

Plant-Pollinator Interactions in Lowbush Blueberry Agroecosystems

by

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Abstract

Lowbush blueberry (*Vaccinium angustifolium* Aiton, Ericales: Ericaceae) is a shrub native to northeastern North America. Wild bees have co-evolved with lowbush blueberry and are effective pollinators. The objective of this thesis was to describe plant-pollinator interactions in lowbush blueberry ecosystems in Maritime Canada. The specific objectives were 1) to evaluate correlations between plant richness, abundance, and diversity and bee visits to lowbush blueberry 2) determine which wild bees collected pollen from lowbush blueberry and 3) which non-crop flowers wild bees collected pollen from over the season. Interactions were characterized at 28 site-years. Observations of bee visits to flowers were recorded during transect walks and a subset of wild bees were captured for DNA metabarcoding of bee-associated pollen.

Significant correlations between plant metrics and bee visits to blueberry were rare. During bloom, andrenid bee visits to blueberry were positively correlated to plant richness and diversity in the field. Bumble bee visits to lowbush blueberry were positively correlated to the mean bloom abundance (excluding blueberry) in the field. Other wild bee visits to blueberry were positively correlated to bloom abundance in the fall. Bees were observed on 55 of the 113 plant taxa recorded. The most abundant wild bees observed on blueberry flowers were the long-lived, social *Bombus* spp. and *Lassioglossum* spp. as well as the short-lived, solitary *Andrena* spp. At some sites, wild bees were observed on blueberry more frequently than honey bees. All wild bee taxa captured carried *Vaccinium* spp. pollen. In fact, 96% of captured bees carried *Vaccinium* spp. pollen, and 52% of captured bees had only *Vaccinium* spp. in their pollen loads. During bloom 86% of wild bee pollen loads had three or fewer plant genera. There were very few significant differences among bee taxa for the number of plant genera present in pollen loads. DNA metabarcoding of bee-associated pollen revealed more plant-pollinator interactions than observations during transect walks. The most common non-blueberry plant families in pollen loads during bloom were Rosaceae, Sapindaceae, and Asteraceae. Asteraceae was the most common plant family in summer and fall pollen loads.

This study identified the wild bees present in lowbush blueberry fields. Observations and bee-associated pollen revealed the plant taxa wild bees visited throughout their lifecycle. These data identified plants that support wild bees in lowbush blueberry agroecosystems.

List of Abbreviations and Symbols Used

°C	degree Celsius
α	alpha significance
ASV	amplicon sequence variant
BOLD	barcode of life database
bp	base pair
BLAST	Basic local alignment search tool
COI	cytochrome <i>c</i> oxidase I gene
DADA2	divisive amplicon denoising algorithm
DNA	deoxyribonucleic acid
fig	figure
h	hour
H	Shannon's diversity index
ITS2	Internal Transcribed Spacer Unit 2
JCD	Julian calendar date
m	meter
M	molar
m ²	square meter
matK	Maturase K
mg	milligram
min	minute
mL	milliliter
mM	millimolar
n	Sample size
NB	New Brunswick
NGS	next generation sequencing
NS	Nova Scotia
NCBI	National Centre for Biotechnology Information
<i>P</i>	p-value
PCR	polymerase chain reaction
PEI	Prince Edward Island
rbcl	Ribulose biphosphate carboxylase large subunit
r_s	Spearman's rank order correlation
s	seconds
spp.	species
U	unit
v	version
w/v	weight/volume
$\times g$	g-force (for relative centrifugal force)
μ L	microlitre
μ M	micromolar
χ^2	Chi-square

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Chapter 1 – Introduction

Lowbush blueberry agroecosystems

Lowbush blueberry, also known as wild blueberry, is an important crop in northeastern North America where the woody shrub occurs naturally. The lowbush blueberry crop is a mixture of *Vaccinium* (Ericales: Ericaceae) species, mostly *Vaccinium angustifolium* Aiton and *V. myrtilloides* Michx., however, in many fields, *V. angustifolium* predominates (Jones *et al.*, 2014). Fields are developed by clearing forests to remove plant competition and increase sunlight to promote the growth of existing stands. Fields are comprised of patches of genetically unique individuals, often referred to as clones, which spread via rhizomes. The perennial plant is managed on a two-year production cycle. In the vegetative or sprout year, plants that were pruned the previous fall undergo vegetative growth and flower buds develop. In the crop or bearing year, plants flower, fruit are harvested, and plants are pruned.

The white or light pink petals are fused together to form a bell-shaped flower that typically hangs down (Hall *et al.*, 1979). The flower contains both male and female reproductive organs with 10 stamens fused together in two groups of five (Hall *et al.*, 1979). The style protrudes farther than the anthers and this morphology limits pollen falling on the receptive surface of the stigma (Bell *et al.*, 2009). Research using bagged flowers to prevent open pollination, and experiments with hand pollination, show that the crop is largely self-infertile (Wood, 1968a; Hall *et al.*, 1979; Usui *et al.*, 2005). The large, heavy pollen is not amenable to wind pollination (Free, 1970) and, therefore, the crop is mostly cross-pollinated by insects. The poricidal anthers require shaking for pollen to be released, limiting the number of effective pollinators. Bees that use their flight muscles to vibrate flowers, known as buzz pollinators (Buchmann, 1983) are adapted to collect pollen from these specialized flowers.

Lowbush blueberry is a mass flowering crop with an early and short bloom period. Bloom time varies among regions. In central and northern Nova Scotia (NS) lowbush blueberry begins to flower between 376 and 409 growing degree days (with T-base of 0°C), typically mid to late May (White *et al.*, 2012) and flowering lasts for three to four weeks (Drummond, 2019a). The two-year production cycle, early bloom time, and poricidal anthers are important factors in the pollination of lowbush blueberry.

The bees of lowbush blueberry agroecosystems

Bees (Hymenoptera: Apoidea) are the main pollinators of lowbush blueberry (Finnamore & Neary, 1978). Bees are characterized by the branched hairs on their body and the fact that, with only a few exceptions, they are phytophagous, provisioning their larvae with pollen, nectar, or plant oils and consuming pollen and nectar as adults. Bees are divided into nine families, of which six are found in Canada; the Family Melittidae is rarely found in eastern Canada, while the other five, Megachilidae, Apidae, Andrenidae, Halictidae, and Colletidae are common (Packer *et al.*, 2007).

Several bee surveys have been conducted in lowbush blueberry production regions (Boulanger *et al.*, 1967; Finnamore & Neary, 1978; Sheffield *et al.*, 2003; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; Cutler *et al.*, 2015; Drummond *et al.*, 2017b; McCallum *et al.*, 2021), which have found a diverse wild bee community. Cutler *et al.*, (2015) documented 95 wild bee species in lowbush blueberry fields in NS. Members of the genera *Bombus* (Hymenoptera: Apidae), *Andrena* (Hymenoptera: Andrenidae), *Halictus* (Hymenoptera: Halictidae), *Lasioglossum* (Hymenoptera: Halictidae), *Megachile* (Hymenoptera: Megachilidae), and *Osmia* (Hymenoptera: Megachilidae) have all been found in lowbush blueberry fields (Boulanger *et al.*, 1967; Finnamore & Neary, 1978; Sheffield *et al.*, 2003; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; Cutler *et al.*, 2015; Drummond *et al.*, 2017b; McCallum *et al.*, 2021). Generally, halictid, andrenid, and bumble bees dominate the bee community at sites sampled throughout eastern Canada and Maine.

Prior to the 1960s, pollination in blueberry fields was performed by wild bees. Since that time, it is common practice to stock *Apis mellifera* Linnaeus (Hymenoptera: Apidae), western honey bee hives in fields during bloom (Kinsman, 1993). Honey bees lack the ability to buzz pollinate but their use has been shown to increase fruit production (Aras *et al.*, 1996). Seventy-two percent of growers surveyed in Prince Edward Island (PEI) used commercial honey bee hives for pollination in 2015 (Collum & Hanes, 2015). *Megachile rotundata* Fabricius, the alfalfa leafcutting bee, and *Bombus impatiens* Cresson, the common eastern bumble bee, are also used as managed pollinators in lowbush blueberry fields.

Pollination by bees in lowbush blueberry production

Pollinators are a major input in lowbush blueberry production (Asare *et al.*, 2017; Yarborough *et al.*, 2017). It is critical to determine which bees are effective pollinators of the crop. Documenting the pollinating species locally is the first step in preservation and/or management (Danks, 1994). Desirable traits of a lowbush blueberry pollinator include early emergence, ability to fly in spring conditions, capable of buzz pollination, fidelity to lowbush blueberry flowers, and movement among flowers of different clones for cross pollination.

Some wild bees exhibit many of these desirable traits. Bumble bees are capable of buzz pollination (Sampson, 1993; Javorek *et al.*, 2002; Cardinal *et al.*, 2018), are known to fly in cool weather (Drummond, 2016), have shown high fidelity to blueberry flowers (Whidden, 1996), and carry large amounts of *Vaccinium* spp. in pollen loads (Moisan-Deserres *et al.*, 2014). Some species from the genus *Andrena* have emergence times that are well-matched to blueberry bloom (Boulanger *et al.*, 1967; Sheffield *et al.*, 2003; Cutler *et al.*, 2015), and many species are capable of buzz pollination (Sampson, 1993; Javorek *et al.*, 2002; Cardinal *et al.*, 2018). Some andrenids captured in blueberry fields carried a high proportion of Ericaceous pollen (Mackenzie & Eickwort, 1996; Sheffield *et*

al., 2003; Tuell *et al.*, 2009; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015). Drummond (2016) found that andrenid bees flew further distances between flowers than honey bees, indicating andrenids may be more likely to move pollen among clones. In addition, pollen carried by andrenid and other non-apidae bees may be more available for pollination as they carry it on hairs or abdominal scopa rather than packed on hind legs (Woodcock *et al.*, 2013). Members of the genus *Lasioglossum* are common in lowbush blueberry fields (Cutler *et al.*, 2015). Moisan-DeSerres *et al.*, (2014) found that while small halictids did not carry large amounts of pollen, a large proportion of it was blueberry.

Lowbush blueberry fruit set in the absence of managed pollinators has been found to be in the range of 30-55% (Desjardins & de Oliveira, 2006; Stubbs & Drummond, 1997; Drummond, 2012; Asare *et al.*, 2017; Bushmann & Drummond, 2020). On a per bee basis, fruit set was increased 1.6 times more by a native bee than a honey bee (Asare *et al.*, 2017). *Bombus* spp. queens and *Andrena* spp. visited 6.5 and 3.6 flowers, respectively, in the time a honey bee visited one flower (Javorek *et al.*, 2002). Drummond (2016) found that *B. impatiens*, *Osmia atriventris* Cresson and *Andrena carlini* Cockerell deposited more blueberry pollen per visit than honey bees. This is consistent with other crops where positive associations with fruit set and wild bee-flower visits were found (Garibaldi *et al.*, 2013).

In addition to the behavioral traits that make wild bees well-suited to lowbush blueberry pollination there are also financial benefits. Pollination is the highest input cost in lowbush blueberry production (Yarborough *et al.*, 2017; Peter Burgess, Director of Wild Blueberry Producers of Nova Scotia, personal communication). The average rental price for a honey bee hive in NS in 2022 was \$178.51 (Jason Sproule, Provincial Apiculturist, personal communication of data he collected via a 2022 NS honey bee producer survey). The “Best Management Practices Guide for Honey Bee Pollination of Wild Blueberries in Atlantic Canada”, recommends a minimum stocking density of 7.4 hives per hectare

(Bennett & Byers, 2023). The average cost to purchase a *Bombus* quad in PEI in 2015 was \$246.00 (Collum & Hanes, 2015) and the list price for a bumble bee quad from Koppert Canada in 2023 was \$369.98 (received a quote from info@koppert.ca).

Bee declines

Declines in wild and managed bee populations have been reported (Biesmeijer *et al.*, 2006; Goulson *et al.*, 2008; Bartomeus *et al.*, 2013; Burkle *et al.*, 2013). The potential drivers of bee declines include loss of semi-natural habitats which provide floral and nesting resources, increased use and/or misuse of pesticides, pathogens, climate change, or various combinations of these stressors (reviewed in Potts *et al.*, 2010). Bee declines and the subsequent loss of pollination services is a concern in agriculture. A study in the United States found pollinator limitation in five of the seven crops studied, including highbush blueberry (*Vaccinium corymbosum* L. (Reilly *et al.*, 2020)). Bee phylogenetic diversity decreased with increasing agricultural land cover (Grab *et al.*, 2019), which is a concern as bee diversity has been linked to stability of pollination over space and time (Genung *et al.*, 2017; Winfree *et al.*, 2018; Lemanski *et al.*, 2022).

Impact of floral enhancements on wild bees

Plants offer food rewards, in the form of nectar and/or pollen to attract bees. Nectar is the main source of carbohydrates and pollen contains proteins, lipids, amino acids, and vitamins. When a bee visits a flower it either intentionally collects pollen or may accidentally pick it up when consuming nectar. The pollen is transported as bees move among flowers collecting food, facilitating plant reproduction. Bees may collect pollen from many plant species (polylecty) or a small number of plant species (oligolecty). Bees that are multivoltine or social require food throughout the season. In general, longer-lived bees are polylectic, or generalist foragers, gathering pollen and nectar from different plant species throughout the season. The shorter-lived bees may be polylectic or oligolectic, where they feed on a subset of available plants, often from one plant

family. Oligolectic bees' emergence is timed with their preferred floral host(s) (Danforth *et al.*, 2019).

Food availability impacts bee populations (Roulston & Goodell, 2011). Crop visitor richness and visitation rate decreased as distance from natural and semi-natural areas increased (Ricketts *et al.*, 2008; Garibaldi *et al.*, 2011). Floral plantings increased pollinators in adjacent tomato fields in California (Morandin & Kremen, 2013), and increased bee visits to strawberry crop in Scotland (Fletham *et al.*, 2015). Blaauw and Isaacs (2014) found that three years after planting floral enhancements pollination services increased in highbush blueberry in Michigan while floral enhancements increased bee visits to lowbush blueberry four years post-establishment in Maine (Venturini *et al.*, 2017b). Other studies have found floral strips have no impact on crop pollination (Campbell *et al.*, 2017; Nicholson *et al.*, 2020). Three meta-analyses looking at the impacts of floral strips on wild bee abundance and diversity and pollination services (Zamorano *et al.*, 2020; Albrecht *et al.*, 2021; Lowe *et al.*, 2021), found that generally field edge plantings increased pollinator richness and abundance at the field edge (Zamorano *et al.*, 2020; Lowe *et al.*, 2021), but that there was no consistent impact on crop pollination nor yield (Zamorano *et al.*, 2020; Albrecht *et al.*, 2021; Lowe *et al.*, 2021).

One explanation for the variable bee response to floral plantings is that bees are ecologically diverse. Studies examining plant-pollinator interactions in common garden studies, where each plant species is planted in a patch, found that floral preference differed among bee taxa (Tuell *et al.*, 2008; Dibble *et al.*, 2020a, 2020b). Williams *et al.*, (2015) tested wildflower mixes in Florida, Michigan, and California and found floral preference differed among regions. The plant species or mix of plant species used may also impact bee response. In some cases, visits to sown flowers designed to promote bees, were infrequent (Wood *et al.*, 2015, 2017, 2018a; Venturini *et al.*, 2017b; Gresty *et al.*, 2018; Nichols *et al.*, 2019; MacLeod *et al.*, 2020). Venturini *et al.*, (2017b) found 37%

of bumble bee pollen loads were from flowers planted at the edge of lowbush blueberry fields in Maine.

Floral resources in lowbush blueberry agroecosystems

Alternative forage use by wild bees in lowbush blueberry fields has been investigated (Stubbs *et al.*, 1992; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; McCallum & McLean, 2017; Venturini *et al.*, 2017b), using either observation of bee visits to flowers or microscopic analysis of pollen loads. Bumble bees visited buckwheat (*Fagopyrum esculentum* Moench), goldenrods (*Solidago* spp.), brambles (*Rubus* spp.) and rhodora (*Rhododendron canadense* (L.) Torr.) pre- and post-blueberry bloom in New Brunswick (NB) (McCallum & McLean, 2017). Bumble bees often collected pollen from Fabaceae and solitary bees were often observed on Asteraceae in Maine (Venturini *et al.*, 2017b). Bushmann & Drummond (2015) observed wild bees (excluding bumble bees) visiting Rosaceae, Asteraceae, and Ericaceae in Maine. Moisan-Deserres *et al.*, (2014) found that wild bees collected pollen from alder (*Alnus incana* L. (Moench)), brambles, mountain holly (*Ilex mucronata* L.), bog Labrador tea (*Ledum groenlandicum* Oeder), and dandelions (*Taraxacum* spp.) during blueberry bloom in Quebec. Stubbs *et al.*, (1992) provides a list of plants that bees that visited lowbush blueberry during bloom in Maine visited before and after bloom.

Identification of the lowbush blueberry bee community, knowledge of their life history traits, and their plant interactions can help narrow the list of potential floral enhancements. Lowbush blueberry has a diverse wild bee community that has co-evolved with the native crop and the other plant species in the system. Native bees tend to be better pollinators in a crop's natural range (Gibbs *et al.*, 2016) and prefer to forage on native plants (Morandin & Kremen, 2013). The fact that tillage is not used in the system may be an advantage for ground nesters (Svensson *et al.*, 2000), the most common nesting type found in lowbush blueberry fields (Sheffield *et al.*, 2003; Moisan-

DeSerres *et al.*, 2015). These favorable agroecosystem characteristics could make the introduction of floral resources that support bees more successful.

Characterizing plant-pollinator interactions

Understanding the relationship between flowering plants and bees is central for developing strategies to maintain or increase bee abundance and/or diversity and/or pollination services. Plant-pollinator interactions can be recorded through observation when walking a transect or viewing a floral patch for a standard time (Tuell *et al.*, 2008; Williams *et al.*, 2015; Lundin *et al.*, 2019; Dibble *et al.*, 2020a, 2020b). Another approach is to identify the pollen collected by bees. Identification of pollen grains from bee bodies by comparison to a reference pollen library has been used to investigate pollen use in lowbush blueberry fields (Stubbs *et al.*, 1992; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; Venturini *et al.*, 2017b), however, the method is time consuming and requires expertise (Bell *et al.*, 2016; Smart *et al.*, 2017).

Molecular tools to characterize plant-pollinator interactions

DNA barcoding is a process where taxonomic identification is performed by sequencing a short, standardized region of the genome (Hebert *et al.*, 2003). A suitable barcode has a highly conserved region, allowing for amplification and sequencing from a range of taxa while at the same time, has sufficient nucleotide variation to discriminate among taxa, ideally at the species-level, with low intraspecific variation. The barcode of choice in animals is a portion of the cytochrome c oxidase I (COI) mitochondrial gene (Hebert *et al.*, 2003). Sheffield *et al.* (2018), estimated that 95% of the bee fauna of Canada have been barcoded.

It has been difficult to find a universal plant barcode (Hollingsworth *et al.*, 2011).

Portions of the ribulose biphosphate carboxylase large subunit (*rbcl*), maturase (*matK*), internal transcribed spacer 2 (ITS2), trnL intron and intergenic spacer *trnH-psbA* genes

have been used. The performance of each barcode differed, and no single barcode was ideal (Hollingsworth *et al.*, 2009). The Consortium for the Barcode of Life (CBOL) working group proposed using *rbcL* and *matK* as standard plant barcodes (CBOL Plant Working Group, 2009). Others have advocated that ITS2 should be the standard barcode (Yao *et al.*, 2010; Li *et al.*, 2011). A study comparing the performance of ITS2, *rbcL* and *matK* on the vascular plants of Canada found all three barcodes performed well at genus-level assignments (Braukmann *et al.*, 2017). The ITS2 barcode offers two advantages for this work. The first is that the barcode is approximately 350 bp, a length compatible with next generation sequencing (NGS), and secondly it has been used in previous studies where it was found to amplify a range of plant taxa with taxonomic resolution at the genus- and sometimes species-level (Keller *et al.*, 2015; Sickel *et al.*, 2015; Bell *et al.*, 2017). Reliable identification using DNA barcoding depends on a complete and accurate reference database. This has resulted in the creation of international and local reference databases. In Canada, Braukmann *et al.*, (2017) undertook a major project to barcode the vascular plants of Canada “Database of Vascular Plants of Canada (VASCAN)”, available on BOLD (www.boldsystems.org - can access by using the search term "DS-VASCAN", in the public record database).

The identification of mixed samples using a combination of DNA barcoding and NGS is known as DNA metabarcoding. The process of DNA metabarcoding involves extraction of DNA from a mixed sample (such as pollen) and the use of polymerase chain reaction (PCR) and NGS to amplify and sequence many barcodes simultaneously. DNA metabarcoding has been used as a high-throughput method to identify the plant composition of honey (Vere *et al.*, 2017), pollen (Keller *et al.*, 2015; Richardson *et al.*, 2015; Gous *et al.*, 2019), and to create plant-pollinator interaction networks (Macgregor *et al.*, 2019; Arstingstall *et al.*, 2021).

Metabarcoding allows for the floral visits of individual bees to be characterized.

Observations are typically conducted at the group or species-level due to difficulties in

tracking individuals. Metabarcoding has identified more plant-pollinator interactions than visual observations (Pornon *et al.*, 2016, 2017; Arstingstall *et al.*, 2021) or microscopy (Keller *et al.*, 2015; Richardson *et al.*, 2015a; Smart *et al.*, 2017; Macgregor *et al.*, 2019). Studies testing if pollen DNA metabarcoding is quantitative have shown mixed results. Some studies have found a weak relationship between sequencing read number and known pollen taxa composition (Richardson *et al.*, 2015a; Bell *et al.*, 2016, 2018), while others have found quantitation to be accurate (Richardson *et al.*, 2021; Polling *et al.*, 2022), particularly for the most abundant taxa in the pollen (Bänsch *et al.*, 2020). The degree to which pollen grain counts and sequencing read counts agree has been found to depend on the taxonomic composition of the pollen load (Bell *et al.*, 2018; Piñol *et al.*, 2019; Bänsch *et al.*, 2020), the marker used (Richardson *et al.*, 2015b; Bell *et al.*, 2018), and may depend on the level of taxonomic assignment (Richardson *et al.*, 2021). DNA metabarcoding of pollen loads could introduce quantitative biases during the extraction, amplification, and sequencing of DNA (Lamb *et al.*, 2019). Therefore, in this thesis data are presented as presence/absence.

Overview of the structure of thesis and research presented

This thesis is divided into five chapters. In Chapter Two, I investigate associations between plant abundance, richness, and diversity in and around lowbush blueberry fields and bee visits to lowbush blueberry flowers. Chapter Three focuses on the interactions between bees and lowbush blueberry. Chapter Four describes plant-pollinator interactions throughout the season with an emphasis on non-blueberry flowers. References are compiled at the end of the thesis. Data presented in each chapter were collected from the same 28 site-years and sampling dates. On each sampling date (n=204) plant surveys, bee observations, and bee collection occurred. During bloom four 100 m transects were walked and post-bloom one 100 m transect was walked. During bloom there were 77 site-date visits and 308 100 m transect walks. Post-bloom there were 127 site-date visits and 127 100 m transect walks.

There is an appendix with supplementary information for each chapter (Appendices A-C). Large supplementary files and raw data can be found at the following link: <https://doi.org/10.5683/SP3/K6PWUP>, herein referenced as Rutherford (2026). The list of files online can be found in Appendix D.

I use the term “wild bees” throughout the thesis. My definition of wild bees follows that of Bushmann & Drummond (2015), to include all solitary, semi social, and eusocial bees that are free-living and not reared for pollination services. It should be noted that not all wild bees are native. In cases where the term native bee is used, it is because I am referencing a publication where they made a distinction between wild and native bees, or they used different terminology. In some cases, producers had managed *B. impatiens* in the field. It is not possible to differentiate between managed and non-managed *B. impatiens* and so they were counted as wild bees.

I did not measure pollination or fruit yield. At times during the thesis, I state that an increase in bee visits has the potential to increase yield. Mallinger *et al.*, (2021) found increased bumble bee visits to highbush blueberry were correlated with increased yields. Bushmann & Drummond (2020) found that bumble bee and honey bee abundances were significantly correlated to increased fruit set in lowbush blueberry in Maine. Eaton & Murray (1997), also found that fruit set was correlated to total pollinator abundance in three of five years in lowbush blueberry in Maine and Maritime Canada.

Lowbush blueberry fields contain a mixture of *Vaccinium* species with *V. angustifolium* dominating, therefore, I use the Latin name *V. angustifolium* when referencing the crop. Whenever lowbush blueberry **pollen** is discussed, it is at the genus-level. DNA barcoding is not able to distinguish among several species of *Vaccinium*. The ITS2 barcode for *V. angustifolium* and *V. myrtilloides* does not have sufficient barcode gap for species-level identification (for example, see BOLD sequence ID FPOON321-16.ITS2 for *V.*

angustifolium and FPOON051-16.ITS2 for *V. myrtilloides*). In this thesis the term blueberry is used to refer to lowbush blueberry.

Another term that is used throughout the thesis is “flowering plant community”. The flowering plants recorded in surveys did not include grasses, rushes, and sedges. These flowering plants were not recorded because they are difficult to identify and are usually wind pollinated. The pollen data were not filtered to remove grasses, rushes, or sedges.

Objectives and hypotheses

This research aimed to increase our understanding of plant-pollinator interactions in lowbush blueberry agroecosystems. Interactions were characterized using observations of bee visits to flowers and DNA metabarcoding of bee-associated pollen.

The first objective was to determine if plant species richness, abundance or diversity within and at the edge of lowbush blueberry fields is associated with wild bee visits to lowbush blueberry flowers. To achieve this objective, it was necessary to characterize the flowering plant community in and around the fields and observe bee visits to flowers in and at the edge of blueberry fields. I hypothesized that lowbush blueberry fields with more flowering plant species, both in terms of the number of species (richness) and number of bloom (abundance) will have more wild bees, and this in turn will increase wild bee visits to lowbush blueberry flowers both at the field edge and within the field.

The second objective was to determine which wild bees visit lowbush blueberry. I used wild bee species visits to lowbush blueberry and the presence of lowbush blueberry pollen on wild bees as a proxy for pollination. I hypothesized, based on previous research, that wild bees from the genera *Andrena*, *Lasioglossum*, and *Bombus* will be the most common visitors of lowbush blueberry flowers and that lowbush blueberry pollen will be found in pollen loads of bees from those three genera. I was also

interested in investigating if wild bees differed in their fidelity to lowbush blueberry flowers. The number of plant genera found in individual pollen loads was used as an indicator of fidelity. I hypothesized that *Andrena carolina* Viereck, a *Vaccinium* spp. specialist, would have many individuals with pure *Vaccinium* spp. pollen loads. I hypothesized that the other wild bees would have mixed pollen loads and that some would contain *Vaccinium* spp. pollen.

The third objective was to identify the flowering plants, other than lowbush blueberry, that wild bees visit and collect pollen from throughout the season. Bee observations and DNA metabarcoding of bee-associated pollen was used to determine the flowers wild bees visited. I wanted to test if different wild bee families collected pollen from different plant families. I hypothesized that Asteraceae, Fabaceae and Rosaceae would be the most common alternative forages. I also hypothesized that bees from the family Andrenidae would have a higher proportion of individuals with Ericaceae pollen than other wild bee families.

This work will contribute to our understanding of plant-pollinator interactions in lowbush blueberry agroecosystems.

Chapter 2: Do Increased Floral Resources in and Around Lowbush Blueberry Fields Increase Bee Visits to Lowbush Blueberry Flowers?

Abstract

Bees (Hymenoptera: Apoidea) are the main pollinators of lowbush blueberry (*Vaccinium angustifolium* Aiton, Ericales: Ericaceae). This research aimed to determine if there were associations between the plant community within and around lowbush blueberry fields and bee visits to lowbush blueberry flowers in Maritime Canada. Twenty-eight site-years were surveyed for plant richness, diversity, and abundance. Bee group visits to flowers were recorded during transect walks at field edge and within field during bloom and at field edge post-bloom. Correlation analyses were used to evaluate associations between plant richness, abundance, and diversity, and bee group visits to lowbush blueberry at field edge and within field. Honey, andrenid, and bumble bees were the most frequent bee groups observed. During bloom, 93% of bee visits recorded were to blueberry. During bloom, andrenid bee visits to blueberry were positively correlated to plant richness and diversity in the field. Bumble bee visits to blueberry were positively correlated to the mean bloom abundance (excluding blueberry) in the field. Other wild bee visits to blueberry were positively correlated to bloom abundance in the fall. Total wild bee visits to blueberry were not significantly different among sites. Bees were observed on 55 of the 113 plant taxa recorded. Bees were observed on a subset of flowering species which were common to most sites and therefore specific floral resources may be more important in supporting wild bees than the number of plant taxa.

Introduction

Many plants depend on insect pollinators, especially bees, for reproduction. Wild bees contribute to the pollination of many crops (Reilly *et al.*, 2020), and are often more effective and/or efficient crop pollinators than the commonly used western honey bee (*Apis mellifera* Linnaeus, Hymenoptera: Apidae) (Garibaldi *et al.*, 2013). Proximity to natural or semi-natural landscapes (Ricketts *et al.*, 2008) and availability of flowering resources (Williams *et al.*, 2015) are important factors in supporting the wild bees that contribute to crop pollination.

Lowbush blueberry (*Vaccinium angustifolium* Aiton, Ericales: Ericaceae) is a naturally occurring shrub in northeastern North America. In Maine and eastern Canada, the crop is managed on a two-year production cycle, alternating between a vegetative year when pruned plants grow and produce new shoots and buds, and a fruit year when buds produce flowers and berries. Fruit production is increased with cross-pollination (Wood, 1968; Hall *et al.*, 1979; Usui *et al.*, 2005). Wood *et al.* (1968), found fruit set increased significantly from 42% with self-pollination to 67% with controlled cross-pollination. Lowbush blueberry agroecosystems have a diverse community of wild bees known to be crop pollinators (Finnamore & Neary, 1978; Javorek *et al.*, 2002; Sheffield *et al.*, 2003; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015, 2020; Cutler *et al.*, 2015; Drummond, 2016).

Non-crop plants at the edge of or within lowbush blueberry fields are considered weeds. Weeds reduce blueberry yields (Yarborough *et al.*, 2017), and can interfere with harvest (Jensen & Specht, 2002). However, non-crop flowers can benefit bees. Different habitat types around lowbush blueberry fields predicted wild bee abundance; some habitats, such as deciduous and mixed forests, were correlated to an increase in abundance of wild bees in the field (Groff *et al.*, 2016). Increased semi-natural habitat at 1000 and 2000 m outside of highbush blueberry fields (*Vaccinium corymbosum* L.) in Vermont, United States of America (USA), increased wild bee crop visitation, bee abundance, and

species richness (Nicholson *et al.*, 2017). As agriculture cover increased in the area immediately (300 m) outside of highbush blueberry fields in Southern New Jersey, USA, wild bee pollination services decreased (Benjamin *et al.*, 2014). Yarborough *et al.* (2017) found that woody weeds within the field in the vegetative year increased wild bee density in the fruit year. Drummond *et al.*, (2017a) found that increased wildflower diversity (as measured by Shannon-Wiener diversity) at the field edge increased native bee foraging density in lowbush blueberry fields in Maine, USA. Targeted floral plantings next to lowbush blueberry fields in Maine (Venturini *et al.*, 2017), increased crop visitation rates four years post-establishment. Highbush blueberry fields adjacent to perennial wildflower plantings had significantly greater wild bee visits to the crop and increased fruit set compared to control fields three years post-establishment in Michigan, USA (Blaauw & Isaacs, 2014). Other studies found no differences in wild bee visits to highbush blueberry between fields with enhanced and unenhanced borders in Michigan (Wood *et al.*, 2018a) and Oregon, USA and Michigan (Nicholson *et al.*, 2020).

The objectives of this research were to determine if there were associations between floral richness, abundance, or diversity within, at the edge, or outside of lowbush blueberry fields and bee visits to lowbush blueberry flowers. To accomplish this objective the flowering plant community was surveyed along transects within lowbush blueberry fields during bloom and at the edge of the field over the season. The area outside of the field was surveyed in two of three sampling years. Interactions between bee groups and plant species over the season were recorded. It was hypothesized that a positive association would be found between plant richness, abundance and diversity and bee visits to lowbush blueberry flowers. I expected that increased flowers would provide habitat and food for wild bees which would increase wild bee abundance and thus increase bee visits to the crop.

Methods

Site description

During the 2017, 2018, and 2019 seasons, data were collected from sixteen, nine, and three commercial lowbush blueberry fields respectively, from the provinces of New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PEI) (Fig. 2.1). The three NS sites sampled in 2019 had been sampled in 2017 (28 site-year). Site locations can be found in Fig. 2.1 and Supplementary Table 2.1.

Sites were selected that differed in terms of the plant community at the field edge (i.e., some had little plant diversity at the edge, while others were adjacent to meadows). Sampling began at the start of blueberry bloom and continued every two to three weeks throughout the growing season (early May to late October). Four, 100 m x 2 m transects were established at each site: one at the field edge (0 m) and one at each of 25, 50, and 100 m increments into the field, parallel to the 0 m transect. During blueberry bloom, transect walks ($20 \text{ m}^{-2} \text{ min}^{-1}$) were conducted for each of the four transects. After bloom, data were collected at the field edge only. During bloom there were 77 site-date visits and 308 100 m transect walks. Post-bloom there were 127 site-date visits and 127 100 m transect walks.

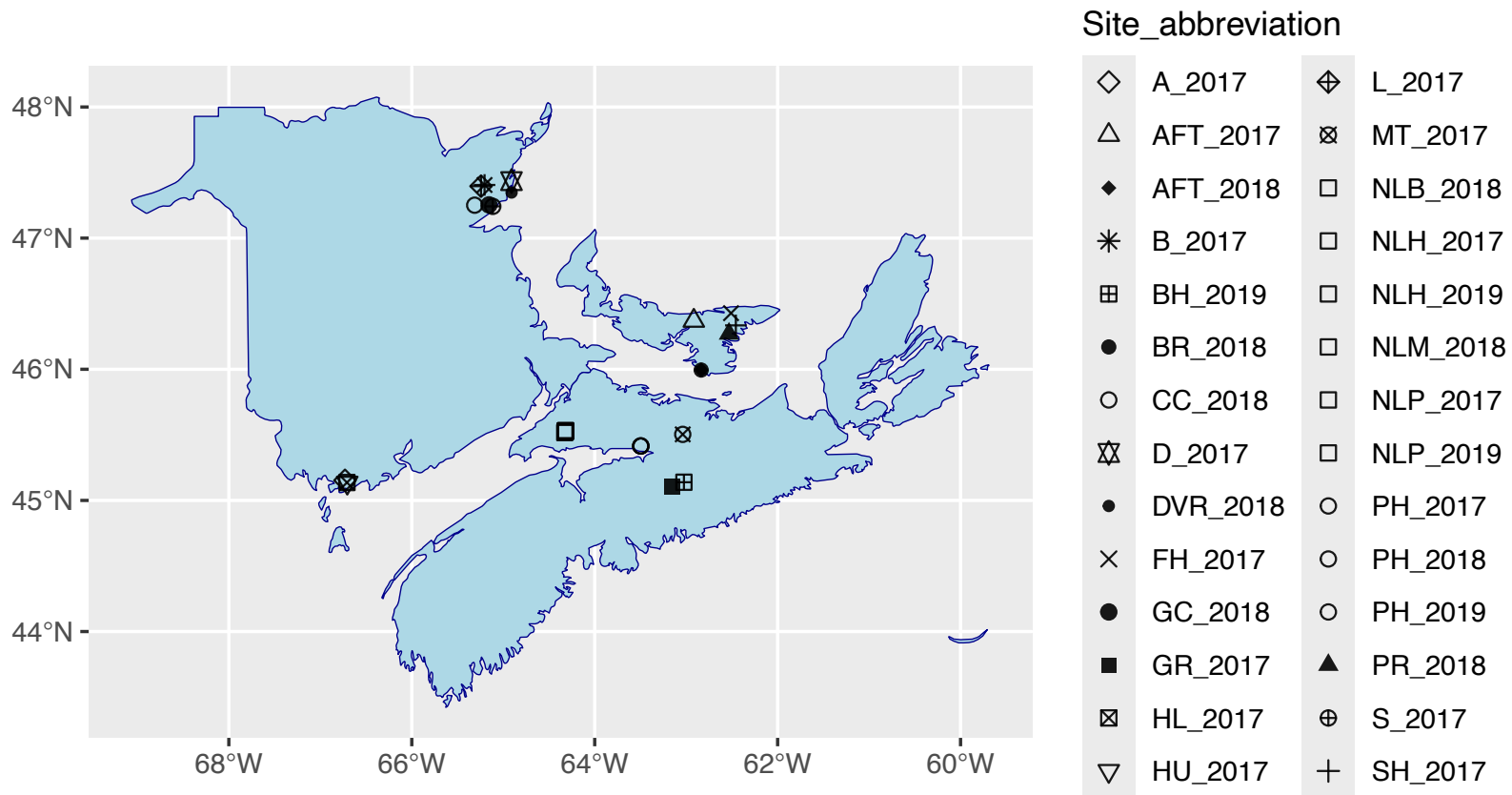


Fig 2.1. Map showing locations of lowbush blueberry sites sampled.

Bee observations

Bees were observed between 1000 and 1600 h on days where the temperature was $\geq 14^{\circ}\text{C}$ with low windspeeds, no precipitation, and at least partial sun. Attempts were made to vary the time of day and order of transect walks. During transect walks every observed bee visit to a flower was recorded. Plants were identified as described below. Bees were assigned to one of six groups: (1) honey bees; (2) bumble bees; (3) andrenid bees; (4) metallic bees; (5) small black bees; (6) other. For statistical analyses the less frequently observed metallic, small black, and other bees were combined into an “other wild bees” group.

Flowering plant community survey

The plant community was characterized by recording all flowering plants and their approximate abundance in the transects (all three seasons) and in the area outside the field adjacent to the 0 m transect (2018 & 2019 seasons only). Plants were identified to species where possible, with genus-level identification for: *Galium* spp., *Hieracium* spp., *Oxalis* spp., *Potentilla* spp., *Rosa* spp., *Rubus* spp., *Stellaria* spp., *Viola* spp., and *Vaccinium* spp. *Solidago* spp. and *Euthamia* spp. were grouped as “goldenrods”. Plant naming was according to the United States Department of Agriculture (USDA) PLANTS database available at: <https://plants.usda.gov/>. Plant abundance was estimated using a floristic index defined by Carvell *et al.*, (2004) using the following categories: (1) rare (1–25 flowers); (2) occasional (26–200 flowers); (3) frequent (201–1000 flowers); (4) abundant (1001+ flowers); (5) super abundant (>5000 flowers). A flower “unit” was defined as an inflorescence. Mean flower abundance was calculated by averaging the median flower number from the floristic index category.

Statistical analyses

Interaction data were divided into three time-points: (1) blueberry bloom (17 May - 29 June); (2) summer (30 June - 31 Aug.) and (3) fall (1 Sept. - 19 Oct.). Graphs were

generated in R (R Core Development Team) using the R package ggplot2 v3.1.0 (Wickham *et al.*, 2020). The number of bees from each bee group visiting lowbush blueberries (bees $20 \text{ m}^{-2}\text{min}^{-1}$) at the field edge (0 m) and the mean number of each bee group visiting blueberries in the field (average of 25, 50 and 100 m transects) were calculated for each site x sampling date during bloom.

Plant richness (number of taxa), mean abundance, and Shannon's diversity index (H) were calculated for each site x sampling date during bloom for the field edge (0 m), the field (mean of 25, 50 and 100 m transects, herein referred to as within the field) and area outside the field (2018 & 2019 seasons only), as well as for each site over the summer and fall (0 m only) using R package vegan v2.5-7 (Oksanen *et al.*, 2020).

Spearman's rank order correlation, calculated using R package Hmisc v4.1.1 (Harrell, 2020) was used to test if plant richness and the mean abundance of bloom at the field edge were correlated within each season (bloom, summer, and fall) and among seasons ($n=21$) at $\alpha=0.05$. Spearman's rank order correlation ($\alpha=0.05$) was also used to test associations between bee group visits to blueberry and plant metrics. Some sites were removed prior to correlation analysis due to: (1) few bee observations (<6 wild bees over the bloom period, $n=3$) or (2) because data were available for only one sampling date during bloom ($n=4$), leaving 21 site-years. Seventy-two planned correlations were performed for the bloom period to test if the number of andrenid, bumble, honey or "other wild bees" visiting lowbush blueberry at the field edge or within the field were correlated to plant richness, abundance of bloom, or H at the field edge ($n=54$), within the field ($n=54$), or the area outside the field ($n=23$). Forty-eight correlations were performed to test if the number of andrenid, bumble, honey or "other wild bees" visiting lowbush blueberry at the field edge or within the field during bloom was correlated to plant richness, mean abundance, or H at the field edge over the summer ($n=21$) or fall ($n=21$).

A generalized linear model (PROC GLIMMIX; SAS v9.4; negative binomial distribution, log link) was used to test for differences among sites for wild bee visits to blueberry during bloom. Visits were averaged over transects and dates were used as reps.

Results

Bee observations

Bees from each of the six bee groups were observed visiting flowers in lowbush blueberry agroecosystems (Fig. 2.2). Bumble bees and small black bees were observed at all site-years, while andrenid bees and honey bees were observed at 27 of the 28 site-years. Metallic bees and other bees were less frequent and found at 13 and 20 site-years, respectively. A list of the bee groups observed at each site-years is presented in Supplementary Table S2.2. Andrenid bees were almost exclusively observed during blueberry bloom with very few observations post-bloom (Fig. 2.2). Bumble bees were less common during bloom and numbers increased into the summer months (Fig. 2.2). Honey bee numbers peaked during bloom, when hives are brought into the field for pollination services (Fig. 2.2). Honey bees observed in the post-bloom period were primarily from sites where the blueberry producer was also a beekeeper and therefore bees were present at the property throughout the season. There were three sites each year for which this was the case. Metallic, small black, and other bees were observed in small numbers throughout the season (Fig. 2.2).

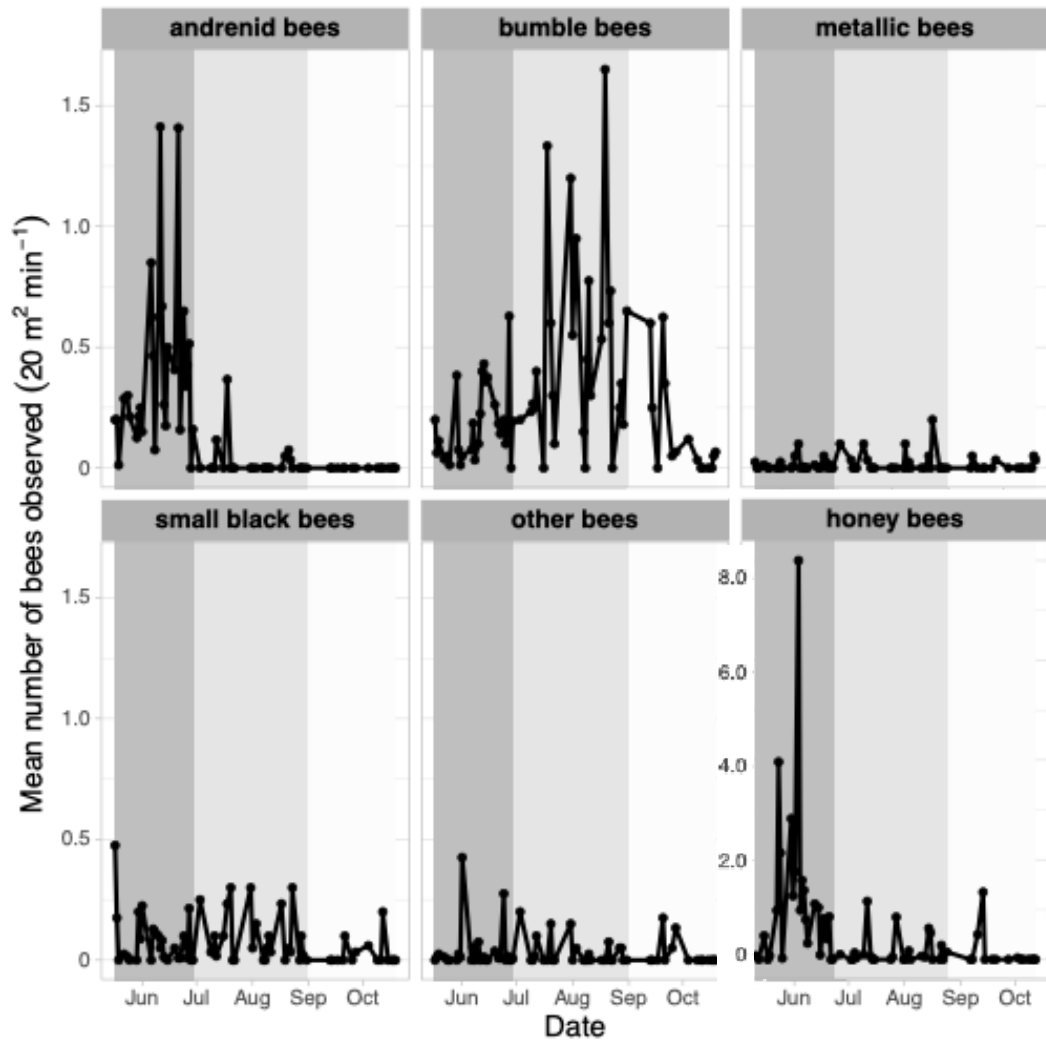


Fig. 2.2. Mean number of bees observed at lowbush blueberry (*Vaccinium angustifolium* Aiton) sites over the season by bee group. The mean number of observed bees from each of the six bee groups was calculated for each Julian calendar date (JCD) over the season for 28 site-years. The shaded background of the plots represents the three seasons: (1) grey, bloom (JCD 137-180; 17 May –29 June); (2) light grey, summer (JCD 181-243; 30 June – 31 Aug.) and (3) white, fall (JCD 244-292; 1 Sept. 19 Oct.). Note that the y-axis scale for the honey bee plot is different than the y axis scale for the other five bee groups.

Flowering plant community survey

In general, the number of flowering plant species peaked during the summer months (Fig. 2.3). Sites with more plant taxa during the bloom period had more plant taxa in the summer ($r_s(19)=0.72$, $P<0.001$) and fall ($r_s(19)=0.56$, $P=0.008$). The number of plant taxa in the summer and fall were also correlated ($r_s(19)=0.79$, $P<0.001$). Mean plant richness and mean bloom abundance were correlated during bloom ($r_s(19)=0.52$, $P=0.015$), however, mean plant richness and the mean abundance of bloom at the field edge over the summer and fall, were not significantly correlated.

Common non-blueberry taxa flowering at the field edge during bloom were: bunchberry (*Cornus canadensis* L.; 18 sites), violets (*Viola* spp.; 15), common sheep sorrel (*Rumex acetosella* L.; 14), common dandelion (*Taraxacum officinale* F.H. Wigg.; 11), rhodora (*Rhododendron canadensis* (L.) Torr.; 11), hawkweeds (*Hieracium* spp.; 11), and cinquefoils (*Potentilla* spp.; 10). In the summer months, common plants at the field edge were: goldenrods (23 sites), common St. John's Wort (*Hypericum perforatum* L.; 18), brambles (*Rubus* spp.; 13), sheep laurel (*Kalmia angustifolia* L.; 12) parasol whitetop (*Doellingeria umbellata* (Mill.) Nees; 12), and hawkweeds (16). In the fall goldenrods (21), parasol whitetop (8), calico aster (*Symphyotrichum lateriflorum* (L.) A. Love & D. Love; 9), and New York aster (*Symphyotrichum noveli-belgii* (L.) G.L. Nesom; 5) were common.

The average number of non-blueberry flowering species in the field during bloom per site was three (Fig. 2.3). At some sites, there was an abundance of non-blueberry taxa in the field (Fig. 2.4, i.e., FH 2017, HL 2017, MT 2017). Non-blueberry flowers in the field during bloom included: common sheep sorrel (19 sites), bunchberry (17), violets (11), cinquefoils (9), and hawkweeds (9).

Surveys of the area outside of the field were completed in 2018 and 2019 (a total of 12 sites). Some sites had few plants blooming outside of the field while others had many

co-flowering plants (Fig. 2.3). Flowering plants recorded in the area outside of the field during bloom included: hawkweeds (12 sites), goldenrods (12), cinquefoils (10), vetch (*Vicia cracca* L.; 10), common St. John's Wort (10), and bunchberry (10). In the summer, hawkweeds (12 sites), goldenrods (11), vetch (10), cinquefoils (19), common St. John's Wort (10), and fleabane (*Erigeron* spp.; 9) were frequent. In the fall, goldenrods were found at all sites (12). Calico aster (8), and parasol whitetop (8) were also frequent. The complete plant survey can be found in Rutherford (2026).

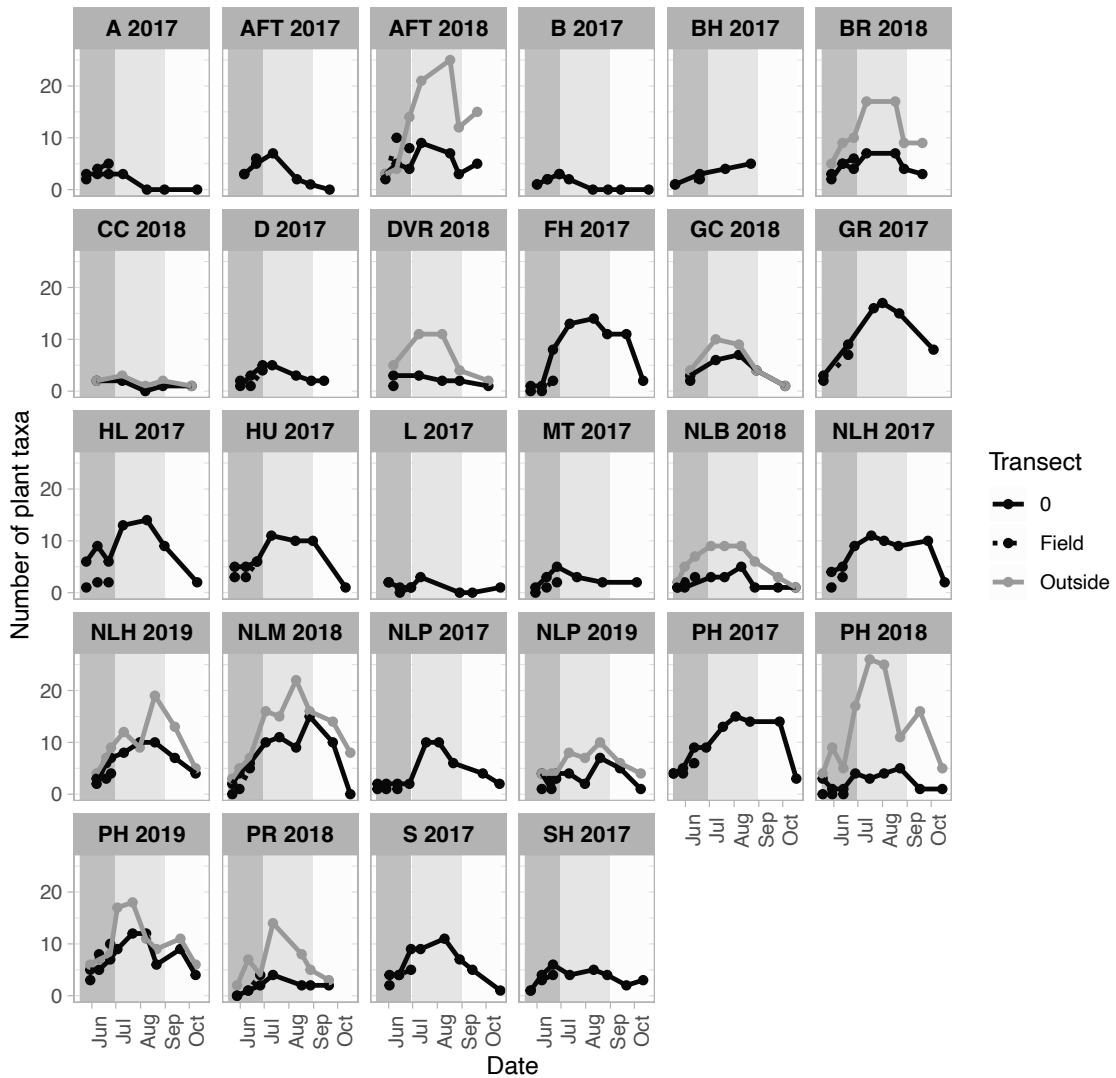


Fig. 2.3. Plant species richness in and around lowbush blueberry (*Vaccinium angustifolium* Aiton) fields. The number of non-blueberry plant taxa for each site-years at the field edge (0 m transect) over the season, in the field (mean of 25, 50 and 100 m transects) during bloom, and the area outside the field (in 2018 and 2019 only) over the season. The shaded background of the plots represents the three seasons: (1) grey, bloom (JCD 137-180; 17 May - 29 June); (2) light grey, summer (JCD 181-243; 30 June - 31 Aug.) and (3) white, fall (JCD 244-292; 1 Sept. - 19 Oct.).

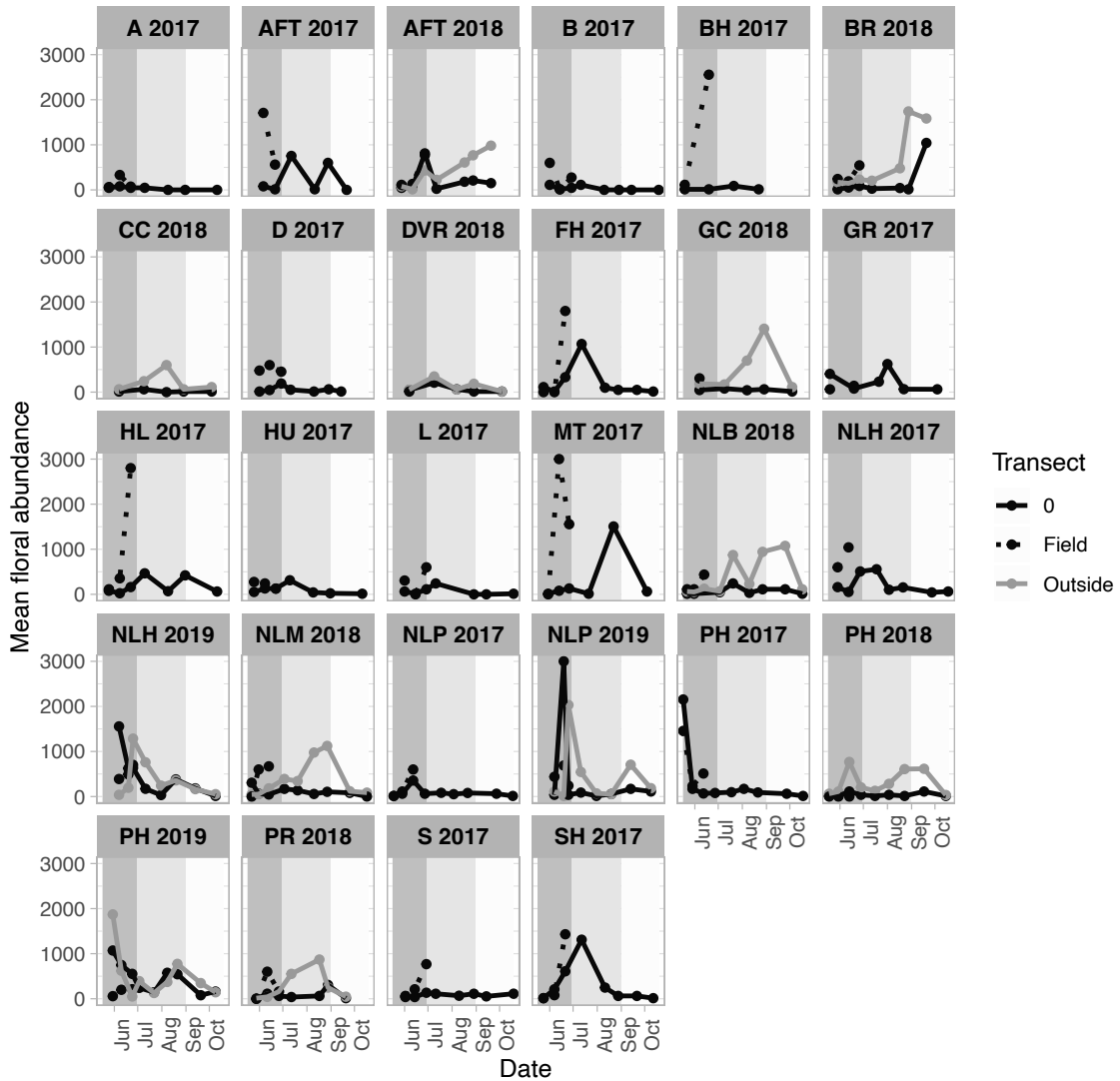


Fig. 2.4. Mean floral abundance in and around lowbush blueberry (*Vaccinium angustifolium* Aiton) fields. The mean abundance of non-blueberry plant bloom as calculated by averaging the median floristic index for each site-years at the field edge (0 m transect) over the season, in the field (mean of 25, 50 and 100 m transects) during bloom, and the area outside of the field (in 2018 & 2019 only) over the season. The shaded background of the plots represents the three seasons: (1) grey, bloom (JCD 137-180; 17 May - 29 June); (2) light grey, summer (JCD 181-243; 30 June - 31 Aug.) and (3) white, fall (JCD 244-292; 1 Sept. - 19 Oct.).

Summary of bee visits to flowers

In total 4,637 bee visits to flowers were recorded during blueberry bloom, which averaged 1.8 bees 20 m⁻² min⁻¹. Lowbush blueberry represented the bulk of total floral visits (93%) and floral visits for each bee group: andrenids (95%), honey bees (95%), bumble bees (90%), other bees (82%), metallic bees (74%), and small black bees (74%). Non-blueberry visits (312 total; 0.2 bees 20 m⁻² min⁻¹) were infrequent.

During summer, 740 bees were observed which averaged to 0.9 bees 20 m⁻² min⁻¹. All bee groups were observed: bumble bees (56.7%), honey bees (22.8%), small black bees (10.8%), andrenids (4.5%), other bees (3.6%), and metallic bees (1.6%). Honey bees are typically removed from blueberry fields post-bloom. Honey bee observations dropped from an average of 1.0 bee 20 m⁻² min⁻¹ during bloom to 0.2 bees 20 m⁻² min⁻¹ in the summer. Seventy percent of the summer honey bee observations were at sites where the blueberry producer was also a honey bee keeper.

During fall, 146 bees were observed, which averaged to 0.4 bees 20 m⁻² min⁻¹. Fall bees included: bumble bees (42.5%), honey bees (41.1%), other bees (8.2%), small black bees (5.5%), and metallic bees (2.7%). No andrenid bees were observed during the fall. All honey bee observations during the fall were at honey bee keeper sites.

A total of 113 plant taxa were recorded over the project. Bees were observed on approximately half of the plants (55) which belonged to 20 of the 32 plant families recorded. Four plant families accounted for >95% of the observed interactions (n=5,523): Ericaceae (80%), Asteraceae (11%), Fabaceae (3%), and Rosaceae (3%).

Correlation between flowers and bees

Correlation between bee group visits to lowbush blueberry and plant richness, abundance, and diversity were evaluated. Plant data were parsed into three seasons: (1) bloom, (2) summer, and (3) fall, and three locations: (1) field edge, (2) within the field,

and (3) outside of the field. Bee group visits to blueberry were divided into visits recorded at (1) the field edge, and (2) within the field. Flowers blooming during summer and fall would not impact bee visits to the crop that season. The logic for the post-season correlations was that season-long bees, like bumble bees, would benefit from summer and fall resources and would increase their visits to lowbush blueberry in subsequent seasons. Given the time-line and two-year production cycle, correlations between summer and fall plant metrics and wild bee visits to the crop in the subsequent bloom was not feasible. The assumption was made that the plant metrics in the summer and fall of the current season were similar to two years previous.

Bloom

During the bloom period there were no significant correlations between bee groups visiting blueberry flowers and plant richness, mean bloom abundance or H for the field edge or the area outside of the field (Table 2.1). The mean number of andrenids observed on blueberry flowers in the field was correlated to plant richness in the field and to H in the field (Table 2.1). The mean number of bumble bees observed on blueberry flowers in the field was correlated to floral bloom abundance in the field (Table 2.1). The average number of wild bees observed visiting blueberry flowers during bloom was not significantly different among sites ($P=0.11$).

Post-bloom; summer

The number of andrenids observed on blueberry flowers at the field edge during bloom was correlated to plant richness at the field edge and to H at the field edge in the summer (Table 2.2). Interestingly, the numbers of “other wild bees” observed on blueberry flowers at the field edge and in the field and the numbers of honey bees observed on blueberry flowers at the field edge were negatively correlated to the mean abundance of plant bloom at the field edge during the summer (Table 2.2).

Post-bloom; fall

The number of andrenids visiting lowbush blueberry flowers at the field edge was positively correlated to the number of plant taxa at the field edge and to the floral abundance at the field edge in the fall (Table 2.3). The mean number of “other wild bees” observed on lowbush blueberry flowers in the field and at the field edge were positively correlated to the abundance of bloom at the field edge in the fall.

Table 2.1. Correlation results for bloom. Spearman's rank correlation between bee group visits (andrenids (An), bumble bees (BB), honey bees (HB), and "other wild bees" (OWB; a group containing metallic, small back, and other bees) to lowbush blueberry (*Vaccinium angustifolium* Aiton) within the field (mean of bee visits to blueberry in the 25, 50, and 100 m transects) and at the field edge (0 m) and plant richness (number of plant taxa), Shannon's diversity (H), and mean floral bloom abundance at the field edge (0 m), within the field, and in the area outside the field during bloom (17 May - 29 June). Numbers indicate Spearman's ranked correlation coefficient (r_s), with P -value in brackets. Significant correlations ($\alpha \leq 0.05$) are shown in bold.

	An 0 m	An field	BB 0 m	BB field	HB 0 m	HB field	OWB 0m	OWB field
0 m taxa	0.14 (0.28)	0.22 (0.11)	0.049 (0.72)	0.12 (0.37)	-0.043 (0.76)	0.13 (0.31)	-0.073 (0.60)	0.029 (0.83)
0 m floral abundance	0.20 (0.13)	0.15 (0.27)	0.036 (0.98)	0.082 (0.55)	0.021 (0.87)	-0.027 (0.84)	0.038 (0.78)	0.0064 (0.96)
0 m H	0.10 (0.46)	0.16 (0.25)	0.12 (0.37)	0.084 (0.54)	0.018 (0.90)	0.16 (0.26)	-0.046 (0.74)	-0.034 (0.81)
Field taxa	0.16 (0.26)	0.33 (0.014)	0.0026 (0.98)	0.096 (0.49)	-0.018 (0.90)	0.12 (0.39)	0.012 (0.93)	0.17 (0.22)
Field floral abundance	0.0047 (0.97)	0.050 (0.72)	0.13 (0.35)	0.33 (0.014)	-0.100 (0.47)	0.029 (0.83)	-0.17 (0.21)	0.035 (0.80)
Field H	-0.050 (0.72)	0.31 (0.024)	-0.069 (0.62)	0.067 (0.63)	-0.13 (0.36)	0.0057 (0.97)	-0.12 (0.40)	0.22 (0.11)
Outside the field taxa	-0.022 (0.92)	0.14 (0.53)	-0.075 (0.73)	0.098 (0.65)	-0.14 (0.52)	0.021 (0.92)	-0.16 (0.46)	-0.59 (0.79)
Outside the field floral abundance	-0.27 (0.21)	-0.22 (0.31)	-0.065 (0.77)	0.033 (0.88)	-0.0035 (0.99)	0.017 (0.94)	-0.094 (0.67)	-0.27 (0.22)
Outside the field H	0.058 (0.79)	0.21 (0.34)	-0.0041 (0.99)	0.091 (0.68)	0.00025 (0.99)	0.19 (0.38)	-0.013 (0.95)	0.27 (0.22)

Table 2.2. Correlation results for summer. Spearman’s rank correlation between bee group visits (andrenids (An), bumble bees (BB), honey bees (HB), and “other wild bees” (OWB; a group containing metallic, small back, and other bees) to lowbush blueberry (*Vaccinium angustifolium* Aiton) within the field (mean of bee visits to blueberry in the 25, 50, and 100 m transects), and at the field edge (0 m) and plant richness (number of plant taxa), Shannon’s diversity (H), and mean floral bloom abundance at the field edge (0 m) during the summer (30 June - 31 August). Numbers indicate Spearman’s ranked correlation coefficient (r_s), with P -value in brackets. Significant correlations ($\alpha \leq 0.05$) are shown in bold.

	An 0 m	An field	BB 0 m	BB field	HB 0 m	HB field	OWB 0 m	OWB field
0 m taxa	0.50 (0.020)	0.049 (0.83)	0.073 (0.75)	-0.082 (0.72)	0.035 (0.88)	0.16 (0.48)	0.15 (0.52)	0.30 (0.18)
0 m floral abundance	-0.065 (0.78)	0.24 (0.30)	-0.26 (0.26)	-0.28 (0.22)	-0.44 (0.047)	-0.044 (0.85)	-0.57 (0.0069)	-0.56 (0.0086)
0 m H	0.48 (0.028)	-0.17 (0.45)	0.30 (0.19)	0.22 (0.33)	0.32 (0.16)	0.17 (0.47)	0.51 (0.018)	0.47 (0.033)

Table 2.3. Correlation results for fall. Spearman’s rank correlation between bee group visits (andrenids (An), bumble bees (BB), honey bees (HB), and “other wild bees” (OWB; a group containing metallic, small back, and other bees) to lowbush blueberry (*Vaccinium angustifolium* Aiton) within the field (mean of bee visits to blueberry in the 25, 50 and 100 m transects), and at the field edge (0 m) and plant diversity (number of plant taxa), Shannon’s diversity (H), and mean floral bloom abundance at the field edge (0 m) during the fall (1 September - 19 October). Numbers indicate Spearman’s ranked correlation coefficient (r_s), with P -value in brackets. Significant correlations ($\alpha \leq 0.05$) are shown in bold.

	An 0 m	An field	BB 0 m	BB field	HB 0 m	HB field	OWB 0 m	OWB field
0 m taxa	0.48 (0.028)	0.034 (0.88)	0.23 (0.32)	0.26 (0.26)	-0.0022 (0.99)	0.11 (0.62)	0.097 (0.68)	0.13 (0.59)
0 m floral abundance	0.61 (0.0033)	0.15 (0.53)	0.27 (0.24)	-0.16 (0.50)	0.37 (0.099)	0.37 (0.10)	0.53 (0.013)	0.46 (0.036)
0 m H	0.17 (0.45)	-0.086 (0.71)	0.20 (0.39)	0.39 (0.084)	-0.15 (0.52)	-0.064 (0.78)	-0.11 (0.52)	0.035 (0.88)

Discussion

Bee groups observed visiting flowers in lowbush blueberry agroecosystems were similar to those reported in previous bee surveys of lowbush blueberry fields in NS (Sheffield *et al.*, 2003; Cutler *et al.*, 2015), Quebec (Moisan-Deserres *et al.*, 2014; Moisan-DeSerres *et al.*, 2015b), and Maine (Stubbs *et al.*, 1992; Bushmann & Drummond, 2015). Bee phenology (Fig. 2.2) matched previous work (Cutler *et al.*, 2015).

Many of the plant taxa recorded in this study have been previously documented in and around lowbush blueberry fields in Maine (Stubbs *et al.*, 1992; Drummond *et al.*, 2017a). A recent weed survey of lowbush blueberry fields in NS identified 211 weeds, of which 139 were herbaceous or woody perennials (Lyu *et al.*, 2021). This study identified 113 flowering plants with considerable overlap to Lyu *et al.* (2021), indicating the flowering plant community in these fields is similar to other lowbush blueberry fields in NS. Drummond *et al.* (2017a) found an average of 11 taxa per site in lowbush blueberry fields in Maine. Like this plant survey, Drummond *et al.* (2017a) found the number of taxa increased over the season (from May to July) with no correlation between the number of flowering plant species and floral density. In this study, both plant richness and the mean floral abundance at the field edge were lower in the early spring, peaked in early summer, and decreased in late summer into the fall (Figs. 2.2 & 2.3). This flowering phenology has been observed in other work. For example, Wood *et al.* (2018), found that the short-season bees that pollinate tart cherry and highbush blueberry in Michigan, USA were not able to make use of herbaceous flowering strips and were collecting pollen from woody spring plants. Nichols *et al.* (2022) tested various seed mixes and suggested that more research is needed on what plants could support early flying spring bees. Similarly, Timberlake *et al.* (2019) investigated floral phenology and found that nectar was in short supply in early spring and late summer.

Non-blueberry flowers within lowbush blueberry fields during bloom was positively correlated to andrenid bee and bumble bee visits to lowbush blueberry (Table 2.1). Yarborough *et al.* (2017) found that an increase in woody weed abundance in the sprout year increased wild bee densities in the fruit year. Flowers present in the field might be attracting bees, serving as alternative forages.

Andrenid bee visits to lowbush blueberry at the field edge were positively correlated with plant richness and H at the field edge over the summer. Although andrenid bees were not often observed during the summer (Fig. 2.2), a few were observed in late June and early July. It is possible that they benefit from flowers at the site just after blueberry bloom. “Other wild bee” visits to lowbush blueberry in the field and field edge was negatively correlated to floral abundance in the summer (Table 2.2). This group of bees (a combination of metallic, small black and other bees) had a lower proportion of crop visits, albeit more than three-quarters of observations were to blueberry. It is possible that an increase in alternative forages distracts these groups from the crop (Nicholson *et al.*, 2019). Another study (Guezen & Forrest, 2021), reported that *Megachile* spp. visits to fruit and vegetable crops were negatively associated with non-crop floral abundance which they attributed to pollinator movement or dilution.

The positive correlation between andrenid bees and fall floral resources was interesting as andrenid bees were not observed during the fall (Fig. 2.2), therefore, it is unlikely that they benefit from floral resources at this time. This positive correlation might be because there was a positive correlation between plant taxa during the fall with both plant taxa during bloom and summer. Diversity and abundance of plants in the fall had a positive impact on “other wild bee” visits to lowbush blueberry (Table 2.3). In social bees, new queens emerge and mate in the late summer to fall, overwinter, and then re-emerge the following spring to start a colony. Red clover blooming late in the season increased bumble bee queen and male densities in Sweden (Rundlöf *et al.*, 2014). Dicks *et al.*, (2015) found that spring pollen was important for colony formation and late

summer pollen was important for continued rearing of larvae in bumble bees in England. Nectar supply during September was found to be a strong predictor of *B. terrestris* L. colony density in the following year in the United Kingdom (Timberlake et al, 2021). Some of the bees in the “other wild bee” group were season-long bees (Fig. 2.2) that, like bumble bees, would benefit from fall resources and would increase their visits to lowbush blueberry in subsequent seasons.

The few significant correlations indicated that specific bee group visits to lowbush blueberry changed depending on plant richness, abundance, and diversity. The fact that all bee groups did not respond the same suggests that further research should not group wild bees together; a view also supported by the work of (Guezen & Forrest, 2021).

In many cases, plant richness, abundance of bloom, and diversity were not correlated to wild bee groups visiting lowbush blueberry flowers. Examination of the plant-pollinator interactions reveals that approximately half the plant species and two thirds of the plant families recorded at the sites were visited by bees. This suggests that increasing species richness and/or abundance is not sufficient. Testing if specific flowering plants increase wild bee visits to blueberry flowers might be more appropriate. For example, Campbell *et al.* (2017), found that wild insect visits to crop flowers in cider apple orchards was positively related to dandelion abundance in the laneways. Warzecha *et al.*, (2018), tested four seed mixes in Germany and found that the presence of key plant species was more important for attracting wild bees and hoverflies than plant diversity. In this study, plants from the families Ericaceae, Asteraceae, Fabaceae, and Rosaceae were visited most often and were utilized by many bee groups. These plant families have been suggested as alternative forages for lowbush blueberry bees (Bushman & Drummond, 2015; McCallum & McLean, 2017; Moisan-Deserres *et al.*, 2014; Stubbs *et al.*, 1992) and further testing on interactions with plants from these families is needed.

There was no difference in the number of wild bees visiting lowbush blueberry among sites during bloom. Previous studies found increased bee visits to highbush blueberry (Blaauw & Isaacs, 2014) and lowbush blueberry (Venturini *et al.*, 2017) three to four years post-floral enrichment. The 21 site-years used in this analysis may not have had enough contrast in the bee and plant communities to detect relationships. Two meta-analyses evaluating agri-environmental schemes in Europe found that the most successful enhancements to increase species richness (Mrja *et al.* 2019) and species richness and abundance (Scheper *et al.* 2013), were when the enhancements created contrast (i.e., the addition of a diverse, dense flower patch in a simple landscape that is limited in resources).

Other studies have also found that increasing floral resources do not reliably increase bee visits to a crop. A study looking at four crops (including highbush blueberry) found that increasing floral resources did not always increase bee visits to the crop (Nicholson *et al.*, 2020). Similarly, Wood *et al.* (2018), found that floral enhancements did not increase bee visits to highbush blueberry or cherry flowers in Michigan, USA. Another possibility is that bees observed in and around lowbush blueberry fields have a strong association with the crop. Fijen *et al.* (2019) found that pollinator response to landscape complexity in leek fields depended on the bees' association with the crop, with dominant pollinators being less dependent on semi-natural habitats.

Plant data from the area outside of the field may have revealed more information on the plant-pollinator interactions at each site. Bobiwash *et al.* (2018) found that bees collected in highbush blueberry fields carried pollen from plant species outside of the field. Benjamin *et al.* (2014) found that as agricultural land around highbush blueberry fields in Michigan, USA, increased, pollination services by wild bees decreased. Correlations were not performed on plant data from the area outside of the field in the summer and fall due to low sample sizes. Other factors that impact wild bee communities, which were not considered in this study, include pesticide use (Tuell &

Isaacs, 2010), nesting site availability (Nicholson *et al.*, 2019), and field size (Stubbs *et al.*, 1992; Eaton & Murray, 1997; Isaacs & Kirk, 2010).

Conclusions

Flowering plants within the field during bloom increased andrenid and bumble bee group visits to lowbush blueberry flowers. This suggests that non-blueberry flowers in the field increase some bee group visits to the crop and that a monoculture of lowbush blueberry may not maximize pollination. Floral bloom abundance at the field edge during summer was negatively correlated to “other wild bees” visits to blueberry in the field and at the field edge while in the fall floral bloom abundance at the field edge was positively correlated to “other wild bees” visits to blueberry in the field and at the field edge. Fall floral resources were important for longer-lived, social bees and increasing floral resources in the fall, increased their visits to lowbush blueberry in subsequent seasons. Given that all bee groups were not correlated in the same way to crop visits, future work should characterize floral interactions using a bee group approach. Most correlations were not significant suggesting that increasing the number of taxa, the number of blooms, and flowering plant diversity in general does not increase wild bee group visits to lowbush blueberry. Alternative forages from the plant families Fabaceae, Asteraceae, Rosaceae, and Ericaceae are candidates for further research to test if the presence of specific plant taxa are correlated to increased wild bee visits to lowbush blueberry flowers.

Chapter 3: Plant-Pollinator Interactions in Lowbush Blueberry Agroecosystems During Bloom

Abstract

Lowbush blueberry (*Vaccinium angustifolium* Aiton, Ericales: Ericaceae), relies on bees for pollination. Floral traits include early bloom and poricidal anthers which require vibrations to release pollen. Many producers rent honey bee (*Apis mellifera* Linnaeus, Hymenoptera: Apidae) hives during bloom to increase the pollination force. Wild bees are also present in blueberry fields and many buzz pollinate. This study aimed to identify which wild bees visited and collected pollen from lowbush blueberry using observations and DNA metabarcoding of bee-associated pollen. At some sites, wild bees were observed on lowbush blueberry more frequently than honey bees. Metabarcoding of the internal transcribed spacer 2 (ITS2) region was used to identify the plant composition of pollen from 451 captured bees. Ninety-six percent of bees carried *Vaccinium* spp. pollen, and 52% of bees had only *Vaccinium* spp. in their pollen loads. The most abundant wild bees observed were the long-lived, social *Bombus* spp. and *Lassioglossum* spp. as well as the short-lived, solitary *Andrena* spp. Wild bees were rarely observed on non-crop flowers, and 86% of wild bees had pollen loads with three or fewer plant genera. This research demonstrated that all wild bee taxa in blueberry agroecosystems visit blueberry flowers and collect blueberry pollen. This suggests that efforts to support wild bees can support blueberry pollination and reduce reliance on managed bees.

Introduction

Lowbush blueberry (*Vaccinium angustifolium* Aiton, Ericales: Ericaceae), also known as wild blueberry, is an important crop in northeastern North America where the woody shrub is native. In Canada, most commercial production occurs in the eastern provinces of Canada including Quebec (QC), New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PEI) with an estimated farm gate value of 121.6 million Canadian dollars in 2023 (Agriculture and Agri-Food Canada, 2025). The perennial plant is managed to bloom and produce fruit every second year. Bloom typically begins mid to late May (White *et al.*, 2012) and lasts three to four weeks (Drummond, 2019a).

Cross pollination by insects, primarily bees (Finnamore & Neary, 1978), increases fruit production (Wood, 1965, 1968b; Usui *et al.*, 2005; Bell *et al.*, 2009). Many producers stock their fields with western honey bee hives (*Apis mellifera* L., Hymenoptera: Apidae) during bloom (Kinsman, 1993). The common eastern bumble bee (*Bombus impatiens* Cresson, Hymenoptera: Apidae), and less frequently, the alfalfa leaf cutter bee (*Megachile rotundata* Fabricius, Hymenoptera: Megachilidae), are managed pollinators used in some fields. Surveys of lowbush blueberry fields found a diversity of wild bees (Boulanger *et al.*, 1967; Finnamore & Neary, 1978; Sheffield *et al.*, 2003; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; Cutler *et al.*, 2015; Drummond *et al.*, 2017b; McCallum *et al.*, 2021) and so, in most fields, a combination of wild and managed bees pollinate the crop.

Wild bees from the genera *Andrena*, *Halictus*, *Osmia*, and early emerging *Bombus* queens are more effective pollinators of lowbush blueberry than honey bees (Javorek *et al.*, 2002; Drummond, 2016). On a per bee basis, fruit set increased 1.6 times more by a native bee than a honey bee (Asare *et al.*, 2017). Wild bees are suited to lowbush blueberry pollination in that they forage in cooler weather (Drummond, 2016), typical during bloom, and many are capable of buzz pollination (Sampson, 1993; Cardinal *et al.*,

2018), where the bee uses vibrations generated by their thoracic muscles to release pollen from the blueberry flower's poricidal anthers.

The average rental price for a honey bee hive in NS in 2022 was \$178.51 (Jason Sproule, Provincial Apiculturist, personal communication of data he collected via a 2022 NS honey bee producer survey). A quote from Koppert Canada (received 2023-10-25), listed one bumble bee quad at \$369.98. Surveys of blueberry producers in Maine (Hanes *et al.*, 2015) and PEI (Collum & Hanes, 2015), found that growers recognize that native bees contribute to pollination. The role of native bees may become increasingly valuable as growers are concerned about the effectiveness, cost, and availability of managed pollinators (Collum & Hanes, 2015).

Field observations have traditionally been used to characterize plant-pollinator interactions. This methodology is labour intensive and requires personnel with expertise to identify the plant and bee species. The collection of insect-associated pollen has detected more interactions with less sampling efforts (Forup *et al.*, 2008; Bosch *et al.*, 2009; Pornon *et al.*, 2016). Complex pollen mixtures collected from insects can be analyzed using microscopy (Moisan-Deserres *et al.*, 2014) or with DNA metabarcoding (Bell *et al.*, 2017). DNA barcoding is the identification of species by sequencing a short, standardized region of the genome (Hebert *et al.*, 2003). The development of high-throughput next generation sequencing (NGS) allows mixed samples to be barcoded through a process known as DNA metabarcoding. Plant DNA metabarcoding has been used to identify allergens (Kraaijeveld *et al.*, 2015), composition of honey (Danner *et al.*, 2017; Vere *et al.*, 2017), provisions of solitary bees (Sickel *et al.*, 2015), and plant-pollinator interactions (Bell *et al.*, 2017).

Several surveys of the wild bee community in lowbush blueberry agroecosystems have been done over the years, see above. Less is known about the degree to which wild bees play a role in pollination. Using observation, Bushmann & Drummond (2015) found that

Andrena spp. accounted for more than 77% of visits to lowbush blueberry flowers in Maine. When they examined the pollen loads of five species of andrenids they found *Vaccinium* made up a large proportion (Bushmann & Drummond, 2015). On average, *Andrena carolina* Viereck (Hymenoptera: Andrenidae), pollen loads were 99.23% Ericaceae (Bushmann & Drummond, 2015). Moisan-Deserres *et al.* (2014) used microscopy to identify the pollen loads of insects in lowbush blueberry fields in Quebec. They found large pollen loads on bumble bees and andrenid bees and although the pollen loads of halictid bees were small, in some cases a large proportion was blueberry (Moisan-Deserres *et al.*, 2014). Two species of andrenids, *A. carolina* and *A. bradleyi* Viereck, had almost pure *Vaccinium* pollen loads (Moisan-Deserres *et al.*, 2014). Managed *B. impatiens* have been reported to collect pollen from lowbush blueberry (Whidden, 1996; Drummond, 2012). DNA metabarcoding has not been used to identify which wild bees collect pollen from lowbush blueberry.

This study characterized plant-pollinator interactions in lowbush blueberry fields during bloom using both observations and DNA metabarcoding. The objective was to identify which wild bee species visit and collect pollen from lowbush blueberry flowers. Characterizing pollen loads of wild bees during bloom would identify which wild bees have the greatest potential to increase pollination services in lowbush blueberry. The hypothesis is that wild bees from the families Andrenidae, Apidae, and Halictidae visit and collect pollen from lowbush blueberry flowers. I predicted *Andrena carolina*, previously reported to be a specialist, will have high proportions of blueberry pollen, but that most of the bees would have mixed pollen loads.

Methods

Site description

During the 2017, 2018, and 2019 seasons, data were collected from sixteen, nine, and three commercial lowbush blueberry fields in Maritime Canada (28 site-years). Site locations can be found in Fig. 2.1 and Supplementary Table 2.1. Four 100 m x 2 m

transects were established at each site: the 0 m transect was placed at the field edge with the most floral diversity and one at each of 25, 50, and 100 m increments into the field, parallel to the 0 m transect. Transect walks, ($20 \text{ m}^{-2} \text{ min}^{-1}$), were conducted at each site on two to three sampling dates during bloom (mid to late May until mid to late June). During bloom there were 77 site-date visits and 308 100 m transect walks.

Floral survey

Flowering plants in all transects were identified to species where possible. Genus or plant taxa groupings were used for: *Amelanchier* spp., *Galium* spp., *Hieracium* spp., *Oxalis* spp., *Potentilla* spp., *Rosa* spp., *Rubus* spp., *Salix* spp., *Stellaria* spp., *Viola* spp., and *Vaccinium* spp. *Solidago* spp. and *Euthamia* spp. were grouped as “goldenrods”.

Bee observations

Bees were observed between 1000 and 1600 h on days where the temperature was $\geq 14^{\circ}\text{C}$ with low windspeeds, no precipitation, and at least partial sun. Attempts were made to vary the time of day and order of transect walks for site visits. Every observed instance of a bee foraging on a flower was recorded. Bees were assigned to one of six groups: (1) honey bees; (2) bumble bees; (3) andrenid bees; (4) metallic bees; (5) small black bees; (6) other. Subsets of the wild bees were collected for DNA barcoding and DNA metabarcoding for identification of bee and of bee-associated pollen, respectively. We attempted to collect the bees in proportion to what was observed. During transect walks, when a bee was selected for collection, we paused the timer, collected the bee, and then continued with observations. Honey bees were not collected.

Pollen removal

Individual bees were processed on sterilized peach paper in a laminar flow hood. Materials were sterilized with ultraviolet light prior to processing. Forceps and scalpels were rinsed with sterile water and 70% ethanol and flamed between samples.

Bumble bees

Bumble bees groom the pollen they collect onto the corbiculae of their hindlegs.

Forceps and scalpels were used to remove the right hind leg and its associated pollen to a NucleoSpin homogenization tube containing Bead Type E (Macherey-Nagel, Allentown, PA, USA) and 300 μ L of molecular grade water and placed on ice.

Non-bumble bees

For non-bumble bees, pollen was often observed on legs, abdominal scopa and/or was distributed over the body. Forceps and a scalpel were used to remove both hind legs which were transferred to a NucleoSpin homogenization tube containing Bead Type E. The bee body was then placed back in the original 50 mL collection tube with 500 μ L of molecular grade water and pollen was washed from the body with vortexing for 30 s, after which 300 μ L of the pollen/water mixture was added to the homogenization tube (which contained the legs) and placed on ice.

A blank was included with every set of extractions. For example, one blank was included for every 23 bees processed (a tube which had no bee but had forceps and scalpel dipped and molecular grade water added).

DNA extraction

Bee tissue and pollen were homogenized using the Bead Ruptor 4 (Omni International, Kennesaw, GA, USA), on setting 4 for 2 min. DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB)-based method (Doyle & Doyle, 1990). All centrifugation steps were performed in an Eppendorf 5425 centrifuge (Millipore Sigma Canada Ltd., Oakville, ON), at 13 800 $\times g$. An equal volume of 2X CTAB buffer (4% w/v CTAB, 2.8 M NaCl, 40 mM EDTA, 200 mM Tris-HCl (pH 8.0)) was added to the homogenate and incubated with 0.2 mg of proteinase K (Macherey-Nagel) at 56°C for 60

min. Samples were then centrifuged to collect the undigested exoskeleton and beads. The top aqueous layer (~300 µL) was transferred to a new, sterile microcentrifuge tube and incubated with 0.1 mg of RNase A (Thermo Fisher Scientific, Toronto, ON) at room-temperature for 20 min. Samples were then mixed with an equal volume of chloroform and centrifuged for 10 min. The top aqueous phase was transferred to a new, sterile microcentrifuge tube, mixed gently with 2/3 volume of cold isopropanol and precipitated at 4°C for 60 min. Samples were centrifuged for 10 min, the supernatant was poured off, the pellet was washed with 500 µL of 70% ethanol and centrifuged for 5 min. Finally, the supernatant was poured off, the pellet was dried for approximately 10 min and resuspended in 50 µL of molecular grade water.

Two targets were amplified from each DNA extract using polymerase chain reaction (PCR). For bee identification, a portion of the cytochrome *c* oxidase I (COI) gene was amplified. A portion of the internal transcribed spacer 2 (ITS2) gene was used to identify the composition of the mixed pollen samples.

Polymerase chain reaction (PCR) and sequencing – cytochrome c oxidase I (COI)

The 20 µL PCR reaction contained 1X Phusion High-Fidelity Buffer with 1.5 mM MgCl₂ (Thermo Fisher Scientific), 200 µM dNTP (Promega UltraPure deoxynucleotide triphosphates, Thermo Fisher Scientific), 0.5 µmol of each primer (Integrated DNA Technologies, Coralville, IA), 0.4 U of Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific) and 0.5 µL (~25 ng) of DNA. PCRs were performed in a T100™ Thermal Cycler (Bio-Rad Laboratories, Mississauga, ON) with the following cycling conditions: initial denaturation at 98°C for 2 min, followed by 10 cycles of denaturation at 98°C for 10 s, annealing at 52-57°C (annealing temperature increased by 0.5°C each cycle) for 10 s, and extension 72°C for 20 s, followed by 25 cycles of 98°C for 10 s, 57°C for 10s, and 72°C for 20 s, and a final extension of 72°C for 10 min. Initially, the primer combination of LepF and LepR (Hebert *et al.*, 2004) was used. In cases where this primer pair failed or yielded amplicons where the best sequence similarity was to *Wolbachia*

spp. (a genus of Gram-negative bacteria from the Ehrlichiaeaceae family which is a known and common internal parasite which has been amplified with this primer pair in other studies) (Smith & Fisher, 2009; Smith *et al.*, 2012), the MLepF primer (Hajibabaei *et al.*, 2006) was used in combination with the LepR primer. The same reaction and thermal cycling conditions were used. For *Bombus* spp., when samples failed amplification, they were repeated with the forward primer BarbeeF (Françoso & Arias, 2013) in combination with LepR. Reaction conditions were the same as described above with the following cycling profile: initial denaturation at 98°C for 2 min, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 10 s, and extension at 72°C for 20 s, with a final extension of 72°C for 10 min. Primer sequences are in Table 3.1.

Table 3.1. Primer sequences used for bee DNA barcoding and pollen DNA metabarcoding.

Primer name	Sequence	Reference	Amplicon size (bp)
LepF	5' - ATTCAACCAATCATAAAGATATTGG	Hebert <i>et al.</i> , 2004	658
LepR	5'-TAAACTTCTGGATGTCCAAAAAATCA	Hajibabaei <i>et al.</i> , 2005	
MLepF	5'-GCTTTCCCACGAATAAATAATA	Hajibabaei <i>et al.</i> , 2006	407
BarbeeF	5'-CAACAAATCATAAAAATATTGG	Francoso <i>et al.</i> , 2013	655
ITS-S2F	5'-ATGCGATACTTGGTGTGAAT	Chen <i>et al.</i> , 2010	Variable ~300 - 400
ITS4	5'-TCCTCCGCTTATTGATATGC	White <i>et al.</i> , 1990	

The LepR primer was used in combination with LepF, MLepF, and BarbeeF.

PCR products were electrophoresed on 2% agarose gels, stained with GelRed™ (Biotium, Fremont, CA, USA) and visualized on a Gel Doc™ EZ Imager (Bio-Rad Laboratories). Amplicons were sequenced using Sanger sequencing at McGill University and Génome Québec Innovation Centre on an ABI 3730xl.

Sequencing data processing and analysis – cytochrome c oxidase I (COI)

Cytochrome c oxidase I (COI) bee barcodes were edited when downloaded from the Nanuq website (Génome Québec Innovation Centre; <https://genomequebec.com/en/nanuq/>) by using the advanced setting to trim good quality sequence and to mask bases with quality scores of less than 15. The fasta file of

sequences were queried against the Barcode of Life Database (BOLD) full length record barcode database.

Polymerase chain reaction (PCR) and sequencing – internal transcribed spacer 2 (ITS2)

A portion of the internal transcribed spacer 2 (ITS2) gene was used to identify the composition of the mixed pollen samples using primer pair ITS2-S2F (Chen *et al.*, 2010) and ITS4R (White *et al.*, 1990) (Table 3.1) modified for NGS compatibility (Comeau *et al.*, 2017). Each sample was amplified in duplicate with the following thermal cycling conditions: initial denaturation at 98°C for 2 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 64°C for 15 s, and extension at 72°C for 15 s, with a final extension of 72°C for 5 min. PCR amplicons were pooled prior to quality control and sequencing at Integrated Microbiome Resource at Dalhousie University for NGS on an illumina MiSeq System using 2 X 300 bp PE v3 chemistry (for details see Comeau *et al.*, 2017).

Next generation sequencing data processing and analysis internal - transcribed spacer 2 (ITS2)

The bioinformatics pipeline used in this analysis relied heavily on published workflows of “Microbiome Helper” (Comeau *et al.*, 2017) which can be found at https://github.com/LangilleLab/microbiome_helper/wiki and a DADA2 ITS2-specific workflow available from the authors of DADA (https://benjjneb.github.io/dada2/ITS_workflow.html).

Raw sequences were de-multiplexed and then processed with Divisive Amplicon Denoising Algorithm (DADA2 v1.14; Callahan *et al.*, 2016). DADA2 was run as an R script (v3.6) (R Core Development Team) using its R package (dada2 v1.14). Primer sequences were removed with Cutadapt (v1.11) (Martin, 2011). During the primer trimming step, reads where no primer sequence was found were removed, using the

“–discard-untrimmed” command. Reads less than 50 nt were also removed at this step. The DADA2 “filterAndTrim” command was used to remove sequences with: ambiguous bases (Ns; required by DADA2); with EE scores less than two; and to trim both forward and reverse reads. Read trim length was selected based on quality plots generated using FastQC (v0.11.4) (available at <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Error learning, dereplication, denoising, and merging of paired reads steps were performed with default settings. During the denoising step the pool parameter was set to “TRUE”. Amplicon Sequence Variant (ASV) tables were merged prior to chimera removal.

Reference Database and taxonomic classification

An effort to sequence the vascular plants of Canada resulted in ITS2 sequences from 3044 plant species (~60% of vascular plants in Canada) now part of the Barcode of Life Database (BOLD) plants of Canada project (Braukmann *et al.*, 2017). This includes samples from four National Parks in Atlantic Canada (Braukmann *et al.*, 2017).

A custom ITS2 reference database was created by downloading ITS2 sequences from the plants of Canada project on 2020-03-24 under project: DS-VASCAN on the BOLD website. Some manual modifications were made to this database 1) records that were identified as problem sequences during the IDTAXA classifier training phase, often due to issues with taxonomic naming, were either re-named or deleted and 2) a few taxa were added. The final database used for taxonomic classification can be found at Rutherford (2026). Details on the manual modifications are in Appendix B.

Taxonomic classification was done using IDTAXA (Murali *et al.*, 2018) which is part of the DECIPHER package (v2.14.0) (Wright, 2015), (available through: <http://DECIPHER.codes>). The training component was run with five iterations. Sequence classification was performed with both strands and a threshold of 0.7.

QIIME2

QIIME2 software v2020.8 (Bolyen, 2019) was used to filter ASVs, and to perform family- and genus-level plant taxa summaries. The ASV table was filtered using a 0.1% abundance filter. After filtering, there were 1,891 ASVs of which 1,722 (91%) were identified to the genus-level at a threshold 0.7. The ASVs with unresolved genus-level taxonomic assignment were used as a query in a Basic Local Alignment Search Tool (BLAST; Camacho *et al.*, 2009) search from the command line against the custom ITS2 database or as a query in a BLAST search on National Centre for Biotechnology Information (NCBI) against the nt database. BLAST scores and knowledge of the local flora were used to determine if BLAST results were reasonable and if so, the taxonomy file was updated manually (described in Appendix B). After this process 1,787 of the 1,891 (94.5%) ASVs were assigned at the genus-level.

For all downstream analysis, ASV tables were converted from read number to presence/absence (Lamb *et al.*, 2019). All figures were generated in R (v3.6) package ggplot2 (Wickham *et al.*, 2020).

Statistical Analysis

Statistical analyses were performed with species that had more than 10 individuals: *Andrena carlini* Cockerell, *A. carolina*, *A. nivalis* Smith, *Bombus bimaculatus* Cresson, *B. impatiens*, *B. perplexus* Cresson, *B. ternarius* Say, as well as the genus *Lasioglossum*. A generalized linear mixed model (PROC GLIMMIX, SAS 9.4) with the Laplace method, Poisson distribution, and log link was used to test if the number of plant genera in pollen loads differed among the eight bee taxa. The model included bee taxa as a fixed effect with site and location (field edge versus within the field) as random effects. Means were compared by Fisher's protected lsd when $P < 0.05$.

Spearman's rank order correlation calculated using the R Hmisc package v4.1.1; (Harrell, 2020) at $\alpha = 0.05$ was used to test if the proportion of *Vaccinium* spp. in an individual pollen load was correlated to the number of plant taxa at the site (the number of plant taxa at the field edge and within the field) for the site x date of bee capture (n=390). The proportion of *Vaccinium* spp. pollen was calculated by taking the presence of *Vaccinium* spp. pollen (0 or 1) and dividing it by the total number of plant genera in the pollen load.

Results

Bee observations

Bees were observed at an average rate of 1.8 bees $20 \text{ m}^{-2} \text{ min}^{-1}$. A total of 4,637 plant-pollinator interactions were recorded of which 4,325 (93.3%) were to blueberry flowers. Breaking-down the visits to blueberry flowers by bee group: 2,665 (61.6%) were by honey bees, 999 (23.1%) were by andrenid bees, 467 (10.8%) were by bumble bees, 122 (2.8%) were by other bees, 55 (1.3%) were by small black bees, and 17 (0.4%) were by metallic bees. For each of the six bee groups, visits to blueberry flowers represented the bulk of observed interactions: andrenid bees (95%), honey bees (95%), bumble bees (90%), other bees (82%), metallic bees (74%), and small black bees (74%). Wild bees accounted for more visits to blueberry flowers than honey bees at some sites Fig. 3.1.

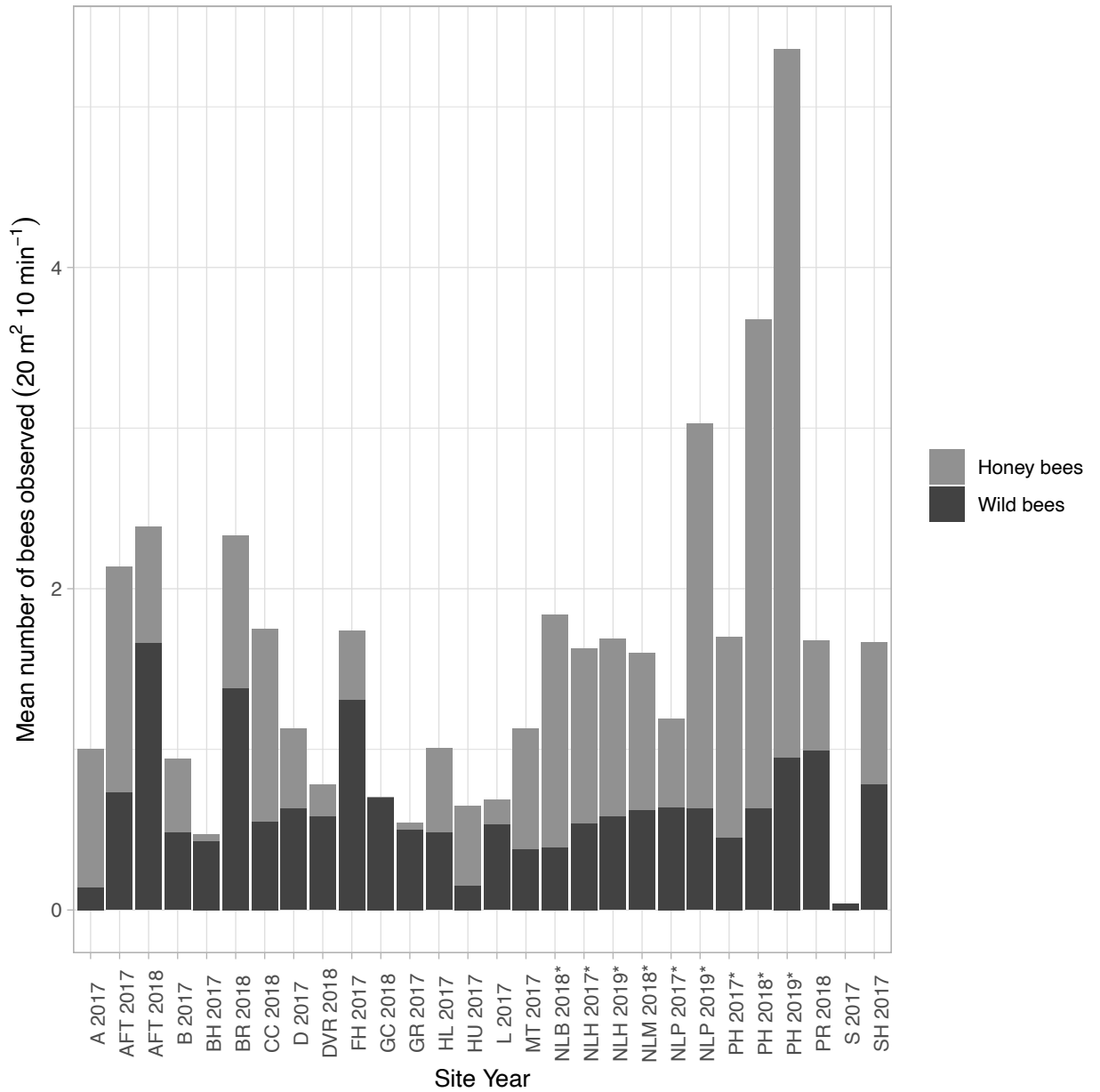


Fig. 3.1. Mean number of honey bees (*Apis mellifera* Linnaeus) and wild bees observed visiting lowbush blueberries (*Vaccinium angustifolium* Aiton), at each site-years during crop bloom. To calculate the mean number of wild bee visits to lowbush blueberry during bloom, the number of observations from each of the five wild bee groups (andrenid bees, bumble bees, metallic bees, small black bees and other bees) from each of the four transects (0, 25, 50, and 100 m) from each sampling date was summed and divided by the total number of transect walks for the site. Sites where the blueberry producer was also a beekeeper are denoted with an asterisk (*).

Bees, including honey bees, were observed visiting non-blueberry flowers at an average rate of 0.2 bees 20 m⁻² min⁻¹ (n=312) during blueberry bloom. Wild bees were observed 167 times visiting 21 non-blueberry taxa which included: sheep laurel (*Kalmia angustifolia* L. (29) 17%), hawkweeds (*Hieracium* spp. (27) 16%), black chokeberry (*Aronia melanocarpa* (Michx.) Elliot (26) 15.6%), and corn spurry (*Spergula arvensis* L. (25) 15%). A complete list of plant-pollinator interactions can be found at Rutherford (2026).

Barcoded bees

Four hundred and fifty-one wild bees were captured during crop bloom. Twenty-five bees with ambiguous DNA barcode identities were removed prior to analyses. Barcode-identified bees were from the following families: Andrenidae n=225 (52.8%); Apidae (primarily *Bombus* spp.) n=133 (31.2%); Halictidae n=58 (13.6%); Colletidae n=6 (1.4%); and Megachilidae n=4 (1%). This composition roughly matches that of wild bee groups observed in lowbush blueberry fields. Sequences of CO1 genes can be found at Rutherford (2026). Taxonomic assignment for all bees is in Table S4.7. Species-level assignment of bees captured during bloom are in the bloom interaction matrix, Table S4.2 Rutherford (2026).

Pollen loads

Sixty plant genera were found in the pollen loads of wild bees during bloom. Plant genera found in the pollen loads of 10 or more captured bees are presented in Fig. 3.2. A complete list of plant genera in pollen loads during bloom are presented in Supplementary Table S3.2. Whenever lowbush blueberry **pollen** is discussed, it is at the genus-level. DNA barcoding is not able to distinguish among several species of *Vaccinium*. Pollen from *Vaccinium* spp. was identified on most bees (Fig. 3.2). Two hundred and thirteen bees (52%) had pollen loads with only *Vaccinium* spp. pollen. Other plant genera that were common in pollen loads were from early flowering trees

and shrubs/bushes (cherry (*Prunus* spp.), maples (*Acer* spp.) elders (*Sambucus* spp.), rhododaras (*Rhododendron* spp.), willows (*Salix* spp.), birches (*Betula* spp.), laurels (*Kalmia* spp.), bay berry (*Morella* spp.), and brambles (*Rubus* spp.)). Most of the trees and shrubs were growing at the field edge or outside of the field. Strawberries (*Fragaria* spp.), hawkweeds (*Hieracium* spp.), and dandelions (*Taraxacum* spp.) were found both in the field and at the field edge while sorrels (*Rumex* spp.) were found mostly in the field.

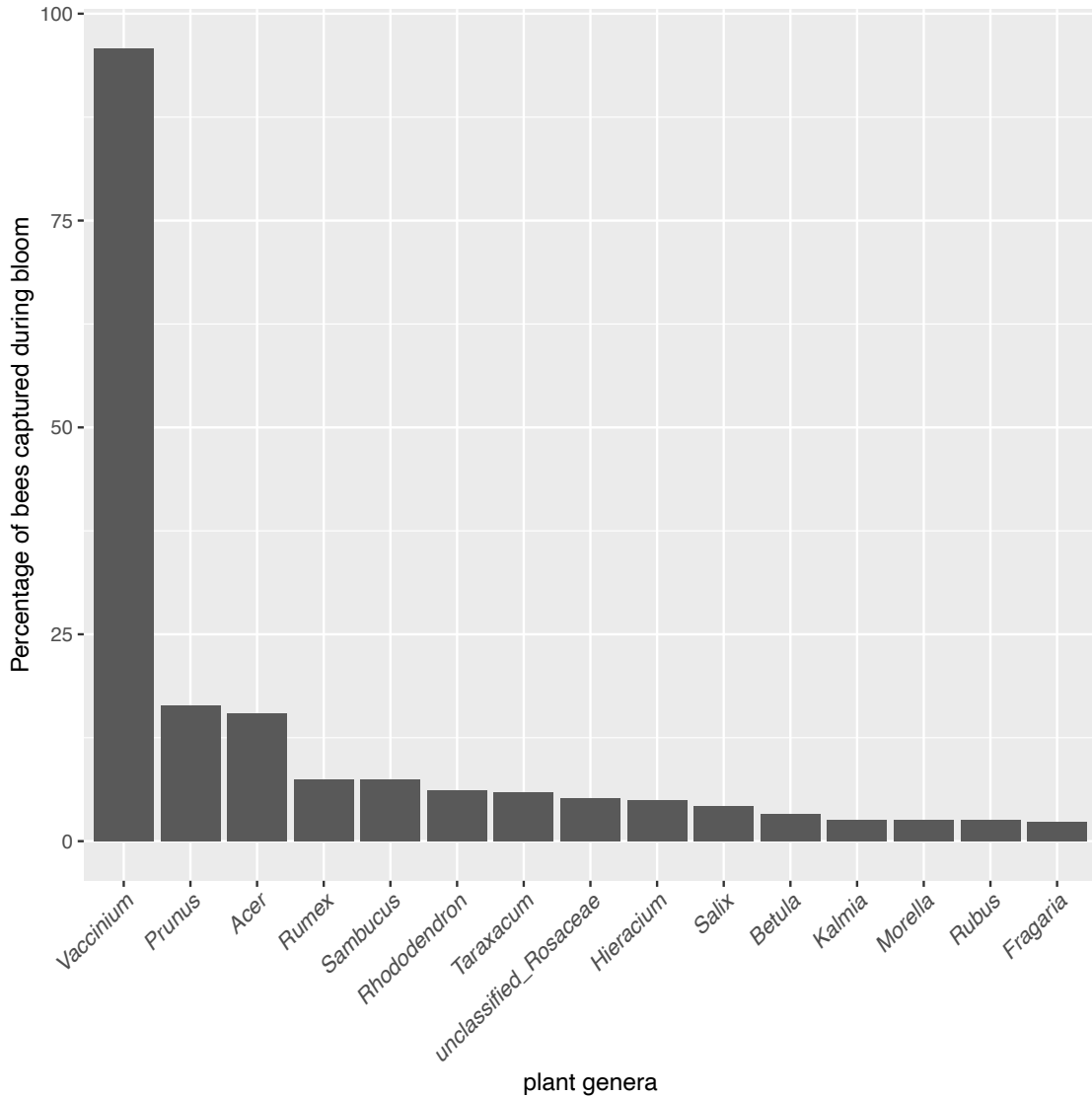


Fig. 3.2. Most frequent (found in ≥ 10 bees) plant genera found in pollen loads of wild bees ($n=426$) captured in lowbush blueberry (*Vaccinium angustifolium* Aiton), fields during bloom.

The pollen loads of individual wild bees captured during blueberry bloom included few plant genera (Fig. 3.3). About half of the bees had pollen loads with a single plant genus, while 86% had three or fewer plant genera. Andrenidae was the family with the most bees that had pollen from a single genus, however, they were also the family that had individuals with many plant genera, the maximum being 13. There were very few individuals from any family that had pollen loads with more than five genera.

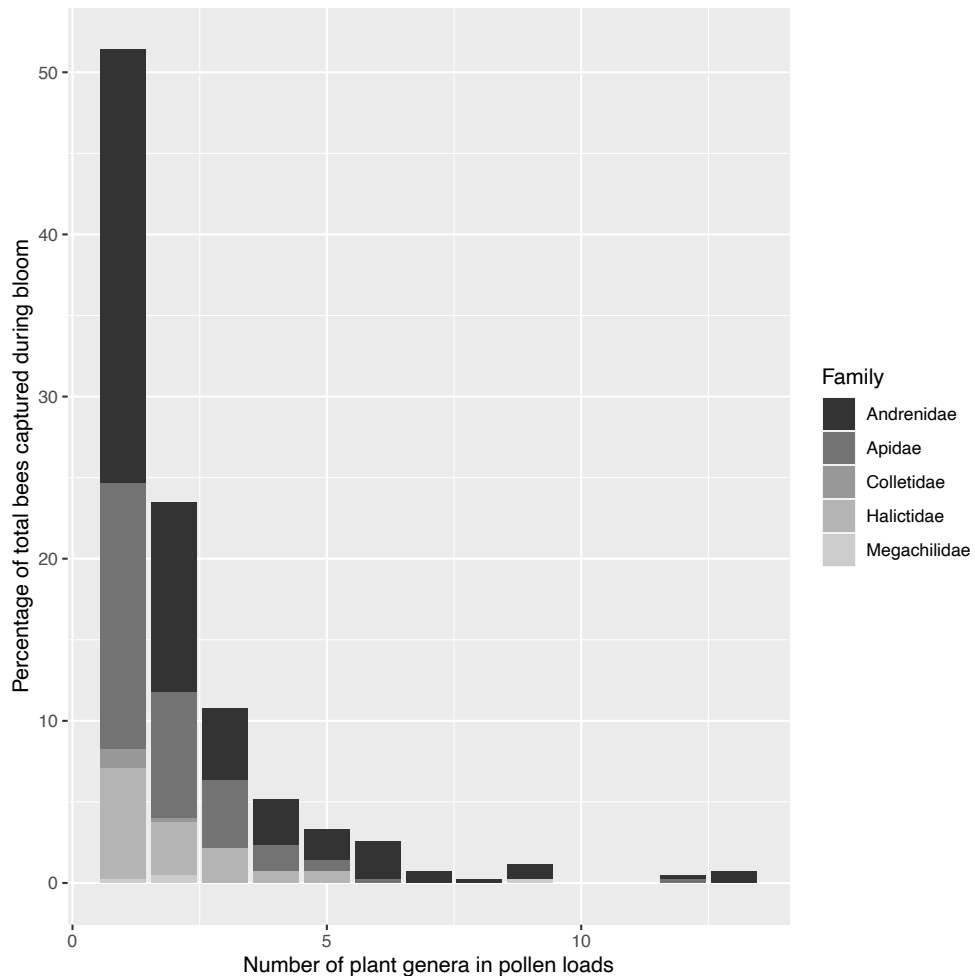


Fig. 3.3. Number of plant genera found in the pollen loads of wild bees (n=426) captured in lowbush blueberry (*Vaccinium angustifolium* Aiton) fields during bloom. Bars are shaded to show bee family.

Analysis using a general linear mixed model showed that for most bee groups there were no differences in the average number of plant genera in pollen loads (Fig. 3.4). *Andrena carlini* had more genera in pollen loads than all the other taxa, except *A. nivalis*. *Andrena carolina* had fewer plant genera in pollen loads than the other taxa except *B. ternarius*, and *B. bimaculatus*.

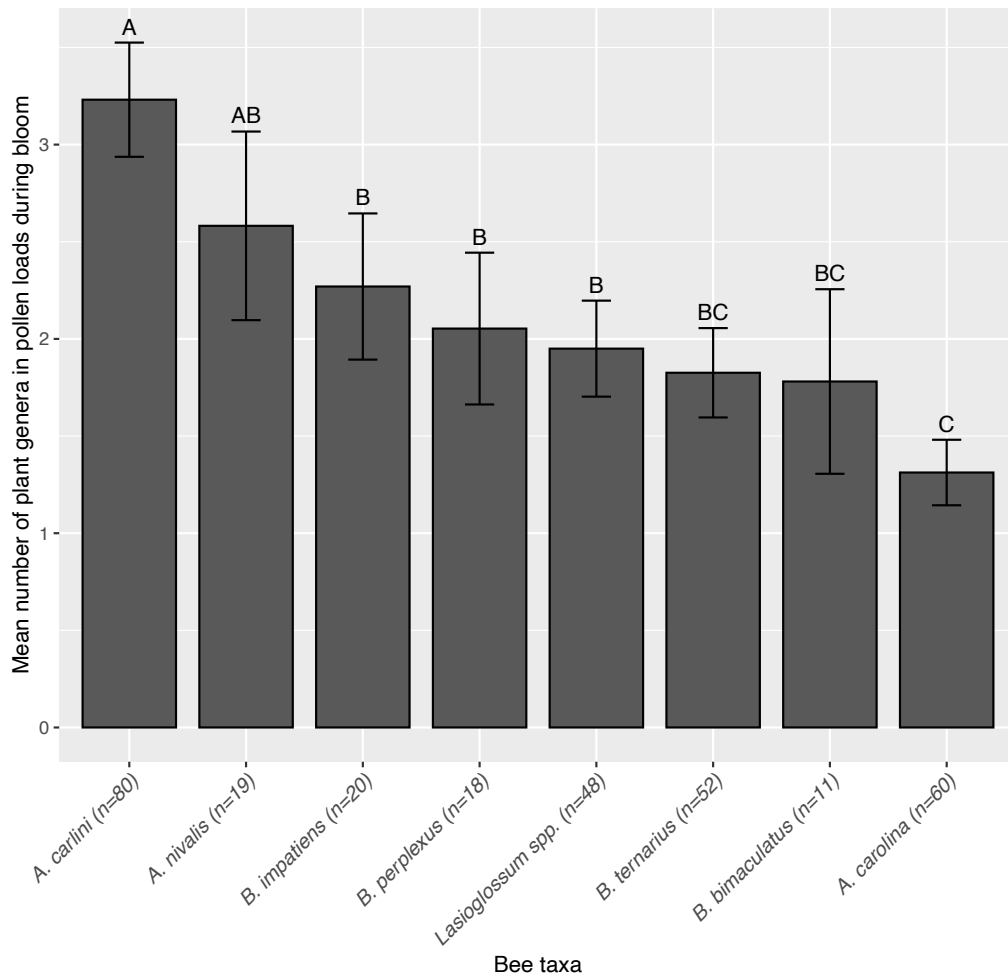


Fig. 3.4. Mean number of plant genera in pollen loads from the eight most frequently captured bee taxa in lowbush blueberry (*Vaccinium angustifolium* Aiton) fields during bloom. Bars represent standard error of the mean. Genera names were abbreviated for simplicity but represent *Andrena* and *Bombus*. Bars with the same letter are not significantly different according to Fisher's protected lsd test at alpha = 0.05.

Correlation analysis was used to test if the proportion of blueberry pollen changed as the number of co-flowering plants in the field and at the field edge increased. The results showed that the relationship was significant, but the correlation coefficient was very small ($r_s(387) = -0.11, P = 0.023$). This indicated that the number of co-flowering plants at the field edge and in the field, had minimal impact on the proportion of blueberry pollen found in wild bee-associated pollen.

Discussion

It was expected that *Vaccinium* spp. pollen would be common, given that collection took place in lowbush blueberry fields during bloom. However, the number of bees and the identity of bees with pure blueberry pollen loads was surprising. The hypothesis that *A. carolina* would be the main species with pure blueberry pollen loads was false. *Andrena carolina* did have the most individuals with pure *Vaccinium* spp. pollen loads, however, there was no significant difference in the mean number of genera between *A. Carolina* and *B. bimaculatus* and *B. ternarius*. Moisan-Deserres *et al.* (2014) used microscopy to analyze the pollen loads of insects in lowbush blueberry fields in Quebec and found that 624 of the 627 captured insects (bees as well other pollinating insects) carried some *Vaccinium* spp. pollen.

Wild bees accounted for more visits to lowbush blueberries than honey bees at some sites (Fig. 3.1). Wild bees may be providing the bulk of pollination services in some fields especially when pollination equivalencies, calculated by Javorek *et al.* (2002), are considered. In the time it takes one honey bee to pollinate one blueberry flower, a *Bombus* spp. queen pollinates 6.5 flowers and an *Andrena* spp. pollinates 3.6 flowers (Javorek *et al.*, 2002). Previous studies found that honey bees do not collect large amounts of *Vaccinium* spp. pollen (Colwell *et al.*, 2017; Dufour *et al.*, 2020) which could be because they are not able to buzz pollinate. Honey bees accounted for 61% of observed visits to lowbush blueberry flowers however, because honey bees were not captured, it is unknown if *Vaccinium* spp. was present in their pollen loads.

This study confirms, through metabarcoding data, that *Andrena* spp. collect pollen from lowbush blueberries. The two most frequently captured andrenids, *A. carlini* and *A. carolina*, differed in the mean number of plant genera in pollen loads (Fig. 3.4). Moisan-DeSerres *et al.*, (2014) found that pollen loads of andrenid bees contained large proportions of *Vaccinium* spp. and that *A. carolina* exhibited monolecty to the crop. Bushmann & Drummond (2015) also found that *A. carolina* was a blueberry specialist and that, like our findings, on average *A. carlini* carried less ericaceous pollen than *A. carolina*. The other frequently captured andrenid bee, *A. nivalis*, has been found to have large pollen loads with high proportions of *Vaccinium* spp. pollen (Moisan-Deserres *et al.*, 2014). Lowbush blueberry is largely self-incompatible (Wood, 1968; Hall *et al.*, 1979; Usui *et al.*, 2005), and therefore, transfer of non-self-pollen increases fruit set in most clones. Andrenid bees were found to travel farther distances between blueberry flower visits than honey bees, bumble bees and *Osmia* spp. (Drummond, 2016) which could mean they transfer more pollen among clones.

Captured *Lasioglossum* spp. behaved as generalists (Fig. 3.4). In the observation data *Lasioglossum* spp., were part of the small black bee group, which had a higher percentage of bees observed on non-crop flowers. *Lasioglossum* spp. are common in lowbush blueberry fields, (Cutler *et al.*, 2015) but were not often found on blueberry flowers (Bushmann & Drummond, 2015). Moisan-DeSerres *et al.*, (2014) found small halictids (including *Lasioglossum* spp.), had smaller pollen loads, however, a large proportion was blueberry.

The four most abundant *Bombus* spp. captured during bloom carried *Vaccinium* spp. pollen. There was no significant difference in the mean number of plant genera in pollen loads among the four *Bombus* spp. (Fig. 3.4), and, as a genus, they collected pollen from a variety of plants. This was expected as *Bombus* spp. are known to be generalist foragers. Drummond (2012), found about one-third of managed *B. impatiens* returning

to the hive carried blueberry pollen as part of mixed pollen loads. The results of this study are similar to Whidden (1996), who found that *B. impatiens* showed high fidelity to *Vaccinium* spp., and Moisan-Deserres *et al.* (2014), who found that native *Bombus* spp. had large pollen loads containing *Vaccinium* spp. pollen. *Bombus* spp. forage for longer periods during the day, in cool weather and precipitation (Chch, 1972; Drummond, 2016) and deposited more lowbush blueberry pollen grains per visit than honey bees (Javorek *et al.*, 2002; Drummond, 2016). Bumble bees may improve honey bee pollination by releasing pollen for honey bees to access. Drummond *et al.* (2016) found that when a honey bee visits a blueberry flower that had previously been visited by a bumble bee, the amount of pollen that the honey bee deposits on the next blueberry flower it visits increases.

Genung *et al.*, (2017) reported that a small proportion of the bee community accounts for the bulk of the pollination services. The abundance of specific bees may be more important than bee species richness for ecosystem service delivery (Winfree *et al.*, 2015). In the current study, the three species of *Andrena*, and the four species of *Bombus* represent the dominant crop pollinators. Increasing their populations may increase pollination services.

Bee taxa that were captured less frequently, such as those from the families Colletidae and Megachilidae, also carried *Vaccinium* spp. pollen. Bee diversity contributes to stability of pollination services (Brittain *et al.*, 2013; Drummond *et al.*, 2017b), making the case that occasional pollinators should also be supported. Drummond *et al.* (2017b) surveyed wild bees associated with lowbush blueberries in Maine over a 29-year period and found that the total wild bee abundance fluctuated two- to three-fold among years, however, predicted fruit-set over the same sampling period was more stable, likely explained by the diversity of bees in the system.

Wild bees visited and collected pollen from non-crop flowers during lowbush blueberry bloom. Bees were observed visiting 22 of the 70 flowering plants and pollen loads contained 59 non-blueberry genera. This was more than the 34 plant species identified in insect pollen loads by Moisan-Deserres *et al.*, (2014). Additional plant taxa in this study could be due to regional differences, or the sensitivity of metabarcoding versus microscopy (Keller *et al.*, 2015; Richardson *et al.*, 2015a; Smart *et al.*, 2017; Macgregor *et al.*, 2019). Moisan-DeSerres *et al.*, (2014) similarly found that many plant taxa were present on a few individuals (Table S3.2). The alternative plants used by wild bees during bloom in this study (Fig. 3.2), match previous findings (Stubbs *et al.*, 1992; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015). Pollen data indicated cherry, maples, and sorrels were visited by many bee groups. Further research to investigate these plants as alternative forage for bees during bloom is needed, especially in alternate years when blueberry is not blooming.

There is concern that co-flowering plants interfere with crop pollination (Lander *et al.*, 2011) via competition for pollinator visits. The proportion of *Vaccinium* spp. found in pollen loads was negatively correlated ($r_s(387) = -0.11, P=0.023$) to the number of co-flowering plant species. The correlation was significant but very weak, which indicated that the number of co-flowering plants had minimal impact on the proportion of *Vaccinium* spp. pollen found in wild bee-associated pollen. Graham *et al.*, (2023) found no correlation between landscape diversity and pollen load diversity in highbush blueberry fields in Michigan. Drummond (2016) found that lowbush blueberries were more attractive to bumble bees than five other co-flowering species (apple (*Malus pumila* Mill.), shadbush (*Amelanchia* spp.), choke cherry (*Prunus virginiana* L.), bunch berry (*Cornus canadensis* L.), and rhodora (*Rhododendron canadense* L. (Torr.))). Co-flowering hedgerows increased native bees in tomato (*Solanum lycopersicum* L.) fields in California (Morandin & Kremen, 2013); increased bee visits to strawberry (*Fragaria* spp.) in Scotland (Feltham *et al.*, 2015); and had no impact on seed set in broad bean (*Vicia faba* L.) in Germany (Eckerter *et al.*, 2022). Increased co-flowering plants in the

landscape increased pollinator visits to sweet cherry (*Prunus avium* L.) flowers in orchards in Australia (Gilpin *et al.*, 2022). However, models have predicted that co-flowering plants will distract pollinators (Nicholson *et al.*, 2019).

Co-flowering plants may also negatively impact crop yields by increasing heterospecific pollen deposition (Campbell & Motten, 1985). The impacts of heterospecific pollen transfer on fruit set depends on many factors such as the relatedness of pollen donor and recipient, if the recipient plant is self-incompatible, and if so, the mechanism of self-incompatibility (see Morales & Traveset, 2008). This study did not quantify the proportion of blueberry pollen in individual pollen loads (i.e., is non-blueberry pollen a major or minor component of the pollen load). Analysis of pollen grains using microscopy found that Ericaceous or *Vaccinium* spp. dominated wild bee pollen loads during blueberry bloom in Quebec (Moisan-Deserres *et al.* 2014), and andrenid pollen loads in Maine (Bushman & Drummond, 2015).

Co-flowering plants could provide valuable food in the sprout year when blueberry flowers are not blooming. Co-flowering plants and mixed pollen loads may be important for a balanced diet and contribute to bee health. Microcolonies of *B. terrestris* L. fed a trifloral diet of Ericaceae, Rosaceae, and Fabaceae pollen performed better than those fed only Ericaceae pollen (Moerman *et al.*, 2017).

Given the weak relationship between co-flowering plants and blueberry pollen and the ubiquity of *Vaccinium* spp. in pollen loads, it is unlikely that co-flowering plants are having a negative impact on crop pollination at these sites. However, when planting floral enhancements, crop visitation and/or pollen load composition should be monitored.

Conclusions

All wild bee species observed and captured in lowbush blueberry fields visited and collected pollen from lowbush blueberry flowers. At some sites, wild bees accounted for more visits to lowbush blueberry flowers than honey bees. Given their abundance and superior pollination effectiveness, wild bees are contributing more to blueberry pollination than honey bees at some sites. The most abundant bees captured were the long-lived, social *Bombus* spp. (*B. bimaculatus*, *B. impatiens*, *B. perplexus*, and *B. ternarius*), and *Lassioglossum* spp. as well as the short-lived solitary *Andrena* spp. (*A. carlini*, *A. carolina*, and *A. nivalis*). The most frequent plant genus found in wild bee pollen loads was *Vaccinium* spp. Over half of the captured bees had pure *Vaccinium* spp. pollen loads and bees from the five captured families all had individuals with pure *Vaccinium* pollen loads. Pollen loads from captured wild bees contained few genera and generally the number of plant genera in pollen loads was not different among bee groups. The number of non-blueberry taxa in pollen loads was weakly correlated to the number of co-flowering plant taxa blooming at the site.

Chapter 4: Key Floral Resources for Wild Bees in Lowbush Blueberry Agroecosystems

Abstract

The demand for bee-mediated pollination services in lowbush blueberry (*Vaccinium angustifolium* Aiton) fields is high. The mass flowering crop blooms for a few weeks every second year while bees require floral resources throughout their lifecycle. The objective of this study was to identify plant-pollinator interactions using observation and DNA metabarcoding in lowbush blueberry agroecosystems in Maritime Canada. Specifically, I wanted to identify non-blueberry plants used by wild bees and to test if pollen collection differed among bee families at different times throughout the growing season. Bee visits to flowers were recorded during transect walks at the field edge throughout the season as well as in the field, during bloom. A subset of wild bees was collected, and their associated pollen was sequenced using DNA metabarcoding of the internal transcribed spacer 2 (ITS2) amplicon. This study found that metabarcoding revealed more plant-pollinator interactions than observations. During blueberry bloom Ericaceae (*Vaccinium* spp.) was the most common plant in observations and pollen loads. The most common non-blueberry plant families in pollen loads during bloom were Rosaceae, Sapindaceae, and Asteraceae. Asteraceae was the most common plant family in summer and fall pollen loads. Bee families differed in their pollen collection only during bloom and only for Adoxaceae, Ericaceae, and Sapindaceae. Understanding bee pollen use has implications for supporting wild bees.

Introduction

Bees' sole source of food is flowers. Nectar is the main source of carbohydrates and pollen contains multiple nutritional components, including protein, lipids, amino acids, vitamins, and carbohydrates, each of which vary in composition and/or concentration among plant taxa (Roulston & Cane, 2000; Somerville, 2001). Female bees consume nectar for energy and collect pollen and nectar to provision their offspring. As a bee moves among flowers collecting food, pollen is incidentally moved, facilitating plant reproduction.

Declines in wild bee populations have been linked to decreased floral resources (Biesmeijer *et al.*, 2006; Roulston & Goodell, 2011; Burkle *et al.*, 2013; Scheper *et al.*, 2014) which threatens plant reproduction and limits yield in pollinator-dependant crops (Reilly *et al.*, 2020). One strategy to conserve bees is to provide more food. In agricultural ecosystems, planting flowers near or at the edge(s) of crop fields has been promoted as a way to attract and increase wild bees. Establishment of floral enhancements adjacent to lowbush blueberry fields in Maine increased bee visits to the crop (Venturini *et al.*, 2017b). In Michigan floral plantings adjacent to high bush blueberry (*Vaccinium corymbosum* L.) fields increased wild bee abundance in the field and increased percent fruit set three years post-establishment (Blaauw & Isaacs, 2014). Similarly, Drummond *et al.* (2017) found that increased floral diversity at the field edge increased wild bee foraging density during bloom in lowbush blueberry fields in Maine.

The practice of planting flowers to support bees is increasing, however, more information on the appropriate flowers is needed. The attractiveness of floral resources has been found to vary among bee taxa (Nichols *et al.*, 2019; Dibble *et al.*, 2020b), region (Williams *et al.*, 2015), and year (Dibble *et al.*, 2020b). Some studies have found that bees rarely visit and/or collect pollen from flowers in enhanced borders (Wood *et al.*, 2017; Gresty *et al.*, 2018). When pollen from bumble bees was examined, only 37% was from sown flowers planted adjacent to blueberry fields (Venturini *et al.*, 2017b). It is

also important to consider which plants can establish in the system. For example, the soils in and around lowbush blueberry fields are sandy and acidic (Agriculture and Agri-Food Canada, 2025). The study of suitable floral enhancements requires a local, agroecosystem-specific approach.

Alternative forage use by wild bees in lowbush blueberry fields has been investigated (Stubbs *et al.*, 1992; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015; Drummond *et al.*, 2017a; McCallum & McLean, 2017; Venturini *et al.*, 2017), using either observation or microscopic analysis of pollen loads. The bulk of this work has been conducted in the Canadian province of Quebec or the state of Maine (located in Northeast United States of America). Their research found that bumble bees visited buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae), goldenrods (Asteraceae), brambles (*Rubus* spp.; Roseaceae) and rhodora (*Rhododendron canadense* (L.) Torr.; Ericaceae) pre- and post-blueberry bloom, while red clover (*Trifolium pratense* L.) (Fabaceae), vetch (*Vicia* spp.; Fabaceae), alfalfa (*Medicago sativa* L.; Fabaceae), and St. John's Wort (*Hypericum* spp.; Hypericaceae) were visited for the longest duration by bees (McCallum & McLean, 2017). In lowbush blueberry fields in Maine bumble bees often collected pollen from Fabaceae and solitary bees were often found on Asteraceae (Venturini *et al.*, 2017b). The most frequent plants Bushmann & Drummond (2015) observed wild bees (excluding bumble bees) visiting were Rosaceae, Asteraceae, and Ericaceae. Moisan-Deserres *et al.*, (2014) found that wild bees collected pollen from alders (*Alnus incana* L. (Moench); Betulaceae), brambles, mountain holly (*Ilex mucronata* L.; Aquifoliaceae), bog Labrador tea (*Ledum groenlandicum* Oeder; Ericaceae) and dandelions (*Taraxacum* spp.; Asteraceae) during blueberry bloom in Quebec.

DNA metabarcoding combines taxonomic identification using DNA barcoding (Hebert *et al.*, 2003) and next generation sequencing (NGS) technology. In the case of plant-pollinator interactions, DNA is extracted from bee-associated pollen. DNA barcodes

from the mixture are amplified using polymerase chain reaction (PCR) and sequenced simultaneously. The species composition is identified by comparing the barcodes to a database containing reference sequences. DNA metabarcoding has been found to reveal more interactions between plants and pollinators than observation (Pornon *et al.*, 2017) and microscopy (Keller *et al.*, 2015; Smart *et al.*, 2017; Macgregor *et al.*, 2019). For example, Pornon *et al.*, (2016) found that DNA metabarcoding revealed 2.5 times more interactions than observation. Richardson *et al.*, (2015a) found that pollen metabarcoding using internal transcribed spacer 2 (ITS2) identified 19 plant families in pollen from honey bee hives while microscopy identified eight. DNA metabarcoding requires less time and expertise and may be more affordable than identification of pollen loads by microscopy (Sickel *et al.*, 2015; Bell *et al.*, 2017).

Bipartite networks are used to characterize interactions between two trophic levels. The nodes represent the species (or some taxonomic rank) and the link between two nodes represents the strength of the interaction. The bipartite package can be used to visualize interactions, compute ecological indices, and compare networks (Dormann *et al.*, 2008). In this study, bipartite networks were constructed for both observation and DNA metabarcoding for three seasons (bloom, summer, and fall) to visualize common interactions to determine wild bee pollen use in lowbush blueberry agroecosystems over the season. This information can help guide which floral enhancements support wild bees.

The objective of this study was to identify plant-pollinator interactions using observation and DNA metabarcoding in lowbush blueberry agroecosystems throughout the season in Maritime Canada. Specifically, I wanted to identify non-blueberry plants used by wild bees. I also wanted to test if pollen collection differed among bee families at different times throughout the growing season. It was expected that DNA metabarcoding would identify more plant-pollinator interactions than observations. I expected that during blueberry bloom a higher proportion of andrenid bees would carry Ericaceae pollen than

other wild bee families. It was expected that plants from the families Asteraceae, Fabaceae, and Rosaceae would be frequent in pollen loads.

Methods

During the 2017, 2018, and 2019 seasons, data were collected from sixteen, nine, and three commercial lowbush blueberry fields, respectively, in Maritime Canada. Site locations can be found in Fig. 2.1 and Supplementary Table 2.1. Sampling began at the start of blueberry bloom and continued every two to three weeks throughout the growing season (early May to late October). Four, 100 m x 2 m transects were established at each site: one at the field edge (0 m) and one at each of 25, 50, and 100 m increments into the field, parallel to the 0 m transect. During blueberry bloom, transect walks ($20 \text{ m}^{-2} \text{ min}^{-1}$) were conducted for all four transects. After bloom, data were collected at the field edge only. During bloom there were 77 site-date visits and 308 100 m transect walks. Post-bloom there were 127 site-date visits and 127 100 m transect walks.

Floral survey

Flowering plants in all transects were identified to species where possible. Genus or plant taxa groupings were used for: *Amelanchier* spp., *Galium* spp., *Hieracium* spp., *Oxalis* spp., *Potentilla* spp., *Rosa* spp., *Rubus* spp., *Salix* spp., *Stellaria* spp., *Viola* spp., and *Vaccinium* spp. *Solidago* spp. and *Euthamia* spp. were grouped as “goldenrods”. In the 2018 and 2019 seasons the area beyond the field (about 100 m immediately adjacent to the 0 m transect) was also surveyed.

Bee observations

Bees were observed between 1000 and 1600 h on days where the temperature was $\geq 14^{\circ}\text{C}$ with low windspeeds, no precipitation, and at least partial sun. Attempts were

made to vary the time of day and order of transect walks for site visits. Every observed instance of a bee foraging on a flower was recorded. Bees were assigned to one of six groups: (1) honey bees; (2) bumble bees; (3) andrenid bees; (4) metallic bees; (5) small black bees; (6) other. Subsets of the wild bees were collected for DNA barcoding and DNA metabarcoding for identification of bee and of bee-associated pollen, respectively. We attempted to collect the bees in proportion to what was observed. During transect walks, when a bee was selected for collection, we paused the timer, collected the bee, and then continued with observations. Honey bees were not collected.

Molecular methods

The methods for pollen removal, DNA extraction, polymerase chain reaction (PCR), sequencing, and sequencing data processing and analysis are identical to those described in Chapter 3. The reference database is described in Chapter 3 with details in Appendix B. The ITS2 reference database, amplicon sequence variant (ASV) table, taxonomy file, and metadata are all at Rutherford (2026).

QIIME2

QIIME2 software v2020.8 (Bolyen, 2019) was used to filter ASVs using a 0.1% abundance filter. Family- and genus-level plant taxa summaries were done for three seasons: (1) blueberry bloom; 17 May - 29 June (2) summer; 30 June - 31 August and (3) fall; 1 September - 19 October.

Bipartite networks

Bipartite networks for pollen and observation data were generated using R package (R Core Development Team) bipartite v2.16 (Dormann *et al.*, 2008) using the “plotweb” function. For comparison, only wild bees are shown in the observation networks. Observation networks including honey bees are in Supplementary figures S4.1 – S4.3

(Rutherford, 2026). For simplification of discussion and visualization, interactions between plant family and bee family (pollen) or bee group (observation) are presented. An advantage of DNA barcoding is increased taxonomic resolution and so interaction matrices at the species-level are presented in Supplementary Tables S4.1 - S4.6 (Rutherford, 2026).

Chi-square tests

A chi-square test of association was performed in SAS 9.4 using PROC FREQ to determine if the number of individuals carrying plant families differed among bee families. When chi-square analysis showed significant differences, post-hoc, pairwise, chi-square tests were performed with a Bonferroni multiple means corrected alpha value of 0.017.

All figures were generated in R (v3.6) package ggplot2 (Wickham *et al.*, 2020).

Results

Plant-pollinator interactions during bloom

During bloom 1,827 wild bees were observed from five bee groups and 426 bees from five families were captured. The most common wild bees in both observation and metabarcoding networks were from the family Andrenidae followed by bumble bees (family Apidae). Bees were observed visiting 12 plant families and pollen loads were comprised of 29 plant families (Fig. 4.1). Ericaceae was the most common plant family bees were observed visiting and found in bee-associated pollen (Fig. 4.1). This was expected as observations and collection took place within and at the edge of lowbush blueberry fields. Bees were also frequently observed visiting Asteraceae, Rosaceae, and Caryophyllaceae (Fig. 4.1). The most common plant families identified in pollen loads in addition to Ericaceae were Rosaceae, Sapindaceae, Asteraceae, Adoxaceae, Polygonaceae, and Salicaceae (Fig. 4.1).

Many plant families in bloom networks were rarely visited by bees or found in pollen loads (Fig. 4.1). Eight and 13 plant families had less than or equal to five visits or instances in pollen loads, respectively. Metabarcoding revealed interactions with more plant families than observations and in some cases, those plant families were found on many bees. Plant families that were not present in observation data at all, or very rarely, but were found in more than five individual pollen loads included Sapindaceae, Adoxaceae, Polygonaceae, Salicaceae, Myriaceae, Betulaceae, Fagaceae, Aquifoliaceae, Oleaceae, and Ranunculaceae (Fig. 4.1).

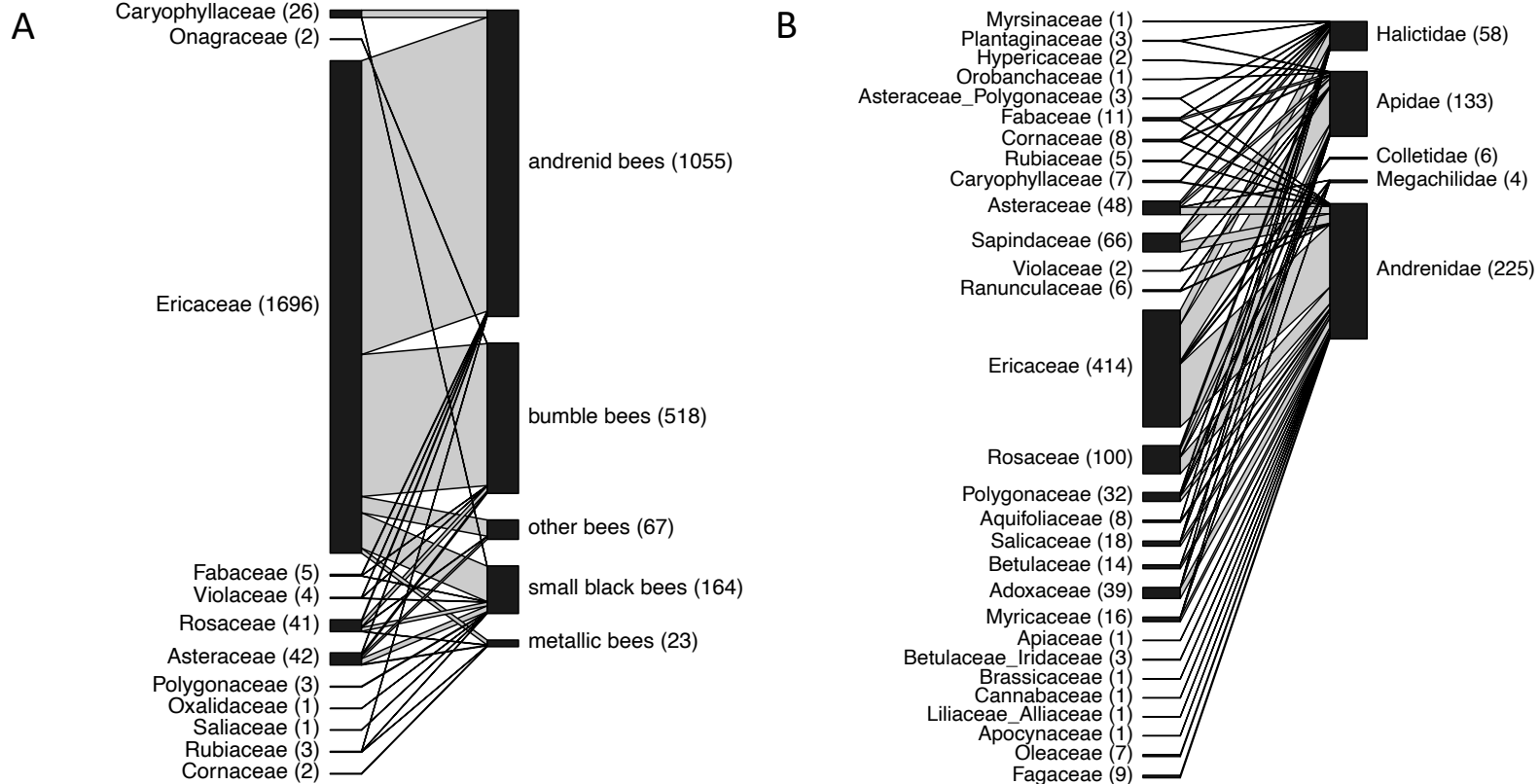


Fig. 4.1. Bipartite graph detailing plant-pollinator interactions in lowbush blueberry fields (*Vaccinium angustifolium* Aiton) for all site-years ($n=28$) during crop bloom. Panel A shows plant family and bee group interactions observed during transect walks. For bee group, bar height indicates the number of bees (also shown in brackets), and for plants, bar height indicates the total number bees observed on the plant (also shown in brackets). Link width indicates the number of interactions. Panel B shows plant family and bee group interactions from DNA metabarcoding. For bee family, bar height indicates the total number of plant taxa found on all bees in that taxon (the number of bees collected from that taxon is shown in brackets). For plant family, bar height indicates the number of bees containing that plant family in their pollen (also shown in brackets). Link width indicates the number of interactions, i.e., the number of individuals carrying that pollen type.

Chi-square analysis revealed that bee families differed in their use of Adoxaceae, Ericaceae, and Sapindaceae pollen during bloom (Table 4.1). Further analysis showed that Andrenidae had significantly higher proportion of individuals with Adoxaceae pollen than Apidae or Halictidae. Halictidae had significantly fewer individuals with Ericaceae pollen than Apidae or Andrenidae. Halictidae had significantly fewer individuals with Sapindaceae pollen compared to Apidae, while Andrenidae was intermediate and not significantly different from Apidae or Halictidae. (Fig. 4.2).

Table 4.1. Chi-square statistics for plant family pollen use in bee families (Apidae, Andrenidae, and Halictidae) during lowbush blueberry (*Vaccinium angustifolium* Aiton) bloom.

Plant family	df	χ^2	P-value
Adoxaceae	2	25.3183	<0.0001
Asteraceae	2	3.2755	0.1944
Ericaceae ^a	2	14.4705	0.0007
Polygonaceae	2	0.5022	0.7780
Rosaceae	2	2.0570	0.3575
Salicaceae	2	4.2799	0.1177
Sapindaceae	2	6.2721	0.0435

^a indicates that 25% of the cells had expected counts of less than 5 and therefore the Likelihood Ratio Chi-Square test was used. The number of bees used for analysis was Andrenidae n=225; Apidae n=133; Halictidae n=58.

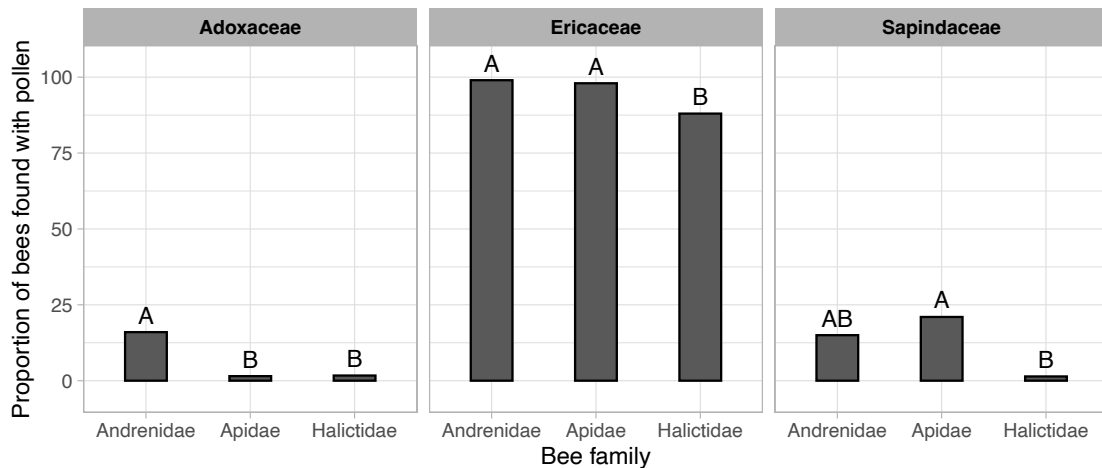


Fig. 4.2 Post-hoc Chi-square analysis to test for differences in proportion of individuals carrying pollen from plant families during lowbush blueberry bloom. Within plant family bars with the same letter are not significantly different $\alpha \leq 0.017$ (Bonferroni corrected alpha).

Plant-pollinator interactions during summer

During summer 571 wild bees were observed from five bee groups and 134 bees from four families were captured (Fig. 4.3). Bumble bees accounted for 73% of the observations (Fig. 4.3). Most (85%) of the captured bees were Apidae (Fig. 4.3), and 101 of the 104 captured Apidae were *Bombus* spp. (Table S4.4).

During summer, bees were observed on 18 plant families and DNA metabarcoding identified pollen from 24 plant families (Fig. 4.3). The most common plant family in the observation and metabarcoding data during the summer was Asteraceae (Fig. 4.3). Many plant families were infrequent in both observation and metabarcoding data. Eight and 16 plant families had less than or equal to five visits or instances in pollen loads, respectively. Plant families with more than five observations or that were found in more than five pollen loads were Asteraceae, Rosaceae, Fabaceae, Hypericaceae, Ericaceae, Boraginaceae, Plantaginaceae, Orobanchaceae, Rubiaceae, Lamiaceae, and Caprifoliaceae (Fig. 4.3). Fifty-five bees (41%) carried Fabaceae pollen; the bulk, (n=47)

were bumble bees (Table S4.4). *Trifolium* spp. and *Vicia* spp. were found on 25% and 24%, respectively, of captured bumble bees (Table S4.4). Over half the bees captured in the summer (58%) had goldenrod pollen while *Hieracium* spp. and *Doellingeria* spp. were also frequent (Table S4.4). Rosaceae pollen was found on 37 bees (30%) and was from four genera: *Spiraea* spp. (13), *Rubus* spp. (14), *Rosa* spp. (11) and *Potentilla* spp. (2) (Table S4.4). Pollen from the family Hypericaceae was found on 41 individuals (34%), all from the genus *Hypericum* (Table S4.4).

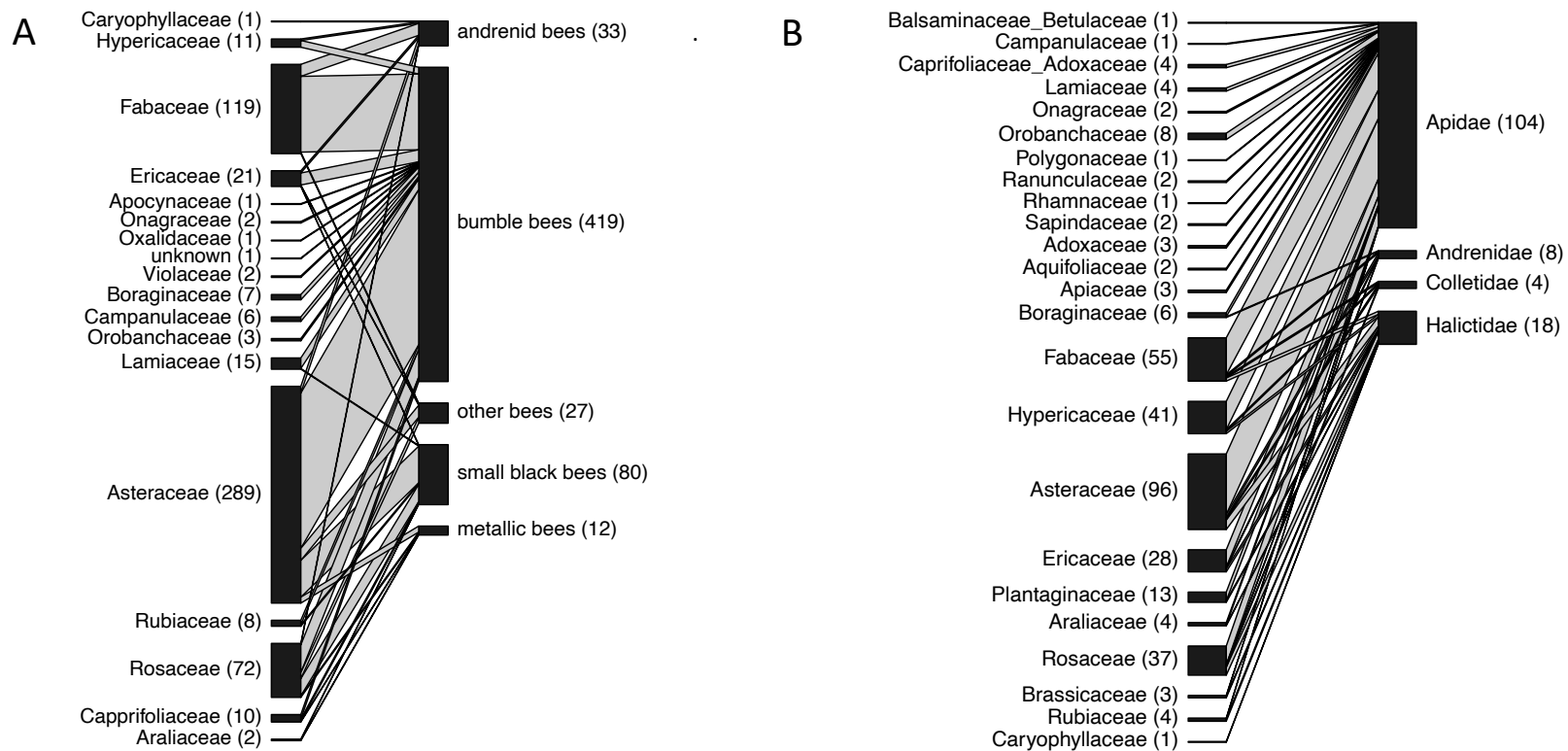


Fig.4.3. Bipartite graph detailing plant-pollinator interactions in lowbush blueberry fields (*Vaccinium angustifolium* Aiton) for all site-years ($n=28$) during the summer (30 June - 31 August). Panel A shows plant family and bee group interactions observed during transect walks. For bee group, bar height indicates the number of bees (also shown in brackets), and for plants, bar height indicates the total number of bees observed on the plant (also shown in brackets). Link width indicates the number of interactions. Panel B shows plant family and bee group interactions from DNA metabarcoding. For bee family, bar height indicates the total number of plant taxa found on all bees in that taxon (the number of bees collected from that taxon is shown in brackets). For plant family, bar height indicates the number of bees containing that plant family in their pollen (also shown in brackets). Link width indicates the number of interactions, i.e., the number of individuals carrying that pollen type.

There were no significant differences between the proportion of individuals from the families Apidae and Halictidae carrying pollen from the six plant families tested over the summer (Table 4.2).

Table 4.2. Chi-square results for comparison of proportion of individuals carrying pollen from common plant families during the summer between the family Apidae and Halictidae.

Plant Family	df	χ^2	P-value
Asteraceae ^a	1	0.4078	0.5231
Fabaceae	1	3.3278	0.0681
Ericaceae ^a	1	0.0104	0.9189
Hypericaceae	1	2.2729	0.1316
Plantaginaceae ^a	1	0.0046	0.9462
Rosaceae	1	2.0016	0.1571

^a indicates that 25% of the cells had expected counts of less than 5 and therefore the Likelihood Ratio Chi-Square test was used. The number of bees used from each family for the analysis was Apidae n=104; Halictidae n=18.

Plant-pollinator interactions during fall

During fall, 86 wild bees were observed from four bee groups and 23 bees from two families were captured. Bumble bees were the most common bee observed and captured. Halictidae were not captured in the fall which was expected given that their populations decrease in late summer (Cutler *et al.*, 2015). Interestingly two Andrenidae were captured during fall. Using DNA barcoding they were identified as *Andrena hirticincta* Provancher. They were likely classified as “other bees” during observations. Bees were observed on two plant families and nine plant families were present in pollen loads (Fig. 4.4). The most common family in fall observations and pollen loads was Asteraceae (Fig. 4.4). All 23 fall-captured bees carried goldenrod pollen (Table S4.6), and sixty-five (76%) of fall observations were to goldenrods (Table S4.5). The next most common aster was *Symphyotrichum* spp. which was found in 14 pollen loads (Table S4.6).

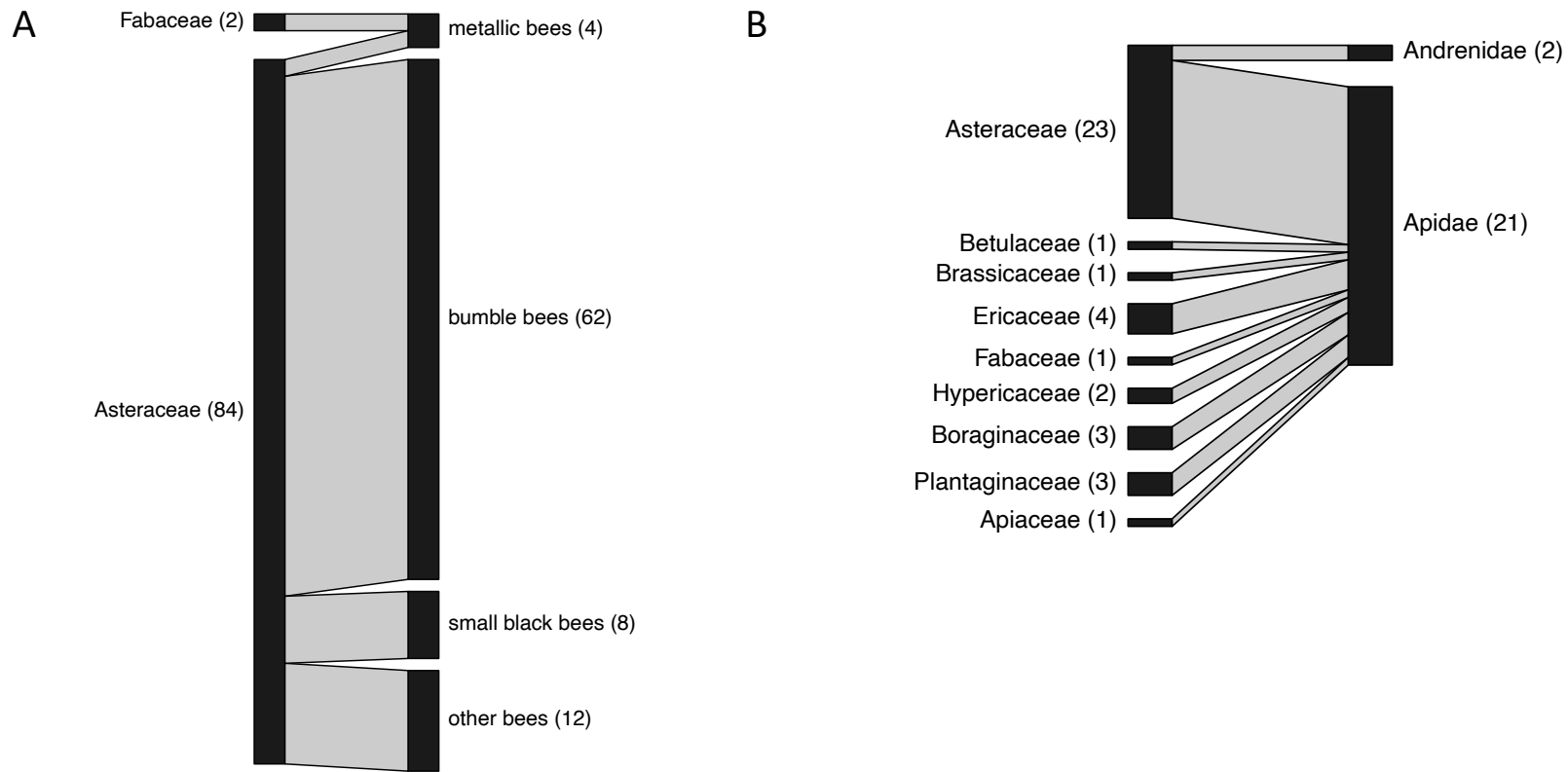


Fig. 4.4. Bipartite graph detailing plant-pollinator interactions in lowbush blueberry fields (*Vaccinium angustifolium* Aiton) for all site-years ($n=28$) during the fall (1 Sept. – 19 Oct.). Panel A shows plant family and bee group interactions observed during transect walks. For bee group, bar height indicates the number of bees (also shown in brackets), and for plants, bar height indicates the total number bees observed on the plant (also shown in brackets). Link width indicates the number of interactions. Panel B shows plant family and bee family from bees collected for pollen analysis. For bee family, bar height represents the number of pollen taxa found in that bee family. The number of bees collected from each family is shown in brackets. For plant family, bar height indicates the number of bees containing that plant family in their pollen (also shown in brackets). Link width indicates the number of interactions.

Bee Barcoding Summary

Of the 621 bees captured, 514 and 481 were successfully identified to the genus- and species-level, respectively (Table 4.3 and Supplementary Table S4.7). The most abundant genera captured were *Bombus* and *Andrena* followed by *Lasioglossum* (Table 4.3). Some COI barcodes had a best match to *Wolbachia* spp. (a genus of Gram-negative bacteria from the Ehrlichiaaceae family), other non-Hymenopterans, or fungi which presumably resulted from the presence and unintended co-amplification of COI sequences from bee-associated organisms. *Wolbachia* spp. COI barcodes have been generated in other studies using insect primers (Smith & Fisher, 2009; Smith *et al.*, 2012). Bees that were unsuccessfully barcoded were inaccessible to re-do PCR, therefore, images of the bee taken pre-extraction were used for family- or genus-level identification where possible or left as “unassigned”.

Table 4.3. Summary of bees captured in lowbush blueberry (*Vaccinium angustifolium* Ait.) fields.

Family	Genus	No. individuals	Percent of bees captured
Andrenidae	<i>Andrena</i>	237	38.0
	<i>Calliopsis</i>	1	0.2
	<i>Pseudopanurgus</i>	2	0.3
Apidae	<i>Bombus</i>	249	40.0
	<i>Ceratina</i>	1	0.2
	<i>Epeolus</i>	1	0.2
	<i>Melissodes</i>	2	0.3
	<i>Nomada</i>	5	0.8
	Colletidae		10
Halictidae	<i>Augochorella</i>	10	1.6
	<i>Dufourea</i>	1	0.2
	<i>Halictus</i>	8	1.3
	<i>Lasioglossum</i>	56	9.0
	<i>Sphecodes</i>	1	0.2
Megachilidae		4	0.6
Unassigned		33	5.0
TOTAL		621	

Pollen metabarcoding summary

After quality control and data processing using the Divisive Amplicon Denoising Algorithm (DADA2) pipeline there were 613 samples with 6,919 features (amplicon sequence variants; ASVs). ASVs with less than 100 reads were removed, which resulted in a filtered dataset of 1,891 ASVs. Taxonomies were assigned to 43% and 91% of ASVs at the species- and genus-level, respectively using the IDTAXA classifier at a 70% confidence threshold. The taxonomy of unclassified ASVs was resolved, in many cases, using the Basic Local Alignment Search Tool (BLAST; Camacho et al 2009) to the National Centre for Biotechnology Information (NCBI) nucleotide database and filtering results based on the local flora (Munro *et al.*, 2014). All ASVs in the taxonomy file used for QIIME2 analysis had family-level taxonomic information, albeit 10 were ambiguous (i.e., were one of two families), see Appendix C and Supplementary Table S3.1 for details.

Collapsing the ASV table by taxonomic assignment in QIIME2 resulted in 144 plant taxa, with 54%, 89% and 97% identified at the species-, genus- and family-level, respectively. Unresolved taxonomies at the family- and genus-level are reported in Table 4.4 and shown in Figs. 4.1 and 4.3 but were not used for further analyses (i.e., were not included in chi-square analyses).

Plants recorded in the survey or found in pollen loads are listed in Table 4.4. This table also shows whether that plant was involved in a bee interaction (observation, pollen, or both). Table 4.4 allows for observation and metabarcoding results to be compared at a lower taxonomic level than the bipartite networks (Figs. 4.1, 4.3 & 4.4). A total of 243 plant taxa and 5 unknown plants are listed in Table 4.4, albeit some are represented at both the genus- and species-level. For example, *Vicia cracca* L. and *Vicia tetrasperma* (L.) Schreb, were recorded in plant surveys, however, in pollen loads vetch (*Vicia* spp.) was identified at the genus-level., therefore the genus *Vicia* and the two species of vetch are all included in Table 4.4. There are five plants unclassified at the family-level and four unclassified at the genus-level. The 248 plants are from 43 families. Of the 248 plants, 113 were found in pollen loads only, 24 in observation only, and 31 were found in both interactions. Eighty were not identified in any type of bee interaction.

Forty of the plant taxa were found in the area beyond the field. Twenty-four plant taxa were found in pollen loads but were not found in the survey.

Metabarcoding revealed unobserved interactions. In summer, Plantaginaceae was found on 13 bees (11%) but was not present in observed interaction data. During bloom, Aquifoliaceae, Adoxaceae, Betulaceae, Fagaceae, Myricaceae, Oleaceae, and Ranunculaceae were all found on more than five individual bees and were not present in observation interaction data. Some plant families differed in frequency between observation and metabarcoding networks. For example, during summer there were 11 (1.9 %) observed visits to Hypericaceae while 41 bees (34%) had Hypericaceae in pollen loads. This same pattern is found with Fabaceae (20% observation versus 45% metabarcoding), Ericaceae (3.6% vs. 23%), Asteraceae (51% vs. 79%), and Rosaceae (13% vs. 30%) in the summer.

Table 4.4. Complete list of plants surveyed at sites or found in bee-associated pollen from lowbush blueberry fields throughout the project and their association with bees.

Plant family	Plant taxa	Interaction
Adoxaceae	<i>Sambucus</i> spp.	*
	<i>Sambucus racemosa</i> L.*	
	<i>Viburnum nudum</i> L.*	*
	<i>Viburnum nudum</i> L. var. <i>cassinoides</i> (L.) Torr. & A. Gray* <i>Viburnum opulus</i> L.	*
Apiaceae	<i>Aegopodium podagraria</i> L.* ^b	
	<i>Angelica sylvestris</i> L.* ^b	
	<i>Carum carvi</i> L.	*
	<i>Daucus carota</i> L.*	*
	<i>Ligusticum scoticum</i> L.	*
Apocynaceae	<i>Apocynum androsaemifolium</i> L.*	●
	<i>Apocynum cannabinum</i> L.* ^b	
Aquifoliaceae	<i>Ilex mucronata</i> (L.) Powell, Savolainen & Andrews	*
	<i>Ilex verticillata</i> (L.) A. Gray	*
Araliaceae	<i>Aralia</i> spp.	*
	<i>Aralia hispida</i> Vent.*	○
	<i>Aralia nudicaulis</i> L.*	

Plant family	Plant taxa	Interaction
Araliaceae	<i>Panax trifolius</i> L.*	
Asparagaceae	<i>Maianthemum stellatum</i> (L.) Link*	
	<i>Maianthemum trifolium</i> (L.) Sloboda* ^b	
Asteraceae	<i>Achillea</i> spp.	*
	<i>Achillea millefolium</i> L.*	
	<i>Anaphalis margaritacea</i> (L.) Benth*	●
	<i>Antennaria howellii</i> Greene subsp <i>neodioica</i> (Greene) Bayer*	
	<i>Antennaria neglecta</i> Greene*	
	<i>Arctium minus</i> Bernh.*	
	<i>Centaurea</i> spp.	*
	<i>Centaurea nigra</i> L.*	*
	<i>Cirsium arvense</i> (L.) Scop*	*
	<i>Conyza canadensis</i> (L.) Cronquist*	
	<i>Coreopsis lanceolata</i> L.* ^b	
	<i>Doellingeria</i> spp. or <i>Solidago</i> spp.	*
	<i>Doellingeria umbellate</i> (Mill.) Nees*	●
	<i>Echinacea angustifolia</i> DC.* ^b	
	<i>Erigeron</i> spp.*	*
	<i>Eupatorium perfoliatum</i> L.	*
	<i>Euthamia graminifolia</i> (L.) Nutt.	*
	<i>Eutrochium</i> spp. or <i>Eupatorium</i> spp.	*
	Goldenrods*	○
	<i>Hieracium</i> spp. or <i>Crepsis</i> spp.	*
	<i>Hieracium</i> spp.*	●
	<i>Jacobaea vulgaris</i> Gaerth.*	●
	<i>Krigia</i> spp. or <i>Leontodon</i> spp.	*
	<i>Lactuca canadensis</i> L.*	
	<i>Leucanthemum vulgare</i> Lam.*	●
	<i>Matricaria discoidea</i> DC.* ^b	
	<i>Nabalus trifoliolatus</i> Cass.*	
	<i>Prenanthes</i> spp.	*
	<i>Prenanthes alba</i> L.	*
	<i>Prenanthes trifoliolata</i> (Cass.) Fernald*	*
	<i>Rudbeckia hirta</i> L.*	●
	<i>Scorzonerooides autumnalis</i> (L.) Moench*	○
	<i>Solidago</i> spp.	*

Plant family	Plant taxa	Interaction
Asteraceae	<i>Solidago nemoralis</i> Aiton	*
	<i>Sonchus</i> spp.* ^b	
	<i>Sonchus arvensis</i> L.*	
	<i>Symphotrichum</i> spp.	*
	<i>Symphotrichum lateriflorum</i> (L.) A. Love & D. Love*	○
	<i>Symphotrichum novae-angliae</i> (L.) G.L. Nesom* ^b	
	<i>Symphotrichum novi-belgii</i> (L.) G.L. Nesom*	○
	<i>Taraxacum</i> spp.	*
	<i>Taraxacum officinale</i> F.H. Wigg.*	○
	<i>Tragopogon pratensis</i> L.*	
	<i>Tripleurospermum maritimum</i> (L.) W.D.J. Koch	*
	<i>Tripleurospermum</i> spp.	*
	<i>Tussilago farfara</i> L.*	
	Betulaceae	<i>Betula</i> spp.
<i>Corylus</i> spp.		*
Boraginaceae	<i>Phacelia</i> spp.	*
	<i>Phacelia tanacetifolia</i> Benth.	●
	unclassified Boraginaceae	*
Brassicaceae	<i>Brassica nigra</i> (L.) W.D.J. Koch* ^b	
	<i>Erysimum cheiranthoides</i> L.* ^b	
	<i>Hesperis matronalis</i> L.* ^b	*
	<i>Raphanus</i> spp.	*
	<i>Raphanus raphanistrum</i> L.* ^b	
Campanulaceae	<i>Campanula rapunculoides</i> L.* ^b	*
	<i>Lobelia inflata</i> L.*	○
Cannabaceae	<i>Humulus japonicus</i> Siebold & Zucc.	*
Caprifoliaceae	<i>Diervilla lonicera</i> Mill.*	○
Caryophyllaceae	<i>Cerastium</i> spp.	*
	<i>Cerastium fontanum</i> Baumg. subsp. <i>vulgare</i> (Hartm.) Greuter & Burdet *	○
	<i>Moehringia lateriflora</i> (L.) Fenzl.* ^b	*
	<i>Saponaria officinalis</i> L.* ^b	
	<i>Silene vulgaris</i> (Moench) Garcke*	
	<i>Spergula arvensis</i> L.*	○

Plant family	Plant taxa	Interaction
Caryophyllaceae	<i>Spergularia marina</i> (L.) Griseb ^{*b}	
	<i>Spergularia rubra</i> (L.) J. Presl & C. Presl ^{*b}	
	<i>Spergularia salina</i> J. Presl & C. Presl ^{*b}	
	<i>Stellaria</i> spp.	●
	<i>Stellaria</i> spp. or <i>Sagnia</i> spp.	*
	unclassified Caryophyllaceae	*
Convolvulaceae	<i>Calysegia sepium</i> ^{*b}	
Cornaceae	<i>Cornus canadensis</i> L.*	●
	<i>Cornus rugosa</i> Lam.*	
	<i>Cornus</i> spp.	*
Ericaceae	<i>Chamaedaphne</i> spp.	*
	<i>Chamaedaphne calyculata</i> (L.) Moench	*
	<i>Epigaea repens</i> L.*	*
	<i>Gaultheria procumbens</i> L.*	*
	<i>Gaylussacia baccata</i> (Wangenh.) K. Koch*	●
	<i>Kalmia angustifolia</i> L.*	●
	<i>Kalmia polifolia</i> Wangenh	*
	<i>Kalmia</i> spp.	*
	<i>Monotropa uniflora</i> L.	*
	<i>Oxycoccus microcarpus</i>	*
	<i>Oxycoccus</i> spp.	*
	<i>Pyrola chlorantha</i> Sw.*	○
	<i>Rhododendron</i> spp.	*
	<i>Rhododendron canadense</i> (L.) Torr.*	●
	<i>Rhododendron groenlandicum</i> (Oeder) K.A. Kron & W.S. Judd*	
	<i>Vaccinium</i> spp.*	●
	<i>Vaccinium corymbosum</i> L.	*
<i>Vaccinium pallidum</i> Aiton	*	
<i>Vaccinium vitisidaea</i>	*	
Fabaceae	<i>Geranium bicknellii</i> Britton*	
	<i>Lathyrus japonicus</i> Willd*	
	<i>Lotus</i> spp.	*
	<i>Lotus corniculatus</i> L.*	●
	<i>Lotus tenuis</i> Waldst. & Kit. Ex Willd	*
	<i>Lupinus</i> spp.	*

Plant family	Plant taxa	Interaction
Fabaceae	<i>Lupinus polyphyllus</i> Lindl.* ^b	
	<i>Medicago</i> spp.	*
	<i>Medicago lupulina</i> L.*	*
	<i>Medicago sativa</i> L.*	
	<i>Melilotus</i> spp.*	●
	<i>Trifolium</i> spp.	*
	<i>Trifolium arvense</i> L.*	●
	<i>Trifolium aureum</i> Pollich*	
	<i>Trifolium campestre</i> Schreb.*	○
	<i>Trifolium hybridum</i> L.*	●
	<i>Trifolium incarnatum</i> L.*	●
	<i>Trifolium pratense</i> L.*	●
	<i>Trifolium repens</i> L.*	*
	<i>Vicia</i> spp. or <i>Lathyrus</i> spp.	*
	<i>Vicia cracca</i> L.*	○
	<i>Vicia sativa</i> L.*	
	<i>Vicia tetrasperma</i> (L.) Schreb.*	○
unclassified Fabaceae	*	
Fagaceae	<i>Fagus grandifolia</i> Ehrh.	*
	<i>Quercus ilicifolia</i> Wangenh.	*
	<i>Quercus palustris</i> Munchh.	*
Hypericaceae	<i>Hypericum</i> spp.	*
	<i>Hypericum canadense</i> L.*	○
	<i>Hypericum ellipticum</i> Hook	*
	<i>Hypericum maculatum</i> Crantz	*
	<i>Hypericum perforatum</i> L.*	●
<i>Sisyrinchium montanum</i> Greene*		
Iridaceae	<i>Galeopsis tetrahit</i> L.*	
Lamiaceae	<i>Glechoma hederacea</i> L.* ^b	
	<i>Monarda</i> spp.* ^b	
	<i>Prunella vulgaris</i> L.*	●
Liliaceae	<i>Erythronium americanum</i> Ker Gawl.*	
	<i>Linum catharticum</i> L.*	
	<i>Clintonia borealis</i> (Aiton) Raf.* ^b	
Malvaceae	<i>Tilia</i> spp.	*
Myricaceae	<i>Comptonia peregrina</i> (L.) J.M. Coult.*	*
	<i>Morella pensylvanica</i> (Mirb.) Kartesz	*
Myrsinaceae	<i>Trientalis</i> spp.	*

Plant family	Plant taxa	Interaction
Myrsinaceae	<i>Trientalis borealis</i> Raf.*	*
Oleaceae	<i>Fraxinus nigra</i> Marshall	*
	<i>Fraxinus pennsylvanica</i> Marshall	*
Onagraceae	<i>Chamerion angustifolium</i> (L.) Holub*	
	<i>Chamerion</i> spp.	*
	<i>Cypripedium acaule</i> Aiton* ^b	
	<i>Epilobium ciliatum</i> Raf.*	
	<i>Epilobium palustre</i> L.*	○
	<i>Oenothera</i> spp.* ^b	*
	<i>Oenothera biennis</i> L.*	○
Orchidaceae	<i>Platanthera lacera</i> (Michx.) G. Don*	
	<i>Spiranthes cernua</i> (L.) Rich.* ^b	
Orobanchaceae	<i>Euphrasia</i> spp.	*
	<i>Euphrasia nemorosa</i> (Pers.) Wallr.*	
	<i>Euphrasia subarctica</i> Raup	*
	<i>Melampyrum arvense</i> * ^b	
	<i>Melampyrum lineare</i> Desr.*	●
Oxalidaceae	<i>Oxalis</i> spp.*	○
Papaveraceae	<i>Capnoides sempervirens</i> (L.) Borkh.*	
Plantaginaceae	<i>Linaria</i> spp. or <i>Nuttallanthus</i> spp.	*
	<i>Linaria</i> spp.	*
	<i>Linaria vulgaris</i> Mill.*	
	<i>Nuttallanthus canadensis</i> (L.) D.A. Sutton*	*
	<i>Plantago lanceolata</i> L.	*
	<i>Plantago major</i> L.* ^b	
	<i>Veronica</i> spp.	*
	<i>Veronica arvensis</i> L.* ^b	
	<i>Veronica officinalis</i> L.*	*
Polygonaceae	<i>Polygonum</i> spp.* ^b	
	<i>Rumex acetosella</i> L.*	●
	<i>Rumex crispus</i> L.* ^b	
	<i>Rumex obtusifolius</i> L.* ^b	
	<i>Rumex</i> spp.	*
Ranunculaceae	<i>Anemone quinquefolia</i> L.*	
	<i>Aquilegia vulgaris</i> L.* ^b	
	<i>Caltha palustris</i> L.	*

Plant family	Plant taxa	Interaction
Ranunculaceae	<i>Ranunculus</i> spp.	*
	<i>Ranunculus repens</i> L.*	
	<i>Thalictrum</i> spp.	*
	<i>Thalictrum revolutum</i> DC.	*
Rhamnaceae	<i>Frangula alnus</i> Mill.	*
Rosaceae	<i>Amelanchier</i> spp.*	○
	<i>Aronia arbutifolia</i> (L.) Pers.	*
	<i>Aronia melanocarpa</i> (Michx.) Elliott*	○
	<i>Fragaria</i> spp.	*
	<i>Fragaria vesca</i> L.	*
	<i>Fragaria virginiana</i> Duchesne*	○
	<i>Geum canadense</i> Jacq.* ^b	
	<i>Malus baccata</i> (L.) Borkh.	*
	<i>Malus pumila</i> Mill.* ^b	
	<i>Potentilla argentea</i> L.	*
	<i>Potentilla</i> spp.*	●
	<i>Prunus</i> spp.	*
	<i>Prunus virginiana</i> L.	*
	<i>Prunus pensylvanica</i> L. f.*	
	<i>Rosa</i> spp.*	●
	<i>Rubus idaeus</i> L.	*
	<i>Rubus</i> spp.*	●
	<i>Sibbaldiopsis tridentata</i> (Aiton) Rydb.*	
	<i>Sorbaria sorbifolia</i> (L.) A. Braun* ^b	
	<i>Sorbus</i> spp., <i>Amelanchier</i> spp. or <i>Malus</i> spp.	*
	<i>Sorbus</i> spp.	*
	<i>Spiraea</i> spp.	*
	<i>Spiraea alba</i> Du Roi*	○
<i>Spiraea chamaedryfolia</i> L.	*	
<i>Spiraea latifolia</i> (Aiton) Borkh*	○	
<i>Spiraea tomentosa</i> L.*		
Unclassified Rosaceae		*
Rubiaceae	<i>Galium mollugo</i> L.* ^b	
	<i>Galium</i> spp.*	●
	<i>Houstonia caerulea</i> L.*	●
Ruscaceae	<i>Maianthemum canadense</i> Desf.*	
Salicaceae	<i>Salix</i> spp.*	●

Plant family	Plant taxa	Interaction
Sapindaceae	<i>Acer spicatum</i> Lam.	*
	<i>Acer saccharum</i> L.*	*
	<i>Acer</i> spp.	*
	<i>Aesculus</i> spp.*	
Scrophulariaceae	<i>Buddleja</i> spp.* ^b	
	<i>Verbascum thapsus</i> L.* ^b	
Violaceae	<i>Viola</i> spp.*	●
Caprifolaceae or Adoxaceae	unknown Caprifolaceae or Adoxaceae	*
Liliaceae or Alliacea	<i>Allium</i> spp. or <i>Erythronium</i> spp.	*
Asteraceae or Polygonaceae	<i>Tragopogon</i> spp. or <i>Rumex</i> spp.	*
Balsaminaceae or Betulaceae	<i>Alnus</i> spp. or <i>Impatiens</i> spp.	*
Betulaceae or Iridaceae	<i>Alnus</i> spp. or <i>Iris</i> spp.	*
unknown	tiny yellow flower*	
unknown	white flowers*	
unknown	purple stems*	
unknown	little herb* ^b	
unknown	little purple flowers*	○

Notes: Plants observed through survey in lowbush blueberry sites are designated with an (*), plants which were observed outside the field only (2018 and 2019 season) are designated with a superscript b (^b). Observed bee visits to flowers are designated with an open circle (○). Plant taxa found only in bee-associated pollen are designated with a star (*). Plant taxa found in both bee-associated pollen and observed bee visits have a filled circle (●). It is possible to have different levels of taxonomic resolution for bee observations and pollen identification. For the plant survey and bee observations the following plants were grouped: *Galium* spp.; *Hieracium* spp.; *Oxalis* spp.; *Potentilla* spp.; *Rosa* spp.; *Rubus* spp.; *Stellaria* spp.; *Solidago* spp. & *Euthamia* spp. as “Goldenrods”; and *Viola* spp. For the pollen identification some amplicon sequence variants (ASVs) were identified to species-level and others to only genus-level (i.e. *Eupatorium perfoliatum* and *Eupatorium* spp. were both identified in pollen loads). Plants with ambiguous identification in the survey or ambiguous identification at the family-level in the pollen are at the bottom of the table.

Discussion

Bloom

Ericaceae, Asteraceae, and Rosaceae were common in both observation and metabarcoding networks (Fig. 4.1). Plants from these three families have been reported as alternative forages used by wild bees during lowbush blueberry bloom (Stubbs *et al.*, 1992; Moisan-Deserres *et al.*, 2014; Bushmann & Drummond, 2015). Other plant families found in bloom pollen loads in this study have been found to be alternative forages in lowbush blueberry fields in Maine (i.e., willows (*Salix* spp.; Salicaceae) and maples (*Acer* spp.; Sapindaceae) in Stubbs *et al.* (1992)), or Lac-St-Jean region of Quebec (i.e., alders (*Alnus* spp.; Betulaceae), elderberry (*Sambucus* spp. (Adoxaceae), and mountain holly (*Ilex* spp. Aquifoliaceae) in Moissan DeSerres *et al.* (2014)).

These findings are also consistent with work from Wood *et al.* (2018) where they examined pollen loads of wild bees in Michigan known to visit highbush blueberry and found woody plants such as *Vaccinium* spp., *Salix* spp., and *Prunus* spp. were common. Managed *Bombus impatiens* Cresson (Hymenoptera: Apidae) pollen loads contained cherries (*Prunus* spp.), oak (*Quercus* spp.), Rosaceae, and willows. during highbush blueberry bloom in Michigan (Graham *et al.* 2023). During highbush blueberry bloom in British Columbia bumble bees often had pollen loads that contained Rosaceae and Fabaceae pollen (Bobiwash *et al.*, 2018).

This study found that early flowering trees including maples (Sapindaceae), beeches (Fagaceae), birches (Betulaceae), and shrubs such as willows (Salicaceae), elders and sambucus (Adoxaceae), lilacs (Oleaceae), bayberry (Myricaceae), and mountain holly (Aquifoliaceae) support spring flying bees. These trees and shrubs could be planted as a hedge or wind break at field edges. Mature hedgerows increased wild bees and pollination services in tomato fields (*Solanum lycopersicum* L.) in California (Morandin & Kremen, 2013). Strawberry fields in southern Germany with connected hedgerows had increased pollinator abundance as well as increased strawberry (*Fragaria x ananassa*

(DUCH.) weights compared to fields without hedgerows (Castle *et al.*, 2019). Typically, sown flower strips are a perennial mix that bloom after most bees emerge (Campbell *et al.*, 2017; Ouvrard *et al.*, 2018). Hedgerows have been found to extend the flowering period of floral enhancements (von Königslöw *et al.*, 2022).

When alternative floral resources bloomed before broad bean in Germany, seed set increased (Eckerter *et al.*, 2022). The main pollinator of broad bean was *Bombus terrestris* L. which used *Prunus* spp., *Acer* spp. and *Salix* spp. before crop bloom (Eckerter *et al.*, 2022). McCallum and McLean (2017), focused on alternative forages used by bumble bees in lowbush blueberry fields in New Brunswick before and after crop bloom. They found that bumble bees visited rhodora (*Rhododendron canadense* (L.) Torr.), and dandelion (*Taraxacum officinale* F.H. Wigg.) before bloom. Andrenid flight time is well matched to crop bloom (Cutler *et al.*, 2015), however, some members of the genus *Andrena* emerge in April and May (Sheffield *et al.*, 2003). Peak *A. regularis* Malloch (Hymenoptera: Andrenidae) emergence was pre-blueberry bloom in northeastern North America (Boulanger *et al.*, 1967). Further work is needed to determine if the addition of early flowering trees and shrubs used by bees in this study would increase the abundance of blueberry pollinators that emerge pre-bloom.

Early flowering herbaceous perennials used by bees in this study were mostly asters including hawkweeds (*Hieracium* spp.) and dandelions (Fig. 4.1). The only genus from the family Polygonaceae was a sorrel (*Rumex* spp.). Hughes (2012) and Bushmann & Drummond (2015) observed bees visiting sheep sorrel (*Rumex acetosella* L.) in lowbush blueberry fields in NS and Maine, respectively.

The three bee families tested did not differ in the proportion of individual bees that carried Asteraceae, Polygonaceae, Rosaceae or Salicaceae pollen which suggests that these common plant families are used by many wild bees. Bee families differed in their use of two other frequently used plant families, Adoxaceae and Sapindaceae.

Andrenidae had significantly more individuals with Adoxaceae pollen than Apidae or Halictidae (Fig. 4.2). Twenty-two of the 25 individuals that carried *Sambucus* spp. pollen and seven of the eight individuals that carried *Viburnum* spp. pollen, were *Andrena carlini* Cockerelle (Supplementary Table S4.2) This suggests that Adoxaceae pollen is used by *A. carlini* but may not be a high priority for bee conservation in general. Girard *et al.*, (2012) found *Sambucus* spp. in pollen from honey bee hives in lowbush blueberry fields in Quebec. Apidae had significantly more individuals with Sapindaceae pollen than Halictidae (Fig. 4.2). *Acer* spp. was found in the pollen loads of Andrenidae (n=34), particularly *A. carlini* (n=26), as well as *Bombus* spp. (n=28) but was rarely used by Halictidae (n=4; Table S4.2). *Acer* spp. are wind pollinated but the pollen is high in protein (Liolios *et al.*, 2015). Maple trees were found at field edges in this study. Bee foraging distance is related to body size with larger bees foraging disproportionately farther than smaller bees (Greenleaf *et al.*, 2007). Small-bodied Halictidae might not fly to trees at the field edge or beyond the field. In this study, Betulaceae and Fagaceae were used by Apidae and Andrenidae but not Halictidae (Fig. 4.1). Further work is needed to determine if trees, in terms of location, usually at the edge of a field, or their height, are within the flight zone of small-bodied bees.

Andrenidae and Apidae had significantly higher proportions of individuals with Ericaceae pollen than Halictidae (Fig. 4.2). Bushmann & Drummond (2015) found that most *Andrena* spp. were observed more often on lowbush blueberry flowers than most *Halictus* spp. and most *Lasioglossum* spp. Moissan-DeSerres *et al.*, (2014) found that the proportion of *Lasioglossum* spp. pollen loads that were blueberry varied among species.

Ericaceae was the most common plant family in observed and metabarcoding bloom interactions (Fig. 4.1). Ericaceae is an important floral resource for wild bees in lowbush blueberry agroecosystems. Most blueberry fields are managed to bloom every second year. Some blueberry fields are managed on a split cycle, where each year half of the field is in the sprout year while the other half is in fruit. This production system has the

potential to stabilize Ericaceae pollen supply. Venturini *et al.*, (2017a) found that split cycle management systems had slightly higher densities of tumuli, which are mounds of soil that surround ground nest entrances.

Botanical composition of sprout-year bee-associated pollen could reveal valuable information regarding alternative forages for wild bees. The southeastern blueberry bee (*Habropoda laboriosa* Fabricius, Hymenoptera: Apidae) has been identified as an oligolect of *Vaccinium* spp., however, surveys conducted outside of commercial rabbiteye blueberry fields found that it gathered pollen from five plant genera from four plant families (Pascarella, 2007). Bushmann & Drummond (2015) found that andrenids gathered pollen from sheep laurel (*Kalmia angustifolium* L.) and black huckleberry (*Gaylussacia baccata* (Wangenh.) K. Koch) and suggested that these Ericaceae plants could be a pollen source at the end of blueberry bloom. In this study bees visited and collected pollen from other Ericaceous plants such as, *Kalmia* spp. and *Rhododendron* spp. (Table S4.1 and S4.2).

Post-bloom

There were no significant differences among the proportion of individuals from the families Apidae and Halictidae carrying pollen from the six plant families tested during the summer (Table 4.2), suggesting the same common plant families support these two bee families during summer.

Asteraceae was the most common plant family in pollen loads and observations in summer and fall (Figs. 4.3 & 4.4). Previous work has found that bees collect pollen from asters after lowbush blueberry bloom (Stubbs *et al.*, 1992; McCallum & McLean, 2017; Venturini *et al.*, 2017b). Wood *et al.*, (2018b) found that *Solidago* spp. dominated pollen loads of solitary and social bees in August and September in Michigan. Venturini *et al.*, (2017b) observed bumble bees on New England aster (*Symphotrichum novae-angliae* (L.) G. L. Nesom) in late September and early October.

Fabaceae was the second most common plant family recorded in summer observations and pollen loads. Wood *et al.*, (2021) found bumble bees were generalists, however, they collected between 30 and 60% of their diet from Fabaceae. Legumes are heavily used, especially by bumble bees (Campbell *et al.*, 2017; McCallum & McLean, 2017; Venturini *et al.*, 2017b). Rosaceae and Hypericaceae were frequently visited by bees in the summer (Fig. 4.3). Wild bees were often recorded on Rosaceae in Maine (Bushmann & Drummond, 2015) however, their survey was completed by early July and so didn't completely overlap with the timing of this study. The only genus from the family Hypericaceae was St. John's wort (*Hypericum* spp.) which is a common weed in lowbush blueberry fields in NS (Lyu *et al.*, 2021) and was found in 45% of blueberry fields surveyed in Maine (Drummond, 2019b). Like this study, Drummond (2019b) found that bumble bees were the most abundant bee observed on St. John's wort.

Bees visit a sub-set of available flowering plants

Many plants recorded in the survey were not visited by bees nor identified in pollen loads (Table 4.4). Some plant-pollinator interactions identified with metabarcoding were rare. During bloom, summer, and fall 16/60, 20/64, and 7/17 plant taxa at the genus-level were found on only one bee (Tables S4.2, S4.4 & S4.6). Moisan-DeSerres *et al.*, (2014) and Wilson *et al.*, (2021), also found that many plant taxa were present on few individuals or contributed to a small proportion of the total pollen load. The goal of this study was not to determine why bees visited and collected pollen from certain plants. Some previously reported factors include flower configuration, origin (i.e., native vs. non-native) (Dibble *et al.*, 2020b), and floral area (Tuell *et al.*, 2008). Bumble bees preferentially forage on plants with increased protein or amino acid content (Hanley *et al.*, 2008). Individual bumble bees have been shown to prefer pollen with higher protein content (Ruedenauer *et al.*, 2016). Ouvrard *et al.*, (2018) calculated a floral resource density by combining pollen or nectar quantities per flower unit and the flower unit density. The floral resource density was significantly correlated with insect visits for both

pollen ($r = 0.93$, $p < 0.001$) and nectar ($r = 0.98$, $p < 0.001$) (Ouvrard *et al.*, 2018). Furthermore, Moerman *et al.* (2017) found that *B. terrestris* L. microcolonies fed different diets differed in pollen efficiency (weight of larvae / weight of pollen collection) but that it was not related to pollen diversity (i.e., one monofloral diet was just as effective as a difloral and trifloral diet), indicating that floral plantings based on diversity alone may not offer the appropriate resources.

Some of the most frequently used plant families in this study have nutritious pollen. Pollen from the Fabaceae family is high in protein and contains essential amino acids (Crailsheim *et al.*, 1992; Hanley *et al.*, 2008; Forcone *et al.*, 2011). For example, white clover (*Trifolium repens* L.) pollen is high in protein, contains essential amino acids, and is digestible by honey bee larvae (Crailsheim *et al.*, 1992). *Prunus* spp. (Vanderplanck *et al.*, 2014) and other members of the Rosaceae family (Forcone *et al.*, 2011) have pollen that is high in crude protein.

The most common plant bees interacted with during summer and fall was Asteraceae. Aster pollen can be low quality (Hanley *et al.*, 2008; Forcone *et al.*, 2011). However, some species of the Asteraceae family have good quality and quantity of nectar (Hicks *et al.*, 2016) or have medicinal or protective properties, For example, sunflower (*Helianthus annuus* L., Asteraceae) pollen has been found to suppress infection by a gut pathogen in *B. impatiens* (Figuroa *et al.* 2023). *Bombus impatiens* colonies had shorter lifespans when fed only sunflower pollen compared to colonies fed broad bean (*Vicia faba*, Fabaceae), rapeseed (*Brassica napus* L., Brassicaceae) or Cucurbitaceae pollen (McAulay & Forrest, 2019). When sunflower pollen was diluted to 50% (mixed with equal amounts of pollen from the other three families), lifespan was the same as the non-sunflower diets (McAulay & Forrest, 2019). No individual bee collected in this study had a pure Asteraceae pollen load (Tables S4.2, S4.4 & S4.6). Also, during summer and fall when Asteraceae pollen was most common, there would be fewer developing larvae.

In this study, 21 of the 23 fall captured bees were *Bombus* spp., a group known to benefit from fall floral resources (Rundlöf *et al.*, 2014; Dicks *et al.*, 2015; Timberlake *et al.*, 2021) and have shorter life spans when consuming only aster pollen (McAulay & Forrest, 2019). Fabaceae was the only other family on which bees were observed in the fall (Fig. 4.4) and was one of the most frequent families found in summer pollen loads (Fig. 4.3). Management of legumes could support fall flying bees. For example, Timberlake *et al.*, (2021), suggests mowing legumes in summer to extend fall bloom.

Blueberry pollen was 13.9% crude protein (Somerville, 2001). This is below the 20% threshold Kleinschmidt *et al.* (1974) found was required to meet the nutritional requirements of honey bee colonies. Blueberry pollen contains essential amino acids (Somerville, 2001) based on amino acid requirements for honey bees (DeGroot 1952). Pollen preference may be related to essential amino acid content (Cook *et al.*, 2003) rather than percent crude protein (Schmidt, 1982). Without knowledge of species-specific bee nutritional requirements, it is difficult to determine the nutritional value of *Vaccinium* spp. pollen. Wild bees have been found with large amounts of *Vaccinium* spp. pollen (Cane & Payne, 1988; Mayer *et al.*, 2012), including *A. carolina* and *A. bradleyi* in lowbush blueberry fields in Maine (Bushman & Drummond, 2015) and Quebec (Moisan-Deserres *et al.*, 2014). In this study 52% of individual bees captured during bloom had pure *Vaccinium* spp. pollen loads. *Vaccinium* spp. pollen may be suitable for their developing larval needs. Specialist bees have been found to develop on low quality pollen on which other specialists failed to develop (Praz *et al.*, 2008). Another possibility is that at least some species of bee larvae are not developing optimally on blueberry pollen. Some bees may overcome possible blueberry pollen nutritional deficiencies by using progressive feeding (i.e., bumble bees) or through pollen mixing (McAulay & Forrest, 2019).

Bees

DNA barcoding identified wild bees from five families, 13 genera (Table 4.1), and 58 species (Supplementary Table S4.7) foraging in lowbush blueberry fields. The goal of this study was to characterize plant-pollinator interactions and not to conduct a general bee survey, which others have done (Sheffield *et al.*, 2003; Cutler *et al.*, 2015; McCallum *et al.*, 2021). This work identified fewer species than the 78 species Sheffield *et al.*, (2003) and the 95 species Cutler *et al.*, (2015) reported in NS blueberry fields. The genus-level composition of captured bees (Table 4.1) was similar to previous surveys of lowbush blueberry fields in Maritime Canada (Sheffield *et al.*, 2003; Cutler *et al.*, 2015; McCallum *et al.*, 2021) and Maine (Bushman & Drummond, 2015).

The relative proportion of captured bees may differ due to differences in sampling method. For example, Cutler *et al.* (2015), found *Lasioglossum* was the most common genus in lowbush blueberry fields. Cutler *et al.* (2015), used pan traps which are biased towards the collection of small bees, such as those in the family Halictidae (Portman *et al.*, 2020; McCallum *et al.*, 2021). Drummond (2017a) found no correlation between bees captured in pan-traps and wild bees foraging on blueberries. Furthermore, Bushmann & Drummond (2015) found differences between the relative abundance of the entire bee community and the subset of bees that were observed on blueberry flowers. In this study, bees were collected if they were found on a flower and so the bee community in this work is more similar to studies that have focused on bees visits to blueberry.

Observations and metabarcoding

Plants on which bees were observed were represented in the pollen data, although not always at the same taxonomic resolution. There are several examples of species-level interactions in the observation data and genus-level interactions in the pollen data

(Table 4.4). This is a known limitation of taxonomic identification using DNA metabarcoding.

Some rare observations (i.e., one bee was observed on *Oxalis* spp.) were not captured in pollen data, which wasn't unexpected. Alternatively, some interactions that were rare in the observation data were more frequent in pollen data. Pollen from trees or shrubs were more frequent in pollen loads than in observations. For example, approximately 2% of observations during bloom were to Rosaceae, while 23% of bees captured during bloom carried Rosaceae pollen (Fig. 4.2). Plants from these taxa were often in the area outside of the field, which explains why they are less frequent in observed interactions. This is consistent with work from Bobiwash *et al.*, (2018) that found plants in the surrounding landscape served as alternative forage.

Metabarcoding bipartite graphs contained interactions with more plant families than observation graphs (Figs. 4.1, 4.3 & 4.4). Some interactions found with metabarcoding were with plants not found in the survey (Table 4.4). In many cases, the interactions seem likely (i.e., oaks (*Quercus* spp.), ash (*Fraxinus* spp.), and mountain ash (*Sorbus* spp.)), given that the plants are present in the region (Munro *et al.*, 2014). In other cases, differences between the survey and pollen data could be explained by incorrect plant identification in the field or errors with the taxonomic classifier. For example, *Fragaria virginica* (survey) vs. *Fragaria* spp. and *Fragaria vesca* (pollen).

Another benefit of metabarcoding individual pollen loads is that it provides information on individual bee foraging behavior. Pooling the interactions at the species-, genus- or family- level can identify foraging patterns or trends. In this study individual wild bees had pollen loads that on average, had few genera (86% had less than three genera in pollen loads during bloom, Fig. 3.3) and bees collected in the summer (n=134) had an average of 3.2 genera per pollen load (data not shown). While individual bees had few taxa in pollen loads, bee groups and bee families collected pollen from many plant taxa

(Figs. 4.1, 4.3 & 4.4). In the summer, *Bombus* spp. (n=101), collected pollen from a total of 23 families (Table S4.4) and Halictidae (n=18) collected pollen from 10 families (Table S4.4). Forrest *et al.*, (2015) found that bumble bees collected pollen from more than 10 plant families. Wood *et al.*, (2021) examined the pollen loads of 4,132 bumble bees from 58 species using microscopy and found broad polylecty (60 plant families) and that each species collected from an average of 10 plant families.

This study demonstrates that DNA sequencing was able to identify more plant-pollinator interactions than observations during transect walks. Metabarcoding identified rare interactions and interactions with plants outside of the survey area. There was a substantial overlap for commonly detected interactions using both techniques. Interactions identified only with metabarcoding seem likely given the plants are in the ecosystem. Together this suggests that DNA metabarcoding enhances or could act as a substitute for visual observations to monitor plant-pollinator interactions.

Conclusions

Wild bee observations and metabarcoding of bee-associated pollen over the season identified flowering plants that bees use in and around lowbush blueberry fields. DNA metabarcoding of bee-associated pollen revealed more plant-pollinator interactions than observations. During bloom, summer, and fall metabarcoding identified 29, 24 and 9 plant families in pollen loads while bees were observed visiting 12, 18 and 2 plant families, respectively. Bees foraged on a subset of the available plant taxa and foraging patterns shifted from primarily early flowering trees and shrubs in the spring to herbaceous perennials in the summer and fall. Asteraceae was the third most common non-blueberry pollen collected during bloom and the most common non-blueberry pollen collected in summer and fall. Fabaceae was the second most common pollen found in summer. Rosaceae was the most common non-crop pollen in bloom and the fourth most common in the summer. Unexpectedly, Hypericaceae was the third most common pollen found in summer, and Sapindaceae was the second most common

pollen found in bloom. The bee community was split between solitary spring flying bees (andrenids) and season-long social bees (bumble and halictid bees). During blueberry bloom, bee families differed in the proportion of individuals with Adoxaceae, Ericaceae, and Sapindaceae pollen. During summer, bee families did not differ in the proportion of individuals carrying pollen from the six families tested.

Chapter 5: Conclusions

This research provides information on plant-pollinator interactions over the season in lowbush blueberry (*Vaccinium angustifolium* Aiton) agroecosystems in Maritime Canada. The interactions captured using observation and high-throughput DNA metabarcoding revealed that wild bees visited and collected pollen from lowbush blueberry flowers during bloom and a subset of other flowering plants over the season. These data can help determine which floral resources are useful in a wild bee management plan for lowbush blueberry producers.

This is the first study to investigate plant-pollinator interactions in lowbush blueberry fields in Maritime Canada using DNA metabarcoding. Metabarcoding revealed more plant-pollinator interactions than observations (Chapters 3 & 4). The bioinformatics pipeline identified the plant pollen to the genus-level for 89% of the amplicon sequence variants (ASVs) and to species-level for 54% of ASVs (Chapter 4). Furthermore, plant families that were more frequent in the observation data were usually found on more individuals (Figs. 4.1, 4.3 & 4.4). Metabarcoding revealed interactions with more plant taxa including plants outside of the field (Table 4.4). Together this indicates that metabarcoding data are reliable, especially at the genus-level, and increased the temporal and spatial scale of plant-pollinator interactions. The methods described in this thesis can be used for future research on plant-pollinator interactions.

Wild bees visit lowbush blueberry

All five bee families found in lowbush blueberry fields (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae) visited and collected pollen from lowbush blueberry (Chapter 3). At some sites, wild bees were observed visiting lowbush blueberry more often than honey bees (Chapter 3, Fig. 3.1), indicating they are contributing more to blueberry pollination, especially when their comparative effectiveness is considered (Javorek *et al.*, 2002; Drummond, 2016). Many of the sites that had more wild bee visits

to lowbush blueberry (Chapter 3, Fig. 3.1) were using management practices to promote wild bees. These included floral plantings in the area outside the field (FH_2017) or cutting trees at the field edges and letting native and naturalized plants regenerate (BR_2018, PR_2018, and AFT_2018). Some of the sites with few wild bee observations to lowbush blueberry (A_2017, HU_2017, S_2017) had no management to support wild bees. In many cases, the sites with more wild bee visits to lowbush blueberry had more plant species (Chapter 2, Fig. 2.3) and/or mean floral abundance (Chapter 2, Fig. 2.4). Wild bees most frequently captured during bloom included: *Andrena carlini* (Hymenoptera: Andrenidae), *A. carolina* Viereck, *A. nivalis* Smith, *Bombus bimaculatus* Cresson (Hymenoptera: Apidae), *B. impatiens* Cresson, *B. perplexus* Cresson, *B. ternarius* Say, and several species from the genus *Lasioglossum* (Hymenoptera: Halictidae).

Andrenid bee phenology is well matched to blueberry bloom (Chapter 2, Fig. 2.2). Ninety-three percent of *Andrena* spp. captured during bloom carried *Vaccinium* spp. pollen (Supplementary Table S4.2). *Andrena carolina* collected pollen from fewer alternative plant taxa than *A. carlini* and *A. nivalis* (Supplementary Table S4.2) and had the most individuals with pure *Vaccinium* spp. pollen loads. The number of plant genera in the pollen loads of *A. carolina* was significantly less than *A. carlini* and *A. nivalis* (Chapter 3, Fig. 3.4).

Bumble bee numbers peaked in the summer (Chapter 2, Fig. 2.1). Most bumble bees flying during blueberry bloom were queens. Ninety-five percent of the *Bombus* spp. captured during bloom carried *Vaccinium* spp. pollen. The number of genera in pollen loads of the three *Bombus* spp. tested did not differ.

Halictid bees, which are part of the small black bees, metallic bees, or the “other wild bees” groups were observed season-long in low but steady numbers (Chapter 2, Fig. 2.2). Eighty-eight percent of the Halictidae captured during bloom carried *Vaccinium* spp. pollen. Halictidae had more observations to non-crop plants (Chapter 4, Fig. 4.1)

and had fewer individuals with pure *Vaccinium* spp. pollen loads compared to Andrenidae and Apidae (Supplementary Table S4.2). Halictidae had significantly fewer individuals with pollen from the family Ericaceae than Andrenidae and Apidae (Chapter 4, Fig. 4.2).

In general, increased plant richness, floral abundance and diversity did not increase bee visits to lowbush blueberry, however, there were a few exceptions. During bloom, andrenid bee visits to blueberry in the field were positively correlated to the number of plant taxa and plant diversity in the field during bloom (Table 2.1). Bumble bee visits to lowbush blueberry in the field during bloom were positively correlated to floral abundance in the field (Table 2.1).

There were very few observations of andrenid or bumble bees visiting non-blueberry flowers in the field. The main non-blueberry flowers bumble bees were observed visiting in the field were black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) and sheep laurel (*Kalmia angustifolia* L.), which were both found in pollen loads (Supplementary Table S4.2). Non-crop pollen from flowers recorded in field were rare in pollen loads, except for sheep sorrel (Polygonaceae; Chapter 4, Fig. 4.1). Most of the non-crop pollen found in bloom pollen loads was from trees and shrubs. So, the significant positive correlations of wild bees to plant metrics in the field (Table 2.1), could be related to the fact that a site with more plant richness, diversity and/or abundance in the field during bloom is more likely to have increased richness diversity and abundance around the field throughout the year and in the sprout year. Co-flowering plants are having limited impact on visits to blueberry. The negative correlation between the proportion of *Vaccinium* spp. in pollen loads and number of co-flowering plants at the field edge and in the field was significant but weak ($r_{s(387)} = -0.11$, $P = 0.023$; Chapter 3).

“Other wild bee” visits to blueberry during bloom at the field edge and in the field was positively correlated to floral abundance at the field edge in the fall (Chapter 2, Table

2.3). Previous research has found that fall resources are beneficial for bumble bees (Rundlöf *et al.*, 2014; Timberlake *et al.*, 2021). Some of the bees in the “other wild bee” group were season-long bees (including metallic bees, small black bees and other bees in Fig. 2.2) that, like bumble bees, would benefit from fall resources. Only twenty-three bees were captured for fall pollen analysis (Chapter 4). Further work is needed to better understand fall floral resources.

Season-long resources are needed

Both early-emerging, short-lived, solitary (Andrenidae), and early-emerging, long-lived, social bees (*Bombus* spp. and Halictidae), were found to pollinate blueberry (Chapter 3). To sustain both groups, continuous floral bloom is needed. Plant diversity and abundance of bloom peaked in the summer (Figs. 2.2 & 2.3). Positive correlations between plant metrics and bee visits to lowbush blueberry were found in spring and fall (Tables 2.1 & 2.2). Previous work has found that there is a lack of phenological overlap between some important pollinating bees and floral enhancements and more plants are needed to support spring- and fall-flying bees (Wood *et al.*, 2017, 2018a; Ouvrard *et al.*, 2018; Timberlake *et al.*, 2019, 2021).

Pollen loads from spring included several tree and shrub species (Fig. 4.1) while summer and fall pollen loads were mainly from herbaceous perennials (Figs. 4.3 & 4.4). To supply season-long floral resources different types of plantings such as hedgerows, meadows, and flower strips could be used. Management practices, such as mowing in summer, to extend fall bloom could also be adopted. Monitoring plant-pollinator interactions pre-bloom and under management practices to extend bloom would be useful.

Alternative forages used by bees

This study provided a list of plants that wild bees visited and collected pollen from over the season in lowbush blueberry agroecosystems in Maritime Canada (Table 4.4). Over

the season, plants from the families Ericaceae, Asteraceae, Rosaceae and Fabaceae were frequently visited.

Bee groups had different phenologies (Fig. 2.2) and responded differently to plant metrics (Tables 2.1 - 2.3). Many plant families were visited by all bee families (Figs. 4.1, 4.3 & 4.4), however, during bloom, bee families differed in the proportion of individuals carrying pollen from some plant families (Fig. 4.2). Therefore, floral enhancements may need to be adjusted depending on the bee taxa of interest.

The most common plant families identified in bloom pollen loads and observations, in addition to Ericaceae, were Rosaceae, Sapindaceae, Asteraceae, Adoxaceae, Polygonaceae, and Salicaceae (Fig. 4.1). During summer, Asteraceae, Fabaceae, Hypericaceae, and Rosaceae were the most common families in plant-pollinator interactions. Asteraceae was the most common plant in fall observations and pollen loads.

Bees visited a subset of available flowers

Bees collected pollen from a sub-set of available plants (Table 4.4). Many seed mixes designed to promote bees have focused on plant diversity with less emphasis on floral use. Investigating plant-pollinator interactions, rather than comparing bee abundance and diversity in enriched versus non-enriched sites reveals what flowers bees are using. Much of what is known about bee nutrition is from work done on honey bees. Nutritional requirements of wild bees are varied and differ from honey bees (Barraud *et al.*, 2022). Continuing to study bee nutrition, pollen nutrient composition, and plant-pollinator interactions can help guide plantings that supply nutritious pollen that bees collect.

The Lowbush blueberry agroecosystem is suited to support wild bees

A large-scale synthesis on the effectiveness of floral borders found that pollination was enhanced when borders contained perennials and had a higher flowering plant diversity (Albrecht *et al.*, 2021). Native bees prefer to forage on native plants (Morandin & Kremen, 2013; Dibble *et al.*, 2020b; Lowe *et al.*, 2022). Previous research has found that flower strips that contain native plants are more effective (Gill *et al.*, 2014; Butters *et al.*, 2022), and native bees tend to be better pollinators in a crops' natural range (Gibbs *et al.*, 2016). Floral plantings adjacent to *Vaccinium* spp. crops take time to positively impact yields (Blaauw & Isaacs, 2014; Venturini *et al.*, 2017b). Lowbush blueberry is a native, perennial shrub. Establishing long-term perennial floral enhancements adjacent to a perennial crop, where land is not rotated is more practical. Also, many plants bees were visiting and collecting pollen from were native, perennial plants and shrubs already present in the ecosystem.

Recommendations for lowbush blueberry producers

Lowbush blueberry producers should survey the plants in and around their fields. It is expensive to plant trees and shrubs, however, many of the flowering plants wild bees visited and collected pollen from were common in lowbush blueberry agroecosystems and were either native or naturalized. Allowing some shrubs to grow by not mowing or spraying might be sufficient to increase some flowering plants. In this study, some of the sites that had more wild bees had cut down or pushed back large trees at the field edge and allowed natural or naturalized plant species to establish.

Other sites that had more wild bees were adjacent to meadows where some of the grasses and wild plants at the edge were not mowed. Allowing for some herbaceous perennials to grow at the field edge or in non-productive areas could also increase flowering resources. Seed mixes that include different legumes (clovers, vetches, trefoil) should be used for strategic field edge plantings.

Important considerations include proximity of floral resources to nesting sites. Smaller fields may be able to maintain flowering plants at the field edge. Larger fields may benefit from a wind break or a strip of flowering shrubs and/or perennials in the field. Strategic management of non-crop flowering plants present in the field could be used (i.e., allow a certain proportion of specific weeds (*Hypericum* spp., Ericaceae or Asteraceae) in some parts of the field to flower). Another important consideration is continuous bloom. Selecting to enrich or strategically plant flowers that bloom when there are few flowers at your site. This could be planting maple trees or alders, staking willows for spring bloom, or strategic mowing of nearby meadow/ditches/established flower strip to extend bloom.

Limitations

Quantification of pollen load composition

Pollen metabarcoding results presented in this thesis were not quantitative. The relative number of reads in a sample was not used to estimate the proportion of that plant taxa in the pollen load. Therefore, it is unknown if plant taxa presented in this thesis are major or minor dietary components. For example, in a study by Wood *et al.*, (2021) almost all *Bombus* spp. carried Fabaceae pollen, but different species carried different proportions. Analysis of pollen loads using microscopy to analyze the proportions of some pollen loads would be needed for quantitative information. Future advances in DNA sequencing technologies that reduce bias such as shotgun metagenome sequencing (Lang *et al.*, 2019) of pollen may allow for results to be presented as relative abundance.

Sampling methods

A subset of bees observed on flowers was collected for DNA metabarcoding. Therefore, bee sampling was dependant on the number of flowers (i.e., low floral abundance at the field edge meant there were few opportunities to capture bees). This likely resulted in sampling bias among sites. This sampling method also resulted in fewer observations

and catches during fall when flowering plants were less abundant (Chapter 2, Figs 2.1 and 2.2). To expand the number of bees captured in fields with fewer floral resources a different sampling protocol is required.

Future work

This study focused on plant-pollinator interactions. Accounting for herbivores, parasites, and parasitoids in plant-interaction networks can provide a more complete picture of how floral resources impact the cropping system (Lundin *et al.*, 2019; Windsor *et al.*, 2021). Balancing the positive impacts of flowering plants on bees and the negative impacts on other production components is important. For example, bees visited and collected pollen from *Rosaceae* spp. (Chapter 4, Figs. 4.1 & 4.3), however, fruit-producing plants can serve as alternative hosts to the spotted wing *Drosophila* (*Drosophila suzukii* Matsumura, Diptera: Drosophilidae) (Klick *et al.*, 2016; Agriculture and Agri-Food Canada, 2025) a pest of lowbush blueberry. Goldenrods were found in lowbush blueberry fields in this study and others (McCully *et al.*, 1991; Lyu *et al.*, 2021) and were frequent in interaction network (Chapter 4, Figs. 4.3 & 4.4), however, goldenrod in the field interfered with harvest (Jensen & Specht, 2002).

All fields sampled in this study were in fruit year. This is one year of the two-year production cycle. Sampling in the sprout year could reveal different plant-pollinator interactions. During bloom the majority of interactions were with *Vaccinium* spp. For some groups, when *Vaccinium* spp. are not blooming, host switching may not present a problem, however, for other taxa, such as *A. carolina*, the lack of *Vaccinium* spp. pollen could be problematic. It would also be beneficial to sample pre-bloom to capture interactions of blueberry pollinators that emerge before crop bloom.

This study sampled many fields over three seasons. Fields were sampled every two to three weeks over the season. Future work could target fewer fields and sample more frequently. The two-to-three-week sampling interval may have been too broad to

capture the flowering period or peak flowering period, or peak nectar and/or pollen production period for some plant taxa.

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Appendices

Appendix A: Supplementary Information – Chapter 2

Table S2.1: Locations of lowbush blueberry fields sampled.

Site abbreviation	Province	latitude	longitude
A_2017	NB	45.152778	-66.730278
B_2017	NB	47.405296	-65.204169
D_2017	NB	47.434610	-64.909772
HU_2017	NB	45.135278	-66.705556
HL_2017	NB	45.136389	-66.708611
L_2017	NB	47.396261	-65.242798
S_2017	NB	47.242276	-65.114298
BH_2019	NS	45.1386094	-63.0243946
GR_2017	NS	45.1015863	-63.1582526
MT_2017	NS	45.5035664	-63.0375103
NLH_2017	NS	45.524444	-64.320833
NLP_2019	NS	45.5301626	-64.3207589
PH_2017	NS	45.4149883	-63.4975725
AF_2017	PEI	46.369722	-62.916389
FH_2017	PEI	46.427778	-62.509444
SH_2017	PEI	46.334722	-62.456944
CC_2018	NB	47.251109	-65.312233
DVR_2018	NB	47.347914,	-64.908736
GC_2018	NB	47.253461	-65.155848
NLB_2018	NS	45.5322409	-64.3175986
NLM_2018	NS	45.516944	-64.327222
PH_2018	NS	45.4145926	-63.4934701
AFT_2018	PEI	45.994167	-62.835833
BR_2018	PEI	45.994444	-62.836389
PR_2018	PEI	46.270833	-62.531944
NLH_2019	NS	45.524444	-64.320833
NLP_2019	NS	45.5301626	-64.3207589
PH_2019	NS	45.4149883	-63.4975725

Supplementary Table S2.2. Bee groups observed at each site-year (n=28) throughout the season.

Site-years	Andrenid bees	Bumble bees	Honey bees	Metallic bees	Small black bees	Other bees
A 2017	X	X	X		X	
AFT 2017	X	X	X		X	X
AFT 2018	X	X	X		X	X
B 2017	X	X	X		X	
BH 2017	X	X	X		X	
BR 2018	X	X	X	X	X	X
CC 2018		X	X		X	X
D 2017	X	X	X		X	X
NLH 2017	X	X	X		X	X
NLP 2017	X	X	X	X	X	X
DVR 2018	X	X	X		X	X
FH 2017	X	X	X		X	
GC 2018	X	X		X	X	X
GR 2017	X	X	X	X	X	X
HL 2017	X	X	X	X	X	X
HU 2017	X	X	X	X	X	
L 2017	X	X	X		X	X
MT 2017	X	X	X		X	
NLB 2018	X	X	X	X	X	X
NLH 2019	X	X	X	X	X	X
NLM 2018	X	X	X	X	X	X
NLP 2019	X	X	X		X	X
PH 2017	X	X	X	X	X	X
PH 2018	X	X	X	X	X	X
PH 2019	X	X	X	X	X	X
PR 2018	X	X	X		X	X
S 2017	X	X	X	X	X	
SH 2017	X	X	X		X	

Appendix B: Supplementary Information – Chapter 3

Reference Database

An effort to sequence the vascular plants of Canada resulted in Internal Transcribed Spacer 2 (ITS2) sequences from 3044 plant species (~60% of vascular plants in Canada) now part of the Barcode of Life Database (BOLD) plants of Canada project (Braukmann *et al.*, 2017). This includes samples from four National Parks in Atlantic Canada (Braukmann *et al.*, 2017).

A custom ITS2 reference database was created by downloading sequences from the plants of Canada project on 2020-03-24 under project: DS-VASCAN on the BOLD website. The database contained taxonomic and representative sequences was filtered to contain the ITS2 marker. After running the IDTAXA classifier, see below, some records were identified as problem sequences during the classifier training phase. This was often due to issues with taxonomic naming. As a result, some records were deleted. These included records for: *Isoetes riparia*, *Betula kenaica*, *Leersia virginica*, *Juncus inflexus*, *Arceuthobium americanum*, *Cyperus bipartitus*, and *Juncus oxymersis*. Records which had taxonomic assignment issues were as follows: record HIMS207312 for *Rumex acetosa* was removed; *Cuscuta coryli* was reassigned to the Cuscutaceae family; record WAT32112, *Lathyrus latifolius* was deleted, record WAT34412 was changed to *Nepeta cataria*, records for the genus *Weigela* spp., HIMS197812 for *Amelanchier canadensis*, *Nabalus* spp. Records containing the genus *Pilosella* were changed to *Hieracium*, *Carex laeviconica* BBYUK212212 was deleted. *Loiseleuria procumbens* changed to *Kalmia procumbens*.

Some sequences were also added to the custom database. ASVs which were unclassified at the genus-level were queried with the basic local alignment search tool (BLAST) on

the NCBI website. (online). ITS2 sequences for *Trifolium pratense* (GenBank Accession number MN601918.1) and *Acer spicatum* (GenBank accession number MW029796.1), *Houstonia caerulea* (GenBank AM939464) and *Rumex acetosella* (GenBank accession AF189730.1) were added to the database.

The IDTAXA-compatible reference database was created using code found in the instructions for creating a “taxid” file as part of the Classify Sequences IDTAXA documentation (Wright, 2019).

The final database can be found at (Rutherford, 2026).

Unresolved Taxonomy

The qiime taxa collapse command was used to summarize the features at the genus-level. In total, there were 102 plant genera found in pollen loads. Eighty-five were assigned taxonomy at the genus-level by the IDTAXA classifier at greater than or equal to 0.7, however, 17 taxa (169 ASVs) had ambiguous genus-level identifications. The ASVs with unresolved genus-level taxonomic assignment were used as a query in a Basic Local Alignment Search Tool (BLAST; Camacho et al 2009) search from the command line against the custom ITS2 database or as a query in a BLAST search on National Centre for Biotechnology Information (NCBI) against the nt database. This resolved the taxonomy of 65 ASVs (Table S3.1). In the end 17 of the 102 taxa, or 104 of the 1,891 ASVs were unassigned at the genus-level (Table S3.1). *Solidago* spp. and *Euthamia* spp. hits were grouped together as Goldenrod. The final taxonomy file can be found at Rutherford, (2026).

Table S3.1. Updated classification using Basic Local Alignment Search Tool.

Unclassified genera after first round of QIIME summary	Number of ASVs with the classification	Taxonomy Resolved and the number of ASVs shown in brackets
Unclassified at Phylum, Class, Order	54	<i>Acer</i> spp. (5) <i>Alnus</i> spp. or <i>Impatiens</i> spp. (2) <i>Alnus</i> spp. or <i>Iris</i> spp. (1) <i>Viburnum</i> spp. or <i>Lonicera</i> spp. (6) <i>Medicago</i> spp. (1) <i>Tragopogon</i> spp. or <i>Rumex</i> spp. (2) <i>Rhododendron</i> spp. (1) <i>Sambucus</i> spp. (2) <i>Rumex</i> spp. (5) <i>Allium</i> spp. or <i>Erythronium</i> spp. (1) <i>Stellaria</i> spp. or <i>Sagina</i> spp. (1) Unclassified Boraginaceae (1) <i>Linaria</i> spp. or <i>Nuttallanthus</i> spp. (1) <i>Cornus</i> spp. (1) <i>Spiraea</i> spp. (23) goldenrods (1)
Unclassified Asteraceae	35	<i>Krigia</i> spp. or <i>Leontodon</i> spp. (13) goldenrods (13) <i>Eutrochium</i> spp. or <i>Eupatorium</i> spp. (3) <i>Tragopogon</i> spp. or <i>Rumex</i> spp. (4)* <i>Hieracium</i> spp. or <i>Crepsis</i> spp. (1) <i>Doellingeria</i> spp. or <i>Solidago</i> spp. (1)
Unclassified Brassicaceae	1	<i>Raphanus</i> spp. (1)
Unclassified Caryophyllaceae	17	All were <i>Stellaria</i> spp. or <i>Sagina</i> spp. Left as unclassified Caryophyllaceae
Unclassified Ericaceae	8	<i>Vaccinium</i> spp. (5)

		<i>Rhododendron</i> spp. (2) <i>Chamaedaphne</i> spp. (1)
Unclassified Fabaceae	20	<i>Vicia</i> spp. or <i>Lathyrus</i> spp. (19) Unclassified Fabaceae (1)
Unclassified Plantaginaceae	5	<i>Linaria</i> spp. or <i>Nuttallanthus</i> spp. (5)
Unclassified Rosaceae	29	<i>Spiraea</i> spp. (4) <i>Sorbus</i> spp., <i>Amelanchier</i> spp., <i>Malus</i> spp. – leaving as unclassified Rosaceae (25)

Supplementary Table 3.2: Frequency of plant taxa found in pollen loads of bees captured during lowbush blueberry bloom.

Family	Genus	count	Percent bees captured (%)
Ericaceae	<i>Vaccinium</i>	408	94.7
Rosaceae	<i>Prunus</i>	70	16.43
Sapindaceae	<i>Acer</i>	66	15.49
Adoxaceae	<i>Sambucus</i>	32	7.51
Polygonaceae	<i>Rumex</i>	32	7.51
Ericaceae	<i>Rhododendron</i>	26	6.1
Asteraceae	<i>Taraxacum</i>	25	5.87
Rosaceae	<i>Unclassified Rosaceae</i>	22	5.16
Asteraceae	<i>Hieracium</i>	21	4.93
Salicaceae	<i>Salix</i>	18	4.23
Betulaceae	<i>Betula</i>	14	3.29
Rosaceae	<i>Rubus</i>	11	2.58
Ericaceae	<i>Kalmia</i>	11	2.58
Myricaceae	<i>Morella</i>	11	2.58
Rosaceae	<i>Fragaria</i>	10	2.35
Adoxaceae	<i>Viburnum</i>	9	2.11
Aquifoliaceae	<i>Ilex</i>	8	1.88
Asteraceae	<i>Hieracium/Crepsis</i>	8	1.88
Cornaceae	<i>Cornus</i>	8	1.88
Fagaceae	<i>Fagus</i>	7	1.64
Oleaceae	<i>Fraxinus</i>	7	1.64
Rosaceae	<i>Aronia</i>	7	1.64
Fabaceae	<i>Lupinus</i>	6	1.41
Ericaceae	<i>Oxycoccus</i>	5	1.17

Family	Genus	count	Percent bees captured (%)
Myricaceae	<i>Comptonia</i>	5	1.17
Caryophyllaceae	Unclassified	4	0.94
	Caryophyllaceae		
Rubiaceae	<i>Houstonia</i>	4	0.94
Ranunculaceae	<i>Ranunculus</i>	4	0.94
Rosaceae	<i>Sorbus</i>	4	0.94
Asteraceae	<i>Goldenrod</i>	3	0.7
Asteraceae	<i>Leucanthemum</i>	3	0.7
Caryophyllaceae	<i>Cerastium</i>	3	0.7
Fabaceae	<i>Vicia/Lathyrus</i>	3	0.7
Fagaceae	<i>Quercus</i>	3	0.7
Betulaceae/Iridaceae	<i>Alnus/Iris</i>	3	0.7
Asteraceae/Polygonaceae	<i>Tragopogon/Rumex</i>	3	0.7
Caryophyllaceae	<i>Stellaria</i>	3	0.7
Ericaceae	<i>Chamaedaphne</i>	3	0.7
Ericaceae	<i>Gaylussacia</i>	2	0.47
Fabaceae	<i>Lotus</i>	2	0.47
Fabaceae	<i>Trifolium</i>	2	0.47
Hypericaceae	<i>Hypericum</i>	2	0.47
Violaceae	<i>Viola</i>	2	0.47
Ranunculaceae	<i>Caltha</i>	2	0.47
Apiaceae	<i>Carum</i>	1	0.23
Asteraceae	<i>Rudbeckia</i>	1	0.23
Brassicaceae	<i>Hesperis</i>	1	0.23
Caryophyllaceae	<i>Moehringia</i>	1	0.23
Caryophyllaceae	<i>Stellaria/Sagina</i>	1	0.23
Ericaceae	<i>Epigaea</i>	1	0.23

Family	Genus	count	Percent bees captured (%)
Myrsinaceae	<i>Trientalis</i>	1	0.23
Apocynaceae	<i>Apocynum</i>	1	0.23
Rubiaceae	<i>Galium</i>	1	0.23
Orobanchaceae	<i>Melampyrum</i>	1	0.23
Plantaginaceae	<i>Linaria</i>	1	0.23
Plantaginaceae	<i>Plantago</i>	1	0.23
Plantaginaceae	<i>Veronica</i>	1	0.23
Cannabaceae	<i>Humulus</i>	1	0.23
Rosaceae	<i>Malus</i>	1	0.23
Liliaceae/Alliaceae	<i>Allium/Erythronium</i>	1	0.23
TOTAL		426	1

Appendix C: Supplementary Information - Chapter 4

Supplementary Table S4.7. Bee identification using DNA barcoding of cytochrome oxidase c subunit one (COI) for bees captured in lowbush blueberry fields

Family	Taxa	No. individuals
Unassigned		33
Andrenidae	<i>Andrena</i> spp.	42
	<i>Andrena carlini</i>	80
	<i>Andrena carolina</i>	61
	<i>Andrena ceanothi</i>	1
	<i>Andrena dunningi</i>	1
	<i>Andrena hirticincta</i>	3
	<i>Andrena kalmiae</i>	2
	<i>Andrena melanochora</i>	1
	<i>Andrena nivalis</i>	19
	<i>Andrena persimulata</i>	6
	<i>Andrena rufosignata</i>	11
	<i>Andrena vicina</i>	5
	<i>Andrena wilkella</i>	4
	<i>Andrena wscripta</i>	1
	<i>Calliopsis andreniformis</i>	1
<i>Pseudopanurgus solidaginis</i>	2	
Apidae	<i>Bombus</i> spp.	28
	<i>Bombus bimaculatus</i>	14
	<i>Bombus borealis</i>	1
	<i>Bombus impatiens</i>	41
	<i>Bombus perplexus</i>	29
	<i>Bombus ternarius</i>	126
	<i>Bombus terricola</i>	3
	<i>Bombus vagans</i>	7

Family	Taxa	No. individuals
Apidae	<i>Ceratina calcarata</i>	1
	<i>Epeolus scutellaris</i>	1
	<i>Melissodes desponsa</i>	1
	<i>Melissodes illata</i>	1
	<i>Nomada</i> spp.	5
Colletidae	<i>Colletes inaequalis</i>	4
	<i>Hylaeus modestus</i>	5
	<i>Hylaeus ornatus</i>	1
Halictidae	<i>Augochlorella aurata</i>	10
	<i>Dufourea novaeangliae</i>	1
	<i>Halictus confusus</i>	2
	<i>Halictus ligatus</i>	1
	<i>Halictus rubicundus</i>	5
	<i>Lasioglossum</i> spp.	1
	<i>Lasioglossum acuminatum</i>	7
	<i>Lasioglossum albipenne</i>	4
	<i>Lasioglossum comagenense</i>	13
	<i>Lasioglossum coriaceum</i>	1
	<i>Lasioglossum cressonii</i>	2
	<i>Lasioglossum dreisbachi</i>	1
	<i>Lasioglossum ephialtum</i>	1
	<i>Lasioglossum foxii</i>	3
	<i>Lasioglossum inconditum</i>	1
	<i>Lasioglossum leucomomum</i>	5
	<i>Lasioglossum leucozonium</i>	1
<i>Lasioglossum pectorale</i>	1	
<i>Lasioglossum quebecense</i>	7	
<i>Lasioglossum seillean</i>	2	

Family	Taxa	No. individuals
Halictidae	<i>Lasioglossum sheffieldi</i>	1
	<i>Lasioglossum timothyi</i>	2
	<i>Lasioglossum versans</i>	2
	<i>Lasioglossum versatum</i>	1
Megachilidae	<i>Chelostoma campanularum</i>	1
	<i>Coelioxys rufitarsis</i>	1
	<i>Hoplitis producta</i>	1
	<i>Megachile centuncularis</i>	1
Total		621

Appendix D – Additional general electronic supplementary files

ASV_sequences.fa

A fasta file containing the sequences of all internal transcribed spacer 2 (ITS2) amplicon sequence variants (ASVs) from dada2 pipeline.

This is the file that was used to assign taxonomy for the pollen loads

ASV_table.txt

Final ASV table from dada2 pipeline

This table shows how many ASVs (pollen) are associated with each bee.

bees_CO1_all_samples.txt

A file containing sanger sequences for Cytochrome oxidase *c* subunit one gene, the DNA barcode used to identify the captured bees.

This file was used as input into Barcode of Life Database (BOLD) to identify the bees

BOLD_DSVASCAN_decipher_ITS2_database.fa

A fasta file containing ITS2 sequences downloaded from Barcode of Life DSVASCAN project. See thesis Appendix B for a description of modifications. The database was modified to be compatible with the decipher classifier.

This was the reference database used to assign taxonomy to the unknown ASVs from ITS2 sequences.

metadata.txt

A text file with metadata for each bee captured and sequenced (i.e., site, date of capture, location of capture, bee taxonomy)

PlantData.tab

Plant taxa and abundance records from plant surveys done during transects walks and field edge surveys.

plant_pollinator_interactions_observations.txt

all plant-pollinator interactions recorded during transect walks

This was used to summarize bee observations and to calculate bee visits to lowbush blueberry and alternative forages.

Supplementary Figure S4.1

Bipartite graph showing plant-pollinator interactions during bloom, including honey bees.

Supplementary Figure S4.2

Bipartite graph showing plant-pollinator interactions during summer, including honey bees.

Supplementary Figure S4.3

Bipartite graph showing plant-pollinator interactions during fall, including honey bees.

Table S4.1 bloom_interactions_observation.tab

A table containing species level plant-pollinator interactions for observations during transect walks during bloom.

Table S4.2 bloom_interactions_pollen.tab

A table containing species level plant-pollinator interactions for bee-associated pollen from bees collected during bloom.

Table S4.3 summer_interactions_obs.tab

A table containing species level plant-pollinator interactions for observations during transect walks during summer.

Table S4.4 summer_interactions_pollen.tab

A table containing species level plant-pollinator interactions for bee-associated pollen from bees collected during summer.

Table S4.5 fall_interactions.tab

A table containing species level plant-pollinator interactions for observations during transect walks during fall.

Table S4.6 fall_interactions_pollen.tab

A table containing species level plant-pollinator interactions for bee-associated pollen from bees collected during fall.

taxonomy.txt

plants ITS2 taxonomy file generated from decipher classifier for bee pollen loads. Some manual updates were done to fill in ASVs that were not assigned at the genus-level.