effects of particular traits, though, e.g., manipulation of flower size or reward amount, would be useful for determining the relative importance of the various mechanisms discussed here.

Flower Colour

In addition to flower size, flower colour was also shown to be important in influencing bumblebee visitation. For instance, when considering contrast between the colour of the flower and its surroundings, a clear trend of bumblebees favouring flowers whose colours contrast those of foliage is evident. Notably, Spaethe et al.'s study on colour and flower size, seems to support this idea, as evident with their finding of considerably shorter 'search times' for flowers whose colours are more distinct from their surroundings (Spaethe et al., 2001). Additional research for bumblebee flower choice of blue versus red artificial flowers demonstrates that bees demonstrate preference for one flower type (often blue) only when flowers are presented to the bees against a background resembling natural foliage, while this selective behaviour was not observed when they forage from flowers presented to them against a generic, monotone, green background meant to mimic foliage (Forrest & Thomson, 2009). The authors suggested that their findings indicate that bumblebees have no difficulty distinguishing both red and blue flowers from the monotone, green background, but had trouble distinguishing the red flowers from the foliage background (Forrest & Thomson 2009). This may indicate that the presence of foliage itself, rather than the green colour alone, contributes to colour-based selectivity in bumblebees due to difficulty in locating flowers.

Other studies propose a different mechanism which influences colour-based discrimination of flowers by bumblebees: their ability to learn to associate flower colour with floral reward abundance. A study involving artificial strawberry flowers found that bumblebees are capable of learning to associate specific flower colours as well as shapes with optimal nutritional rewards (Symington & Glover, 2024). This is supported by studies which suggest that while both bumblebees and honeybees have an inherent preference for blue and green flowers, they can learn to differentiate between different flower colours associated with differing levels of nutritional rewards (Bauer et al., 2017; Hempel de Ibarra et al., 2014). This may suggest that bumblebees in Bauer et al's study learned to identify flowers based on colour.

In some studies on flower colour and bumblebees, however, this relationship was less clear. For instance, a two-year (2014-2015) study on pollinators (including bumblebees) of *Medicago sativa* plants with different colours found that in 2015, bumblebees were less likely to visit 'less reflective' yellow and white flowers, which were defined by their capacity to reflect UV light, than purple flowers, which were considered to be more 'distinct' from their background (Bauer et al., 2017). However, in 2014, less reflective flowers showed a higher number of bumblebee visits (Bauer et al.,

2017). Bauer et al. (2017) also note that variations in visitation may be attributed to individual plants. In particular, they note that one plant with yellow flowers received many more pollinator visits than any other plant in 2015, which indicates the role of outliers in influencing the data in a study due to chance events. As a result, these findings may not be indicative of general trends, which calls into question the true effect of reflectivity in flowers on bumblebee visitation. Therefore, these findings may suggest more research, with a focus on understanding why certain individual flowers are so much more attractive to other pollinators, and whether this is relevant to flower colour, or some other factor, such as floral resource abundance, is needed to better understand this phenomenon.

Flower Shape

Some studies suggest that flower shape, in addition to size and colour, is important to bumblebee attraction. Bumblebees preferentially visit symmetrical flowers, i.e., those with petals of equal lengths (del Carmen et al., 2018; Møller 1995). This may be because symmetrical flowers tend to produce more pollen (Møller 1995), though other studies suggest that bumblebees have an inherent preference towards symmetrical versus asymmetrical flowers, even when there is no difference in floral reward (Rodriguez et al., 2004), which indicates multiple possible mechanisms associated between the relationship between flower symmetry and bumblebee visitation.

Flower shape can also influence accessibility to bumblebees. One notable example involves artificial constriction of the corolla tube making the plant less accessible to bumblebee pollinators as a whole, resulting in fewer bumblebee visitors to constricted versus control flowers (del Carmen et al., 2018). Flower size was generally found to be positively correlated with pollinator body size, with bumblebees showing preference for larger flowers (Gomez et al., 2008; Toji et al., 2021). However, a study by Brunet et al. 2015 suggested that bumblebees only prefer larger flowers when increased flower size corresponds to increased pollen rewards. This suggests that for some traits, the underlying mechanism for why bumblebees prefer certain flowers over others may differ from case to case, with increased floral rewards being the underlying factor in some cases, and properties of the bees being the governing factor in other scenarios.

Flower Number

Both number of flowers per inflorescence and number of inflorescences per plant influence bumblebee attraction (Gustafson et al., 2022). For instance, the 2014-2015 *Medicago sativa* study demonstrated that plants in the second year of the study with fewer racemes per plant, and fewer flowers per raceme, were less likely to be visited by bumblebees (Bauer et al., 2017). Floral display size, as defined by number of flowers per raceme, was the trait most consistently associated with the attraction of pollinating bees, including bumblebees (Bauer et al., 2017), a finding consistent with previous studies (e.g., Karron & Mitchell, 2011; Harder & Barrett, 1995). This has been attributed to

the fact that larger floral displays result in increased floral rewards (Makino & Sakai, 2007; Brunet et al., 2015), such as pollen and nectar (Makino & Sakai, 2007). Further research on bumblebees and artificial plants suggests that bumblebees, at least initially, show a preference for artificial plants with a larger 'display size' consisting of a greater number of artificial flowers, regardless of differences in flower rewards between plants, before eventually learning which plants are more rewarding (Makino & Sakai, 2007). Likewise, additional research on bumblebees and both live *Aquilegia coerulea* and artificial flowers found that the bees made more visits to plants with larger display sizes, where an equal amount of pollen was available, but ultimately chose more pollen-rich flower regardless of display size when pollen rewards varied (Brunet et al., 2015). This suggests that bumblebees only exhibit a strong preference for plants with more flowers and inflorescences when these correspond with nutritional reward abundances. Insect herbivory was also shown to reduce flower production, ultimately leading to lower levels of pollinator visitation (Moreira et al., 2020). In addition, increased intensity of early season herbivory by *T. tetrophthalmus* led to fewer inflorescences per plant, which was further linked to fewer bumblebee visitors (Gustafson et al., 2022), suggesting that bumblebees in particular are sensitive to herbivory-induced inflorescence reduction. These findings help prove that the number of flowers per inflorescence and number of inflorescences per plant are important to bumblebee attraction.

Traits Related to Flower Accessibility

Floral Orientation

Flower orientation (horizontal vs vertical) was another trait which influenced bumblebee foraging behaviour through its impact on accessibility to bumblebees. A study by Pattrick et al. (2023) found that bumblebees increasingly prefer to forage from more accessible horizontal flowers which they could land on over vertical flowers which they had to hover next to, as they expend more energy on foraging over time. By landing on horizontal flowers, the bees were able to maximize net energy gain from pollination, thereby explaining the preference of bumblebees for these horizontal flowers (Pattrick et al., 2023). These findings therefore suggest that energy conservation is an important consideration for foraging bumblebees, with horizontal flowers being more desirable their ability to act as platforms for bees to perch on while foraging. However, this study is limited in that it used artificial flowers over live ones, which may not accurately reflect how bumblebees would behave in the wild, where factors such as flower colour and odour can come into play. Furthermore, this appears to be the only study on this topic, which limits the current understanding of the importance of floral orientation on bumblebee foraging behaviour. The scientific community may therefore benefit from exploring this topic more, with a focus on live flowers as opposed to artificial ones, where other floral traits may come into play.

Other factors related to flower accessibility

As seen in the previous section focusing on visual and olfactory cues, the relationship between many floral traits associated with this category and bumblebee attraction can also be related to accessibility of flowers to bumblebees. Namely, traits such as flower colour relative to that of foliage (Spaethe et al., 2001) or flower shape (del Carmen et al., 2018) can influence whether bumblebees are able to detect or access flowers. So, some traits associated with visual or olfactory cues can also be correlated with accessibility to bumblebees. This demonstrates that multiple mechanisms can influence the relationship between flowers, their traits and foraging decisions in bumblebees feeding on these flowers. This calls into question the distinction between visual and olfactory traits versus accessibility traits. By investigating the relationships between floral traits and bumblebee foraging further, scientists can better understand how all of these factors interact, and have a more complete understanding of underlying mechanisms between these interactions.

Conclusions drawn from findings and knowledge gaps

In analysing the effects of floral traits on bumblebees and other bees, a number of research gaps are apparent. First, I have only identified 39 foraging choice studies involving bumblebees that investigate relevant floral traits. In addition, while a wide variety of floral traits have been covered by current research, relatively few studies involving bumblebee choice experiments exist for each trait, with relatively few host plants per trait being represented by research. As a result, it can be difficult to assess whether flower choice behaviours in bumblebees are consistent across host plant species, or if the species of host plant comes into play. Indeed, in some cases, two studies of the same floral trait, but using different plant species, reach contradictory conclusions about the role of that trait in bumblebee attraction. For example, Spaethe et al., (2001)'s study seems to suggest that larger flowers are associated with less difficulty in bumblebees finding these flowers, while Kariyat et al., (2021) instead seemed to suggest a more complex relationship between flower size and foraging behaviour which is mediated by plant genetics and relevant reward composition. By conducting a larger number of bumblebee choice studies for each trait, or incorporating a larger number of traits into each study, scientists can better understand how bumblebee flower choice behaviour responds to these different traits. Furthermore, of the existing literature, most studies focus on traits such as floral resource abundance and corolla traits, with relatively few focusing on other traits such as plant height and flower orientation. As a result, the scientific community is especially limited in understanding how bumblebees respond to these traits in host plants across different plant species. Future research should focus on conducting further investigation into the roles of different floral traits in influencing bumblebee choice across a wider range of host plants.

Second, few studies, such as Herrera et al., (2006), compare the differential effects of floral traits on bumblebees vs. other bees, especially other wild bee species. By understanding how different species of bees react to a range different floral traits in comparison to bumblebees in the same

experimental study, one can more readily compare bumblebees to other species, and subsequently identify whether foraging behaviours are unique to the *Bombus* genus, or if they are conserved across taxa. Studying the foraging behaviours of different bee taxa in comparison to bumblebees in relation to floral traits may also reveal different mechanisms influencing flower choice which are shared between bumblebees and other species.

Third, while some studies explain the relationship between attractiveness to bumblebees or other bees and floral traits, this is not as clear for studies looking at others. This may indicate a knowledge gap in the understanding of underlying phenomena behind why bumblebees make the foraging decisions they do based on certain floral traits. Ultimately, by focusing on these areas, understanding of bumblebee foraging ecology can be greatly improved.

Many floral traits discussed in this study are also influenced by environmental conditions (ex. Gustafson et al., 2022; Leach et al., 2021). Therefore, in addition to looking at how bumblebee foraging behaviour is influenced by floral traits, I also sought to investigate how these floral traits are influenced by the environment.

How do ambient temperature, water availability, soil quality and insect herbivory influence Bumblebee foraging, and how does this impact flower choice in bumblebees? How does this compare to other bees?

Environmental conditions are well known to influence plant growth, with this often having an impact on traits relevant to bumblebee attraction (e.g., Salman et al., 2022; Samocha & Sternberg, 2010;Aguirre et al., 2020; Agrawal et al., 2003; Arnold et al., 2014a; Haas & Lortie, 2020; Mu et al., 2024; David et al., 2019; Höfer et al., 2021; Kuppler et al., 2021; Waser & Price, 2016a; Dai et al., 2022). In this review, I focused on insect herbivory, soil fertility, soil moisture and air temperature. Each driver impacted, and in many cases either enhanced or restricted these traits as outlined below, prompting bumblebees to either be more attracted to plants grown under these conditions or less, depending on the effect each driver had. In some cases, environmental drivers even had a bimodal effect on herbivory, with variations in these environmental conditions either making plants more attractive to bumblebees, or less, depending on the circumstances.

Insect herbivory

Insect herbivory was generally shown to negatively affect various floral traits relevant to bumblebee pollinator attraction. Studies looking at *Capsodes infuscatus* herbivory of *Asphodelus aestivus* (Samocha & Sternberg, 2010) and *Tetraopes tetrophthalmus* herbivory of *Asclepias syriaca*

(Gustafson et al., 2022) respectively found that increased intensity of herbivory reduces nectar volume and the total number of flowers per plant while increasing nectar sucrose (Gustafson et al., 2022). Bumblebees in the study, in turn, showed a preference for plants with more flowers per inflorescence (Gustafson et al., 2022). However, the 2010 study on *Capsodes infuscatus* herbivory of the mediterranean genotype of *Asphodelus aestivus* also suggests that herbivory has no impact on total nectar sugar content despite a reduction in nectar volume as a result of herbivory (Samocha & Sternberg 2010). These findings suggest that nectar sugar concentrations respond to insect herbivores differently in different plant species. The underlying phenomena explaining these differences may therefore be of interest for further scientific study. Nonetheless, the reduction in nectar abundance, a trait key for bumblebee attraction, re-affirms the idea that insect herbivory is detrimental to physical/chemical floral traits related to bumblebee attraction. As a result, both of these studies indicate a clear correlation between insect herbivory and both floral reward and some morphological traits associated with bumblebee visitation, which demonstrates a plausible explanation for the preference for non-herbivorized plants in bumblebees.

In addition to alterations to physical traits, insect herbivory can also affect the chemical properties of flowering plants in ways which impact bumblebee foraging behaviour. In *A. syriaca*, insect herbivory increased volatile chemicals in flowers, with olfactory experiments showing that bumblebees strongly prefer plants that did not suffer from insect herbivory (Gustafson et al., 2022). This may suggest that bumblebees are able to detect herbivory stress in plants based on chemical cues, and preferentially avoid these plants. Additional studies seem to support this correlation between herbivory and alterations to floral chemistry, with research on *Pieris brassicae* and *Spodoptera littoralis* caterpillar herbivory on *Brassica rapa* (Schiestl et al., 2014) and *Bemisia tabaci* herbivory of *Mattholia livia* (Salman et al., 2022) further finding that the emission of volatile compounds is altered by herbivory. Furthermore, studies on *Manduca sexta* herbivory of *Nicotiana tabacum* (Aguirre et al., 2020) and *Sollaum peruvianum* (Kessler et al., 2009) found evidence of alterations to chemical composition of pollen resulting from herbivory. These findings reveal that chemical cues, much like morphological traits, can help bumblebees detect and avoid plants exposed to herbivory.

Mechanical damage from insect herbivory has also been shown to increase the production of defensive chemicals in plants, in turn leading to decreased allocation towards reproduction (Agrawal et al., 2003; Struckman et al., 2019; Arnold et al., 2014a) which includes the aforementioned physical and chemical traits associated with bumblebee attraction. For example, research on *A. syriaca* demonstrates that plants experiencing insect herbivory invest more into the production of chemical defences, namely cardenolides, resulting in lower investment into flower production (Struckman et al., 2019), potentially reducing pollinator attraction. This phenomenon may therefore further explain why bumblebees prefer non-herbivorized plants.

While folivory (consumption of leaf tissue) is the most widely researched form of insect herbivory in studies looking at the effects of insect herbivory on plant-bumblebee pollinator interactions, insect herbivory in flowering plants can also occur in the form of florivory, or the partial or complete consumption of flowers (Haas & Lortie, 2020). Plants themselves can, in certain cases, adapt to florivory by over-producing flowers such that there is no net loss in plant reproduction in herbivorized versus healthy plants, as was demonstrated in a study involving nitidulid beetle*, Carpophilus melanopterus,* florivory in *Yucca dilamentosa* plants (Huth & Pelmyr, 1997). However, pollinators, including bumblebees, can nonetheless be impacted by florivory. In a study involving 'flower-naïve' (ie. those with no previous experience with foraging from flowers) *Bombus terrestris* bumblebees, bees demonstrated an inherent preference for bilaterally symmetrical flowers (Rodriguez et al., 2004), which are associated with no herbivory damage, whereas asymmetrical flowers are associated with partial consumption of flowers by insect herbivores (Haas & Lortie, 2020). This may indicate that bumblebees inherently prefer symmetrical flowers that are undamaged by florivor. As a result, these findings highlight the importance of florivory, particularly partial florivory by insect herbivores, in shaping bumblebee foraging patterns. Furthermore, the inherent preference for bilaterally symmetrical flowers suggests that bumblebees have evolved to instinctively prefer symmetrical flowers. This therefore suggests that innate preferences in bumblebees can shape their interactions and foraging decisions associated with flowers exposed to insect florivory. Ultimately, however, these findings support the notion that insect herbivory largely has a negative effect on bumblebee visitation.

Soil Fertility

The addition of fertilizers to soil in many cases led to increases in the values of traits related to the attraction of pollinators, including bumblebees, and ultimately increased bumblebee pollination. Notably, this trend was observed in *Impatiens capensis* (Leach et al., 2021; Soper Gorden & Adler, 2013), *Saussurea nigrescens* (Mu et al., 2024) and *Cucumis sativus* (Cardoza et al., 2012) plants. In *I. capensis*, a two-year (2015-2016) study found that leaf, stem and inflorescence biomass positively increased with fertilizer dosage during both years, with flower area and number of open flowers being positively impacted in 2016 (Leach et al., 2021), a finding consistent with previous research on *I. capensis* and both bumblebee and honeybee pollinators (Soper Gorden & Adler, 2013). Subsequently, the species was associated with higher visits by bee pollinators *Bombus terrestris* and other bumblebee species as well as *Apis mellifera* and solitary bees (Leach et al., 2021). Likewise, in *Saussurea nigrescens*, addition of nitrogen-based fertilizer was found to increase above ground-biomass, with nectar volume per flower and number of flowers per capitulum (flower head) increasing with addition of nitrogen in a concentration-dependent manner, where bumblebees' visitation rates similarly increased with nitrogen levels (Mu et al., 2024). In this study, both bumblebees and

honeybees were influenced by these increased rewards resulting from increased soil fertility, however honeybees were most influenced by the number of flowers on a plant, while bumblebees were most influenced by the abundance of nectar, with the authors suggesting that their findings point to a 'balance' between flower and nectar production, resulting in increased visitation by both types of bees (Mu et al., 2024). This shows that while both types of pollinators benefit from increased floral rewards resulting from improved soil fertility, different rewards explain this trend for either bumblebees or honeybees. The study offered differences in body size of either bee type as a possible explanation for this, where smaller honeybees must forage closer to their hives, and therefore seek to maximize the number of visited flowers, while bumblebees are larger and can travel farther to find individual nectar rich flowers. (Mu et al., 2024). This therefore points to the fact that body size plays a role in bumblebee versus honeybee foraging behaviour, with the larger bumblebee species being able to prioritize nectar abundance over collecting nectar from as many flowers as possible closer to the hive. Furthermore, the ability of plants to use the excess nitrogen from fertilizer to improve both nectar and flower production suggests that some plant species, such as *S. nigrescens* are able to invest more resources into floral traits useful for attracting multiple pollinator species, including bumblebees, suggesting the existence of a generalist pollination system in these plants.

Other examples, however, show that the positive correlation between soil fertilization and bumblebee visitation does not always hold true. For example, one study involving pumpkins (*Cumubria maxima*) found that nitrogen enrichment did not significantly impact bumblebee visitation rates despite having a statistically significant positive impact on nectar consumption (Hoover et al., 2012). The study also found that consuming pollen from nitrogen enriched plants resulted in worker bumblebees having shorter lives (Hoover et al., 2012). This may suggest that the costs and benefits associated with nitrogen enrichment of pumpkin nectar to bumblebees cancel each other out, such that bumblebees are not influenced by higher nectar volumes associated with nitrogen enrichment. However, further research is needed to verify this, indicating a knowledge gap in the literature regarding bumblebee foraging and soil fertility.

Studies also suggest that different types of fertilizers can impact plants and bumblebee visitation rates differently. In a three-year study on *C. sativus* plants involving a Vermicompost fertilizer (VC) (potting soil +33% vermicompost) and Osmocote (CF) (potting soil plus Osmocote 14:14:14) based fertilizer on cucumber plants in an environment where bumblebees were the most common pollinator, with other pollinators consisting of honeybees, *Megachilidae* bees as well as occasional *Eucera* and *Xylocopa* bees. VC plants flowered significantly earlier than CF plants in the first year of study, while in the second year, pollinators took less time to find VC compared to CF plants, with a comparable but slightly higher visitation rate to VC versus CF plants (Cardoza et al., 2012). Furthermore, VC plants were heavier than plants in other treatments (Cardoza et al., 2012),

where a larger biomass is an indicator of healthier plants, potentially making said plants more attractive to bumblebees. In addition, there is evidence that bumblebee microcolonies feeding on VC vs CF treated flowers produced significantly more and larger oocytes (Cardoza et al., 2012), indicating that VC treated plants can improve bumblebee reproductive success drastically in comparison to CF plants. This indicates that differences in the type of fertilizer plants are exposed to can impact both the foraging preference of bumblebees as well as the overall reproductive success of bumblebees foraging from either plant type. This may explain why bumblebees, and possibly other pollinators, showed a slight preference for VC versus CF plants. However, the reason for the small differences in visitation frequency in treatment types is less clear and warrants further investigation by the scientific community.

Contrary to evidence of increased reproductive fitness, as suggested by Cardoza et al. (2012), fertilizer treatment has been associated with increased larval mortality in bumblebees. This is evident in a study involving *Suissa pratensis* plants, where increased bumblebee larval mortality was observed in bees fed pollen from fertilizer treated versus control plants in the first four weeks of study (Ceulemans et al., 2017). Interestingly, the same study also found that untreated (control) rather than fertilizer treated plants had higher amino acid concentrations, contrary to the findings of the study by Leach et al. (2021), who used different plant species. This suggests that different plants respond differently to fertilizer treatment. As a result, amino acid poor fertilizer treated *S. pratensis* plants are associated with lower fitness in bumblebees, which can potentially translate to lower bumblebee visitation rates. Relatedly, nitrogen eutrophication or excess nitrogen deposition into soil reduces pH and increases levels of toxic metals including aluminium, leading some plant species, especially those which produce nectar and pollen, to struggle to survive in high nitrogen environments (David et al., 2019). By contrast, low levels of nitrogen deposition may increase the production of flowers, thereby increasing pollinator visitation rates (David et al., 2019). This points to the idea that additional nitrogen is beneficial to plants in relatively small doses, such as those seen in the previously discussed studies, but is detrimental at very high concentrations. However, increased soil nitrogen does not have a uniform effect on floral traits relevant to bumblebees. For example, nectar secretion rates increase with nitrogen enrichment in plant species such as *Antirrhinum majus* and *Ipomopsis aggregata,* but not in *Trifolium pratense, Linum lewisii,* or *Potentilla pulcherrima* (David et al., 2019). This indicates that, much like with amino acids, the effects of nitrogen deposition on nectar secretion are species specific, perhaps because plant species differ in their nitrogen needs. This may explain the different and sometimes contradictory findings of different studies looking at the relationships between nitrogen-based fertilizers, flowers and bumblebees.

Out of the screened literature on the effects of soil fertilizer on bumblebee visitation, five studies found that the effects on visitation were positive, with one of these further suggesting that the

extent of this positive effect is dependent on the type of fertilizer used, while three others instead found or suggested that the effects on bumblebee visitation or fitness were negative. As a result, scientific knowledge on bumblebee foraging ecology would be greatly improved by studies seeking to understand what influences the effect of a given fertilizer on bumblebee foraging in any given situation. interact in their influence on bumblebee flower choice. Ultimately, it is evident that the effect of fertilizer on bumblebee-plant interactions is a topic that is not fully understood, with many knowledge gaps needing to be filled in by future studies.

Soil Moisture

As with soil fertility, soil moisture levels had inconsistent effects on bumblebee visitation. In three studies investigating drought stress, bumblebees preferentially chose to forage from well-watered over drought stressed plants (Höfer et al., 2021; Kuppler et al., 2021; Waser & Price, 2016a). In each of these studies, floral traits including olfactory cues attractive to bumblebees (Höfer et al., 2021), lengths and diameters of different reproductive structures such as petals, flowers and nectar tubes (Kuppler et al., 2021), numbers of inflorescences and flowers (Kupper et al., 2021) in *Sinapis arvensis* as well as nectar sugar and pollen production in *Ipomopsis aggregata* (Waser & Price, 2016a) were positively correlated with soil moisture. This suggests that drought stress has negative consequences for floral traits relevant to bumblebee pollination. Similar findings were observed in drought stress studies looking at other bee species such as honeybees (*Apis mellifera*), squash bees (*Eucera* spp.) (Gamble & Holway, 2024) and orchard mason bees (*Osmia lignaria*) (Rose-Person et al., 2024), suggesting that the impacts of drought-stressed plants on pollinators are conserved across bee species, rather than being unique to bumblebees. Further research suggests that other floral traits associated with plant fitness, such as shoot length and shoot dry mass are positively correlated with soil moisture (Mal, & Lovett-Doust, 2005), which re-enforces the idea of soil moisture being important for healthy plant development and subsequent bumblebee pollinator attraction.

Other studies, which looked at the effects of different soil moisture levels on bumblebee pollination outside the context of drought stress, instead found that more moist soils did not necessarily result in the most bumblebee visits (Dai et al., 2022; Xiang et al., 2009). One study looking at *Gentiana aristata* plants grown in soil with different volumetric water contents (vwc): 25-30%, 35-40% and 50-60%, found that plants exposed to the intermediate treatment bloomed earlier, produced more pollen as well as having the largest number and size of flowers, where these traits had a positive effect on bumblebee visitation rates (Dai et al., 2009). On the other hand, the wettest soil treatment group produced taller plants, which were also associated with increased bumblebee visitation rates, but these plants had fewer and smaller flowers as well as producing the least pollen (Dai et al., 2022). Bumblebees subsequently visited flowers from dry and intermediate treatments more frequently than those in wet habitats (Dai et al., 2022). The study also noted that drier habitats

had higher levels of forbs, which are considered to be an important food source bumblebees (Dai et al., 2022; Xiang et al., 2009), which may further explain why bumblebees were attracted to drier habitat types. This therefore implies that a higher presence of forbs, which thrive in relatively dry soil, can influence where bumblebees forage more often, suggesting that relatively dry habitats with a high abundance of forbs attract the most bumblebee visitors. Furthermore, one of the previously mentioned studies involving *I. aggregata* found that while nectar production did increase linearly with soil moisture, pollen production only did up to 7% water content before decreasing with additional water content (Waser & Price, 2016a), further suggesting that floral traits relevant to bumblebee pollination do not always follow a linear relationship with soil moisture, similarly to the relationship between floral traits and nitrogen.

Ultimately, using different plants or focusing on different environmental conditions can result in variations in how plants, water availability and foraging bumblebees interact. Future studies looking at how all of these factors interact may greatly benefit the current understanding of relationships between soil moisture in plants and the behaviours of their bumblebee foragers.

Air temperature

Studies looking at air temperature and bumblebee and other bee pollination found a negative correlation between pollinator behaviour and heat stress in plants. One such study involving bumblebees and *Borago officinalis* plants found that flowers exposed to warmer temperatures of around 26°C resulted in fewer flowers, smaller flowers and as much as 50% less nectar in comparison to flowers exposed to cooler temperatures of 21°C. Subsequently, bumblebees were able to collect 50% less sugar from plants grown in hot conditions during visits of equal duration. As many as fourfold more flowers in the cooler treatment received bumblebee bee visits compared to those in the warmer treatment, with the majority of bees preferring to visit flowers on plants receiving the cooler temperature treatment as opposed to the warmer one; with flower height and flower area also being major predictors for which flowers received visits (Descamps et al., 2021). Furthermore, of the few bumblebees who visited the warmer flowers first, all individuals switched to cooler flowers on subsequent visits (Descamps et al., 2021). Flowers experiencing different growing temperatures (21° C and 26° C respectively) also demonstrated differences in floral traits relevant to the attraction of bumblebee pollinators. Namely, plants grown at 26° were taller, but produced considerably fewer open flowers per plant, had much smaller total flower areas and had much smaller corollas (Descamps et al., 2021). This suggests that temperature impacts bumblebee foraging preference through its impacts on both floral rewards and pollinator attraction traits.

Stress to plants due to increased heat was cited as the main explanation why bees prefer relatively cooler plants (Scaven & Rafferty, 2013). In particular, increased temperatures associated

with heat stress trigger lower investment into reproduction and can result in lower flower production, lower nectar production, smaller flowers and shorter plants (Scaven & Rafferty, 2013), all of which can result in flowers that are less attractive to pollinators.

The effects of heat stressed plants on pollinators are further evident in other bee species in addition to bumblebees. Notably, a study looking at the bee *Osmia lignaria* and heat stress in *Vaccinium corymbosum*, *Phacelia tanacetifolia* and *Trifolium repens* plants, suggested that consumption of pollen from heat stressed plants resulted in significantly fewer eggs being produced by the bees, and among the eggs laid, a far smaller proportion survived to adulthood (Walters et al., 2024). In the study, plants from each of the above species were randomly assigned one of two temperature treatments: 25 °C and 37.5 °C, with plants being exposed to either treatment for 4 hours. *O. lignaria* bees were placed in cages containing plants of either temperature treatment, but otherwise having similar floral reward abundances, and allowed to forage, feeding on pollen from the plants before reproducing (Walters et al., 2024). Only ten eggs were laid by females fed assigned to plants in the 'heat stressed' 37.5°C treatment, with only three of them surviving to adulthood. In comparison, bees assigned to the 25 °C treatment laid 33 eggs, with 28 surviving to adulthood (Walters et al., 2024). This suggests decreased fitness of *O.lignia* bees exposed to pollen from heat-stressed plants. It was also observed that female *O.lignia* assigned to heat stressed plants foraged considerably less in comparison to those on control plants. This indicates that heat stress in plants leads to decreased nutritional intake in *O.lignia*, resulting in the decreased fitness observed in the study. To date, this is the only study looking at the effects of temperature stress, equivalent to that found in the field, on bee reproduction. This suggests a knowledge gap in the field which may need to be addressed by future studies looking at the relationship between bee foraging and heat stress. Nonetheless, these findings help to prove that other bee species respond similarly to these decreased floral rewards to bumblebees, suggesting that the response to heat-stress and subsequent lower reward quality and quantity is conserved across bee species.

While not the focus of this literature review, research exists that focuses on air temperature and bumblebee foraging directly, studies suggest that increased temperatures lead to increased mortality and lower foraging in bumblebees (Höfer et al., 2021; Botsch et al., 2024). This may further explain why increased temperatures result in lower bumblebee activity.

Experimental protocols

The evaluated studies employed a wide variety of experimental methodology, where studies focused on plants grown in controlled, greenhouse or nethouse conditions (Samocha & Sternberg, 2010; Salman et al., 2022; Aguirre et al., 2020; Agrawal et al., 2003; Schiestl et al., 2014) as well as field studies focusing on plants in their natural environment (Subedi et al., 2011; Gustafson et al.,

2022; Raderschall et al., 2020). In greenhouse and nethouse studies, growing conditions were often heavily regulated to simulate natural growing conditions (Salman et al., 2022) and insect herbivory were tightly monitored to ensure that insects only remained on the plant for a specified amount of time (Schiestl et al., 2014; Salman et al., 2022; Aguirre et al., 2020) or until a certain portion of the leaf area had been consumed (Agrawal et al., 2003). In field studies, plants were instead manipulated under natural conditions, with insect herbivores being added to wild plants (Subedi et al., 2011; Gustafson et al., 2022). Differences in study protocols (field vs greenhouse) have the potential to impact the results of the study in a number of ways. This stems from the fact that field experiments and those conducted in controlled environments such as greenhouses differ considerably in the nature of the experiment (Aziz, 2017). Field studies allow researchers to observe a subject in a natural setting and may therefore better reflect conditions and behaviours seen in actual ecosystems (Aziz, 2017). Controlled experiments, on the other hand, allow for easier manipulation of the experimental subjects, and by extent, the elimination of variables other than the experimental predictor variable which may impact the response variable, which in this case would be bumblebee foraging choice. For example, a study looking at interaction between historic use of fertilizer in agricultural soil, herbivores, bumblebee and pathogens in *Cucurbita* (squash) plants employed both field 'mesocosm' and controlled greenhouse experiments (Davis et al., 2023). The study's justification for the second experiment consisted of the desire to understand the role of powdery mildew in influencing plant-pollinator interactions, where performing this study enabled the scientists to ensure that only treatment plants would receive the mildew treatment, something which was not possible in the field experiment (Davis et al., 2023). This suggests that researchers consider that the study environment plays an important role in the setup of an experiment, and what the researcher hopes to identify. I plan to further investigate this matter in my continued research on this chapter of my thesis in order to better ascertain the role of experimental setup in influencing how bumblebee foraging behaviour responds to environmental changes.

Conclusions drawn from findings and limitations of current literature

From these findings, it is evident that some factors such as herbivory or air temperature have a relatively consistent effect on bumblebee foragers, others, namely soil fertilizer and soil moisture have effects which vary depending on context, with soil fertility in particular showing high variability depending on type of fertilizer used. When considering the effects of insect herbivory on bumblebees, more studies should address the effects of different defensive compounds on bumblebee visitation rates through their effect on bumblebee mortality and reproductive fitness. In the case of the effects of soil fertility on bumblebee foraging, more research should be conducted to fully understand how different types of fertilizers impact bumblebee visitation rates to different extents, and why this is the case. In terms of the effects of soil moisture on bumblebee foraging, future research should seek to understand how bumblebees are able to detect drought stressed vs. non drought stressed plants in

cases of extreme drought, and how soil moisture and the composition of plants, including those nutritionally important to bumblebees, influence their foraging behaviour.

In addition, similar to studies on the effects of floral traits on bumblebee pollination, more studies should compare the effects of the different drivers on bumblebees versus other bee species. By doing so, the scientific community can better understand if bumblebees are unique in their foraging behavioural responses to environmental pressures, or if these behaviours are shared by other bee species. Lastly, no more than ten papers looking at the effects of most of the drivers on bumblebee foraging could be identified, which suggests that there is relatively little knowledge being conducted on the topic. In particular, fewer than five studies on the effects of air temperature on bumblebees were identified, despite these studies identifying the effects of both higher and lower temperatures on bumblebees. This indicates that further scientific research on these topics as a whole is necessary to better understand how bumblebees respond to these environmental stressors. By tackling knowledge gaps associated with these effects, future research on the interactions between bumblebees, host plants and the environment will be addressed.

Chapter 2: How do Parasitism and Environmental Changes Affect Bumblebee Foraging Behaviour?

Introduction

Pollinators are crucial in their role in maintaining both natural ecosystems (Kevan, 1999) and agricultural production (Klein et al., 2007). Among pollinators, bees (Apoidae) are particularly important, with findings suggesting that this taxon is the most effective pollinator of many plant species (Ballantyne et al., 2017). Within bee pollinators, bumblebees (*Bombus*) are a taxon of notable interest due to their ability to use rhythmic vibrations to free pollen from plant species, including commercially important crop species such as tomatoes or blueberries (Cooley & Vallejo-Marín, 2021).

Bumblebees do not forage randomly, and instead make decisions when foraging in order to optimize the quality of extracted resources (Symington & Glover, 2024; Kariyat et al., 2021; Brunet el al., 2015). The amount and composition of floral rewards (i.e., pollen and nectar) are what primarily determine attractiveness to bees (Symington & Glover, 2024; Kariyat et al., 2021; Brunet el al., 2015). Bumblebees predominantly obtain carbohydrates from nectar, which fuel growth and foraging activity, while pollen provides proteins, lipids, amino acids and necessary micronutrients, which are important for growth and larval development (Roulston & Cane, 2000; Vaudo et al., 2015). Different plant species differ in protein and nectar qualities, which can impact the availability of these nutritional resources (Roulston & Cane, 2000; Vaudo et al., 2016), thereby explaining the need for

bumblebees to optimize their behaviours in response to resource availability. However, bumblebees can also be influenced by floral traits which advertise nutrient abundance (Gustafson et al., 2022).

Previous research has established that insect herbivory directly impacts bumblebee foraging decision-making due to its impact on phenotypic plasticity in floral traits associated with bumblebee pollination (Gustafson et al., 2022). Some notable examples of this include alterations to visual and olfactory cues associated with pollination (Hoffmeister & Junker, 2017) as well as floral display traits (Barber et al., 2015). While the effects of herbivory on pollinator attraction are not uniform, herbivory most commonly reduces pollinator attraction (Gustafson et al., 2022). In addition to herbivory of host plants, bumblebee foraging behaviour is further complicated by other factors, such as parasitism (Brown et al., 2003; Gegear et al., 2006), or nutrition (Sadd, 2011; Conroy et al., 2016). While the effects of these factors on pollinating bees is commonly studied in isolation, bees are often exposed to them simultaneously.

One well known bumblebee parasite is the gut trypanosome *Crithidia bombi*, which is very common in bumblebee populations, and has been attributed to various adverse effects to bumblebee health and behaviour (Brown et al., 2003; Gegear et al., 2006). In the bumblebee species *Bombus terrestris*, *C. bombi* has been attributed to lower overall fitness by as much as 40% (Brown et al., 2003). *C. bombi* has further been implicated in lower foraging success in *B. impatiens* (Gegear et al., 2006). In experimental trails, infected bumblebees chose flowers with suboptimal nutritional rewards more frequently across 150 foraging visits in comparison to healthy bees (Gegear et al., 2006). Furthermore, the effects of *C. bombi* were shown to be dose dependent, where bees with relatively high *C.bombi* loads (>1000 *C. bombi* cells) struggled to successfully access floral rewards, while bees with relatively lower *C. bombi* loads (10-1000 cells) and uninfected bees did not. This was attributed to impaired cognition in bees with heavy *C. bombi* loads, with bees with relatively high *C.bombi* loads making considerably more errors when landing on a flower and attempting to find the source of nectar in comparison to bees with relatively low or no infection (Gegear et al., 2005). This suggests that sufficiently high *C. bombi* loads in bumblebees can disrupt their ability to forage efficiently. Since foraging behaviour is linked directly to pollination, an ecologically and economically vital service, it is of great importance to the scientific community to understand the role of *C. bombi* in shaping this behaviour.

The effects of *C. bombi* infection on bumblebees are further impacted by the bee's nutritional status, where diet can mediate the effect of parasite infection on bumblebees (Sadd, 2011; Conroy et al., 2016). In particular, lower levels of pollen and nectar sugar in *B. impatiens* diets seems to lower their *C. bombi* parasite loads (Sadd, 2011; Conroy et al., 2016). In a study by Sadd (2011), which examined the effects of three different concentrations of sugar—50% (high), 20% (medium)

and 12% (low)–on bumblebee parasite infection, bees with the low-sugar treatment had the lowest parasite load, while bees in the medium sugar treatment had the highest parasite load (Sadd, 2011). In Conroy et al. (2016), the authors manipulated both nectar sugar concentration and pollen presence in bumblebee diets to assess the effects of both on *C. bombi* infection. In this study, both the absence of pollen and low sugar concentration significantly lowered *C. bombi* infection (Conroy et al., 2016). These findings suggest that a poor diet can negatively impact *C. bombi* parasite load in bumblebees, which may impact the capacity of *C. bombi* infected bees to make decisions when foraging.

Poor nutrition is known to strongly influence bee survival (Dobeš et al., 2020, Lawson et al., 2020, Di Pasquale et al., 2016). While its effect on foraging behaviour has been less well-studied, dietary stress in species was associated with reduced motivation to forage in bumblebees (Lämsä et al., 2018), though this may have been due to pesticide exposure rather than dietary stress *per se.* Even less well understood is how infection and nutritional stress might interact to influence foraging behaviour. In the case of *C. bombi*, infection is correlated with reduced foraging capacity and selectivity (Gegear et al., 2006). Similarly, bees with a poorer diet may also be less selective in their foraging behaviour in order to achieve the nutritional intake needed to survive while expending as little of their limited energy as possible (Vaudo et al., 2020; Ruedenauer et al., 2016). At acute levels of nutrition stress, lower energy levels likely result in lethargy and reduced overall foraging. Thus, we can imagine multiple outcomes of combined nutritional stress and *C. bombi* infection on bumblebee foraging activity and selectivity. On the one hand, a poorer diet may lead to lower parasite load, reducing parasite-driven impairment and leading to optimized foraging. On the other hand, hunger in nutrition-stressed bumblebees may result in them foraging indiscriminately and, depending on degree of nutrition stress, either increasing or decreasing foraging activity; parasite-induced cognitive impairment is likely to exacerbate both these trends.

In this study, I first asked how low levels of herbivory prior to flowering influenced floral traits in *Symphotrichum novae-angliae* (New England aster; Asteraceae). I then examined how *C. bombi* infection and diet (high vs. low sucrose content in artificial nectar) interactively impact worker bumblebee (*Bombus impatiens*) foraging behaviour, and whether one or both of these influences' bumblebee selectivity for non-herbivorized vs. herbivore-damaged flowers. I predicted that (1) Herbivory by the generalist beetle *Popillia japonica* (Japanese beetle) would impact floral traits associated with bumblebee pollinator attraction, namely plant height, floral area, number of inflorescences, nectar production, pollen production and nectar sucrose concentration and (2) these alterations would result in plants which are less appealing to bumblebee pollinators, resulting in lower visitation to plants and inflorescences, while also reducing time spent foraging in comparison to plants unaffected by *P. japonica* herbivory. Further, I predicted that (3) infection by *C. bombi* would result in impaired capacity of bees to identify optimal floral rewards associated with *P. japonica* herbivory,

leading to reduced selectivity. I also predicted that (4) differences in diet sucrose content prior to foraging would impact foraging capacity of bees, either by reducing *C. bombi* content in the guts of bee workers, thereby increasing capacity to choose optimal floral rewards, or decreasing this capacity, by creating starved workers who have lower overall energy reserves, and are less capable of exerting energy towards foraging.

By conducting this study, I aimed to understand which of the two proposed scenarios will result from interactions between diet, parasitism, plants and environment. Currently, very few comparable studies have been conducted looking at all of these factors acting together on foraging bumblebees. By understanding these interactions, my research can offer novel and valuable insights into the nature of how different environmental factors pertaining to both bees and plants shape this system.

Methods

Study System

Bumblebees

We used worker bees from commercially sourced *B. impatiens* colonies. The species is widespread in eastern North America, and is a generalist, occupying a wide variety of habitats ranging from forests, to farmland and urban environments (Goulson, 2010; Williams et al., 2014). Like most temperate bumblebees, *B. impatiens* has annual colonies, with overwintering queens producing new cohorts of workers in springtime, producing drones and new queens later in the season, and the colony dying off in the late season, with only the newly mated queens surviving the winter to re-start the process the following spring (Goulson, 2010; Cnaani et al., 2006). These bees are generalist foragers, which visit a wide variety of plants spanning multiple families. This feature enables *B. impatiens* to persist in areas with high environmental variation, thereby making them important as both wild and managed pollinators (Goulson, 2010; Williams et al., 2014). The species is also common host to *C. bombi*, with transmission most commonly occurring through horizontal transfer at flowers, where bees consume C. bombi cells when they ingest faeces of infected bees while foraging (Durrer & Schmid-Hempel, 1994; Schmid-Hempel, 1998; Otterstatter & Thomson, 2008). *Bombus impatiens* was selected due to their availability and ecological importance; *B. impatiens* is among the most abundant late-season bees in much of eastern North America (Williams et al., 2014). Furthermore, due to being a native species, using *B. impatiens* removed the risk of introducing potentially invasive species into the local environment should any bees escape the lab or greenhouse.

Plants

I used New England Asters (*Symphyotrichum novae-angliae*), a species of perennial plant which blooms in the late fall. The plant is a late-flowering, herbaceous species found throughout much of

eastern and central North America. It possesses tall, erect stems, lanceolate clasping leaves, and dense inflorescences, which are composed of purple ray florets surrounding yellow disk florets (Flora of North America Editorial Committee, 2006; North Carolina Extension, n.d.).

Insect Herbivore

P. japonica was selected as the insect herbivore used in my study due to their status as invasive species in North America while having a wide range of host plants, which includes >300 species, and feed primarily by eating leaf tissue between leaf veins, leaving a characteristic 'skeletonized' leaf appearance, and aggregating into large groups (Shanovich et al., 2019). They are highly prolific pests whose management has proven difficult, with expenses managing turfgrass alone in relation to the species costing $450 million annually (Shanovich et al., 2019). There is also great concern among farmers in regards to damages to corn and soybean crops (Shanovich et al., 2019). *P. japonica* was first discovered in the United States in a plant nursery in New Jersey, and has since spread to and infested much of the eastern portion of the States and Canada, being established in 28 states and five Canadian provinces (Shanovich et al., 2019). The species has an annual life cycle, with adults emerging from the soil from late June to early July, alternating between feeding, mating and egg production throughout their 4–6-week adult lifespan (Shanovich et al. 2019). Eggs hatch within 10-14 days, with larvae reaching their third instar in fall, and will burrow up to 6 inches into the soil to overwinter, before moving upwards in the soil layer to pupate in the spring, before emerging as adults in the summer (Shanovich et al. 2019). Japanese beetles are discouraged from feeding on plants due to the presence of defensive compounds, rather than being attracted to certain species (Shanovich et al. 2019), which may explain their wide range of host plants.

Parasite

Crithidia bombi is a unicellular, flagellated protozoan organism found within the Trypanosomatidae family. It is an obligate gut parasite of the bumblebee genus (*Bombus* spp.) which targets the hindgut, persisting as a chronic infection (Erler et al., 2012). *C. bombi* spreads between colonies after a worker becomes infected while foraging outside of the colony (which is presumed to be due to contact with flowers exposed to other infected bumblebee workers), and is spread within the colony through networks of workers coming into contact with one another within the colony as well as through contact with infected faeces (Folly et al., 2017). *C. bombi* is a widespread parasite of bumblebees, and is capable of infecting numerous different bumblebee species, including *B. impatiens* and *Bombus terrestris*, making this a parasite one of great concern for the survival of bumblebee populations (Mockler et al. 2018).

Experiment 1: Do flowers grown under different conditions differ in their value to pollinators?

Beetle Treatment

Two-year-old plants were obtained in early August from Toronto Native Plant Supply, and grown in the greenhouse for most of August, before being transported outside near the end of the month in order for the cooler climates to stimulate flowering. Plants were then returned to the greenhouse several days later out of concern for wind damage. Initial budding had started on some of the plants by this point, but none of the plants would flower for at least two to three weeks after the end of the herbivory treatment.

We collected adult *P. japonica* using scent-baited Japanese beetle traps, or by hand from the wild upon emergence in mid-July-late August. We collected these beetles from bushes on York University's Keele Campus. I treated half the plants (n = 39) with beetles, and left the other half (n = 39) untouched by them. I covered plants with transparent mesh bags to prevent beetles from escaping, while ensuring that plants received adequate sunlight for photosynthesis. All plants, regardless of treatment being bagged to ensure that different plant treatments did not differ in level of sunlight exposure. Plants were initially treated with 7 beetles on August 30th, 2025, with all beetles being removed on Monday, September 8th, 2025. Dead beetles were replaced as supplies were available. All beetles were removed on September 6th. Living, dead and missing beetles on each plant were then counted and recorded.

I treated plants with beetles at the end of August simultaneously with transporting the plants outside. Plants were randomly assigned to beetle (n = 39) or no-beetle (n = 39) treatments. I covered both treated and untreated plants with transparent mesh bags in order to prevent beetles from escaping, and to ensure that plants in either treatment did not differ in their exposure to sunlight. For plants in the beetle treatment, I introduced 7 beetles into the bag and left them for just over a week. Plants were initially treated with beetles on 30 August 2025, with all beetles being removed on 8 September 2025. Mesh bags were removed simultaneously with removal of beetles, and plants were treated with suffinol X mineral oil insecticide on Wednesday, September 10th, 2025 to deal with a whitefly infestation.

At the start of this time frame, no visible buds were seen on any of the plants, with green buds forming on some of the plants by the end of this period. Dead beetles were replaced as supplies were available. The number of living, dead and missing beetles on each plant was counted and recorded at the end of the treatment period as beetles were removed. The timing and duration of herbivory was chosen to ensure that 2-3 weeks between herbivore exposure and flowering had elapsed, in order to allow enough time for defensive chemicals to take their effect on floral traits. This duration was chosen, as previous research found that week-long herbivory treatments were adequate in inducing

alterations (Barber et al., 2015), with induced plant defences activating within hours to days, and lasting for extended periods upon activation (Agrawal, 2005).

Quantifying floral traits

Upon bloom, I selected seven plants from the untreated group and ten plants in the beetle-exposed group for nectar volume quantification. I sampled nectar presence and volume from 23 inflorescences across eight untreated plants and 31 inflorescences across nine beetle-exposed plants, respectively. I sampled 27 flower (inflorescence) areas from nine beetle-exposed plants, and 20 from eight untreated plants. Inflorescence number and plant height were assessed at the plant level, with ten plants in either treatment group being sampled; all other traits (nectar volume, and inflorescence area) were assessed at the inflorescence level. Doing so allowed me to account for variation in these floral traits within the same plant.

In order to measure nectar volume, I collected nectar from 5 nectarines on up to three inflorescences per plant using 2 μL microcapillary tubes following a modified version of the protocol described by Hicks et al. (2016). I then calculated the volume of nectar by dividing the portion of the tube containing nectar by the total length of the microcapillary tube, and multiplying this fraction by 2 microliters (total volume of tube). I attempted to measure nectar sugar content and pollen volume of flowers as additional metrics of floral reward quantity and quality, but was not able to successfully assess either, given difficulties with extracting floral rewards (see Appendix B for details).

In order to determine the inflorescence area, I measured the diameter of each sample flower, divided this number by 2 to obtain the radius, and used the radius to calculate the flower area based on the formula: $A=\pi r^2$. Since *S. novae-angliae* produce multiple stems, I used a ruler to physically measure the height of a given plant from the bottom of the tallest stem to the highest point on the stem. Since stems may be bent due to watering, length of the stem, rather than vertical height, served as my measurement of plant height.

Experiment 2: Are healthy bees and infected bees able to choose the optimal floral reward, and how does diet content impact this?

Setting up microcolonies

In order to run bumblebee choice experiments, we set up bumblebee microcolonies. Since an individual bee may or may not forage (i.e., due to stress, hunger, or injury resulting from handling), a microcolony of around four bees would increase the likelihood of at least one bee foraging. Starting one week before the start of the experiment. We transferred sets of four bees which were prepared and inoculated using the above protocol into plastic containers which served as microcolony habitats.

Bees were sorted into microcolonies based on their source colony and *C. bombi* treatment received (either inoculated with the *C. bombi* solution or blank; figure 1). We recorded the colony of origin and *C. bombi* treatment for each microcolony, and labelled each plastic container housing the microcolony with this information. These served as control or uninfected microcolonies during the experiment. Bees were taken from four different source colonies. A total of 51 uninfected and 51 infected microcolonies were used throughout the experiment.

Microcolonies were assigned either a low-quality (15% sucrose) or high-quality (30% sucrose) nectar treatment, with approximately half the microcolonies from each combination of source colony and inoculation status assigned each treatment. The dietary treatment information was recorded on the microcolony deli cup alongside colony and inoculation information. All microcolonies were maintained for 7 days before being used in foraging assays (figure 2.1). During this time, they were kept in dark conditions at 28C, and provided with pollen and the appropriate concentration of sucrose *ad libitum.* Date of preparation and colony of origin were recorded on the deli cup alongside treatment information. Uninfected and infected microcolonies were housed separately, to avoid contamination.

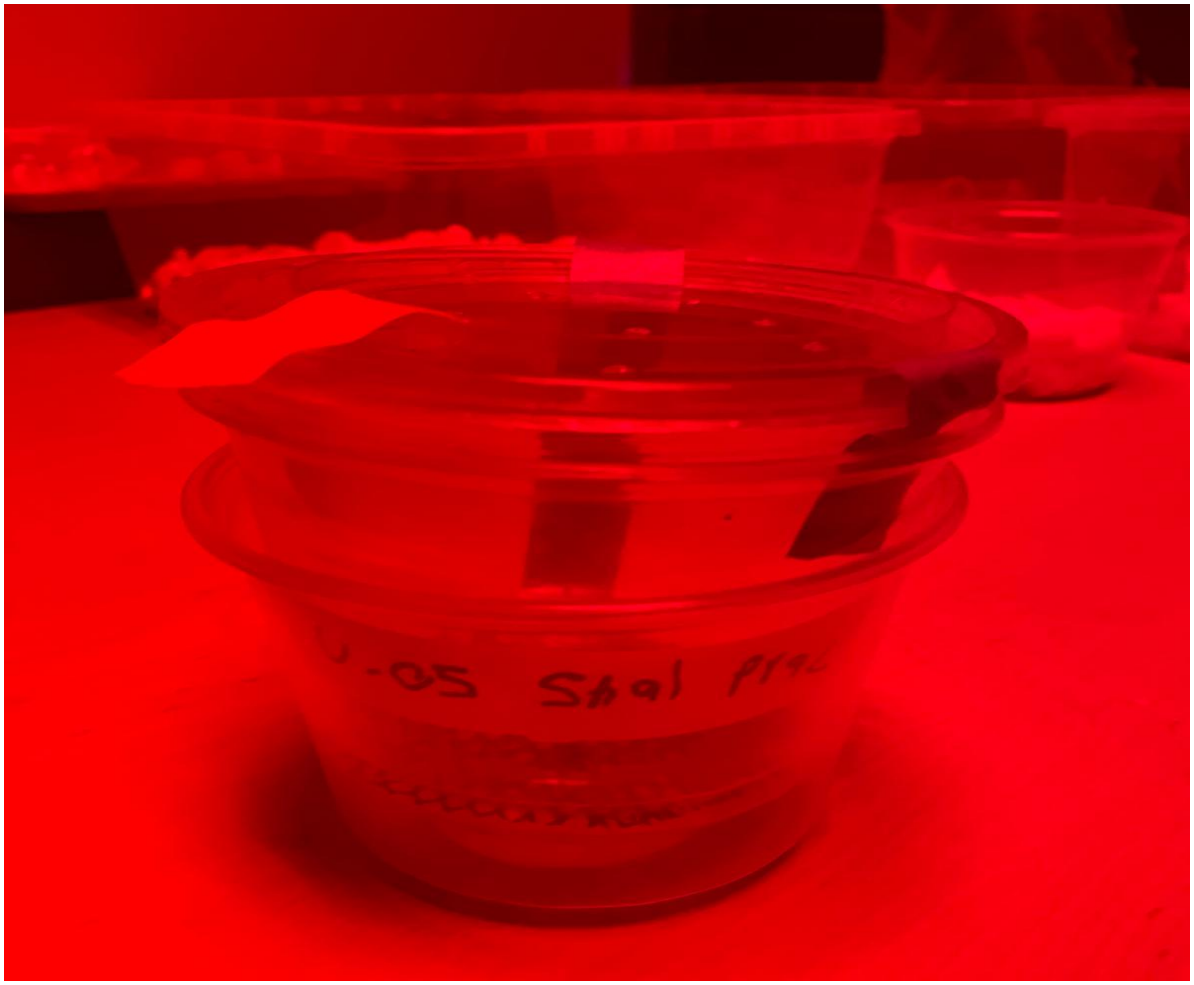

Figure 2.1**: Microcolony trays used to house bumblebee microcolonies.** A deli cup with a mesh bottom was used to house bees. A petri dish filled with 15 or 30% sucrose with a cotton swab placed inside it was placed inside a second deli cup, into which the first one was placed to allow bees to feed on the sucrose through the cotton swab. Sucrose and nectar were replenished throughout the week as needed. A piece of tape was used to label colonies with dietary treatment (15 vs 30% sucrose), *C. bombi* inoculation status (infected or uninfected for inoculated vs uninoculated respectively), source colony and date of preparation.

Nutrition treatment

Half of all microcolonies in either treatment group (*C. bombi* vs control) were given the 'high nutrition' treatment (30% sucrose) while the other half were given the 'low' sucrose treatment (15% sucrose). These levels were based on previous studies and a pilot study indicating that bees still successfully foraged after consuming a 15% sucrose diet for one week (Appendix B). Bees in either treatment were also provided wildflower pollen pellets *ad libitum* to supplement their diet.

Crithidia bombi treatment

In order to create *C. bombi* infected microcolonies, we inoculated *B. impatiens* workers with a known dose of *C. bombi* cells from homogenized gut tissue of infected *B. impatiens* bees from pre-infected colonies reared in the Fitch lab. All inoculated bumblebees were treated with the same *C. bombi* strain to ensure consistency due to variations in effectiveness of different strains in infecting bumblebee hosts. The strain was sourced from wild *B. impatiens* at a single site in Hadley, MA, USA in 2015, and has been maintained *in vivo* in captive *B. impatiens* since then. To produce inoculum, bees were removed from colonies known to be infected with *C. bombi*, then anaesthetised by placing the vials containing the bees into the refrigerator for 10-15 minutes, or until they were no longer moving.

We took one microcentrifuge vial per bumblebee used for inoculum creation in a microcentrifuge rack, and added 300 μL of Ringer's solution to each vial. We gathered the following equipment onto a tray lined with paper towels: two pairs of forceps, two tissue grinders, one pair of dissecting scissors, a squirt bottle of ethanol, a deli cup to squirt into, kimwipes.

Immediately after anesthetizing the bees, we cut off each bee's head with dissecting scissors. This was done quickly, as bees start to regain consciousness as they warm up. Using two forceps, we held the bee down with one forcep, then grasped the final abdominal segment, by the stinger, with the forceps and pulled gently to remove the gut. We placed the gut in a vial, removing the nectar crop before doing so, making sure that all the gut material gets into the solution. We then ground the bee gut solution with the tissue grinder until the gut tissue was well mashed into the solution (no large intact pieces). We then labeled the vial with the bee's source colony ID number and time. Afterwards, we sterilised the forceps and grinders between each bee using 70% ethanol and allowed the ethanol to dry before using it on a new bee (since the ethanol may kill *C. bombi*). Once all the bees were dissected, we used a piece of coloured tape to clearly label the entire rack with the name of the person dissecting the bees, date, the project name, and the time dissection was completed. Next, we waited ~3 hours to allow *C. bombi* to swim out of gut tissue and into the solution. Following the dissection, we placed 10 ul of gut samples into a haemocytometer and placed the slide under a light microscope. Using a hand counter, we counted the *C. bombi* in 5 squares (corners plus center) as seen on the slide. We moved the focus knob on the microscope in order to count *C. bombi* cells, since the entire sample was often not fully visible in a single field of view. We then summed the counts from these five squares and recorded our observations (figure 2.2). We prepared and used a total of 51 *C. bombi infected*, and 51 uninfected microcolonies throughout the experiment.

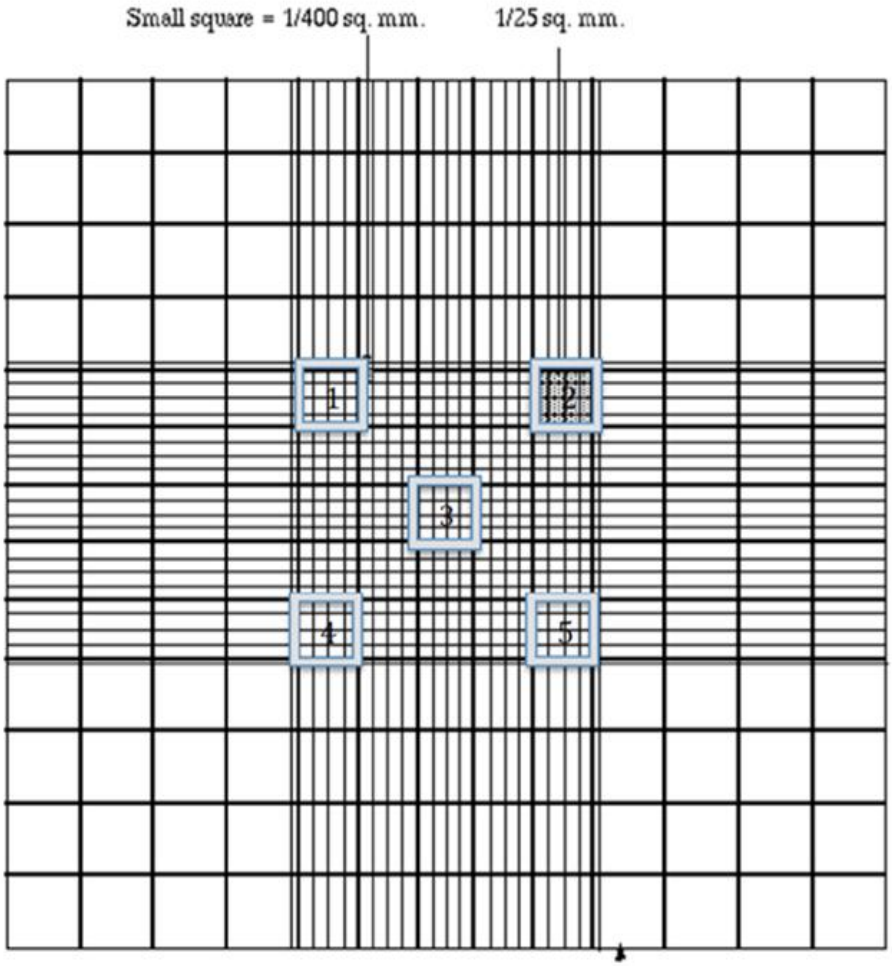

Figure 2.2**: The haemocytometer slide as viewed under a compound light microscope.** The scale of the slide is listed at the top. The slide is divided into a 5 by 5 grid of smaller 4 by 4 squares. Only *C. bombi* cells in the top left (1), top right (2), middle (3), bottom left (4) and bottom right (5) 4 by 4 by 4 squares are counted when estimating *C. bombi* in a gut sample. This helps account for movement of *C. bombi* cells during observation.

In order to inoculate bees with *C. bombi* solution, I firstly removed Ringer's solution and 30% sucrose from the fridge and diluted sucrose solution to 15% as necessary. We selected one or more previously prepared gut samples to use for creating the inoculum, aiming to use samples that have moderate to high *C. bombi* loads (minimum 24 cells; no maximum), where higher counts would produce more inoculum per sample. From the sample(s) selected, we removed 150 ul of supernatant (being careful not to take gut tissue along) and placed it in a clean microcentrifuge tube. An appropriate volume of Ringer's solution was added to the microcentrifuge tube to dilute to 600 *C. bombi* cells per ul. Lastly, we added a volume of 50% sucrose solution equal to the volume of the gut homogenate sample. We homogenized the solution by pipetting or flicking it with one's finger.

At least two hours before inoculating, we removed bees from one or more of four uninfected source colonies housed in the lab and placed each bee in individual vials with air vented lids without

access to food. Once bees were adequately starved, we fed the bees 15 uL of either the previously prepared inoculum solution, or a blank which was prepared by mixing sucrose and Ringer's solution into a vial where the amount of sucrose was equal to the amount used to create the inoculum that day. Bees fed the inoculum solution were part of the 'inoculated' treatment group, while bees fed the blank were part of the 'uninoculated' or *C. bombi* infection control group. A micropipette was used to deposit the solution droplet into the vial containing the bee, and the bee was observed until it had consumed the entire droplet.

Behavioural Trials

For each behavioural trial, a pair of plants, one which received the beetle treatment and one which did not, were placed into a mesh tent 88.9 cm height x 58.42 cm width x 58.42 cm length enclosure. I randomized the position of each plant in the mesh enclosure, placing each plant either in the bottom right corner or top left corner relative to my position as the observer. Plant position was determined using coin flips. I further randomized which microcolony treatment was used in a given trial by assigning each combination of diet, infection statuses, and source colonies a number from 1 to the total number of trials conducted in a given day and used a random number generator to determine the order in which each colony was used. Each microcolony was starved for half an hour prior to the experiment to encourage foraging. At the start of each trial, I placed each pair of plants into the tent. Afterwards, using a sleeve on the side of the tent, I placed the container with each microcolony into the tent and opened the lid, allowing the bees to forage for 15 min. During the trial, I focused on the first bee to start foraging in a given microcolony and recorded the number of visits made by the bee to individual inflorescences on a plant, time spent actively foraging or resting on each plant, and number of visits to each plant. Individual inflorescence visits were defined by whether bees visited and fed from a given inflorescence, and then left the immediate vicinity of the inflorescence, either returning to the same inflorescence immediately afterwards, moving onto a different inflorescence on the same plant, flying to elsewhere in the tent, including the other plant, or resting on the netting or ground. If other bees foraged, I did not record their activity. Time spent foraging was defined as the total amount of time that the first bee to forage spent foraging. This was determined by recording the time spent foraging from each inflorescence, and then adding up all these values. Number of plant visits was defined as the number of times a bee visited a plant, foraged from it, and then left the immediate vicinity of the plant, irrespective of number of inflorescence visits made during this period. This measure was ultimately removed from my statistical analysis later on, due to redundancy with inference visit data. After 15 min, I removed the microcolony, and replaced the plants with another randomly selected pair of plants before introducing another microcolony. Used microcolonies were returned to the lab, provided with sucrose solution and pollen, and maintained at 28 °C until the following day.

Verifying Inoculation and Testing the Effects of Bee Diet on *Crithidia bombi* Load

In order to verify that bees were infected, and determine the number of *C. bombi* cells in different dietary treatments, we dissected and screened the observed bee in each microcolony (first bee to forage) on the day following the foraging assay, using the protocol described above. Bees that were not screened were euthanized at -20C.

Statistical Analysis

Experiment 1: Do Flowers Grown Under Different Conditions Differ in Their Value to Pollinators?

Plant Height

Plant height (cm) was analysed using a linear model, plotting plant height against herbivory treatment. The dead or missing beetle covariate was initially incorporated into the model, but was removed, as doing so resulted in a more robust model (AICc of 185.74 vs 161.21).

Inflorescence counts

Inflorescence counts were analysed using a GLMM with a negative binomial distribution, with inflorescence counts as my fixed effect and plant ID as my random effect. I initially included the dead and missing beetle covariate, but found that removing it resulted in a more robust model: AICc (133.1 vs 130.76).

Flower Area

I modelled flower areas versus beetle herbivory treatment using a linear mixed model. Since I took multiple flower area measurements from each plant, I included plant identity as a random effect. I ran the model both with and without the dead and missing beetle covariate, and found that including the covariate ensured a more robust model as evident with a lower AICc (665.94 vs 662.81). As such, the covariate was kept in the final model.

Nectar presence and volume

I quantified nectar volume using a 2-part hurdle approach, first quantifying nectar presence, and then quantifying nectar volume for flowers where flowers produced a quantifiable volume of nectar.

Nectar presence

I analysed the probability of nectar presence using a generalized linear mixed-effects model with a binomial error distribution with plant treatment as my fixed effect, and plant ID of the plant the measurement was taken from as my random effect. Nectar presence was defined as whether nectar

volume was greater than zero. The covariate of Dead or missing beetles had no effect on model fit (AICc remained constant). The model was fitted using glmmTMB.

Nectar Volume

I analysed nectar volume using a generalized linear mixed-effects model with a Gamma error distribution and a log link, implemented with glmmTMB. I restricted analyses to flowers producing measurable volumes of nectar (>0 ul). I included treatment as my main fixed effect while also including the number of dead or missing beetles as a covariate. I included plant identity as a random effect to account for repeated measures from the same plant. Since the effect of number of dead or missing beetles on a given plant was insignificant ($p = 0.534$) and removing it produced a lower AICc compared to the model with the covariate (-55.27 vs -52.62), the covariate was omitted from the final model.

Experiment 2

Effects of diet on *Crithidia bombi* load

Effect of diet on *C. bombi* infection intensity was analysed using a zero-inflated negative binomial mixed-effects model with diet and colony as fixed effects and sampling date included as a random intercept, and an intercept-only zero-inflation component. The model accounted for under dispersion in parasite counts (dispersion parameter = 0.84) and a high frequency of zero observations.

I analysed inflorescence visits with a GLMM with a poisson error distribution and a log link. I incorporated: dietary treatment, beetle damage treatment, and infection intensity (*C. bombi* cell counts), and their interactions as fixed effects. I included interaction terms in order to determine whether diet impacted foraging behaviour through its effect on *C. bombi* load, as previously hypothesized.

The initial model included inoculation state (either inoculated with *C. bombi* or not), but this was replaced with infection intensity since infection did not hold in all inoculated bees, and infection intensity was predicted to impact foraging behaviour. Plant height (based on height of tallest flower on the plant), the source colony, plant orientation and floral display size (number of open flowers) were also incorporated into the model as fixed effects. Trial was included as a random intercept, to account for paired nature of data (i.e., one beetle-exposed [hereafter B+] and one unexposed [B-] plant per trial), and sampling date was included to account for temporal clustering. Models were fitted using glmmTMB in R.

Stepwise model simplification was then performed on the model in order to optimize it, with AICc values being used to determine the optimal model. Statistically insignificant covariates and

interaction terms were eliminated and ΔAIC was checked to see whether the simplified model was better.

Time the target bumblebee spent on each plant was modelled using a hurdle approach, where probability of visitation was first modelled using a binomial GLMM model with a logit link. Fixed and random effects were the same as for the above models. I next analysed the length of time spent on a plant, including only visited plants, using a GLMM with a Gamma error distribution and a log link. I performed stepwise simplification on the model as described above. All GLMMs were implemented using the glmmTMB package (Brooks et al., 2017).

Results

Experiment 1: Do Flowers Grown Under Different Conditions Differ in Their Value to Pollinators?

Plant Height

Average plant height was 75 cm for beetle-exposed plants (N = 10), and 70.83 for untreated plants (N = 10). The model explained little variation in plant height ($R^2 = 0.04$, adjusted $R^2 = -0.02$) and was not statistically significant overall ($F_{2,17} = 0.7$, $p = 0.4$). Residual variation was substantial (residual SD = 11.19 cm). Beetle damage treatment was not significantly associated with plant height ($\beta = -4.17 \pm 5.003$ SE, $t = -0.833$, $p = 0.416$, df = 1, 18, figure 2.3).

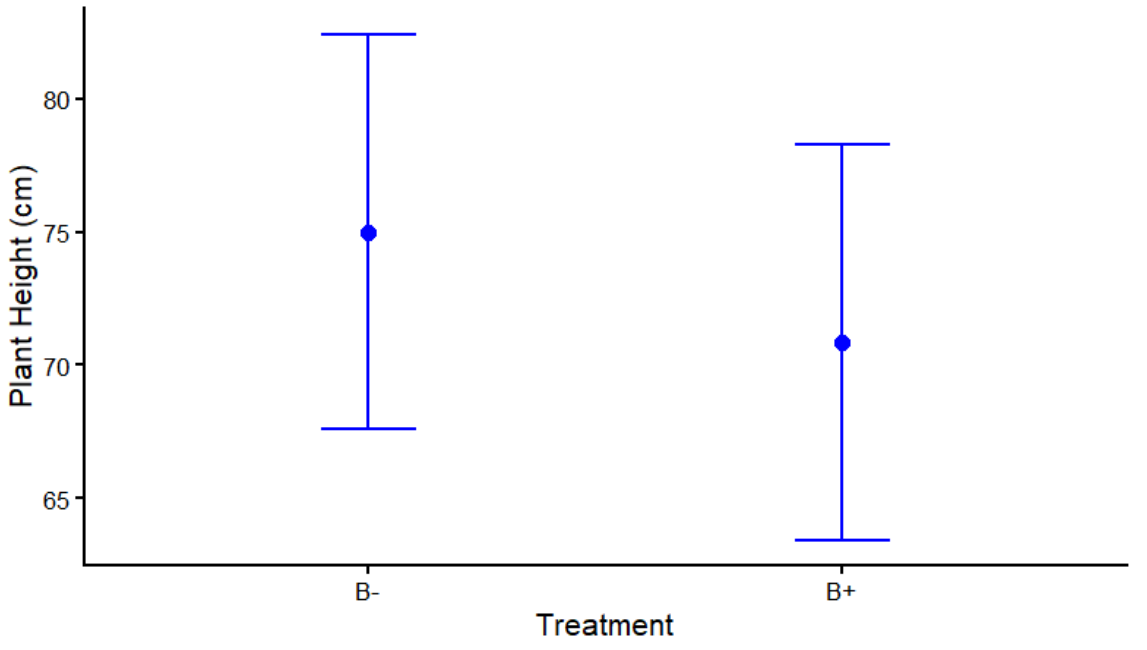

Figure 2.3**: Predicted Differences in plant height in Symphyotrichum novae-angliae plants based on different beetle treatments: either beetle-exposed (B+) or non-beetle-exposed (B-) generated using ggpredict.** Predicted plant height was higher in untreated plants than in beetle-damaged plants (B+), with untreated plants having an average height of 75 (95% CI 67.57 - 82.43) cm and treated plants having an average height of 70.83 (95% CI 63.40 - 78.26) cm. However, differences were not statistically significant due to high overlap in confidence intervals.

Untreated plants (N=10) produced an average of 10.20 inflorescences, while treated plants (N=10) had an average of nine inflorescences blooming. However, this difference was not significant (β = -0.1252 +/- 0.2769, t = -0.452, p = 0.651, df = 1,17, figure 2.4).

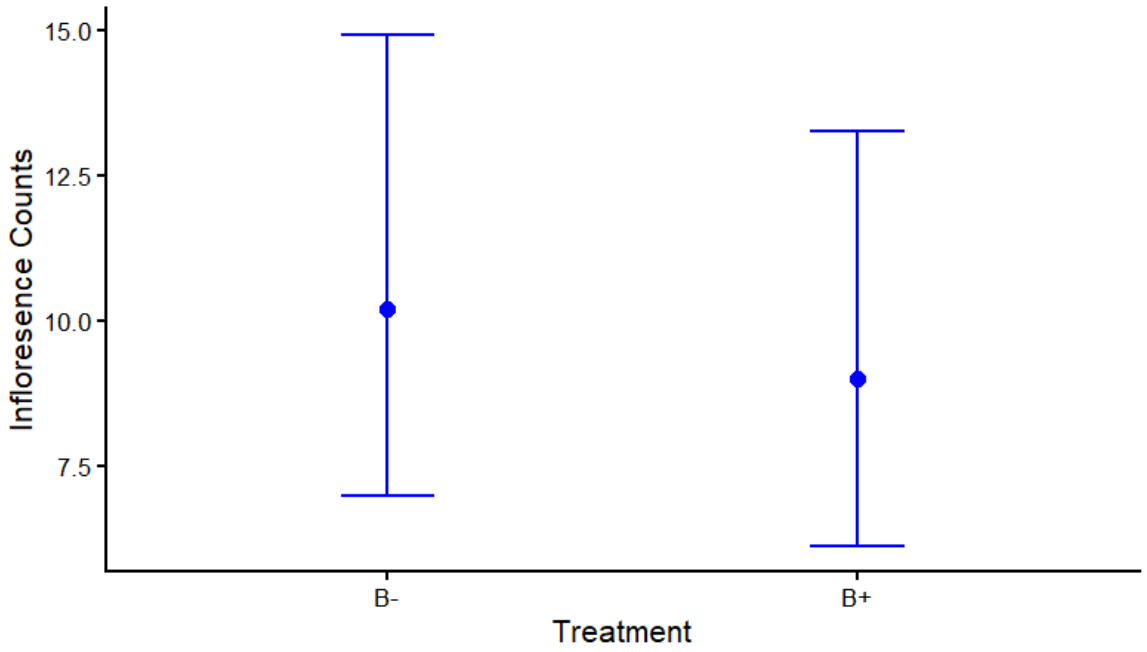

Figure 2.4**: Predicted differences in inflorescence counts based on different beetle treatments for Symphyotrichum novae-angliae plants: either beetle-exposed (B+) or non-beetle-exposed (B-) generated using ggpredict.** Non-beetle-exposed plants produced an average of 10.20 (95% CI 6.97-14.92) inflorescence, while untreated plants produced 9 (95% CI +/- 6.11 - 13.25) inflorescences. The high level of overlap in 95% CIs suggests very similar mean counts, indicating that treatment, when adjusted for dead or missing beetles on a given plant, has no effect on inflorescence counts.

Mean inflorescence area was 901.24 mm^2 for untreated plants and 965.20 mm^2 for beetle-exposed plants, but this difference was not significant (z = 0.603, p = 0.57, df =12.3, figure 2.5).

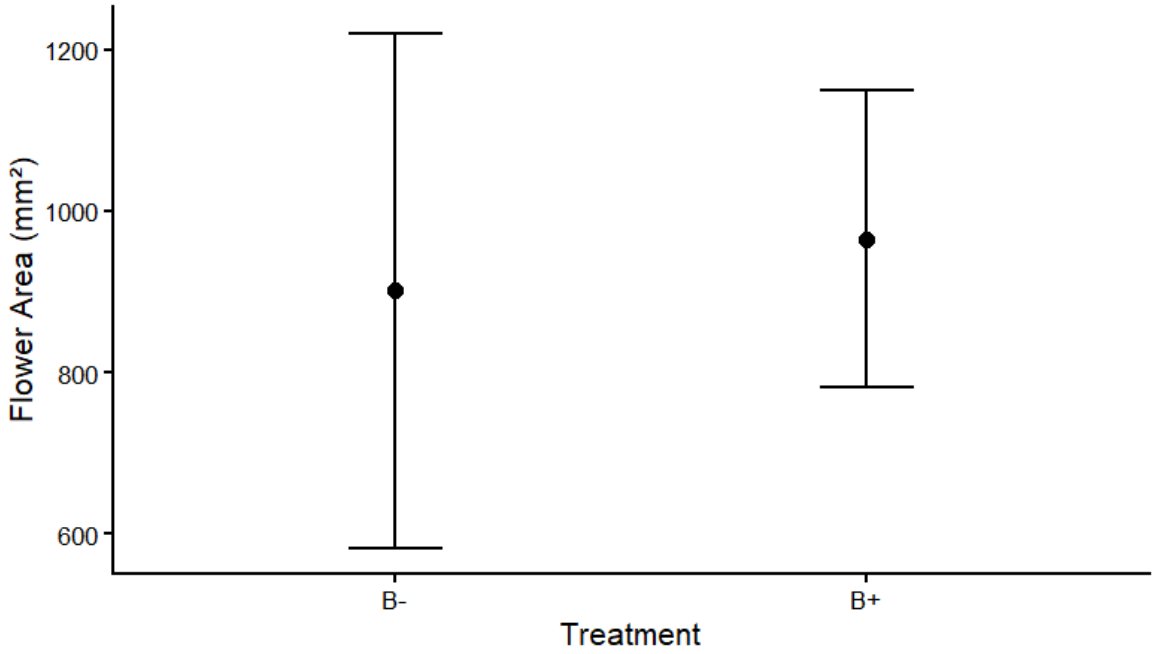

Figure 2.5: **Differences in Inflorescence areas based on different beetle treatments for Symphyotrichum novae-angliae plants: either beetle-exposed (B+) or non-beetle-exposed (B-) based on predicted means generated using ggpredict.** Untreated flowers had an average flower area of 901.24 (95% CI 581.88 - 1220.61 mm^2), while treated flowers had an average flower area of 965.20 (95% CI 781.24 - 1149.14 mm^2), with no real difference being observed between treatments due to high overlap in error bars between treatments.

Nectar Presence

I assessed 55 inflorescences from 20 plants for nectar presence. In untreated plants, an average of 65% produced nectar, while in treated plants 39% produced nectar. However, the odds of nectar being present did not differ between beetle-damaged and undamaged plants ($\beta = -1.01 \pm 0.75$ SE, $z = -1.35$, $df = 1,52$, $p = 0.176$, figure 2.6).

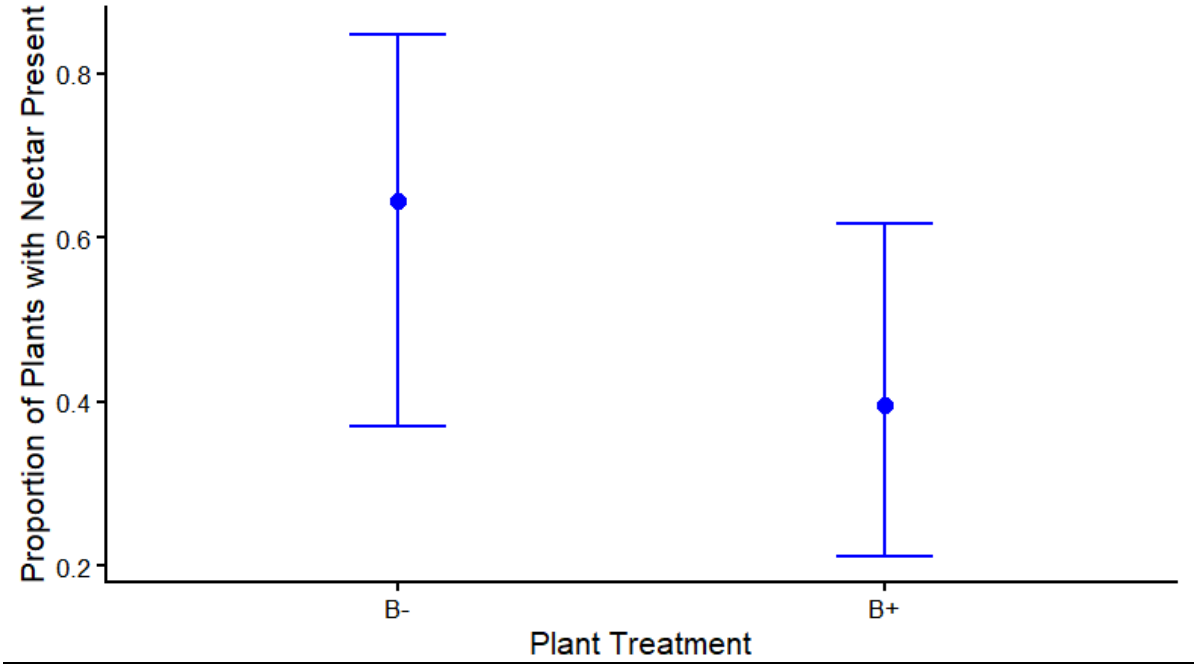

Figure 2.6: **Effects of diet on beetle herbivory on plant treatment based on different beetle treatments for Symphyotrichum novae-angliae plants: either beetle-exposed (B+) or non beetle-exposed (B-) based on predicted means generated using ggpredict**. Among untreated plants, an average of 0.65 (95% CI 0.16-0.95)% of screened plants produced nectar, while in beetle-exposed plants produced an average of 0.39 (95% CI 0.11-0.78) of plants produced nectar based on predicted means. However, the high error bars indicated a high level of uncertainty, suggesting that neither treatment group was significantly different from the other in terms of nectar production.

Nectar Volume

I collected measurable nectar from 27 inflorescences across 16 plants, 14 from B- plants, and 13 from B+ plants. I found no correlation between nectar volume and beetle damage treatment (β = -0.24 ± 0.49 SE, z = -0.5, p = 0.62, df = 1,22, figure 2.7).

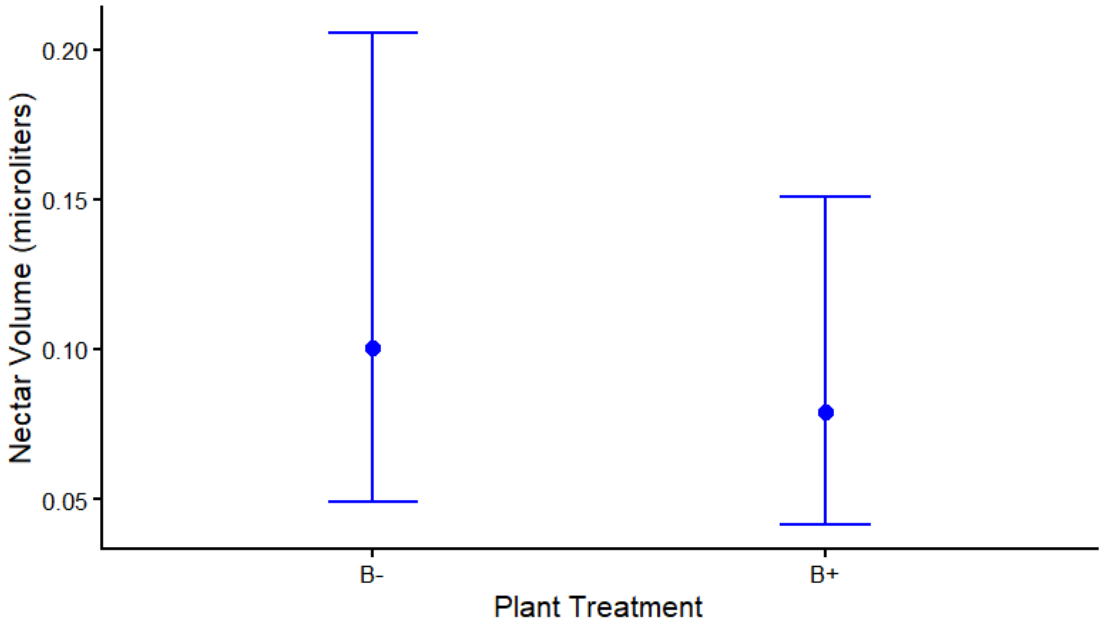

Figure 2.7: **Effects of plant treatment on nectar volume based on different beetle treatments for Symphyotrichum novae-angliae plants: either beetle-exposed (B+) or non-beetle-exposed (B-) based on predicted means generated using ggpredict.** Undamaged plants had a predicted nectar volume of 0.10 µL (95% CI: 0.05–0.21), whereas beetle-damaged plants had a higher point estimate of 0.08 µL (95% CI: 0.04 - 0.15). However, high levels of overlap between confidence intervals indicate that this difference is statistically insignificant.

Effects of diet on *C.bombi* load

Diet (30% sucrose vs. 15% sucrose) had no significant effect on *C. bombi* counts (figure 2.8, $\beta = 0.35 \pm 0.47$ SE, $z = 0.74$, df = 89, $p = 0.46$). However, infection intensity was significantly affected by colony of origin. Individuals from colony U12 exhibited significantly higher *C.bombi* loads than other colonies ($\beta = 1.98 \pm 0.70$ SE, $z = 2.82$, df = 89, $p = 0.0048$, figure 2.9), corresponding to an approximately seven-fold increase in parasite counts. The zero-inflation component of the model indicated a significant baseline probability of structural zeros (intercept = -1.15 ± 0.39 SE, $z = -2.98$, df = 89, $p = 0.0029$), consistent with a substantial proportion of individuals being uninfected. Random intercept variance for sampling date was high (SD = 2.13), indicating considerable temporal variation in infection intensity independent of diet and colony effects.

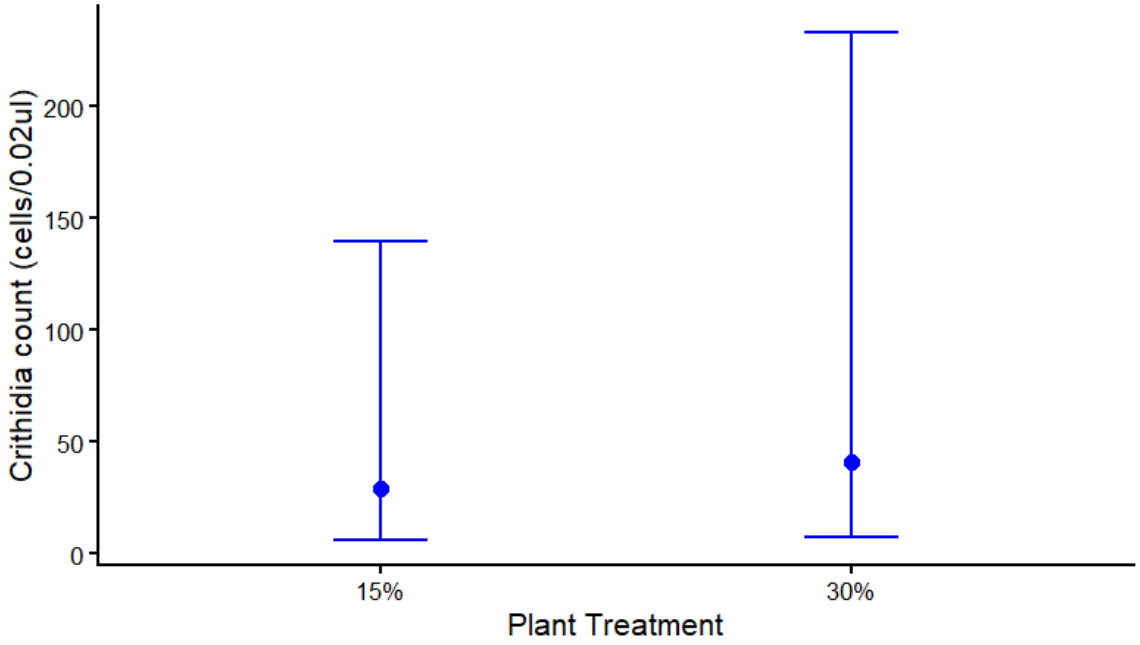

Figure 2.8: **Predicted average *C. bombi* counts for different diet treatments based on predicted means generated using ggpredict. *C. bombi* counts did not differ significantly between dietary treatments**. Bees fed the 15% sucrose diet had an average of 29.11 (95% CI 6.06- 139.81) *C. bombi* cells per 0.02 ul, while bees fed the 30% Sucrose diet had an average of 41.27 (95% CI 7.31 - 233.07) cells per 0.02 ul.

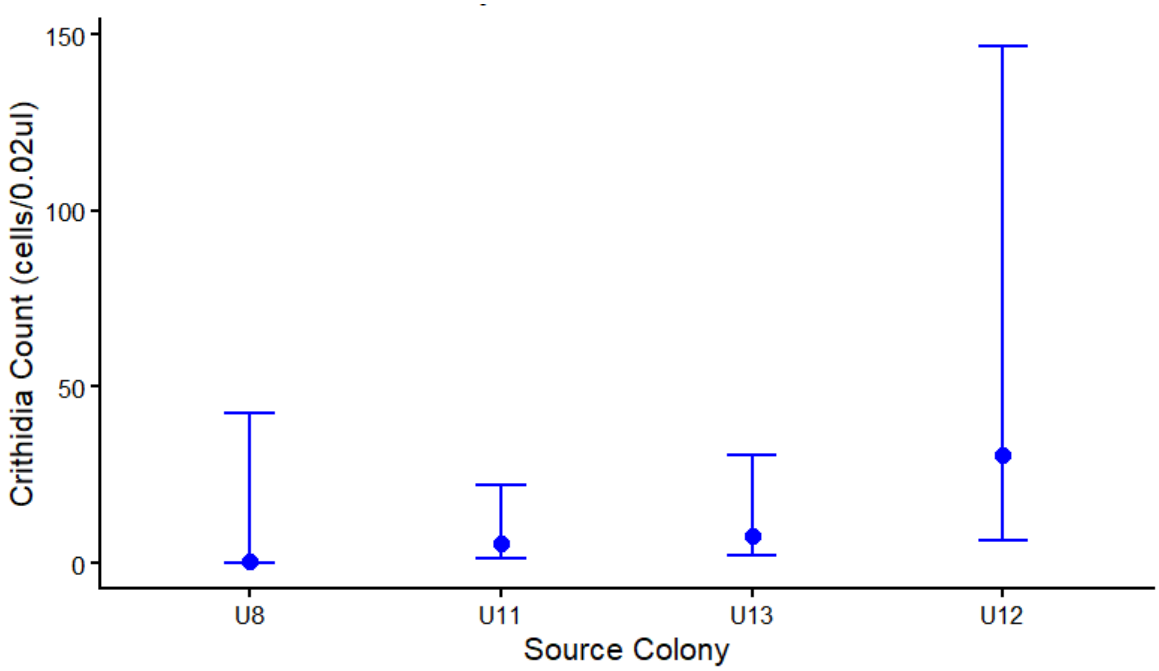

Figure 2.9: **Effects of Source Colony on *C. bombi* counts based on predicted means generated by ggpredict.** Bees from source colony U12 had significantly more *C. bombi* cells per 0.02 microliters on average in comparison to bees from other colonies, with an average of 30.54 (95% CI 6.355 - 146.79) cells. Conversely, bees from colony U8 had an average of 0.44 (95% CI 0 - 42.56) cells, bees from U11 (reference) had 5.79 (95% CI 1.50 - 25.528 cells, and bees from U13 had 7.76 (95% CI 1.97 - 30.53) cells, which did not differ significantly from one another.

Effects of Diet, *C. bombi* infection and nutrition status on foraging and selectivity

Beetle treatment had a significant effect on the probability of a plant being visited (figure 10) but only in bees given the 15% sucrose dietary treatment (figure 10), with bees given the 15% treatment being more likely to visit plants that had been fed on by beetles, while plant height and interaction between diet and plant treatment (figure 10) had marginally nonsignificant effects (table 2.1).

Table 2.1: **Effect sizes of different parameters on probability of visitation** using a generalized linear mixed-effects model with a binomial distribution and a logit link alongside standard errors, Z values, degrees of freedom and p-values. (196 residual df).

	Estimate	**Std. Error**	**Z value**	**df**	**Pr (>|z|)**
Intercept	-25.02	9.29	-2.71	1	0.0067**
Diet	3.49	3.34	1.04	1	0.30
Plant Herbivory Treatment	7.21	3.15	2.29		0.022 *
Infection Strength (*C. bombi* count)	-0.17	0.23	-0.75	1	0.46
Plant Height	0.12	0.07	1.77	1	0.077 .
Number of open inflorescences	0.34	0.24	1.4	1	0.16
Colony U12	-4.48	4.54	-0.99	1	0.32
Colony U13	-1.30	2.65	-0.49	1	0.63
Colony U8	-1.03	3.30	-0.31	1	0.75
Interaction between diet and plant treatment	-5.76	3.06	-1.88	1	0.06 .

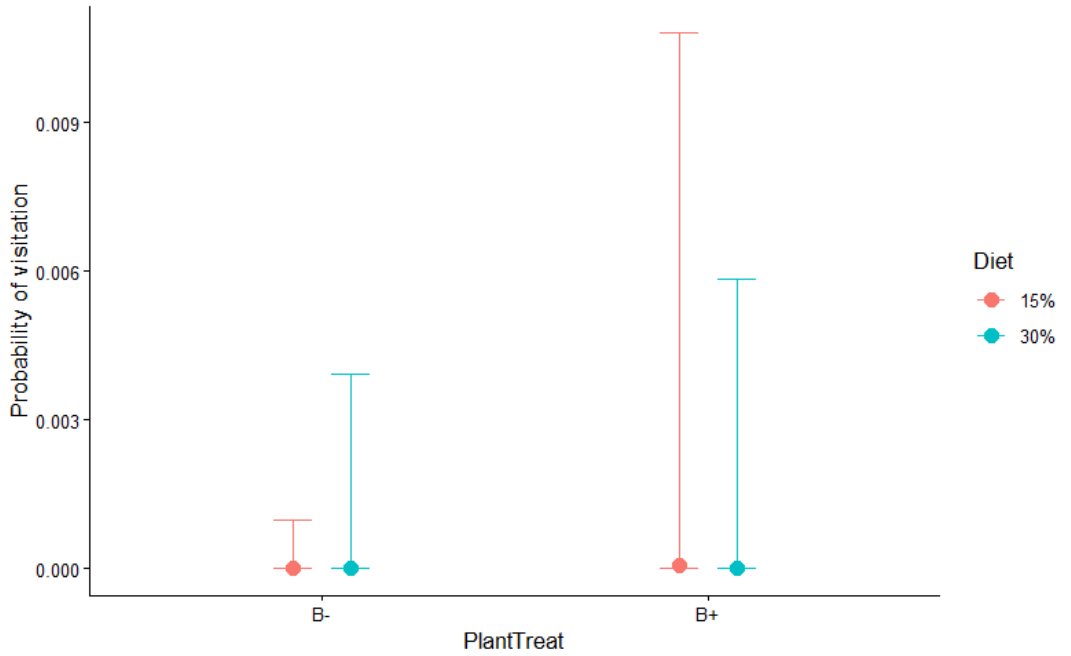

Figure 2.10: **Interaction between herbivory and plant treatment plotted against probability of visiting based on predicted means generated using emmeans.** The trend reveals a weakening, marginally significant negative effect of bees being fed a 30% sucrose diet, and foraging from B+ plants, despite an overall positive effect of insect herbivory on plant visitation. Bees receiving the 15% sucrose diet foraged an average of 0 percent of the time on untreated (B-) plants, while bees fed the 30% diet fed an average of 0 (95 CI 0-0.01) percent of the time from B- plants. Likewise, on beetle-exposed plants, bees in the 15% treatment fed an average of 0 (9 CI 0-0.05) percent of the time, while bees in the 30% treatment fed an average of 0 (95% CI 0 - 0.02) percent of the time. Bees fed the 15% diet were significantly more likely to visit B+ plants versus B- plants (β = -5.67 ± 3.06 SE, z = -1.88, df = 1, p = 0.06), though in all cases probability of visitation was very low.

Inflorescence visitation rate varied from 0-10 across trials, with no visits recorded to either plant in 81 trials, and visits to only one of the two plants recorded in 13 trials. The model indicated considerable variation in inflorescence visits between trials (6.198 +/- 2.49), and negligible variance between dates (1.09 e-08 +/- 00.0001).

Inflorescence visitation rates were strongly affected by beetle treatment, with bees showing a preference for plants which received beetle herbivory (table 2.2, figure 2.14). Likewise, colony identity had a significant effect on visitation, with bees in colony U12 visiting considerably fewer inflorescences in comparison to bees from other colonies (table 2.2, figure 2.11). Floral display size had a strong positive effect on inflorescence visitation, with plants bearing more open inflorescences receiving significantly more visits (table 2.2, figure 2.13). *Crithidia bombi* counts showed a marginally significant negative association with inflorescence visitation (table 2.2, figure 2.12). Neither diet treatment nor any interaction terms influenced inflorescence visitation and none were retained in the best model (table 2.2).

Plant orientation showed a marginally significant negative effect, with right-oriented plants receiving fewer visits, whereas plant height did not significantly influence inflorescence visitation (table 2.2). Despite not reaching statistical significance, these factors were kept in the final model, as removing them resulted in higher AICc values.

Table 2.2: **Effect sizes of different parameters on number of bee visits to individual inflorescences** modelled using a generalized linear mixed-effects model with a poisson distribution and a log link alongside standard errors, Z values, degrees of freedom and p-values.

	Estimate	**Standard Error**	**Z value**	**df**	**Pr (>|z|)**
Intercept	-2.48	0.79	-3.144	1	0.002**
Beetle Herbivory Treatment	0.5	0.185	2.71	1	0.00068**
Bee Infection Strength	-0.11	0.06	-1.735	1	0.08 .
Floral Display Size (# of open inflorescences)	0.11	0.04	2.8	1	0.0016***
Colony U12	-3.32	1.17	-2.81	1	0.0045***
Colony U13	-0.46	0.79	-0.58	1	0.51
Colony U8	0.22	1.00	0.22	1	0.905
Plant Orientation	-0.33	0.2	-1.675	1	0.094

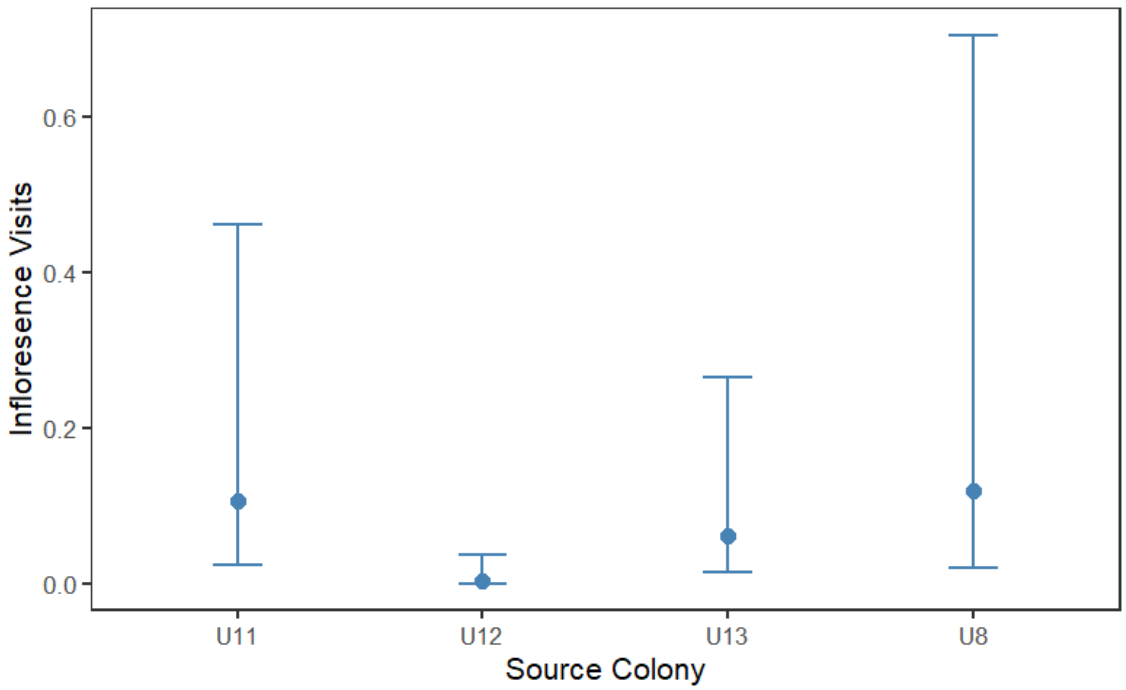

Figure 2.11: **Effect of source colony on bumblebee visitation to individual inflorescences generated using predicted means generated using ggpredict.** Bees from Colony U12 showed a statistically significant negative trend in their plant visitation frequency, making an average of 0 (95% CI 0-0.04) visits, while effects of other colonies were insignificant. Bees from colony U8 made an average of 0.12 (95% CI 0.02 – 0.70) visits, bees from U11 (reference) made an average of 0.11 (95% CI 0.02 – 0.46) visits, and bees from 13 made an average of 0.06 (95% CI 0.01 – 0.26) visits. Bees from U12 made an average of 0.02 visits (95% CI 0 – 0.10), considerably less than bees from other colonies.

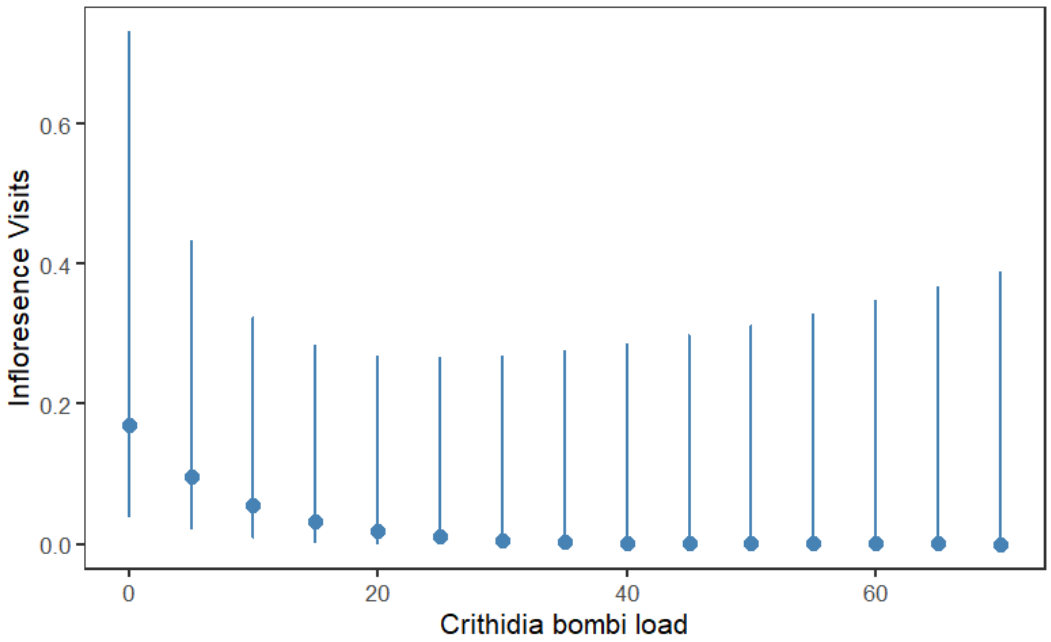

Figure 2.12: **Effects of *C. bombi bombi* infection strength, based on *C. bombi* load, or number of cells per 0.2 microliters on inflorescence visits based on predicted means generated using ggpredict.** Infection strength had a marginally significant, negative effect on inflorescence visitation (ß = -0.11 +/- 0.06, z = -1.735, df= 1,198, p = 0.08).

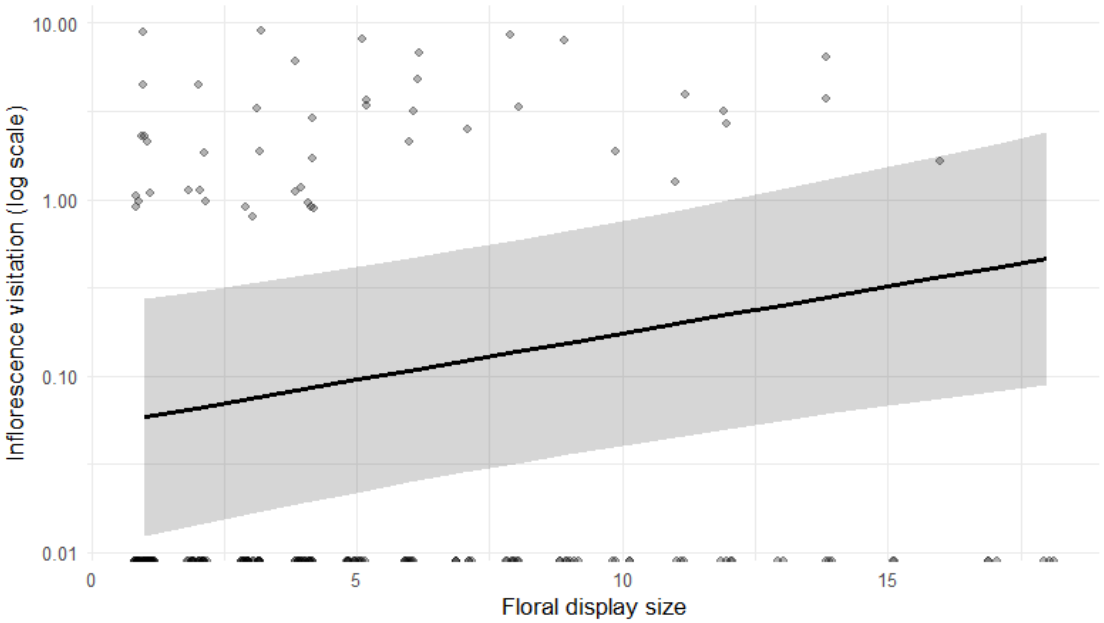

Figure 2.13**: Effect of floral display size (number of open inflorescences) at time of trial plotted on a log scale for ease of visualisation.** Floral display size had a strong, positive effect on inflorescence visitation (ß = 0.11 +/- 0.04, z = 2.8, df= 1,198, p = 0.0016).

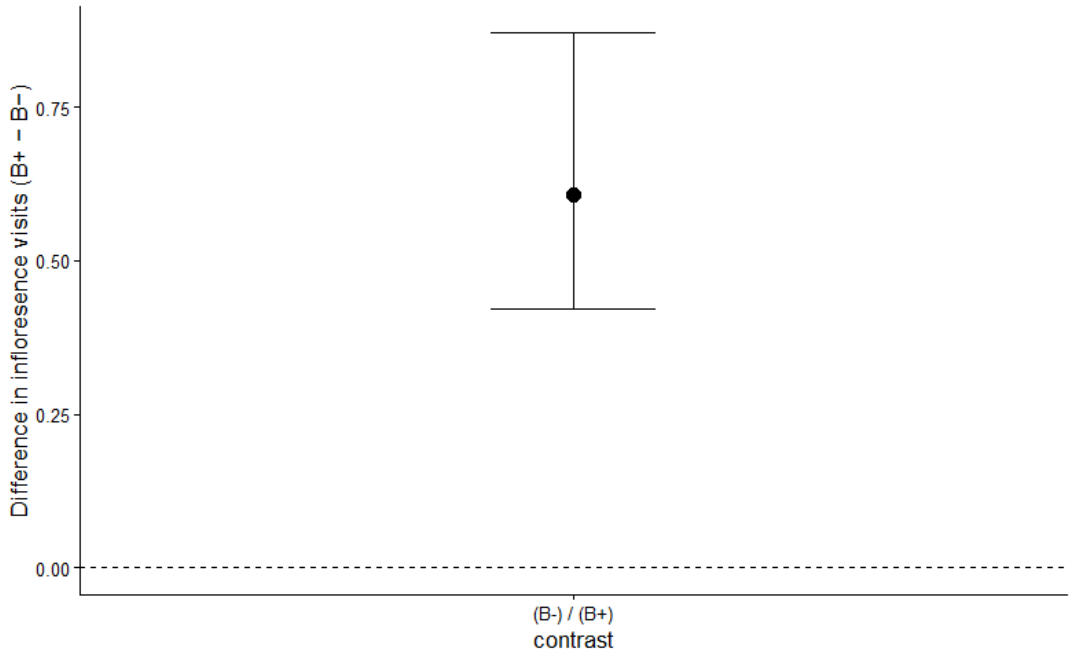

Figure 2.14: **Differences in inflorescence visits between Beetle-damaged and undamaged plants based on predicted means generated using emmeans.** Points show model-estimated contrasts and vertical bars represent 95% confidence intervals. Contrasts whose confidence intervals do not cross zero indicate statistically significant differences. The model accounts for variation among colonies, trials, dates, and open flower number. Estimates are presented on the log scale (exponentiated values correspond to multiplicative differences in visit counts).

Among plants that received visits from bumblebees, *C. bombi* count had a statistically significant negative effect on time spent on plants (table 2.3, figure 15), while plant height had a statistically significant positive effect (table 2.3, figure 16).

Table 2.3:**Effect sizes of different parameters on time bees spent on plant** modelled using a generalized linear mixed-effects model with a poisson distribution and a log link alongside standard errors, Z values and p-values. (40 residual df).

	Estimate	**Standard Error**	**Z value**	**df**	**Pr(>|z|)**
Intercept	2.59	1.066	2.438	1	0.0148*
Infection Strength (*C. bombi* count)	-0.096	0.04	-2.15	1	0.03*
Plant Height	0.03	0.01	2.46	1	0.014*

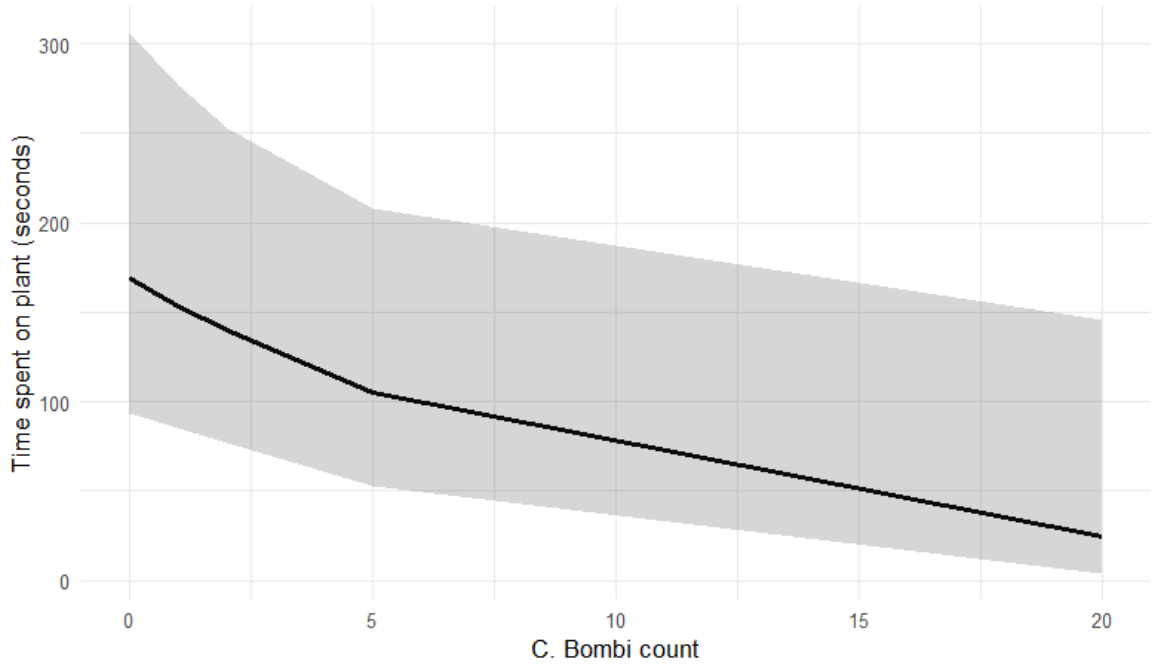

Figure 2.15: **Effects of *C. bombi* infection strength, as defined by number of *C. bombi* cells per 0.2 microliters, on foraging duration based on time spent foraging in seconds represented graphically based on predicted means generated using ggpredict.** *C. bombi* has a strong, negative effect on time spent foraging (ß = -0.096 +/- 0.04, z = -2.1, df= 1,40, p = 0.03).

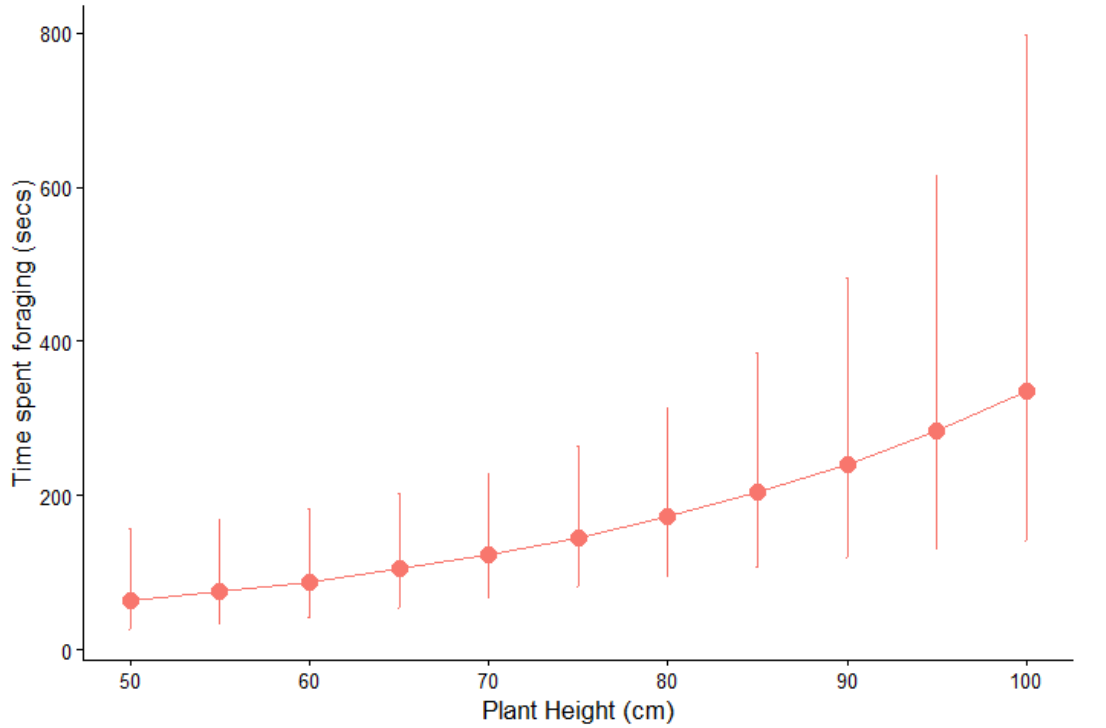

Figure 2.16: **Effect of plant height in centimetres on foraging duration in seconds based on predicted means generated using ggpredict.** Plant height had a strong, positive effect on time spent foraging (ß = 0.03 +/- 0.01, z = 2.46, df= 1,40, p = 0.014).

Discussion

In my study, I sought to understand how environmental changes to host plants in the form of insect herbivory, as well as diet quality and parasitism interact to shape bumblebee foraging behaviours. I hypothesized that herbivory by *P. japonica* would reduce plant attractiveness to bumblebee pollinators due to impacts on floral traits relevant to bumblebee attraction such as plant height, flower number and area as well as the presence and abundance of nectar rewards. Furthermore, I hypothesized that bees infected with the parasite that are fed a less nutritious sucrose diet would be less susceptible to parasites, but also potentially more lethargic due to lower energy reserves.

Contrary to predictions, herbivory did not significantly alter floral display traits such as plant height, floral area or inflorescence, nor did it affect the overall presence or abundance of nectar. This would suggest that these traits are developmentally buffered against herbivore damage (Strauss & Irwin, 2004). Instead, herbivory may have influenced floral traits which were not or could not be quantified in this study, namely nectar chemistry and floral volatile emissions, which likely explain observed differences in bee foraging patterns between plant treatments.

Herbivory did not reduce pollinator visitation as predicted. Instead, herbivore-damaged plants received more visits from pollinators at the inflorescence level, as well as a higher likelihood of visitation by bees fed the less nutritious 15% dietary treatment, which suggests that herbivory instead enhanced floral cues associated with bumblebee attraction, and that this effect was stronger for nutritionally stressed bees. Previous work has revealed that herbivory can alter floral volatile emissions or nectar chemistry (Bruinsma et al., 2014).

Crithidia bombi infection intensity had a limited effect on inflorescence visitation patterns, but was negatively associated with time spent foraging, which suggests that *C. bombi* infection affected foraging efficiency rather than initial decision-making in bumblebees. This is consistent with previous work, which found that *C. bombi* reduces cognitive performance and capacity of bees to forage efficiently (Gegear et al., 2006). However, it is worthwhile noting that bumblebees in my study generally had lower infection levels in comparison with bees in other studies (e.g., Gegear et al., 2006), which may have contributed to the generally weak effect of infection intensity seen in this study.

Differences in diet (15 vs 30% sucrose) had minimal effect on parasite load, which may indicate that sucrose concentrations used in the study were inadequate to elicit changes in bee physiology associated with parasite dynamics, although in other studies these sucrose concentrations did lead to differences in infection intensity (Conroy et al., 2016; Brown & Schmid-Hempel, 2000).

Three-way interactions between diet, herbivory and infection were generally weak, which would suggest that there is little evidence for interactions between these factors in influencing the landscape of bumblebee foraging behaviour. Instead, other factors, such as colony-level variation appear to play a more prominent role, while the effect of herbivory on this behaviour may be explained by herbivory-induced to subtle, or unmeasured floral cues.

Overall, the primary factors investigated in the study (sucrose diet content, herbivory and parasite load) appeared to have less of an effect on bumblebee foraging behaviour than initially predicted. Instead, other factors, such as colony-level variations and plant variation appeared to be more important in determining this behaviour.

Experiment 1:

Despite evidence of insect herbivory having a strong, positive effect on bee visitation, with bees being more likely to visit inflorescences on plants exposed to *P. japonica*, while also making these plants more attractive overall to bees with higher nutritional stress, these did not translate to differences in observed floral traits between beetle-exposed versus untreated plants. This suggests that measured floral traits are not indicative of bee visitation patterns. Instead, these observations may be explained by herbivory induced alterations to other floral traits such as volatile organic compounds (VOCs) (Brunisma et al., 2014) and nectar chemistry, such as nectar sucrose (Cnaani et al., 2006), which could not be reliably quantified due to technical issues. This therefore indicates a limitation within my study design which prevented a complete understanding of how relevant floral traits associated with bumblebee attraction change in response to insect herbivory. This can be remedied by replicating this study with reliable means of measuring both nectar chemistry and VOCs incorporated into the experimental design.

The observed effects of herbivory (or lack thereof) on measured floral traits may be attributed to a number of factors such as timing (Boege & Marquis, R. J, 2005; Quiroz-Pachco et al., 2020) or intensity (Karban & Baldwin, 1997) of herbivory, developmental buffering of these plant traits against herbivory (Strauss & Irwin, 2004) and decoupling of defensive metabolic pathways associated with leaf versus floral tissues (Strauss & Whittall, 2006). While the findings associated with these floral traits do not support the hypothesis of herbivory impacting floral traits, they nonetheless reveal key insights regarding bumblebee pollinator relevant reproductive traits in response to insect herbivory.

Research shows that plants can compensate for damage when it occurs early in development (ie. prior to onset of flowering) (Quiroz-Pacheco et al. 2020). As such, this overcompensation mechanism may explain the lack of differences in plant height between treatments, as plants can recover lost tissue by investing more into growth and reproduction (Paige & Whitham, 1987). In

particular, perennial plants like *S. novae-angliae* are well documented in their ability to reallocate resources to compensate for tissue damage caused by herbivory (Paige & Whitham, 1987). This may explain why height or flower quality/quantity in my *S. novae-angliae* plants were unaffected by Japanese beetle herbivory.

Timing of herbivory is key here, with research showing that herbivory damage occurring prior to critical periods of plant growth associated with development results in inflorescence development and plant growth (i.e., plant height) being unaffected by herbivory due to plants adjusting their development schedule to account for damage, allowing them to produce the same number of flowers, and grow as tall as plants without herbivory damage (Boege & Marquis, R. J, 2005; Quiroz-Pacheco et al., 2020), decoupling of defensive metabolic pathways between floral and leaf tissue.

A further possibility is that *P. japonica* damage in the treated plants may have been insufficient to have any real effect on floral traits. Indeed, research shows that defensive responses to herbivory by *P. japonica* in plants are proportional to damage intensity (Karban & Baldwin, 1997). In my own study, I did not quantify herbivore damage, but anecdotally, even after a week of beetle presence, severe damage was typically limited to only one or two leaves on each plant (personal observation). Furthermore, white flies (Aleyrodidae) were present in the greenhouse and feeding on all plants before and during my experiment. As a result, the additional effect of *P. japonica* damage to the B+ treatment group may have been insufficient to warrant any real effect on floral traits. Therefore, it is possible that beetle-treated plants may have not faced adequate environmental stress to warrant a trade-off between defensive and reproductive traits. (Herms & Mattson, 1992).

An additional consideration is that inducible herbivore-defence responses are often compartmentalised, with the activation of defences associated with leaf tissue having no connection to nectar production (Strauss & Whittall, 2006). This suggests that any insect herbivory my plants were exposed to may have impacted only the leaf tissue without impacting floral traits such as nectar production. This compartmentalization stems from the fact that chewing insect herbivores, such as *P. japonica*, activate the jasmonic acid (JA) metabolic pathway (Lortzing & Steppuhn, 2016), which in many plant species, is decoupled from floral tissues (Radhika et al., 2010). In their study on *Brassica rapa* plants, Radhika et al., (2010) found that JA application directly to flowers increased nectar production, while insect herbivory on leaves had no effect on nectar, despite increasing JA levels in leaf tissue. This is further supported by a study on wild tobacco (*Nicotiana attenuvta*), which found JA signalling in flower and leaf tissue to be regulated independently (Li et al., 2017). Compartmentalization of anti-herbivore defence and plant growth pathways may also explain a lack of differences in floral traits such as inflorescence size and abundance between treatment groups (Agrawal et al., 2003).

Furthermore, traits associated with high pollinator selective pressure, such as number of flowers and flower area may be developmentally buffered, effectively resulting in low phenotypic plasticity due to environmental stressors such as insect herbivory (Strauss & Irwin, 2004). This could indicate that certain floral traits of some species of plants, including *Symphyotrichum novae-angliae* are capable of developmentally buffering certain reproductive traits against relatively low levels of insect herbivory. Instead, research points to the fact that traits of flowers such as the aforementioned nectar chemistry and volatile emissions are the traits most sensitive to herbivory stress (Adler, 2000).

Experiment 2:

This experiment revealed key insights into the nature of interactions between host plants, insect herbivores, bumblebee pollinators and their *C.bombi* parasites. While the findings did not show evidence of three-way interactions between parasites, bee diet and herbivory (owing in part to low overall infection rates, which may have masked effects of diet), they nonetheless show that insect herbivory of host plants and parasitism both influence foraging by bumblebees. While the role of herbivory was contrary to that of the initial hypothesis, which predicted a negative correlation rather than a positive one, the findings supported the notion that parasitism impaired bumblebee foraging capacity. Furthermore, the findings also suggest that other factors, such as colony identity and its interactions with parasitism are also vital in shaping bumblebee foraging patterns.

Herbivory stood out due to its counterintuitive role in attracting bumblebees to plants, with bumblebees being both more likely to visit plants and visiting more inflorescences on plants which received the beetle herbivory treatment. One possible explanation for this is the relationship between herbivory and chemical production associated with pollinator attraction. Herbivory can result in alterations to nectar composition, which can, in some cases, increase nectar sugar concentrations, thereby potentially increasing nectar sugar rewards to pollinators (Bruinsma., et al. 2014). This finding is quite interesting, as other research suggests that herbivory results in lower levels of nectar sucrose (Gustafson et al., 2022), or no effect whatsoever (Samocha & Sternberg, 2010). However, it is less clear whether this has a direct effect on bumblebee visitation, which may make this phenomenon worthwhile exploring in future studies. If *P. japonica* herbivory led to increased nectar sugar content, this could explain why bumblebees tended to visit inflorescences in beetle-treated plants more than those of non-herbivorized plants, contrasting with other herbivory-related phenomena which alter floral chemistry in ways which make the plants less appealing to pollinators, such as the alteration of volatile floral emissions which would instead make herbivorized plants less appealing to pollinators (Bruinsma et al., 2014). On the other hand, evidence of herbivory altering volatile compounds in a manner which positively impacts pollinators is context-dependent, with one study seeming to suggest that some herbivory to plants attracts some pollinator species while repelling others (Burkle, L. A., &

Runyon, 2016). However, these findings are not bumblebee specific, which may present a knowledge gap to be addressed in future research.

Since I could not effectively quantify nectar sugar content as part of this study and volatile emissions were not examined, this presents a gap in the scope of my study. Indeed, the relationship between nectar chemistry and bumblebee attraction is well studied, with multiple notable examples showcasing the importance of nectar chemistry, in addition to abundance, as being important in influencing bumblebee foraging behaviours (Heuel et al., 2025; Byers et al., 2014). For example, a recent study on *B. impatiens* found that bees can both smell and taste these emissions and respond differently based on whether they were smelled or tasted, even responding differently based on differences in tastes associated with these chemicals (Heuel et al., 2025). This relationship between nectar chemistry and bumblebee foraging further supported by an additional example, where research on multiple species of monkeyflowers (*Mimulus*) and their bumblebee pollinator, *Bombus vosnesenskii*, showed that differences in olfactory cues associated with different combinations of monoterpenes between different monkeyflower species differed in their capacity to attract bumblebee pollinators (Byers et al., 2014). Therefore, it is plausible that some of the observed variation in bumble visitation patterns may have been observed due to differences in floral chemistry caused by herbivory.

Regarding the relationship between parasite load and time spent foraging, the negative correlation aligns well with the hypothesis, with previous research suggesting that increased infection intensity decreases energy available to the bee to expend foraging, stamina which enables the bees to maintain their ability to forage, as well the bees' ability to learn to accurately identify flowers (Gegear et al., 2006). Interestingly, parasite load influenced bumblebees' capacity to forage for extended periods, despite not having a direct effect on the number of visits made to inflorescences. While Gegear et al. (2006) conducted their study in a controlled laboratory setting with artificial flowers, my study involved work with live flowers in a tent, which shows evidence of a consistent, negative relationship between foraging ability and *C. bombi* load, irrespective of whether live or artificial flowers are used.

Bees fed the less nutritionally rich 15% sucrose diet were more motivated to forage from the apparently more desirable (and potentially more nutritious) beetle-damaged plants. If these plants offered increased nutritional rewards, such as nectar sugar (Bruinsma et al., 2014), this may indicate that nutritionally stressed bees are more likely to select optimal rewards while foraging (Chittka & Raine, 2006). However, because the effect of herbivory on factors such nectar sucrose content could not be quantified in this study, this cannot be proven definitively. As such, similar future studies where nectar sucrose is successfully quantified may help to verify this. Colony identity had an

unexpectedly strong effect on both foraging behaviour and *C. bombi* infection intensity. Several possibilities may explain these phenomena. First, as previously stated, research suggests that individual colonies differ in their immune responses to parasites (e.g., Brunner et al., 2013; Mockler et al., 2018). These findings are consistent with previous research linking bumblebee colony genotype diversity to *C. bombi* susceptibility (Schmid-Hempel & Reber Funk, 2004) and *C. bombi* infection to impaired foraging capacity (Gegear, et al., 2006). Since bees from colony U12, which had higher parasite loads, visited fewer inflorescences, it is possible that bees from this colony were foraging less due to influence from higher parasite counts in comparison to other colonies. This suggests that differences in immune system function (Brunner et al., 2013) and microflora (Mockler et al., 2018) between colonies may mediate foraging behaviour through their effects on parasite resistance. These findings ultimately indicate that colony genetics play an important role in influencing parasite load, and subsequently the role of parasitism itself in mediating bee behaviour. Second, genetic differences between colonies may directly influence foraging behaviour, irrespective of parasitism. For example, one bumblebee study found that foraging behaviours such as foraging route fidelity (i.e., the tendency of workers to stick to specific foraging routes) varied consistently between colonies (Klein et al., 2017). Likewise, another study found evidence of differences in 'personalities' between colonies, including those associated with foraging, such as exploration, with certain colonies having persistent behaviours within workers even when the environment was standardized (Jandt & Gordon, 2016). Further research looking at fire ants (*Solenopsis invicta*), another species of eusocial, foraging insect, found evidence of variation in foraging behaviour between workers from different colonies which varied as much as tenfold between colonies in some cases (Bockoven et al., 2017). These behavioural variations may therefore offer an alternative explanation for differences between colony U12 and other colonies.

Finally, beyond genetic differences between colonies, another source of variation may stem from differences in colony age. Colony U12 was received on September 3rd, 2025 while colonies U11, U8 and U13 were received on July 29th, July 8th and September 3rd of 2025 respectively. Bumblebee colonies are known to undergo three distinct developmental stages throughout their annual life cycle: the early growth stage, where bees prioritize investment into foraging and brood production, the ergonomic growth stage, where colonies prioritize foraging and the reproductive stage and the reproductive stage, where colonies prioritize production of drones and queens (Schmid-Hempel & Schmid-Hempel, 1993). Furthermore, in a colony of eusocial insects such as bumblebees, it is well established that foraging is performed by older workers (Robinson, 1992). Since colony U12 was relatively young in comparison to the reference colony U11, it may have been in its early growth stage at the time the experiment took place, where many more of its workers were younger, and therefore less likely to forage, which may explain why there were considerably fewer plant visits overall in bees from this colony. However, this would not explain why colony U13, which arrived on

the same day as U12 did not differ significantly from U11 in terms of its foraging behaviour. The differences in behaviour between the two youngest colonies may be explained by other factors, such as differences in colony personality (Jandt et al., 2014) interacting with variations in worker foraging behaviour associated with the age of the source colony and individual workers. As a result, these findings suggest that while colony age may influence foraging behaviour, this factor interacts with other phenomena to shape bumblebee foraging behaviour. Future research should focus on understanding how colony age and labour division between workers interacts with other factors to shape foraging behaviour seen in bumblebee colonies.

The lack of correlation between colony identity and time spent foraging may be at least partly explained by the fact that the colony often influences foraging and exploration activity as opposed to fine-scale behaviours such as duration of time spent on plant (Jandt et al., 2014). These findings suggest the importance of variation in colony level social behaviours such as colony personality (Jandt et al., 2014) or variations in worker foraging capacity associated with age (Schmid-Hempel & Schmid-Hempel, 1993), as opposed to variations in genetic susceptibility to *C. bombi* infection in influencing variation in foraging behaviour between different colonies. Consequentially, this could mean that these behavioural differences between colonies, rather than susceptibility to parasites may be the main driver of inter-colony variation in foraging decision making in bumblebees. As a result, this presents a knowledge gap to be addressed by future research looking at interactions between genetic makeup and subsequent behavioural patterns of individual bumblebee colonies, the susceptibility of each individual colony to parasites such as *C. bombi* and foraging behaviours of bees from these colonies associated with visits to individual plants and inflorescences, as well as time and energy investments at each individual plant or inflorescence.

Unsurprisingly, bees tended to visit more inflorescences on plants with larger floral display sizes (i.e., number of anthetic inflorescences at the time of the trial). There is strong support from literature regarding this phenomenon (Vaughton & Ramsey, 1998; Karron et al., 2004) which aligns with observations, suggesting that bees visit more flowers per plant when there is a large floral display size. Research on native weeds in Mexico shows that this pattern is conserved across pollinator species (Hernández-Villa et al., 2020). Additional research supports that these within–flower behavioural patterns are likely explained by foraging decision-making behaviours in bees, where floral display size is an indicator of nutritional reward availability, thereby supporting the previous hypothesis regarding floral display size and inflorescence visits (Glaettli & Barrett, 2008).

Plant height had a significant effect on visit duration, and a marginally significant effect on the probability of visiting. This trend suggests that taller plants, which typically advertise increased floral rewards (Mitchell et al., 2004; Harder & Barrett, 1996) result in bees spending more time

foraging on these plants. This higher reward availability on taller plants may result in these plants being associated with higher densities of rewards, which can subsequently result in bumblebees investing more time into foraging from these plants, thereby optimizing nutrition while foraging.

Diet and *C. bombi* Load

When considering the reasoning behind diet having no effect on *C. bombi* load, despite previous findings suggesting that a poorer diet equates to lower *C. bombi* load (Conroy et al., 2016), several possibilities may explain this phenomenon. Firstly, bees are known to develop immune responses from pollen intake, which may help to mediate responses to *C. bombi* infection regardless of the effects of sucrose consumption (Alaux et al., 2010). Since both dietary treatment groups were fed an equal amount of pollen, it is possible that pollen proteins may have mitigated infection in either treatment group. This is supported by the fact that a large number of screened bees in either dietary treatment group had low or non-existent *C. bombi* loads. This is further supported by additional research which shows that *C. bombi* infections are commonly regulated through the immune activity of the host, as opposed to solely being dependent on host diet (Brown et al., 2003).

However, it is important to note that infection levels across the board were quite low compared to typical *C. bombi* infection levels reported in other studies. This may have obscured the effect of diet, which needs more variation in infection level to be apparent. Studies looking at bumblebee foraging typically use controlled, relatively high inoculation doses, which provide clear, relatively high, measurable parasite loads (Riddell et al., 2011). Behavioural effects are, in turn, dependent on the parasite load of an infected bumblebee (Schmid-Hempel & Schmid-Hempel, 1993). Since most inoculated bees in my study had relatively low or nonexistent *C. bombi* counts, the parasite load may not have been strong enough for diet to play a role in influencing foraging behaviour.

Conclusion

Together, my findings suggest that bumblebee foraging behaviour in this case was shaped more strongly by plant traits unrelated to herbivory and by colony-level behavioural patterns rather than by herbivory-induced changes to plants or diet-mediated differences in parasite infection. Herbivore damage may have altered floral cues in ways which increase pollinator attraction rather than decreasing it, even in absence of changes to observed floral traits, which suggests that herbivory-induced changes to other floral traits, such as floral chemistry, may have been the main factors which attracted bumblebees to herbivorized plants. More broadly, these findings highlight the complex and hierarchical nature of pollinator foraging decisions, where plant traits, colony characteristics and individual physiological conditions are the most important drivers shaping pollinator behaviour.

Appendices

Appendix A: Pilot study: Determining Nectar Sugar Concentration for Foraging Assays

In choosing a low-quality dietary treatment for bees, I was looking for a sucrose concentration low enough that it could influence behaviour and parasite infection but not so low as to induce lethargy, leading to little or no foraging. In Taylor et al. (2016), the 15% sucrose treatment was shown to result in considerably lower *C. bombi* load in bumblebees. However, there was also an increased mortality in bees given the 15% treatment (Conroy et al., 2016). Since my study focused on bumblebee foraging, even if bees in a hypothetical 15% sugar treatment were to survive, they may be too lethargic to forage. Alternatively, a slightly higher dosage of 20% sucrose could be used if 15% sucrose would result in lethargy.

As a result, I conducted a pilot study to determine whether bees fed 15% sucrose would indeed forage. To do so, I created sets of 3 uninfected bumblebee microcolonies consisting of four bees each, and fed each of them either 15%, 20% or 30% sucrose consistently for a week alongside a steady diet of pollen. After the weeklong period, I released the bees from each set of microcolonies (15%, 20% and 30%) into mesh enclosures containing plants and allowed bees to forage. If bees in the 15% treatment foraged successfully, I would use the 15% solution as my 'low nutrition' treatment. If bees in the 15% solution did not forage, while those in the 20% solution did, then 20% sucrose would be my 'low nutrition' treatment. Bees in the 30% treatment were expected to have adequate nutrition to forage. However, if this was not the case, I would need to adjust both the 'high' and 'low' nutrition treatments accordingly.

After conducting this study, I found that bees in the 30% treatment showed signs of consistent foraging and flight behaviours, as did those in the 20% treatment, albeit with some of the bees becoming weaker and less active towards the end of the 15-minute flight period. In the 15% treatment, one of the three colonies had three or four bees which were lethargic and unable to properly fly at the start of the 15-minute trial. However, the other two colonies appeared to function normally within the 15-minute period. As a result, I decided to keep the 15% dietary treatment as my 'low nutrition' treatment.

Appendix B: Protocol for Attempted Pollen and Nectar Sucrose Collection

I attempted to estimate the total amount of nectar sugar, or sucrose using a Bellinghamm + Stanley Eclipse 45-81 sucrose refractometer (give make and model). Since the sugar concentration was often too high to be properly quantified by the refractometer (max measurable sugar content 50 brix), I diluted the nectar using 1–2 microliters of distilled water, which I placed onto the sensor using

a micropipette. I then obtained the raw percentage of sugar in Brix. To adjust for the added water, I used the formula: **Brix = Measured Brix * (1 + (volume of water added / volume of original sample))** to calculate the actual percentage of sugar in a given nectar sample. Unfortunately, quantifying nectar sucrose this way proved unreliable, as calculated nectar percentages were often over 100% due to the extremely low volumes involved. As a result, nectar sucrose was ultimately removed as a measure of nectar concentration, and nectar sucrose was removed as a measure of floral attractiveness.

Attempts were made to collect pollen from inflorescences including heating inflorescences in a thermal cycler, and rinsing remaining pollen grains using centrifugation. However, due to difficulties in collecting pollen, as well as a strong interest in nectar as opposed to pollen in foraging bumblebees observed during the experiment, these quantifications were ultimately removed from the study protocol.

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