PLANT-POLLINATOR RELATIONSHIPS OF CULTURALLY SIGNIFICANT FOOD AND MEDICINE PLANTS IN THE GREAT LAKES REGION

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Abstract

Wild plant and pollinator populations have experienced declines globally in recent years. While species declines have often been examined for their impacts ecologically and economically, little is known about how species declines will impact humans culturally. All Indigenous cultures in North America use plant species for food and medicine. Plants considered important to a specific culture are known as cultural keystone species, meaning they are significant to cultural identity. With global declines in plant and pollinator species, this study aims to determine the role of wild pollinators to cultural keystone plant species. Availability of information on breeding systems and important pollinators is crucial information for management of plant species, especially if a plant species is experiencing population declines (e.g. at-risk, endangered). A literature review was conducted to determine what is known about the breeding systems of cultural keystone species in eastern North America. The results show 50% of the culturally significant plants lack any information regarding the breeding system. A plant-pollinator network was constructed based on observations in the field on the Three Sisters (corn, beans, squash) garden. The common eastern bumble bee (*Bombus impatiens*) and hoary squash bee (*Xenoglossa pruinosa*) were important pollinators in this system. A breeding experiment including diurnal and nocturnal video recording was conducted to determine the most frequent visitors of sacred Tobacco (*Nicotiana rustica*), and to determine the degree to which *N. rustica* relies on insect pollination. Lastly, a breeding experiment was conducted on common bearberry (*Arctostaphylos uva-ursi*) in eastern Ontario, Canada to determine the plant's reliance on insect pollination. In conclusion, wild pollinators are important to the continued success of cultural keystone species, and therefore to the cultures that use these plants. Including multiple ways of knowing and practicing ethical ecology in the future is part of ensuring biocultural conservation.

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Sincerely,

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Chapter One: Towards a better understanding of the relationships between pollinators, human well-being, and medicine plants in the Great Lakes Region of Eastern North America

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Candidate's contribution:
Shelby Gibson and Dr. Colla conceived of the idea to conduct a systematic review of the breeding systems and pollinators of culturally significant medicine plants in the Great Lakes Region. Shelby Gibson determined the search terms and conducted the review using Scopus and Web of Science. Shelby Gibson conducted a preliminary assessment of all articles, downloaded relevant articles, and read all retained articles. Shelby Gibson led the writing of the manuscript. Shelby Gibson and Sheila Colla both contributed to the editing of the manuscript.

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Abstract

Biocultural conservation theory allows for an examination of complex problems using systems thinking and conserving biological and cultural diversity together. In the Great Lakes Region of eastern North America, wild medicine plants are an important part of what Anishinaabe people understand as *Mini bimaadizi*, or the good life, and of *aki miijim*, traditional food systems. Given the various threats facing wild plant populations and continued global change, this review aims to investigate what is known about the pollination and breeding systems of culturally significant plants in the Great Lakes Region. The overall goal is to determine what proportion of these plants have had basic ecological studies done, and the degree to which culturally significant plants in this region rely on insect pollination. A systematic review of the literature was conducted on breeding systems and pollination of culturally significant plants. Half of all the culturally significant plants included lacked specific information related to reproduction. Most plants that have been studied relied on outcrossing, and the most commonly reported pollinators were in the Apidae family. With the complex interactions of plant and pollinators, and many insect pollinators experiencing population declines, it is important to determine the dependence of culturally significant plants on animal pollination. These findings will be relevant to conservation planning and policy for the future.

Keywords: *Aki miijim*, Biocultural conservation, breeding system, culturally significant plants, ecological-interactions, *Mini bimaadizi*, pollination, plant reproduction

Societal Impact Statement: A diversity of values are needed to maximise the effectiveness of conservation planning, including policies and programs. The results of COP 15 in Montreal, Canada, highlight the importance of a shift towards recognizing multiple ways of knowing in ecology and conservation. This review aims to provide a baseline of what is known about culturally significant plants in the Great Lakes Region of Eastern North America. The results of this research provide insight into the ways in which ecology has been conducted in the past and the importance of changing the way ecology is conducted in the future.

Introduction

Biocultural approaches to conservation are embedded within social-ecological systems and are focused on reducing declines of both biological and cultural diversity (Pretty et al. 2009, Gavin et al. 2015, Berkes et al. 2016, Hill et al. 2019, Winter et al. 2020). Conservation planning is ineffective when it fails to consider the social system in which it operates (Pretty et al. 2009, Ban et al. 2013, Artelle et al. 2019). The standard (colonial) approach to solving ecological problems in a silo limits our capacity to benefit from diverse people, knowledge systems, and solutions (Tuhiwai Smith, 2013, Artelle et al. 2019, Hill et al. 2019, Trisos et al. 2021). The Anishnaabe people of the Great Lakes Region in Northeastern North America have a cultural connection to the land (Densmore, 2005, Brokenleg and Tornes, 2013). *Mino bimaadizi* (Ojibwemowin) (Densmore, 2005), or *Mino bimaadiziwin* (Anishinaabemowin) (Simpson, 2011) which describes 'the good life' refers to a state of cultural and spiritual well-being. Plants are considered sacred and are strong components of maintaining *Mino bimaadizi* (Densmore, 2005, Brokenleg and Tornes, 2013). Anishinaabe people have traditionally collected plants for many reasons including for use as medicine (Densmore, 2005, Brokenleg and Tornes, 2013, KBIC, 2021). Globally, there is a trend of species declines which is occurring simultaneously with losses in Indigenous language and cultural practices (Ens et al. 2016, Artelle et al. 2019). Plant populations are threatened by land use changes and the traditional knowledge associated with the plant is lost due to lack of documentation and inter-generational knowledge transmission (Motaleb, 2010). Ongoing settler-colonial violence, including genocidal land management practices (Brokenleg and Tornes, 2013), have had a severe impact on Anishinaabe people who are undergoing a loss of culture and traditions such as plant knowledge, language, and stories.

Indigenous people in North America have used plant management techniques for millennia that rely on a reciprocal, caretaking relationship with the land (Turner et al. 2013). This includes tending of plant resource sites between human generations (Turner et al. 2013). Biological research on plants has primarily been focused on plants used for major crops and commodities (Padulosi et al. 2004). This has resulted in a lack of research on other plants, including basic ecological studies, mapping of distributions, breeding systems (pollination and seed dispersal), taxonomy, and phylogenetics (Kearns et al. 1998, Padulosi et al. 2004). A recent review of at-risk plants in Ontario found that many plants lacked any basic information related to pollination requirements, pollinator needs, and recovery recommendations (MacPhail et al. 2018). The basic aspects of any plant's reproduction are necessary for conservation planning (MacPhail et al. 2018, Wani et al. 2022).

Of all flowering plants, 87.5% are estimated to be animal-pollinated (308 009 species of angiosperms) (Ollerton et al. 2011). Approximately 80% of all wild flowering plants directly require insect pollination for fruit and seed set (Harder and Barrett, 1996, Kearns et al. 1998, Potts et al. 2010, Potts et al. 2016). Self-compatible plants which reproduce through autogamous self-pollination have a particular set of characteristics such as: low herkogamy (anther-stigma distance) and low dichogamy (temporal separation of mature anthers and stigmas) (Thomann et al. 2013). Self-compatible plants must sometimes employ a mixed mating system using cross-pollination to avoid inbreeding depressions (Ollerton et al. 2011, Thomann et al. 2013). Other plants are unable to self-fertilize or require a pollinator to move pollen from anther to stigma within a flower (Ollerton et al. 2011). Plants requiring insect pollination often have a set of characteristics including large floral display, high scent intensity, large rewards (nectar and pollen), and large corolla size (Thomann et al. 2013).

Bees are important pollinators of wild plant species (Raven et al. 1999, Ollerton et al. 2011, Pots et al. 2010, Potts et al. 2016). Across many parts of the globe, wild pollinator diversity and abundance is in decline (Potts et al. 2010, Colla et al. 2012, Tylianikis et al. 2013, Rodger et al. 2021, Zattara and Aizen, 2021). The main factors affecting global pollinator declines identified are habitat loss and fragmentation, agriculture (pesticides and GMOs), pathogens, invasive alien species, pollinator management, and climate change (Brown and Paxton, 2009, Potts et al. 2010, Potts et al. 2016, Dicks et al. 2021). The reproductive success of wild flowering plants is at risk due to pollinator declines (Bond, 1994, Biesmeijer et al. 2006, Aslan et al. 2013, Thomann et al. 2013, Scheper et al. 2014, Rodger et al. 2021). Globally, 75% of crop plants rely on pollination by animals, in particular, bees (Klein et al. 2007). A global review of crop pollination found that wild bees pollinated crops more effectively than honey bees (Garibaldi et al. 2013). In general, honey bees are not able to replace the pollination services provided by wild bees (Garibaldi et al. 2013, Tylianikis, 2013). While this is more well studied in food crop systems, it also occurs in wild plants (Page and Williams, 2022).

Human health and well-being are influenced by pollination services (Kearns et al. 1998, Potts et al. 2016, Stout and Dicks, 2022). The Assessment Report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) on Pollinators, Pollination, and Food Production found that pollinator declines could impact human well being through loss of wild plant diversity and yield, and cultural practices and traditions surrounding plant and pollinator species (IPBES, 2016, Potts et al. 2016, Dicks et al. 2021). This highlights the importance of research and knowledge sharing that considers the social and ecological systems together.

According to the World Health Organization (WHO) nearly 80% of the world's population relies on traditional medicines, particularly plants, for their main method of health care (WHO, 1993). The surge of interest in using plants as medicine in recent decades (Westfall and Glickman, 2004), has been operating under the assumption that these plants will be available in perpetuity (WHO, 1993). This assumption ignores the threats wild medicinal plant populations are currently facing including increasing overharvest, climate change, and habitat loss (WHO, 1993, Mooney and McGraw, 2007, Erland et al. 2022). Based on these factors the WHO, International Union for the Conservation of Nature (IUCN) and World Wildlife Fund (WWF) convened a meeting in Chiang Mai, Thailand on 21-27 March 1988 to develop a plan to address the security of medicinal plant populations (WHO, 1993). Out of this meeting came "The Chiang Mai Declaration – Saving Lives by Saving Plants" which called upon the United Nations, its agencies, and Member States to work towards medicinal plant conservation.

There is an increasing demand for medicinal plants in North America (Westfall and Glickman, 2004, Smith et al. 2020, Erland et al. 2022). In some cases, a plant may experience significant population decline due to human overharvesting, such as the case with American Ginseng (*Panax quinquefolius*) (Mooney and McGraw, 2007, Erland et al 2022). One conservation strategy which includes introducing cultivated seeds into wild populations can potentially decrease the genetic integrity of the plant population (Grubbs and Case, 2004, Mooney and McGraw, 2007). Breeding system studies must be conducted to further understand the impacts of various conservation strategies and management plans, especially regarding medicinal plants for which demand is rising (Grubbs and Case, 2004, Mooney and McGraw, 2007, Erland et al. 2022). High quality seed is a requirement for the production of a reliable, healthy stand of a particular plant (Pooni et al. 1997). Farming a plant for seed stock often requires pollinator supplementation. In order to properly support development of a healthy supply of seed stock, the reproductive biology of the plant must be well understood (Cane, 2006). The strongest threats to wild medicinal plant populations in Canada are habitat loss, invasive species, overharvesting, and climate change (Karst 2010, Uprety et al. 2012, Erland et al. 2022).

Parties to the Convention on Biological Diversity (CBD) set the 20 “Aichi biodiversity targets” as a conservation framework spanning 2011-2020 (Artelle et al. 2019). The targets have largely been unmet with continued declines in biodiversity globally (Artelle et al. 2019). At the recent UN Biodiversity Conferences in Montreal, Canada, world leaders agreed on a plan to halt biodiversity decline (CBD, 2022). The new action plan acknowledges the rights of Indigenous peoples and their importance as stewards of nature (CBD, 2022). In the 2030 Action Targets there is a goal to protect medicines for sustainable harvesting by Indigenous peoples, ensuring Indigenous knowledge informs relevant conservation decision-making, and that Indigenous people have equitable access to participate in decision-making related to biodiversity on their lands based on relevant rights and treaties (CBD, 2022).

This paper aims to determine to what extent culturally significant plant breeding systems have been studied, and to what extent culturally significant plants rely on pollination by wild bees in the Great Lakes Region of Eastern/Central North America.

Materials and Methods

The colonial history of ecology means that the research questions and who can answer them is based on power dynamics and using a decolonizing framework for the future means having marginalized groups set the research agenda (Tuhiwai Smith, 2012, Trisos et al. 2021).

Tuhiwai Smith (2012) outlines two methods for advancing the Indigenous research agenda which are using a community-based approach and through Indigenous studies departments in the institution. Trisos et al. (2021) highlight five steps for decolonizing ecology which include: decolonize your mind, know your histories, decolonize access, decolonize expertise, and practice ethical ecology in inclusive teams. This study is part of The Finding Flowers project (www.findingflowers.ca) at York University in Toronto, Ontario, Canada. Finding Flowers is an interdisciplinary project which integrates art, ecology, and education using a biocultural lens to study native plant and pollinator conservation. The Finding Flowers project is co-led by Anishinaabe artist Lisa Myers and native bee ecologist Dr. Sheila Colla. Determining a baseline of what is available in the scientific literature on native plant reproduction is a key component of conducting future research on native plant-pollinator relationships. The authors of this paper are non-Indigenous settlers living in Ontario, Canada who are grateful to the original inhabitants of Turtle Island for their stewardship of these lands for millennia and who are committed to learn how to responsibly contribute to the care of this land.

This review was conducted to determine what is known about plant breeding systems in the Great Lakes Region and focusses on Anishinaabe medicine plants. A plant list of 215 species was compiled. The focal plant list was compiled using the following studies: Smith, 1932, Meeker, 1993, Davidson-Hunt et al. 2005, Genuisz, 2015. This paper focusses on plant-pollinator interactions and therefore only angiosperms are included. Further, herbaceous plants are the most commonly utilized in the region (Uprety et al. 2012) and therefore we excluded all other plants (e.g. woody-stemmed shrubs, vines).

In order to investigate what is known about the breeding and pollination systems of the selected plants, we used Scopus and Web of Science databases. Two databases were used in an effort to ensure the search returned all relevant papers. The following search terms were used on both Scopus and Web of Science: (“Scientific name” OR “Common name” AND “breeding system” OR “pollinat*”). The term breeding system is used here because it is a general term which refers to the sexual or asexual characteristics of a population as well as the level of inbreeding or outcrossing (Neal and Anderson, 2005). The search was conducted in September 2022. As each term was searched the results underwent preliminary refinement based on the title and abstract (e.g. where pollination referred to release of pollen into the air with relevance to allergies and not on the act of pollination between plants the paper was not retained). All relevant articles were downloaded to Mendeley. Papers were included in the title and/or abstract included reference to the plant species and also to reproduction OR pollinators. A total of 322 papers were excluded from Scopus and 392 papers were excluded from Web of Science because they did not meet the inclusion criteria. Plants with over 50 results in either search (Scopus or Web of Science) were excluded in order to maintain focus on the lesser studied species by excluding plants with extensive research. The plant species removed for having over 50 studies included *Brassica rapa, Cucurbita maxima, Impatiens capensis,* and *Silene latifolia.* Every downloaded article was read, and some were removed based on irrelevance after reading. A total of 525 articles were retained for further analyses.

All retained papers were skimmed for data on breeding system and pollinators/insect visitors. Data regarding breeding system and known or recorded pollinators or floral visitors were recorded. Breeding system information was collected into seven categories: Apomixis (asexual reproduction), Parthenocarpy (fruit without fertilization), Anemophily (wind pollination), Biparental Inbreeding (pollen from genetically related plants), Autogamy (self-fertilization within flower), Geitonogamy (self-fertilization within plant), Xenogamy (outbreeding – pollen from another plant). Pollinator information was collected into 23 categories: Coleoptera, Diptera, Hymenoptera, Hymenoptera - Solitary bees, Hymenoptera – Bees, Hymenoptera – Other, Hymenoptera – Andrenidae, Hymenoptera – Apidae, Hymenoptera – Colletidae, Hymenoptera – Halictidae, Hymenoptera – Mellitidae, Hymenoptera – Megachilidae, Hymenoptera – Strenotrinidae, Lepidoptera, Orthoptera, Odonata, Homoptera, Hemiptera, Collembola, Psocoptera, Neuroptera, Thysanoptera, Mecoptera. Insect visitors were recorded to order except for Hymenopterans which were recorded to family were possible. In some cases, a study was removed due to its experimental nature which compromised the results of the floral visitors as being representative of what occurs naturally. For example, a study on *A. millefolium* found beetles and flies pollinating control plants (Hamback, 2016). When a floral volatile experiment was conducted, and floral volatile oil from *C. arvense* was sprayed on *A. millefolium*, the host of pollinators changed drastically to one not recorded on the control flowers. The pollinators, then, are not representative of what pollinates the plant in a natural setting and this information was not extracted and retained as part of this study. General comments on pollinators such as “we saw bees (personal observations)” were included in our analyses in the appropriate categories.

Results

Breeding systems of 108 (50%) plants from the plant list have not been studied, while 107 (50%) plants have been studied. Of the plants studied (107), self-compatibility was recorded in 57 (52%) plant species, while self-incompatibility was recorded in 36 (34%) plant species. Of the plants studied (107) the most commonly recorded breeding system was xenogamy with 85

(79%) plant species using this system (**Figure 1**). Autogamy was recorded in 40 plant species (38%) (**Figure 1**). From our list, (107) 54 plant species (50%) were reported to have one breeding system, while 31 (30%) plant species had two breeding systems, 10 (10%) plant species had three breeding systems, and two (2%) plant species had four breeding systems recorded. Of the 85 plant species recorded as xenogamous, 34 (41%) were also recorded as autogamous. A total of 27 (33%) xenogamous plant species were strictly outcrossing. Of 40 autogamous plants recorded, 34 (85%) were also xenogamous.

The most commonly recorded visiting insect order was Hymenoptera (**Table 1**). All Hymenoptera were recorded into one of nine categories: Megachilidae, Colletidae, Halictidae, Adrenidae, Apidae, Other, Bees, Solitary bees, Hymenoptera (**Figure 2**). 'Other' refers to Hymenopterans other than bees. Bees and solitary bees were recorded when the literature stated visitation by "bees", or "solitary bees" occurred with no further taxonomic identification. The most commonly recorded Hymenopteran visitors were in the Apidae family (**Figure 2**). The highest number of papers for a single plant species was 19 (*Fragaria virginiana*), with 108 plants returning zero papers. The average number of papers for the 105 plants studied was five.

Discussion

Limited scientific information is available about many herbaceous plant breeding systems (Cane, 2006, Kevan et al. 1993, Lindell, 1998, Culley, 2000, Palmer et al. 2009, Edens-Meier et al. 2018, Palumbo et al. 2021). Half of the plants included in this literature review have not yet been studied. These findings highlight the importance of conducting biocultural research that focuses on the social components of the system and includes different ways of knowing. Here we are including Anishinaabe way of knowing in order to better understand and inform conservation of social-ecological systems in the Great Lakes region. *Mini bimaadiziwin* is enhanced by access to *aki miijim*, or Indigenous food systems (Pawlowska-Mainville, 2020). This includes access to important medicine plants for sustainable harvesting. Therefore, research focusing on further understanding the reproductive biology of these plants helps to elucidate the human-plant-pollinator relationships. Continued research focus on the agricultural plants perpetuates the understudied state of these culturally significant plant species and plant species of lower economic value.

Even among the plants that have been scientifically studied, there are still knowledge gaps and a need for more research (e.g. Akeroyd and Briggs, 1983, MacPhail et al. 2018); the average number of papers for the plants studied was five. In *Cirsium arvense*, one study concluded that previous determination of the importance of sexual reproduction in the plant was overlooked due to a lack of studies (Heimann and Cussans, 1996). Edens-Meier et al. (2018) refer to findings by Case and Bradford (2009) which indicate that *Cypripedium* species are not well studied in terms of breeding system because populations are scattered, small, or inaccessible, and that insect visitors are infrequent. Because the *Cypripedium*'s are unrewarding to pollinators, they have limited insect visitation which leads to difficulty in understanding their breeding system (Case and Bierbaum, 2013). *Fragaria vesca* (Woodland strawberry) is well-studied (15 papers) because it is a good model system for better understanding commercial *Fragaria* species (Dong et al. 2022). Particularly the availability of plant's draft genome and its short life cycle make it useful as a model system (Dong et al. 2022). This highlights the importance of food plants and their dominance as plants of importance in scientific study. While *Fragaria vesca* is a culturally significant medicine plant, its reason for being well-studied is related to its similarity with other commercial species within the same genus.

Overall, 79% of the plants that had been studied were found to either require or to benefit from outcrossing (xenogamous). This type of breeding system requires a pollinator to move pollen between plants, which highlights the importance of pollinators to reproduction of culturally significant medicine plants. 32% of the plants that had been studied were found to be self-incompatible. These results are similar to those of Rodger et al. (2021) which found in a global assessment of flowering plant's that 33% of species would produce no seeds with the exclusion of pollinators. Similarly, 33% of xenogamous plants were limited to one breeding system – outcrossing. Rodger et al. (2021) concluded that across all flowering plants there was a 0.60 mean Pollinator Contribution (PC) to reproduction in flowering plants. These results provide a level of understanding as to the changes that can be expected under pollinator declines (Rodger et al. 2021). While self-compatibility is common, many species benefit from pollination in terms of seed production, and therefore the impact of pollinator declines on wild plant populations could be significant. It has been suggested that plants will evolve in the short-term to either higher degrees of autogamy or by increasing attractiveness to pollinators (Roles and Kelly, 2011, Thomann et al. 2013). This short-term evolution, however, will not be available in plant populations with inbreeding depression or lack of genetic diversity (Rodger et al. 2021). Rodger et al. (2021) found a higher PC to plants with a longer life span, greater stature, and greater pollinator specificity, and predict that pollinator declines will be stronger causing changes (e.g. decreased abundance) in those plant populations. Highly autogamous plant species will potentially become increasingly abundant. Higher PC in native plants when compared to invasives which showed greater self-capability should also be considered (Rodger et al. 2021).

While a mixed mating system appears to be common, there is a large portion of this group of plants that requires a pollination vector. Biesmeijer et al. (2006) found that in Britain and the Netherlands plants capable of self-pollination had an intermediate affect, plants that require outcrossing by an abiotic factor (e.g. apomixis) were increasing, and plants that were obligate outcrossers requiring insect visitors were in decline. Specifically, a decline was found in bee-pollinated obligately outcrossing plant species. Since Apidae bees were the most commonly recorded insect visitors to the medicinal plants studied here, it would be useful to focus future research efforts on expanding the knowledge base on bee and medicinal plant interactions. Biesmeijer et al. (2006) suggest future research on this group of plants to determine if the plant is in decline due to a loss of pollinators, or if the pollinators are in decline due to a loss of their forage plants, or due to some other causal factor affecting both populations (e.g. habitat alteration, climate change, pesticide use).

Common woodland herb *Maianthemum canadense* is self-incompatible and requires pollination by insects (particularly hoverflies and bees) (Taki et al. 2008). This plant has been found to have a decreased sexual reproductive output due to loss of insect pollinators where pesticides have been used to treat forest pests (Kevan and Plowright, 1995). While populations of *M. canadense* are able to persist in the short-term using clonal reproduction, eventually the lack of insect pollination causes a decrease in genetic diversity in the population (Taki et al. 2008). Taki et al. (2008) found that maintaining even this common forest herb requires consideration and management in a fragmented landscape (e.g. effort to increase forest cover).

38% of plants included in this review that had been studied were found to be autogamous, and 85% of those plants were found to also to be capable of outcrossing (xenogamy). Having a generalized breeding system can give plants an advantage by increasing plasticity in a new or changing environment (Trapp and Hendrix, 1988, Parker, 1991, Powell et al. 2011). Insects play a major role in pollen vectors during plant breeding of boreal forest herbs (Barrett and Helenurm, 1986). While many plants are automatically autogamous, outcrossing significantly increases plant reproductive success (Barrett and Helenurm, 1986). When studied as an invasive plant outside of its native range *Cirsium vulgare* was able to set high levels of seed without high insect visitation (Powell et al. 2011). Powell et al. (2011) concluded that due to its generalist insect visitor guild and autonomous self-pollination, *Cirsium vulgare* was capable of invading an area with a small number of propagules. Parker (1991) concluded that *Amphicarpaea bracteate* mating system (large reliance on self-pollination) is a major factor restricting the plants evolutionary capacity in relation to protection from plant pathogen attack. The lack of pollen movement between populations restricts the opportunity for recombination of alleles (Parker, 1991). Hog peanut does however have plasticity within its mating system which allows the plant to adjust its reproductive strategy based on environmental conditions (Trapp and Hendrix, 1988). To have the ability to outcross between populations, this requires management of plant populations of adequate size and within an appropriate distance for access for relevant pollinators (Taki et al. 2008). This is a key feature of avoiding inbreeding depression in plant populations. Having information about a plant's ability and affinity for outcrossing is necessary in order to properly manage plant populations, especially under pressure by human overharvesting. Linking human medicinal and traditional harvesting and needs with the plant's breeding system means better understanding (a) how the human uses of the plant will affect the plant population and (b) how to properly manage the plant to ensure its continued presence on the landscape for human uses.

Obligate outcrossing animal-pollinated flowering plants have been found to be in decline when the animals that pollinate them are also declining (Biesmeijer et al. 2006). The level of threat of pollinator declines to success of wild flowering plants depends on the contribution of pollinators to seed production in the plant (Rodger et al. 2021). It is unclear to what extent self-compatibility and insect pollination contribute to reproductive success for many plants globally (Rodger et al. 2021). Studies quantifying seed production of plants with pollinators excluded and those with pollinators present are required in order to quantify pollinator contribution to the plant's reproductive success (Rodger et al. 2021). When assessing the risk of extinction due to pollination or dispersal, Bond (1995) recommended examining four key attributes. 1) The plant species dependence on pollination, 2) Whether pollination was dependent on one pollinator species, 3) spatial movement requirements, 4) degree of dependence on seeds for survival (Lofgren, 2002). This suggests that when a plant species depends on generalist visitors for pollination services it is better able to adapt and avoid difficulties due to isolation (Bond, 1995, Lofgren, 2002). Some plant species may be capable of adaptation within their breeding system which allows for survival under the threat of pollinator declines (Thomann et al. 2013). Bee declines are also highest for species with a dependence on a declining plant species (Scheper et al. 2014). This suggests there is an interdependence between declines in wild pollinator and wild plant species (Potts et al. 2016). It has been hypothesized that under pressure of pollinator declines plants will evolve in one of two ways: (1) evolution towards higher automous selfing, or (2) reinforcement of characteristics that support the plant-pollinator relationship (Thomann et al. 2013). Declines of wild pollinators can affect reproduction of wild plant species through reductions in fruit or seed set (Potts et al. 2016) The extent to which wild plant reproduction is impacted will vary based on redundancy within the plant-pollinator network (Potts et al. 2016). Burkle et al. (2013) measured change in plant-pollinator interactions over 120 years at revisited sites in Illinois and found that herbaceous perennial plant species *Claytonia virginica* experienced a 75% reduction in pollinator species between the 1970s and 2009/2010. Burkle et al. (2013) also found that declines in bee species were associated with specialists, parasites, cavity-nesters, and species with weak historic interactions.

Researchers have examined the degree of cultural significance for each plant in previous studies (e.g., Petelka et al. 2022). Future research may investigate the degree of cultural significance of each plant and combine that with our research findings to further elucidate research priorities for medicine plants in the Great Lakes Region. Our results show that there is little information available in the published scientific literature about these culturally important (and ecologically important) plant species. Thus, there is an urgent need to construct new methods of conservation by incorporating different ways of knowing into planning. These findings will be useful in determining future research priorities regarding medicine plants in the Great Lakes Region.

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Figures

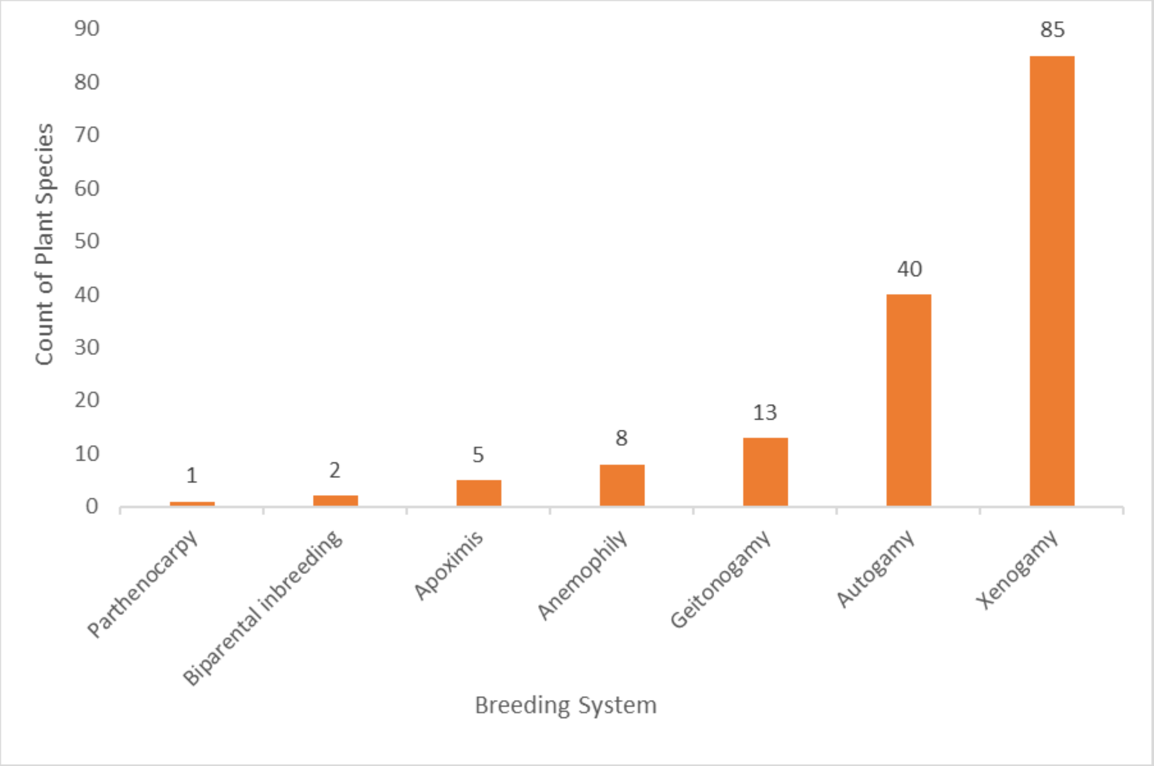

Figure 1. Count of different types of plant breeding systems recorded during a systematic literature review for 215 culturally significant medicinal plant species conducted in Fall 2022.

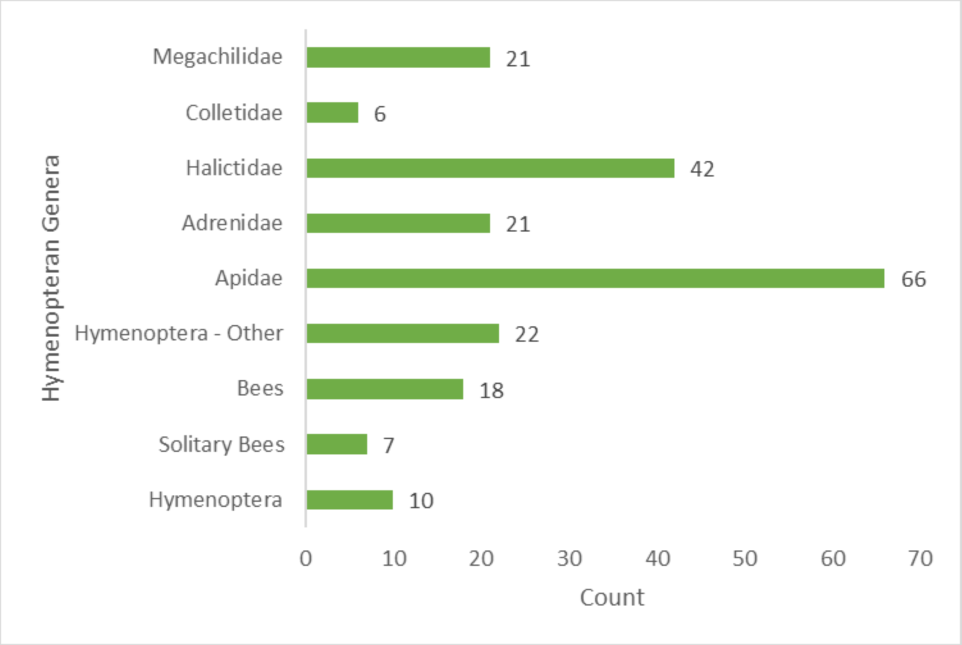

Figure 2. Breakdown of Hymenopteran visitor categories recorded as insect visitors or pollinators of 215 culturally significant medicinal plant species examined during a systematic literature review in Fall 2022.

Tables

Table 1. Total number of culturally significant medicinal plant species each insect order was recorded visiting during a systematic literature review in Fall 2022.

Insect Order	Total Number of Plant Species
Coleoptera	31
Collembola	1
Diptera	59
Hemiptera	8
Homoptera	1
Hymenoptera	213
Lepidoptera	31
Mecoptera	1
Neuroptera	2
Odonata	1
Orthoptera	2
Psocoptera	1
Thysanoptera	7

Chapter Two: Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region

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Candidate's contribution:

Shelby Gibson, Lisa Myers, and Dr. Colla conceived of the ideas and methodology for this manuscript. Shelby Gibson selected the survey sites, prepared garden spaces, organized growing plants for all sites, managed a field assistant, conducted field work, input data, cleaned data, and analyzed the data. Shelby Gibson led the writing of the manuscript. Shelby Gibson and Dr. Colla edited the manuscript. Tom Onuferko provided expert insect identification to the species level for all insects collected during field work.

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Abstract

Understanding the interactions between plants and pollinators within a system can provide information about pollination requirements and the degree to which species contribute to floral reproductive success. Past research has focused largely on interactions within monocultured agricultural systems and only somewhat on wild pollination networks. This study focuses on the culturally significant Three Sisters Garden, which has been grown and tended by many Indigenous peoples for generations in the Great Lakes Region. Here, the plant-pollinator network of the traditional Three Sisters Garden with the inclusion of some additional culturally significant plants was mapped. Important visitors in this system included the common eastern bumble bee, *Bombus impatiens* Cresson (Hymenoptera: Apidae), and the hoary squash bee, *Xenoglossa pruinosa* (Say) (Hymenoptera: Apidae), as determined by their abundances and pollinator service index (PSI) values. Understanding the key pollinators in the Three Sisters Garden links biological diversity to cultural diversity through the pollination of culturally significant plants. Further, this information could be of use in supporting Indigenous food sovereignty by providing knowledge about which wild pollinators could be supported to increase fruit and seed set within the Three Sisters Garden. Our findings can also lead to more effective conservation of important wild pollinator species.

Introduction

Pollination is a mutualistic interaction between two levels of the food web—plants and their pollinators (Jordano, 1987; Carvalheiro et al., 2008; Ings et al., 2009; Willis Chan and Raine, 2023). Network theory has been used in the evaluation of mutualistic interactions, and the interactions are cumulatively referred to as a plant-pollinator network (Jordano, 1987; Ings et al. 2009; Kaiser-Bunbury and Blüthgen, 2015; Jolls et al. 2019). While plant-pollinator network characteristics (such as asymmetry and nestedness) (Bascompte et al. 2003; Bascompte et al. 2006; Montoya et al. 2006) make them theoretically robust, there is potential for anthropogenically driven environmental disturbance to eventually collapse plant-pollinator networks (Kearns et al., 1998; Potts et al., 2010; Brosi and Briggs, 2013; Tucker and Rehan, 2016; Tscharntke, 2021).

Plant-pollinator networks are negatively impacted by various factors including habitat fragmentation and land use changes (Spiesman and Inouye, 2013), conventional agricultural practices (e.g., pesticides), non-native species introductions (Kearns and Inouye, 1998), and increasingly, climate change (Memmott et al., 2007). Documenting these mutualisms and modeling how they respond to change are integral to the conservation and restoration of ecological networks (Memmott, 2009; Kaiser-Bunbury and Blüthgen, 2015; Tucker and Rehan, 2016). Studying plant-pollinator networks helps to fill in baseline information about the ecological role of wild bees and to understand the stability and/or resiliency of the network to environmental change (Tucker and Rehan, 2016). The loss of even a single species can have significant effects on reproductive success of the plants within a system (Brosi and Briggs, 2013). Declines of pollinators within a network can contribute to negative feedback of less floral reproduction, which then in turn contributes to fewer resources for pollinators (Tscharntke, 2021). Wild pollinators provide significant levels of pollination services to crops (Garibaldi et al., 2011), yet the details (e.g., the level of pollinator abundance or diversity required to provide adequate pollination) of these relationships remain relatively unknown (Kovacs-Hostyanszki, 2017; Danforth et al., 2019). Pollination deficits are a threat to global food security (Tscharntke, 2021).

There have been efforts recently to increase food production without increasing the level of environmental harm from agriculture (Pretty, 2014; Tscharntke, 2021; Ramirez and Wright, 2023). Intercropping is a practice that may increase yield and promote sustainable land and resource use (Tscharntke, 2021; Ramirez and Wright, 2023). Intercropping has also been suggested as a method of reducing agricultural causes of pollinator decline (Kovacs-Hostyanszki, 2017; Tscharntke, 2021). The Three Sisters method of cultivation is a polyculture practice (intercropping) involving the growth of multiple crops simultaneously (Eames-Sheavly, 1993; Kuepper et al., 2016).

There is evidence in the forests and prairies of Canada of corn domestication as early as 500 A.D. (Boyd and Surette, 2010) and common bean and squash cultivation in the Woodland period (1000 B.C.–1000 A.D.) (Boyd et al., 2014). The Three Sisters were grown for 500 years pre-contact by the Seneca people in western New York and was referred to as “Diohe'ko”, which translates to “these sustain us” (Lewandowski, 1987). The Haudenosaunee people (people of the long house) of the Eastern United States and Canada have traditionally planted the Three Sisters Garden (Eames-Sheavly, 1993). Broadly, it has been reported that the Three Sisters were grown by all tribes who practiced agriculture in northeastern North America (Lewandowski, 1987). This study aims to better understand the pollinator community and plant-pollinator network in a Three Sisters Garden (TSG). The Three Sisters Garden is composed of corn (*Zea mays* L. (Poales:

Poaceae)), common bean (*Phaseolus vulgaris* L. (Fabales: Fabaceae)), and squash (*Cucurbita* L. sp. (Cucurbitales: Cucurbitaceae)) (Boyd et al., 2014).

Cucurbita plants (pumpkins, squash, gourds) are monoecious and rely on insect pollination; each plant has both pistillate (female) and staminate (male) flowers (Stapleton et al., 2000; Whitaker and Davis, 2012; Brochu et al., 2021, Willis Chan and Raine, 2021). Flowers open at dawn and close by noon each day, and pollination must occur within this window (Nepi and Pacini, 1993, Willis Chan and Raine, 2021). *Xenoglossa pruinosa* (Say) is an oligolectic bee species, foraging only on the flowers of *Cucurbita* crops and wild *Cucurbita* spp. where they are present (Hurd and Linsley, 1964; Willis Chan, 2020; Brochu et al., 2021). The hoary squash bee's natural geographic range has increased over the past 1000 years following the spread of squash planting for agricultural purposes (Brochu et al., 2021). Domesticated squash has been receiving pollination by wild pollinators prior to the introduction of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (Lopez-Uribe et al., 2016). *Phaseolus vulgaris* L. (Fabaceae) is self-compatible, i.e., able to self-pollinate as the flower opens and provides little, if any, nectar (Ibarra-Perez et al., 1999; de Souza Paulino et al., 2023). It is also noted, however, that the reproductive success of the plant (seed yield) can be increased by visits from larger bees (e.g., carpenter bees, bumble bees, etc.) (Ibarra-Perez et al., 1999). *Phaseolus coccineus* L. has been found to set few pods without the presence of insect visitors (Darwin, 1876; Free, 1966; Free and Racey, 1968; Kendall and Smith, 1976). *Zea mays* is wind pollinated and therefore does not rely on insects for pollination; however, insects may visit the flowers (Johnson and Hayes, 1932; Wheelock et al., 2016; Rondeau et al., 2022).

In some cases, sunflowers would be grown along one side of the Three Sisters Garden (Kuepper et al., 2016); it has been reported that this was done to attract pollinators to the garden

(Native Seeds Search, 2020; Rodale Institute, 2020). Other plants, including Hopi tobacco (*Nicotiana rustica* L. (Solanales: Solanaceae)), purple coneflower (*Echinacea purpurea* (L.) Moench (Asterales: Asteraceae)), common milkweed (*Asclepias syriaca* L. (Gentianales: Apocynaceae)), wild bergamot (*Monarda fistulosa* L. (Lamiales: Lamiaceae)), Oswego tea/bee balm (*Monarda didyma* L.), and American vervain (*Verbena hastata* L. (Lamiales: Verbenaceae)), are also planted in some food and medicine gardens (Our Sustenance, 2020; PAN and TRCA, 2020).

The Three Sisters Garden (TSG) is a growing method with long biological and cultural roots, and medicine plants are important to many Indigenous cultures (Densmore, 1928; Lewington, 1990; Padulosi et al., 2004; Genuisz, 2015; PAN and TRCA, 2020). A better understanding of the pollinator community and plant-pollinator network in the Three Sisters Garden will provide information about the wildlife that provides ecological services to this kind of garden and thus the pollinators that are connected to Indigenous food and medicine sovereignty. Intercropping also offers a sustainable agricultural practice that may be useful, specifically in urban agriculture (Ramirez and Wright, 2023).

The objective of this study is to map the plant-pollinator network in the culturally significant Three Sisters garden and determine if and how the pollinator community in the garden differs from the local wild pollinator community based on pan-trap sampling in adjacent, natural sites and in the context of other regional studies. These baseline conditions will be useful for predicting how the Three Sisters Garden system may be impacted by environmental change into the future.

Materials & Methods

Study Sites and Land Acknowledgment

This research was undertaken on the traditional territories of multiple First Nations. The campus of York University is located on the traditional territory of the Anishinabe Nation, the Haudenosaunee Confederacy, the Huron-Wendat, and the Métis. The current treaty holders in this location are the Mississaugas of the New Credit First Nation, and the land is subject to the Dish with One Spoon Wampum Belt Covenant. The eastern Ontario field sites are located on unceded Algonquin territory and are subject to Treaty 27 and Treaty 27 1/4. We acknowledge the generations of caretaking of the land by the many Indigenous peoples who have and still do call these places home. Plot A is located in Arnprior, Ontario, Plot B is located in Pakenham, Ontario, and Plot C is located in Lanark, Ontario (Figure 1).

Planting

Seeds for this study were sourced from Urban Harvest (www.urbanharvest.ca) and included scarlet runner (*Phaseolus coccineus*) and true red cranberry beans (*Phaseolus vulgaris*), delicata (*Cucurbita pepo* L. 'Delicata') and pattypan squash (*Cucurbita pepo* 'Patty Pan'), bloody butcher corn (*Zea mays* 'Bloody Butcher'), purple coneflower (*Echinacea purpurea*), bee balm (*Monarda didyma*), and sunflowers (*Helianthus annuus* L. 'Autumn Beauty' (Asterales: Asteraceae)). Pots with purple coneflower, Oswego tea, and American vervain (*Verbena hastata*) were added to the garden sites since growing these plants from seeds is a multi-year process. Sunflowers and medicine plants were added to replicate the addition of these plants to the traditional TSG system. Seeds (including corn, squash, and beans) were started indoors in April using Organic ProMix and Jiffy pots (4"). The soil was kept moist until germination, and light was provided using a TOLYS 1000W LED light. At each site, the earth was tilled with a shovel, and soil amendments were added as follows: two 85L bags of Organic ProMix and 4 bags of composted sheep manure per plot.

Planting methods followed those described by Six Nations Our Sustenance (2020), which include mounds of soil placed with the centre of each mound 5 ft from the centre of the next mound for a total of 18 mounds. Each mound was 18″ across and 12″ high. Four corn plants were planted in a 6″ square in alternating mounds. Beans were planted 3″ from each corn plant, creating a square in each corn/bean mound. Squash plants were planted in the remaining mounds, with 2 plants spaced 4″ apart (alternating mounds of 'Patty Pan' and 'Delicata' squash). Plots A, B, and C in eastern Ontario were used, and each contained a 30 ft × 30 ft garden plot of the Three Sisters. Various medicine plants were also added to all three plots, where some grew more succesfully than others. Plot A also had purple coneflower, Oswego tea, milkweed, and American vervain growing in the garden. Plots B and C also had purple coneflower, bee balm, and sunflowers.

Insect Interaction Observations, Collection & Curation

In 2020, for four days per week and over eight weeks, sweep-net sampling for the plant-pollinator network was conducted, rotating between morning and afternoon sessions, resulting in a total of four sampling sessions per garden per week at three sites in eastern Ontario (Plots A, B, and C) (total of 96 sampling sessions). Morning sessions were between 8 AM and noon, and afternoon sessions between 1 PM and 5 PM (as in Tucker and Rehan, 2016). Previous work sampling the plant-pollinator network in a garden setting used similar methods (Gotlieb et al., 2011). The garden plot was sampled by walking down each of the rows and up the next row for

20 minutes. Each pollinator on a flower or inflorescence was collected using a sweep net and placed in an insect vial, and the plant species was noted on the vial. It was not determined whether pollen was deposited; rather, a visit to the flower was used to signal an interaction. Vials were placed in a small lunch cooler containing icepacks until the end of the 20-minute session. At the end of each session, the cooler was emptied, with each specimen that could be identified on the wing identified and released (*Apis mellifera* and *Bombus* Latreille spp.). A voucher of each species was collected to represent each species collected in the field (Kearns and Inouye, 1993; Packer et al., 2018). Each specimen not identified in the field was labelled with the floral species, site, and date. Collected specimens were placed in 70% ethanol (Kearns and Inouye, 1993) and pinned at the end of each sampling day throughout the season.

Pan trap sampling was conducted in the natural areas adjacent to the gardens—Pan traps were placed 500 m away from the garden site at two sites. Small plastic coloured bowls (yellow, blue, and white) were placed on the surface of the ground and filled with soapy water. A 100 m transect was used, and pan traps were placed every ~15 m in a repeating order of yellow, blue, and white (for a total of 6 traps per transect). Pan traps were set in place for 24-hr periods on sweep sampling days.

Bee Identification

Vouchered collected specimens are stored at Dr. Sheila Colla's laboratory at York University, Toronto, Canada. The records were identified to species or morphospecies and include preserved, physical, and some imaged specimens (e.g., *Xenoglossa* Smith (Hymenoptera: Apidae) and *Bombus* (Hymenoptera: Apidae)). Pan trap samples were identified to species. Bees were identified to genus (and species for genera that are monotypic in Eastern Canada) using the key of Packer *et al*. (2007). Species-level identifications were made with reference to the keys and taxon concepts of de Silva (2012) for *Coelioxys* Latreille (Hymenoptera: Megachilidae); Gardner & Gibbs (2022) for metallic weak-veined *Lasioglossum* Curtis (Hymenoptera: Halictidae); Gibbs *et al*. (2013) for non-metallic weak-veined *Lasioglossum*; Laverty & Harder (1988) and Williams *et al*. (2014) for *Bombus*; McGinley (1986) for strong-veined *Lasioglossum*; Mitchell (1960) for *Halictus* Latreille (Hymenoptera: Halictidae); Mitchell (1962) for *Melissodes* Latreille (Hymenoptera: Apidae) and *Osmia* Panzer (Hymenoptera: Halictidae); Onuferko (2017, 2018) for *Epeolus* Latreille (Hymenoptera: Apidae); Oram (2018) for *Hylaeus* Fabricius (Hymenoptera: Colletidae); Portman *et al*. (2022) for *Augochlora* Smith (Hymenoptera: Halictidae) and *Augochlorella* Sandhouse (Hymenoptera: Halictidae); Rehan & Sheffield (2011) for *Ceratina* Latreille (Hymenoptera: Apidae); Mitchell (1962) and Rowe (2017) for non-*Osmia* Osmiini; Mitchell (1962) and Sheffield *et al*. (2011) for *Megachile* Latreille (Hymenoptera: Megachilidae); and Stephen (1954) and Mitchell (1960) for *Colletes* Latreille (Hymenoptera: Colletidae). It was not possible to distinguish some females of *Ceratina dupla* Say from *C. mikmaqi* Rehan & Sheffield, so they were treated as a single morphospecies in data analysis, as *Ceratina dupla/mikmaqi*.

Statistical Analysis

Statistical analysis was conducted in R (R version 3.6.2 (2019-12-12) (R Core Team, 2019). The R package ‘vegan’ (Oksanen et al., 2020) was used to run species accumulation estimates using from abundance data (Gardener, 2014). The R package ‘bipartite’ (Dormann et al., 2008) was used to run community (function *networklevel*) and species (function *specieslevel*) level network analyses. Data were pooled together from all three sites. Community level analysis was conducted including calculations of weighted nestedness and weighted connectance (Tucker and Rehan, 2018). Nestedness refers to the level of overlap between generalist and specialist interactions, where values closer to “1” indicate a high degree of overlap and values closer to 0 indicate a low degree of overlap (Tucker and Rehan, 2018; Delmas et al., 2019). Connectance refers to the proportion of possible interactions that have been realized in the network (Kearns et al., 1998; Tucker and Rehan, 2018; Delmas et al., 2019) and is a measure of the community’s ability to respond to change such as species loss (Dunne et al., 2002; Tucker and Rehan, 2016). Species level analysis included estimation and comparisons of weighted degree and Pollination Service Index (PSI) values (Dormann et al., 2008; Tucker and Rehan, 2018). Degree measures the diet breadth of the pollinators or the number of unique interactions of floral visitors (Tucker and Rehan, 2016). The PSI measures the relative importance of each pollinator species to the functioning of the community (1 = the species is critical to ecosystem functioning, 0 = community could function without the species) (Dormann et al., 2008). PSI is calculated by first determining the proportional representation of the plant species visited by a pollinator and second the proportional representation of the bee species that visit a plant (Dormann and Fründ, 2024). Third, these proportions are multiplied to determine the PSI value for each pollinator species (Dormann and Fründ, 2024).

Results

Abundance and Diversity

A total of 310 interactions were observed during the sampling period (July–August 2020) across all sites. Thirty-seven bee species/morphospecies were identified at all sites combined

(Figure 2), with 14 recorded at Plot A, 19 at Plot B, and 22 at Plot C. A total of ten plant varieties were present across all sites combined (Figure 2), with eight species at Plot A, five species at Plot B, and seven species at Plot C. The species accumulation estimate using the Chao 1 and ACE tests was 63 (se.chao1 = 18) for all plots combined. The observed 37 species represent 59% of the ~63 bee species estimated to occur in the plant community. Bees of the following three families were recorded: Apidae, Megachilidae, and Halictidae. No Andrenidae or Colletedae were recorded in the interaction sampling. The most frequent family was Apidae (n = 200), the most frequent genus was *Bombus* (n = 93), and the most frequent species were *Bombus impatiens* (n = 88) and the hoary squash bee, *Xenoglossa pruinosa* (n = 81). The Shannon's Diversity for the garden sampling was 2.37.

A total of 397 bee specimens were collected from pan-trap sampling. Bees of 19 genera and 53 species were collected in the pan trap samples. Bees of five families were collected (Apidae, Megachilidae, Andrenidae, Colletidae, and Halictidae). Halictidae (n = 228) was the most frequent bee family collected and Andrenidae was the least frequent (Table 1). *Halictus* (n = 88) was the most frequent genus, and *Halictus ligatus* (n = 68) was the most frequent species (Table 1). The species accumulation estimate using the Chao 1 and ACE tests was 62 (se.chao1, 6). The observed 53 species represent 85% of the ~62 bee species estimated to occur at the sites. The Shannon's Diversity for the pan trap sampling was 3.22.

Plant-pollinator Network

For the garden sampling, the most common interactions observed across sites were between *Cucurbita pepo* 'Patty Pan' and *X. pruinosa* (53 interactions) and *B. impatiens* (54 interactions) (Figure 2). Weighted connectance was 0.08 and weighted nestedness was 0.47.

Bombus impatiens had the highest degree (6) and *X. pruinosa* (42) and *B. impatiens* (41) had the highest PSI (Table 2).

Cucurbita pepo ‘Patty Pan’ was associated with the highest number of bees (127), followed by *Nicotiana rustica* ‘Hopi’, which was associated with the second highest number (69) (Figure 3). *Phaseolus coccineus* ‘Scarlet Runner’ and *Verbena hastata* were associated with the lowest number of bees (4) (Figure 3). *Nicotiana rustica* ‘Hopi’ was associated with the highest number of bee species (19), followed by *C. pepo* ‘Patty Pan’ (11) (Figure 3). *Phaseolus coccineus* ‘Scarlet Runner’ was associated with only one species of bee (Figure 3). The most common bee species recorded were *B. impatiens* (n = 88) and *X. pruinosa* (n = 81) (Figure 4). Seventeen bee species were recorded only once (Figure 4). There were no interactions recorded for corn flowers.

Discussion

The most abundant visitor to the Three Sisters gardens, and the visitor with the highest PSI, was *X. pruinosa*. The most common interactions were between *X. pruinosa* and *C. pepo* ‘Patty Pan’ and between *B. impatiens* and *C. pepo* ‘Patty Pan’. Based on PSI, *X. pruinosa* is one of the key species within the Three Sisters Garden system. There were only two records of *X. pruinosa* in the pan trap samples, which can be explained by this species’ specialization on *Cucurbita*. *Xenoglossa pruinosa* had the highest PSI at all sites combined as well as at Plots A and B and was most frequently collected while visiting *C. pepo* ‘Patty Pan’ and *C. pepo* ‘Delicata’.

As highlighted by Willis Chan (2020) and Willis-Chan and Raine (2021a, 2021b, 2023), *X. pruinosa* has a close association with *Cucurbita* crops grown for agricultural purposes in Ontario. The range of *X. pruinosa* in Ontario is outside of the range of wild *Cucurbita* (López-

Uribe et al., 2016). The range of *X. pruinosa* has been found to be impacted by agricultural expansion in North America (Pope et al., 2023). The history of the Three Sisters Garden and the remnants of domesticated beans and squash during the woodland period (Boyd et al., 2014), as well as the close association still found today between *X. pruinosa* and *Cucurbita* crops, highlight the importance of continued research focusing on managing threats to *X. pruinosa* in a changing environment to ensure continued pollination services by wild bees to this culturally and economically significant food plant. There were also other bee species interacting with *C. pepo* including *Apis mellifera*, *Augochlora pura* (Say), *Augochlorella aurata* (Smith), *Bombus* spp., *Halictus rubicundus* (Christ), *Lasioglossum* spp., *Megachile brevis* Say, and *Melissodes* spp. Future studies should examine the role of these bee species to pollination of *Cucurbita*.

The diversity of bees collected in the natural environment was higher than that in the TSG system. The Shannon's Diversity Index for the garden samples was 2.37, and for the pan traps was 3.22. Based on the species accumulation estimates, our results represent 59% of bee species estimated to occur in the TSG system, indicating that more extensive sampling is warranted for getting a more accurate representation of the pollinator community. The interaction sampling recorded three bee families whereas pan trap sampling recorded five bee families. Perhaps increased sampling effort would have recorded Andrenidae and Colletidae in the interaction sampling. The absence of *Andrena* Fabricius (Hymenoptera: Andrenidae) (the largest genus of andrenids in Eastern Canada) from our samples may be explained by our sampling having taken place during the summer and *Andrena* being most active in the spring. In addition, pan trapping and sweep netting may not provide a direct comparison as they may sampling different components of the bee community (Roulston et al. 2007; Kuhlman et al. 2021).

Current management recommendation for *X. pruinosa* in agroecosystems are as follows: (1) minimize pesticide exposure, (2) provide nesting sites, (3) maintain yearly field proximity, (4) monitor populations, parasites, and pathogens, and (5) limit deep tillage (Brochu et al., 2021). Therefore, some of the TSG growing methods may already be implementing some of these practices, such as not using pesticides and limiting deep tillage. With information about the importance of *X. pruinosa* to the TSG and current management recommendations, it is possible to both maintain healthy pollinator populations and ensure adequate pollination of culturally significant food plants. In the absence of squash bees, honey bees and bumble bees are known to be effective pollinators of *Cucurbita* crops (McGrady et al., 2021); however, these pollinators are not as effective due to their foraging activity taking place primarily after the early morning pollination window for *Cucurbita* plants (Willis Chan and Raine, 2021). Therefore, using management recommendations for squash bees, such as reducing pesticide exposure (Willis Chan and Raine, 2021c), can increase their populations as well as pollination of *Cucurbita* crops by squash bees.

Based on abundance, *B. impatiens* and *X. pruinosa* were the most common floral visitor bee species in the TSG system. Tucker and Rehan (2016) found *B. impatiens* to be the most abundant species in their recent study as well. *Bombus impatiens* is a bumble bee species with a broad distribution in North America that is also used as a managed pollinator (including outside its native range) (Ratti and Colla, 2010). It has a wide diet breadth, frequently found on both native and introduced plant species (Williams et al., 2009; Colla and Dumesh, 2010; Richards et al., 2011; Colla et al., 2012; Williams et al., 2014). In this study, *B. impatiens* had the highest overall diet breadth of all bee species, which would explain its ubiquitousness in wild and managed systems. Bumble bees are good pollinators of *Cucubita*, able to deposit more pollen grains per stigma and come in contact with the stigma more frequently than squash bees or honey bees (Artz and Nault 2011). Willis-Chan and Raine (2021) found that bumble bees in Ontario are active during the daily crop pollination window, as are the squash bees. Whilehoney bees are found visiting *Cucurbita* flowers, it is likely that the pollen has been depleted and the bees are foraging for nectar (Percival, 1947, Artz and Nault, 2011, Brochu et al., 2020). Pollen supply on staminate flowers has been found to decrease by approximately 60% within the first hour after the flowers open (Brochu et al., 2020; Willis Chan and Raine, 2021). This information further highlights the importance of *X. pruinosa* to the TSG system. Willis Chan and Raine (2021) found that the pollination window of *Cucurbita* in Ontario to be between 6 AM and 8 AM. Future studies investigating the role of *X. pruinosa* to the TSG in Ontario should take this into account during experimental design.

Within a pollination network, high connectance and high interaction diversity are associated with stability and resilience (Tscharntke, 2021). The overall weighted connectance of 0.08 is the same value found in wild bee pollination networks in northern New England (Tucker and Rehan, 2016). The weighted nestedness of 0.47 is similar to this study as well (0.51) (Tucker and Rehan, 2016). The authors concluded that both connectance and nestedness were low, indicating that the pollination network in their study may not be resilient to change and may be impacted by significant disturbances (Tucker and Rehan, 2016).

Based on the similar results of our study, we deduce that the TSG plant-pollinator network may not be resilient to environmental perturbations. Brosi and Briggs (2013) suggest that plant-pollinator networks overestimate the resiliency of pollination networks to perturbations and found that the removal of one pollinator species can affect the quality of reproduction of plant species in the system. Brosi and Briggs (2013) suggest that this is because when a pollinator is removed, the plant-pollinator network finds species within the system that will replace the pollination service to the plants visited by this particular pollinator species, but in doing so the network does not consider the pollinator effectiveness of each species. Pollinator effectiveness refers to how well a species of pollinator moves pollen and sets fruits and seeds for a particular plant (Brosi and Briggs, 2013; McGrady et al., 2021). Therefore, it is important to study not only the plant-pollinator network but also particular interactions between a plant species and its specific pollinators. If *X. pruinosa* were not present, *Bombus* species could provide adequate pollination in terms of pollen grains deposited (McGrady et al., 2021), however, and visits would be within the peak pollination window (Willis Chan, 2021).

The second most frequently visited plant in this study was *N. rustica*. Hopi tobacco is a culturally significant plant to many Indigenous peoples (Brokenleg et al., 2013; Sadik, 2014). *Nicotiana rustica* is a plant reported to produce up to two thirds of its seeds through self-pollination and is pollinated by bees (Mather & Vines, 1952). In this study, *N. rustica* supported the highest diversity of bee species and the second highest abundance of bee visits. *Augochlorella aurata* represented 16% of the records in the pan trap samples from the wild bee families, suggesting growing *N. rustica* may provide important foraging resources to wild bees. While *N. rustica* is self-compatible (Mather & Vines, 1952, Raguso et al., 2003), visits by insects increase its reproductive success (Adler et al., 2012; Gibson et al., 2022). *Augochlorella aurata* belongs to the family Halictidae (sweat bees), which like most other sweat bees, nest in bare soil (Buckley et al., 2019), but it is a species that has also been found to be an important pollinator of *N. rustica* (Gibson et al., 2022).

Management recommendations for Halictidae includes providing appropriate nesting and foraging resources (Buckley et al., 2019). Avoiding tilling the soil is a key factor in providing nesting space for sweat bees (Buckley et al., 2019). Lewandoski (1987) reported that upon observation of European agriculture, the Seneca people who had traditionally grown the Three Sisters Garden were shocked by the “wounding of Mother Earth” occurring, which was a reference to the tillage of the soil. In some cases, however, mild tilling has had a positive impact on ground-nesting bee abundance in agricultural areas (Cusser et al., 2023).

Beans, forming a large component of the Three Sisters Garden, had some of the lowest observed rates of bee visitation. Only *B. impatiens* was observed visiting *P. coccineus*, despite the other genera being present and visiting other plants within the plant community. Overall, bee visitation to both bean varieties was quite low. Beans are mostly self-pollinated with limited floral resources available, and yield is only marginally increased by insect pollination (Ibarra-Perez at al., 1999).

Indigenous and other local communities support pollinator conservation through (1) supporting biocultural (biological and cultural) diversity, (2) landscape management, and (3) diversified farming systems (Hill et al., 2019). Here, we examine one of those diversified farming systems. The Three Sisters is a key feature of northeastern North American Indigenous agriculture (Lewandowski, 1987). The intercropping growing method of the Three Sisters Garden has been found to support not just humans physically and spiritually but also a wide diversity of wild pollinators through foraging and nesting provisions. Supporting the cultivation of culturally appropriate foods, and therefore food sovereignty, simultaneously supports and depends on the conservation of wild bee species.

Conclusions

In this study we aimed to determine the plant-pollinator network in a Three Sisters Garden in the Great Lakes Region. The results of this study highlight *Bombus impatiens* and *Xenoglossa pruinosa* as important pollinators in the TSG system. Three of five bee families found in nearby natural areas were also found in the garden system. One limitation of this study is the lack of sweep net sampling in natural areas, which may be an avenue for future research. Future directions may also include more research efforts focused on the critical role of wild pollinators in culturally significant plants and the applied policies and programs towards promoting their conservation and diversity.

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Figures

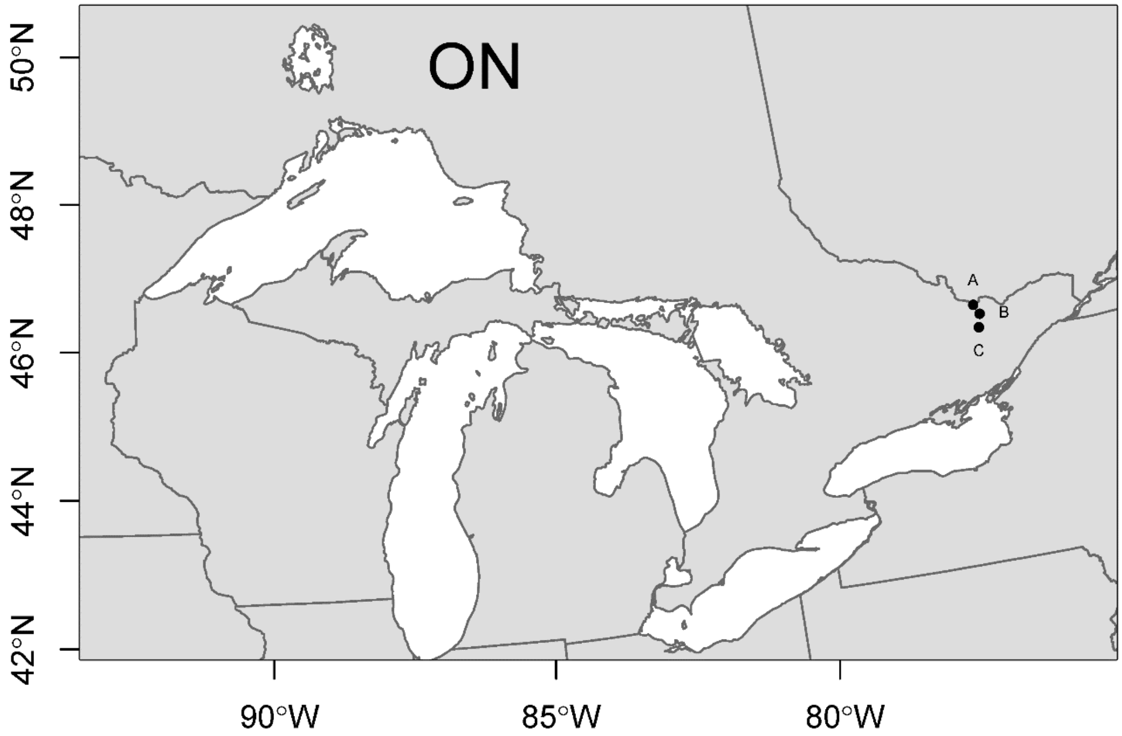

Figure 1. Map showing study sites A, B, and C in the Great Lakes Region of northeastern North America.

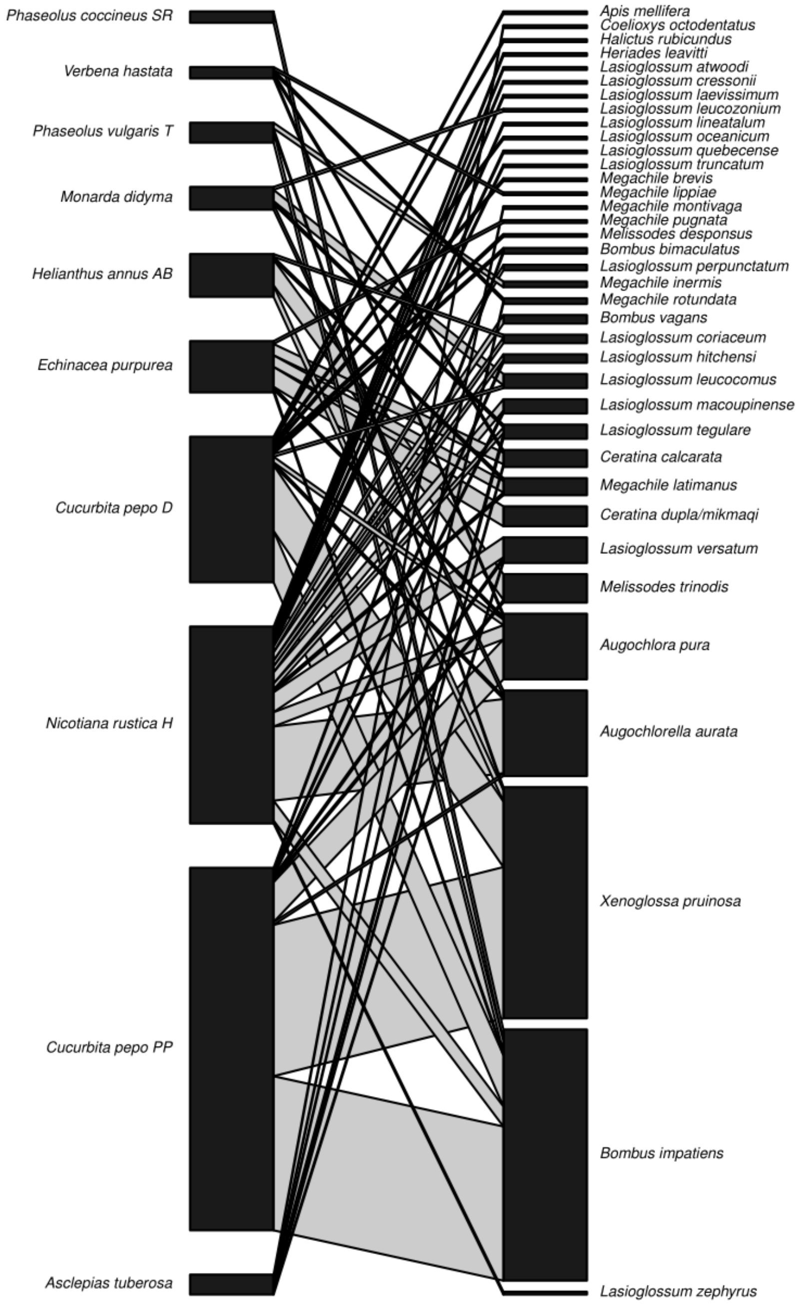

Figure 2. Interaction network displaying plant (species level) and pollinator (species level) interactions (n = 310) observed during sweep netting in the Three Sisters Garden plots in the Great Lakes Region. Interaction line width is proportional to abundance.

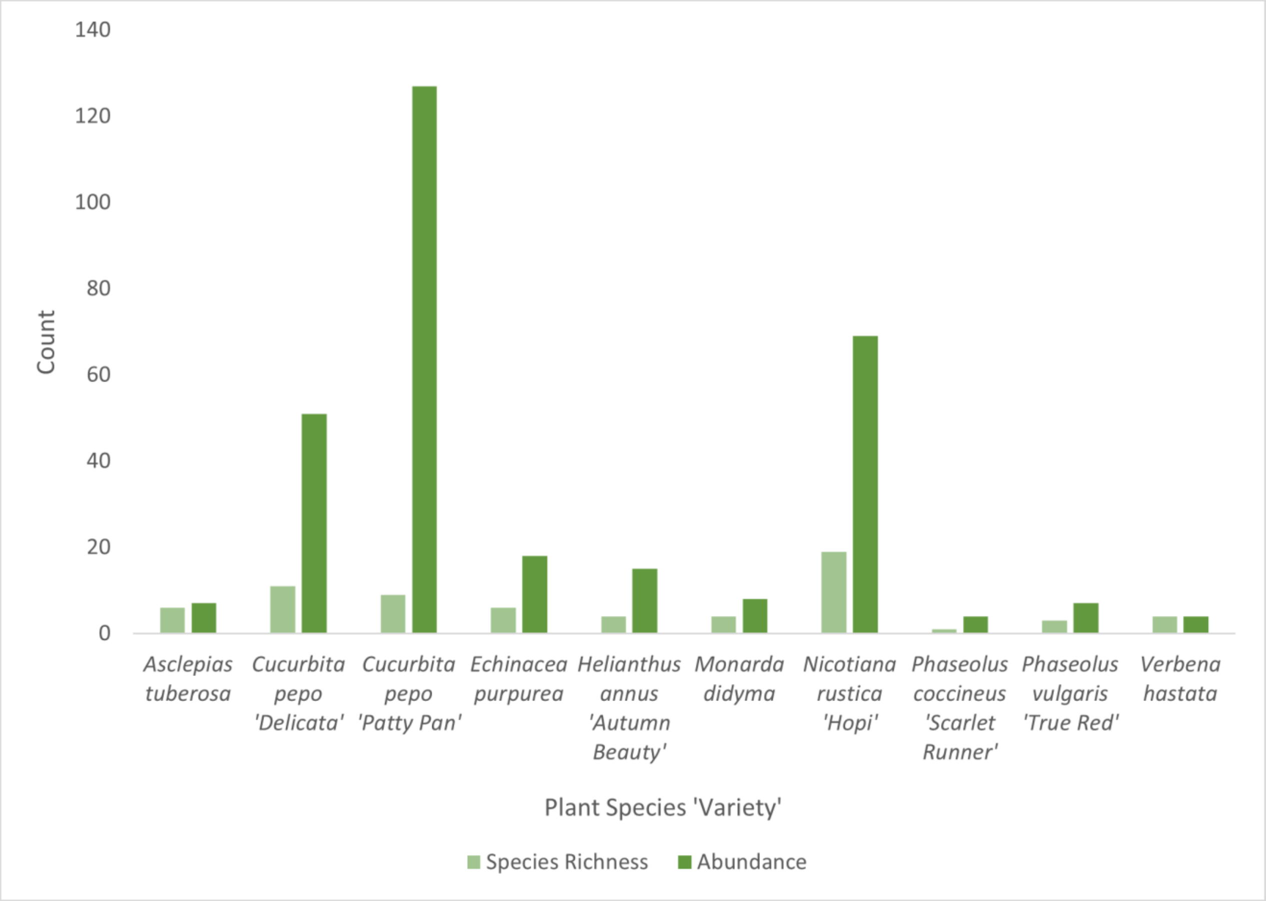

Figure 3. Bee abundance (n = 310) and species diversity collected using sweep netting on each plant species in the Three Sisters garden plots in the Great Lakes Region between July and August 2020.

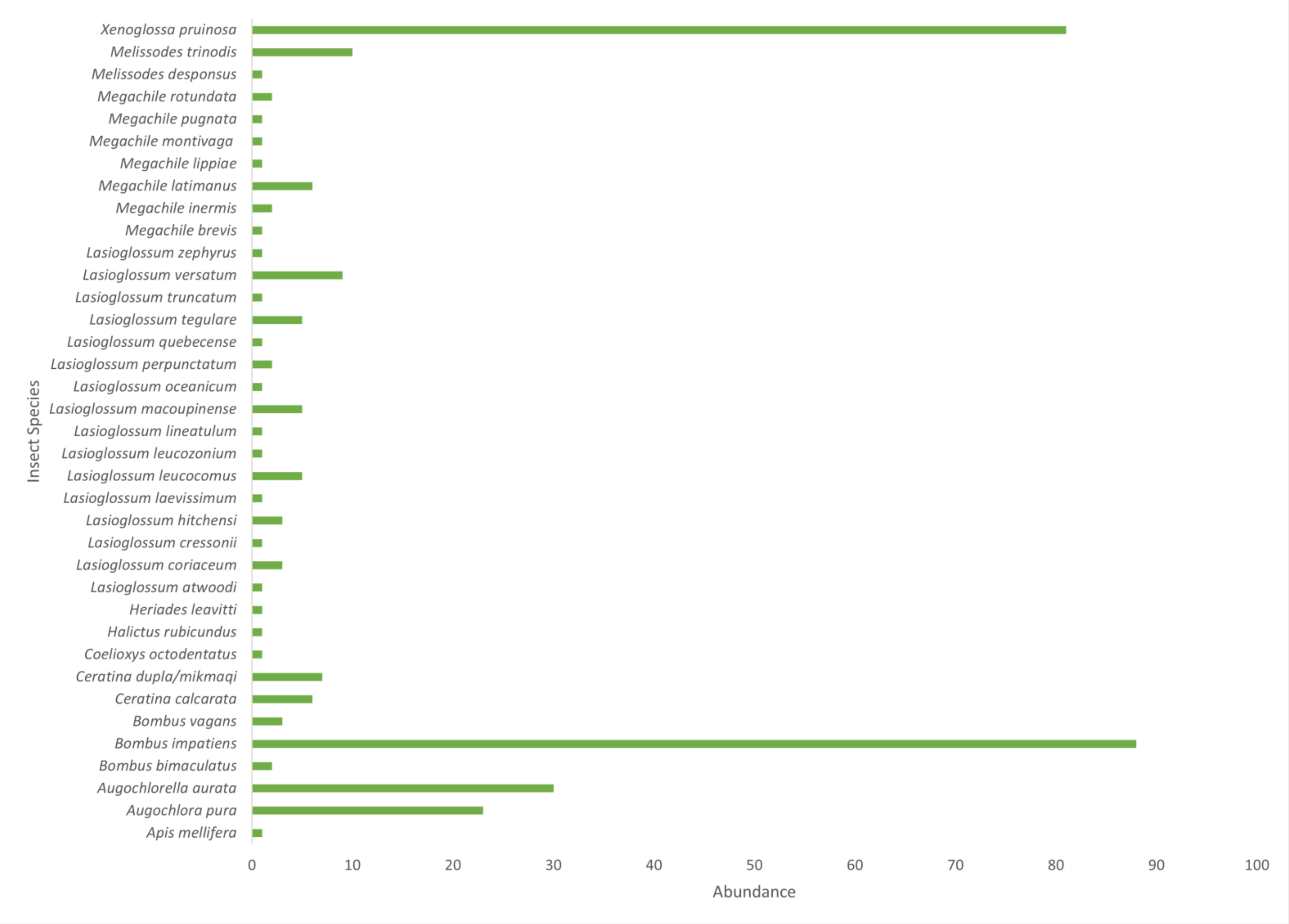

Figure 4. Bee species abundance (n = 310) collected in using sweep netting in the Three Sisters Garden plots in three sites in the Great Lakes Region.

Tables

Table 1. List and abundance of bees collected during pan trap sampling adjacent to Three Sisters Garden plots in the Great Lakes Region.

Andrenidae		**3**
	***Calliopsis* Smith**	**3**
	andreniformis Smith	3
Apidae		**103**
	***Apis* L.**	**2**
	mellifera L.	2
	***Bombus* Latreille**	**37**
	borealis Kirby	1
	griseocollis (De Geer)	11
	impatiens Cresson	9
	rufocinctus Cresson	8
	terricola Kirby	4
	vagans Smith	4
	***Ceratina* Latreille**	**24**
	calcarata Robertson	10
	dupla Say	1
	mikmaqi Rehan & Sheffield	13
	***Epeolus* Latreille**	**1**
	scutellaris Say	1
	***Xenoglossa* Smith**	**2**
	pruinosa (Say)	2
	***Melissodes* Latreille**	**37**
	desponsus Smith	18

	druriellus (Kirby)	1
	illatus Lovell & Cockerell	3
	subillatus LaBerge	13
	trinodis Robertson	1
Colletidae		**6**
	***Colletes* Latreille**	**3**
	latitarsis Robertson	3
	***Hylaeus* Fabricius**	**3**
	mesillae (Cockerell)	1
	modestus Say	2
Halictidae		**228**
	***Augochlora* Smith**	**2**
	pura (Say)	2
	***Augochlorella* Sandhouse**	**62**
	aurata (Smith)	62
	***Halictus* Latreille**	**88**
	confusus Smith	16
	ligatus Say	68
	rubicundus (Christ)	4
	***Lasioglossum* Curtis**	**76**
	coriaceum (Smith)	4
	cressonii (Robertson)	3
	hitchensi Gibbs	11
	imitatum (Smith)	1
	leucocomus (Lovell)	7
	leucozonium (Schrank)	8
	lineatulum (Crawford)	2
	oceanicum (Cockerell)	7

	pectorale (Smith)	4
	perpunctatum (Ellis)	1
	pilosum (Smith)	2
	tegulare (Robertson)	2
	versatum (Robertson)	20
	zephyrus (Smith)	3
	zonulus (Smith)	1
	***Sphecodes* Latreille**	1
	sp.	1
Megachilidae		**56**
	***Coelioxys* Latreille**	**20**
	rufitarsis Smith	20
	***Heriades* Spinola**	**5**
	carinata Cresson	4
	leavitti Crawford	1
	***Hoplitis* Klug**	**4**
	producta (Cresson)	3
	spoliata (Provancher)	1
	***Megachile* Latreille**	**27**
	brevis Say	4
	campanulae (Robertson)	2
	inermis Provancher	2
	latimanus Say	15
	mendica Cresson	1
	rotundata (Fabricius)	3
	***Osmia* Panzer**	1
	distincta Cresson	1

Table 2. Bee species collected in blue, white, and yellow pan traps during sampling in the natural environment adjacent to the Three Sisters Garden in the Great Lakes Region.

Blue
Andrena
Augochlorella
Augochloropsis
Bombus
Ceratina
Coelioxys
Dufourea
Epeolus
Eucera
Halictus
Hoplitis
Hylaeus
Lasioglossum
Megachile
White
Andrena
Apis
Augochlorella
Augochloropsis
Bombus
Ceratina
Coelioxys
Dufourea
Halictus
Hoplitis
Lasioglossum
Megachile
Osmia
Protandrena
Sphecodes
Yellow
Andrena
Augochlorella
Augochloropsis
Bombus

Ceratina
Coelioxys
Halictus
Hoplitis
Hylaeus
Lasioglossum
Megachile
Perdita

Table 3. Species level network statistics of bee species collected using sweep netting in the Three Sisters Garden plots in the Great Lakes Region, including Degree and Pollinator Service Index (PSI). Degree is the diet breadth of the insect species. PSI is the relative importance of each pollinator species to the functioning of the plant community.

Insect Species	**All Sites**	
	Degree	PSI
Apis mellifera mellifera L.	1	0.02
Augochlora pura (Say)	5	0.1
Augochlorella aurata (Smith)	4	0.34
Bombus bimaculatus Cresson	2	0.01
Bombus impatiens Cresson	6	0.41
Bombus vagans Smith	1	0.04
Ceratina calcarata Robertson	3	0.21
Ceratina dupla Say/*mikmaqi* Rehan & Sheffield	1	0.39
Coelioxys octodentatus Say	1	0.14
Halictus rubicundus (Christ)	1	0.02
Heriades leavitti Crawford	1	0.02
Lasioglossum atwoodi Givvs	1	0.04
Lasioglossum coriaceum(Smith)	2	0.02
Lasioglossum cressonii (Robertson)	1	0.02
Lasioglossum hitchensi Gibbs	2	0.02
Lasioglossum laevissimum(Smith)	1	0.02
Lasioglossum leucocomus (Lovell)	2	0.4
Lasioglossum leucozonium (Schrank)	1	0.13
Lasgioglossum lineatulum(Crawford)	1	0.02

Lasioglossum macoupinense (Robertson)	2	0.05
Lasioglossum oceanicum (Cockerell)	1	0.02
Lasioglossum perpunctatum (Ellis)	1	0.03
Lasioglossum quebecense (Crawford)	1	0.02
Lasioglossum tegulare (Robertson)	3	0.15
Lasioglossum truncatum (Robertson)	1	0.02
Lasioglossum versatum (Robertson)	3	0.1
Lasioglossum zephyrus (Smith)	1	0.02
Megachile brevis Say	1	0.02
Megachile inermis Provancher	1	0.3
Megachile latimanus Say	3	0.16
Megachile lippiae Cockerell	1	0.25
Megachile montivaga Cresson	1	0.14
Megachile pugnata Say	1	0.06
Megachile rotundata (Fabricius)	2	0.2
Megachile desponsus Smith	1	0.02
Melissodes trinodis Robertson	2	0.5
Xenoglossa pruinosa Say	4	0.42

Chapter Three: Insect visitation and pollination of a culturally significant plant, Hopi tobacco (*Nicotiana rustica*)

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Candidate's contribution:

Shelby Gibson, Kennedy Halvorson, Lisa Myers and Dr. Colla conceived of the idea and methodology for the manuscript. Shelby Gibson selected the survey sites, organized growing the plants, managed a field assistant, conducted field work, input data, and conducted statistical analysis. Shelby Gibson led the writing of the manuscript. Kennedy Halvorson, Lisa Myers, and Dr. Colla contributed to editing the manuscript.

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Abstract

Nicotiana rustica is a member of the Solanaceae family, and one of 76 species in the genus *Nicotiana* globally. *Nicotiana rustica* originated in South America and is now distributed across North America where it is grown for ceremonial purposes. Flowers of *N. rustica* open in the morning and are receptive to pollen until the following day and are thus exposed to diurnal and nocturnal pollinators. This study investigates the role of diurnal and nocturnal pollinators to reproductive success (seed weight , seed set , seediness, and insect visitation rate) in *N. rustica* in eastern North America using floral bagging techniques. Results of the experiment show that *N. rustica* benefits most from open (open day and night) and day open (closed at night) pollination. Fruit weight is found to be higher with the presence of diurnal pollinators; fruit set and seediness are unaffected. Video recordings show that the most abundant floral visitors are small bees. Understanding the role of diurnal and nocturnal pollinators can lead to a better understanding of *N. rustica* reproduction and how to ensure persistence of this culturally important plant species in a changing landscape.

Keywords: pollination, plant-pollinator, plant reproduction, wild pollinators, pollinator diversity, Indigenous medicine plants

Introduction

Intimately connected to many Indigenous cultures and considered sacred, Hopi tobacco (*Nicotiana rustica* L.) continues to play an important role in traditional, ceremonial, spiritual, social, and medicinal customs across North America (Struthers & Hodge, 2004; Brokenleg & Tornes, 2013; Sadik, 2014). The plant's importance cannot be overstated; Haudenosaunee and Cree creation stories feature tobacco as among the first plants gifted to the earth, and in Anishinaabe culture, *Semaa*/*Asema* (tobacco) is a kind of *bagijigan*, or offering, meant to foster relationships between humanity and the spiritual world (Sadik, 2014; Doerfler et al. 2013). Bagijigan have been referred to as the currency of life and are included at many daily and special events (Doerfler et al. 2013). While commercial tobacco (*N. tabacum*) has sometimes been used for ceremonial purposes, many people in the past decade have been working to re-establish the practice of growing and increasing the availability of traditional tobacco species like *N. rustica*, in an effort to reduce recreational usage, promote ceremonial use, and rekindle sacred relationships (Maron, 2018). Tobacco also plays an important role in Indigenous research methodologies, where offering the plant to the land upon which the research will take place, or to the participants within the study, can be an integral step in conveying the respect and commitment necessary to begin the research in a "good way" (Wilson & Restoule, 2010, pg. 32). Better elucidating the ecology of *N. rustica* and filling knowledge gaps surrounding the plant's reproduction represents one way to help ensure its continued growth on the landscape and promote its cultural use in North America.

Nicotiana rustica L. (Alternate names: wild tobacco, Aztec tobacco, Native tobacco, Zuni tobacco, *Mapacho*, *Semaa*, *Asema*) is a dicot member of the Solanaceae family (USDA NRCS, 2020) (Figure 1). *N. rustica* is a forb/herb with an annual lifecycle, introduced to Canada and the lower 48 states of the United States through Indigenous trading routes (USDA NRCS, 2020; Winter, 2000). *N. rustica* is a very recent hybrid (~0.5million years) from *N. undulata* and *N. paniculata* (Dodsworth et al. 2020). *N. rustica* was reported to produce up to two-thirds of its seeds through self-pollination, although it is also known to be visited by bees (Mather & Vines 1952). Rates of self-pollination were later noted to differ based on the relative distance between the stigma and ring of anthers (heterostathmy or herkogamy) (Breese, 1959). Based on findings by Breese (1959), rates of outcrossing varied between 0 to 70%, and were inferred to be under genetic control responding to changing environmental conditions. In a study in Delhi, India, *N. rustica* flowers were reported to open at 8:00 AM with the release of nectar available to pollinators (Rao & Rangaswamy, 1972). Anther dehiscence occurs a couple of hours prior to anthesis, and self-pollination occurs during the process of anthesis (Seghal and Gifford, 1979). Flowers remain receptive to pollen until the following day (Rao & Rangaswamy, 1972). It has been documented repeatedly in the literature that flowers open >12 h are exposed to both diurnal and nocturnal visitors (Cruden, 1973; Bertin & Willson, 1980; Haber & Frankie, 1982; Jennersten, 1988; Jennersten & Morse, 1991; Guitian et al. 1993; Shykoff & Bucheli, 1995; Miyake & Yahara, 1998; Arizaga et al., 2000; Slauson, 2000; Young, 2002). Some species of *Nicotiana* are known to be night-time pollinated (Adler et al. 2012). However, little is known about the pollination of *N. rustica* such as when pollination occurs.

Pollination effectiveness, along with parental plant genetics and environmental conditions are known to affect the size of *N. rustica* seeds (Pooni et al. 1997). Pollination effectiveness refers to the % of conspecific pollen deposited on stigmas and has a positive correlation with high quality seed production in *N. rustica*. Understanding how to achieve optimal seed production is useful in growing healthy plants, and plants being grown for seed should be grown under optimal controlled conditions (Pooni et al. 1997). The few available breeding system studies performed with *N. rustica* have shown both self-incompatibility (Rao & Rangaswamy, 1972), and self-compatibility (Raguso et al. 2003; Adler et al. 2012). The plant has been shown to have low to intermediate reliance on pollination based on studies of hand-pollination in a greenhouse setting (Adler et al. 2012), however the pollination biology of *N. rustica* remains poorly studied. Raguso et al. (2003) suspected bees or moths as *N. rustica*'s main pollinators based on the plant's morphology, while Tiedge & Lohaus (2017) characterized *N. rustica* as a day-time flowering plant pollinated by bees in the family Apidae, citing Raguso's work and that the plant has (1) flowers open during the day and at night, (2) corollae which are white, yellow, green, or pink, (3) and low scent intensity (Tiedge & Lohaus, 2017). Note, however, Raguso et al. (2003) found scent increased two-fold at night and was predominantly composed of benzaldehyde, a known chemical attractant for both pollinators and florivores (Theis, 2006). With these plant-pollinator relationship predictions based on the currently contested theory of pollination syndromes, it is important to complete a study directly observing the ecological interactions of *N. rustica* (Ollerton et al., 2009; Rosas-Guerrero et al., 2014). There have been no studies that document pollinator activity on *N. rustica* in a field setting with the aim of determining important groups of pollinators for the plant's reproduction.

This study examines the role of diurnal and nocturnal pollinators in the reproductive success of *N. rustica* (fruit weight (g), fruit set (%), and seediness (estimated average number of seeds per pod)). Here it is hypothesized that *N. rustica* benefits most from diurnal pollination, based on the previous work and predictions of Raguso et al. (2003) and Tiedge & Lohaus (2017), as it is known to flower during the day and have floral traits compatible with pollinators active in the daytime. It is predicted that plants that receive diurnal pollination will have higher reproductive success than those that receive nocturnal pollination. Accordingly, insect visitation is predicted to peak during the day.

Results

Bagging Experiment

Fruit weight, fruit set, and seediness all showed significance with pollination treatment (Figure 3). Fruit weight was not independent of pollination treatment which showed significant difference between treatments (P = <2e-16, F = 54.19, df = 3). A post-hoc test showed day open, night open, and open to have significantly higher fruit weight than closed. Day open had significantly higher fruit set than night open , as did open . Day open and open were not significantly different from each other. Fruit set also differed significantly between treatments (P = 0.0281, F = 3.258, df = 3). Open and closed were the only two treatments that differed significantly . Lastly, seediness also showed significant relationship with pollination treatment (P = 0.0109, F = 3.327, df = 4). Day open , night open , and open all differed significantly with the closed treatment. Seediness also was significantly higher in Round 1 than Round 2 (P = 0.000292, F = 7.38, df = 57).

Insect Visitation Rate

A total of 781 plant-flower visitor interactions were documented during 334 hours of video recording. Small bees were the most common visitor (59% of interactions) and bumble bees were the second most common visitor (30%) (Figure 4). The least common visitors were honey bees (1) and butterflies (1) (Figure 4). These visitors were most often caught on the video footage interacting with the flowers of *N. rustica* (Figure 5). The average visitation rate was highest in the late morning sessions, and lowest at night (Figure 6). Voucher specimens collected were identified to species level with the three most common species being *Augochlorella aurata* (28), *Lasioglossum perpunctatum* (12), and *Bombus impatiens* (7) (Table 1). Insect visitation rate differed significantly between pollination treatment (P = 0.0109 , F = 3.327, df = 4, 0.05954). The main difference was Late Morning which was significantly higher than Night (p = 0.0229704). Insect visitation rate also differed significantly between round with Round 1 having significantly higher IVR than Round 2 (P = 1.08e, F = 67.22, df = 734).

Discussion

This study confirms that fruit weight, fruit set and seediness are all increased in a field setting when *N. rustica* is exposed to open pollination. The difference between diurnal and nocturnal pollination is mainly reflected in the difference in fruit weight between flowers open for pollination during the daytime compared to those open overnight. Fruit weight does not differ between the unbagged, continuously open flowers and flowers only open during the daytime, which has been previously reported in the literature, and highlights the importance of diurnal pollinators (Jennersten, 1988). Adler et al. (2012) found that in a greenhouse setting *N. rustica* had an intermediate reliance on pollinators, where the plant was self-compatible but had increased reproductive success when exposed to pollination.

Fruit set and average seediness per pod, however, do not reflect a similar trend. Flowers available to be pollinated overnight versus during the daytime do not significantly differ for the attributes of fruit set and average seediness per pod. The difference in fruit set between unbagged flowers and those continuously bagged to exclude pollinators shows that fruit set is increased significantly by insect pollination. However, there is no clear difference between the timing of pollination, since the flowers that were always available to be pollinated were not found to be significantly different from those that were only available overnight or during the daytime. Neither diurnal nor nocturnal pollinators seem to be the dominant pollinator group.

All treatments with flowers open to visitors have on average higher seediness than treatments excluding pollinators. There is, however, no difference between the remaining treatments, indicating that neither day nor nighttime pollinators are more effective. While *N. rustica* plants were able to produce seeds without pollination (closed treatment), treatments open to pollination have a significantly higher number of seeds, consistent with the observations that the plants are self-compatible, but benefit from insect pollination. One factor affecting seed set may be that since *N. rustica* flowers open in the morning, the flowers are freshest and most receptive to pollen at this time (Young, 2002). It is also possible that continuous nectar secretion during bagging could have led to artificially inflated nectar levels upon unbagging, which may have influenced the results. Seediness was significantly higher earlier in the season, corresponding with a significantly higher IVR at that time as well. This information helps to better characterize the assemblage of pollinators visiting *N. rustica* and contributing to the plant's pollination.

Based on the findings of the video recordings, it may be the case that the diurnal visitors show an increase in fruit weight caused by their sheer abundance. It has been previously reported that while nocturnal visitors may be more effective per-visit (Bertin & Willson, 1979) diurnal visitors show a higher reproductive success due to their abundance and frequency of visitation (Jennersten & Morse, 1991; Young, 2002). Small bees had the highest abundance based on their visitation rate (Kearns & Inouye, 1993), and therefore may be the most effective visitors contributing to pollination (Cárdenas et al. 2020). Small bees were the most commonly documented visitor making up 59% of all recorded interactions. Since visitation rates can serve as a measure of abundance (Kearns and Inouye, 1993), we can conclude that small bees are the most abundant pollinators of *N. rustica.* While video recording was limited to dawn and dusk (crepuscular activity), when assessed in combination with the results of the fruit weight measures, it would appear that small bees are significant pollinators of *N. rustica*. Visitation peaked during late morning which is potentially related to when the anthers dehisced and pollen availability is highest, as has been the case in similar studies (MacPhail and Kevan, 2007). Based on observations, the visits of bees were shorter in duration than those by moths. There was one video session which lasted all night due to a full moon, however it showed no moths during the middle portion of the night. It would appear that the nocturnal pollinators primarily are active just after dusk. A previous study using video recording of nocturnal visitors found a peak of activity between 21:00-24:00 PM (Altizer et al. 1998), a time frame frequently represented by the videos in this study. Awareness of the timing of bee activity may also be important for creating best management practices for growing *N. rustica* in a crop setting where insecticides may be used (Egan et al. 2020). Future studies may record insect visitation throughout the night, and may also investigate the role of temperature and precipitation on insect visitation rates.

Since small bees were the most common visitor, pollination of *N. rustica* could be increased in the field by providing unmanaged land nearby for bee nesting and additional foraging resources, a suggestion made by other studies which have found bees to be the most effective pollinators of a particular plant (MacPhail & Kevan, 2007). The most common species of voucher specimen collected was *Augochlorella aurata.* The conservation status of this species in Ontario is Secure, however globally it has not been evaluated (Schoepf, 2018). *Augochlorella aurata* is a ground-nesting eusocial species (Gibbs et al. 2017). In terms of providing suitable habitat, The Xerces Society recommends leaving bare patches of sandy soil for use by ground nesting bees (Vaghaun et al. 2015). The second most common species collected from *N. rustica* was *Lasioglossum perpunctatum*. *Lasioglossum* is a genus exhibiting a full range of social behavior and often are ground-nesters (Danforth et al. 2019). Notably, these species are both members of the Halictidae family, revealing a pollination network different from Tiedge & Lohaus' Apidae prediction (2017). Providing suitable ground-nesting habitat in the form of bare patches of sandy soil will support wild bee populations as a whole and also ensure effective pollination of *N. rustica.*

Interestingly, although all of the sites in this study were located within a wider agricultural landscape, only one honey bee was seen visiting *N. rustica*. This suggests that the use of managed honey bees to increase pollination of *N. rustica* plants may not be appropriate, and that the plants benefit from wild bee activity. Further research is required to understand why honey bees were not common visitors of *N. rustica*. Since there is a mismatch between conservation science and management actions for bees with regards to bee decline (Potts et al. 2016), it is important to highlight the role of wild bee pollinators on *N. rustica* in the field. This information may be useful for ensuring the continued reproduction of this culturally significant plant which may in turn provide foraging resources for wild bees. Since many wild bees have been found to be in decline, or remain data deficient, understanding the role of wild bees to plants of importance can help to preserve both the plant and the pollinator together (Kearns and Inouye, 1998; Brosi and Briggs, 2013).

Conclusion

The results of this study highlight the importance of wild bees to the pollination of a culturally significant medicine plant, *N. rustica*. During this experiment *N. rustica* was able to set fruit without pollination (closed treatments), confirming that the plant is self-compatible (Mather

& Vines, 1952; Raguso et al. 2003; Adler et al. 2012), including when grown in a field setting in eastern North America. However, fruit weight (g) was significantly increased by any kind of treatment where flowers were unbagged and open to floral visitors, indicating that *N. rustica* benefits from insect pollination. With the Indigenous use of traditional tobacco species like *N. rustica* in ceremony and research increasing (Maron, 2018), this study broadens the general ecological knowledge of *N. rustica* and can help to inform potential strategies for improving growth and harvest of this sacred plant. Those interested in cultivating *N. rustica* may find better success if their management practices consider the habitat needs of small wild bees like *A. aurata* and *L. perpunctatum*, who require bare sandy soil patches for their ground nests, rather than seeking out the more commonly used and commercially available pollination services from managed honey bees. This research also reveals a previously unknown plant-pollinator relationship, that of the Halictidae family and *N. rustica*, and adds to the growing body of literature highlighting the importance of preserving wild pollinators and their respective ecosystem services (both ecological and cultural) on the landscape.

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Author contributions

Conceptualization, S.D.G., L.M., and S.R.C.; Methodology, S.D.G. and S.R.C; Investigation, S.D.G; Formal Analysis, S.D.G.; Writing – Original Draft, S.D.G; Writing – Review & Editing, S.D.G., K.S.H, and S.R.C.; Funding Acquisition, S.D.G., L.M., and S.R.C.; Supervision, S.R.C.

Declaration of interests

The authors declare that they have no conflict of interest.

Figures

Figure 1. Seedling (top left), young plant (top right), plant prior to anthesis (bottom left), and blooming in *N. rustica* (bottom right).

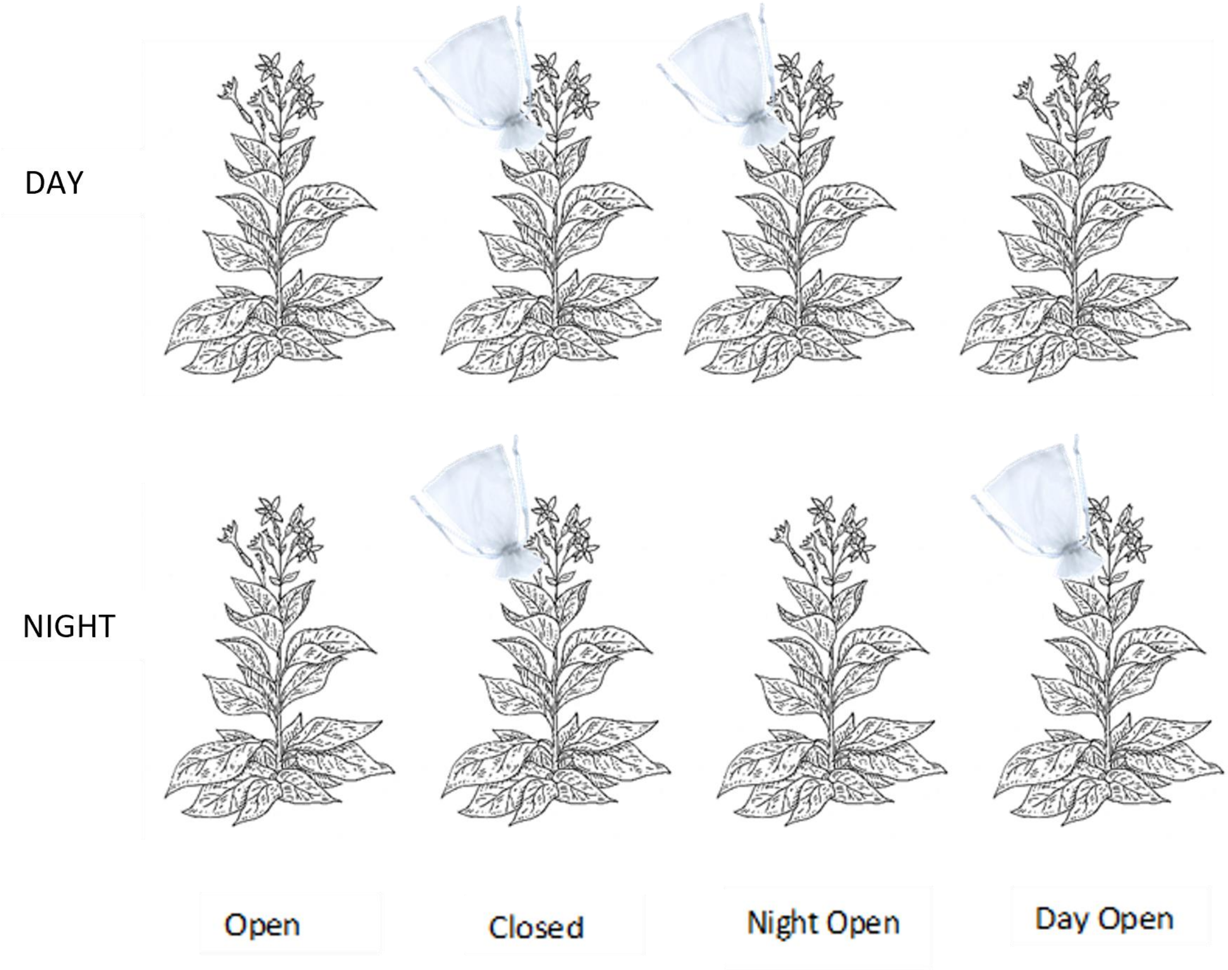

Figure 2. Conceptual diagram showing four treatments (Open, Closed, Day Open, and Night Open) conducted on *N. rustica.*

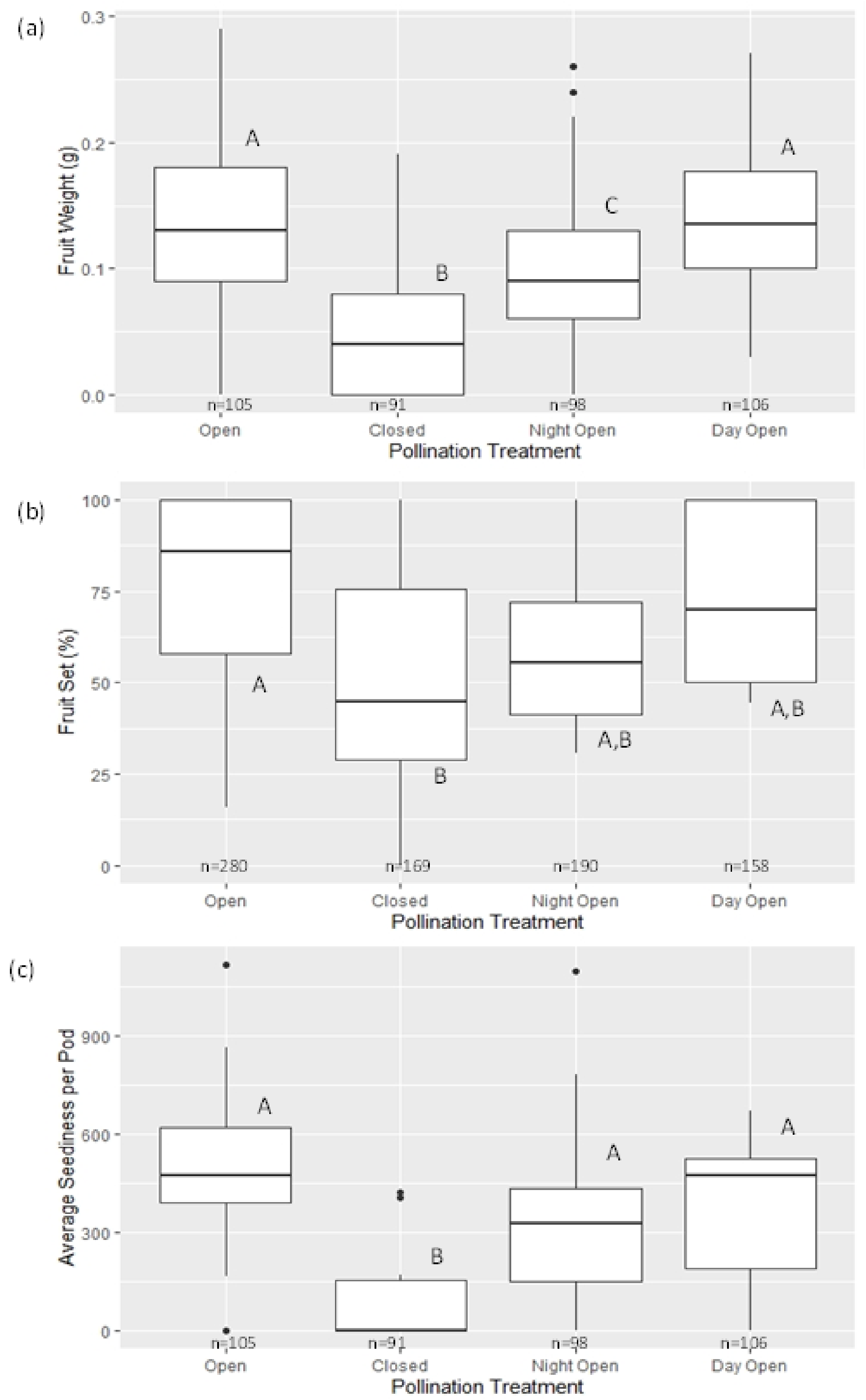

Figure 3. Fruit weight (g) (a), fruit set (%) (b) and average seediness per pod (c) produced by each pollination treatment during the bagging experiment. Means with the same letter are not significantly different from each other (Tukey's HSD test, P>0.05). Sample sizes (no. fruits – a, c; no. flowers – b) are located above treatment labels.

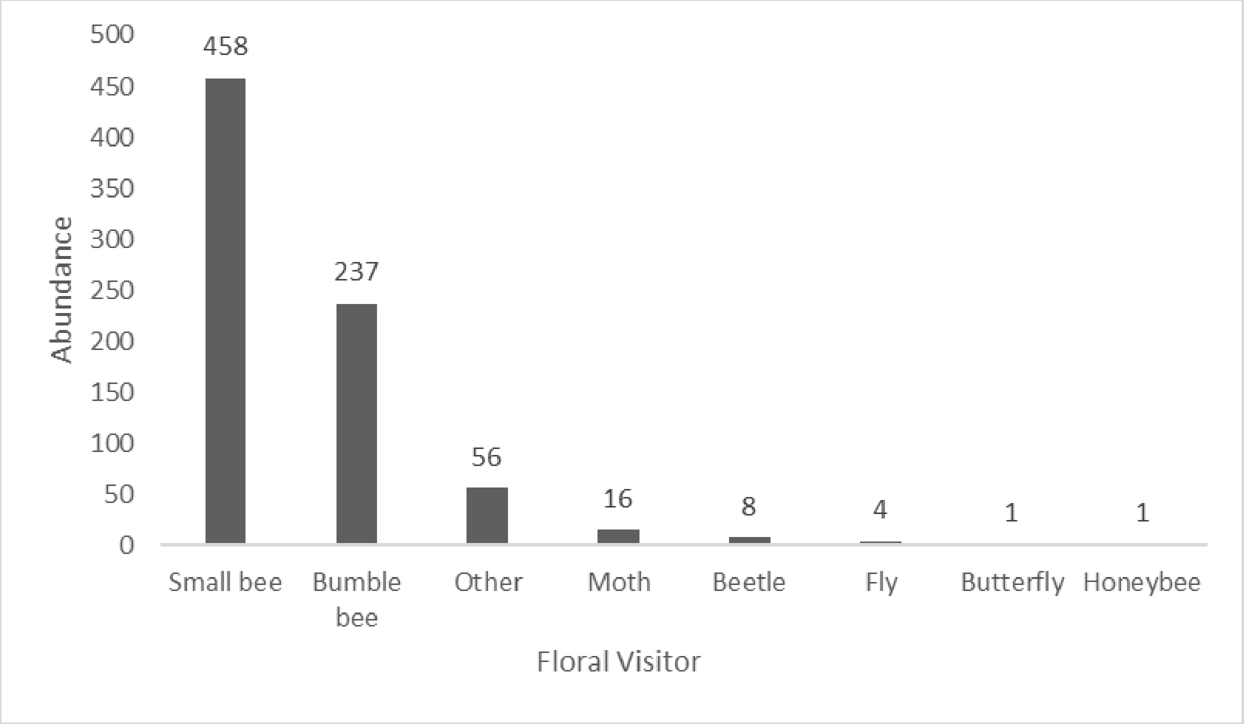

Figure 4. Visits of each floral visitor category recorded during diurnal (274 hrs) and nocturnal (60 hrs) video recording of *N. rustica* between July and September 2020 across all sites.

Figure 5. The most common visitors recording during the insect visitation recordings (Left to right: small bees, bumble bees, moths).

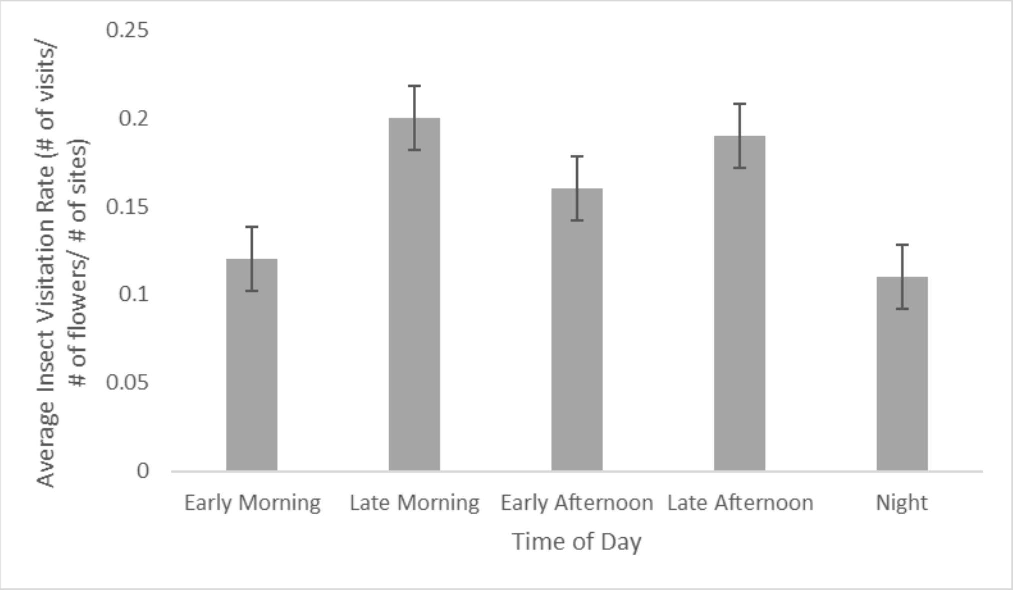

Figure 6. Average insect visitation rate recorded on *N. rustica* during different times of day using a time-lapse video camera between July and September 2020 at all three sites. Significant difference was found between late morning and night ($P>0.05$).

Tables

Table 1. Species list of voucher specimens collected on *N. rustica*.

Visitor	Count
Augochlorella aurata	28
Lasioglossum perpunctatum	12
Bombus impatiens	7
Lasioglossum tegulare	5
Augochlora pura	3
Lasioglossum michiganense	3
Lasioglossum versans	3
Bombus vagans	3
Halictus ligatus	1
Lasioglossum oblongum	1
Halictus rubicundus	1
Megachile frigida	1
Sphecodes heraclei	1

STAR★Methods:

Resource Availability

Lead contact

Requests for further information and resources can be directed to and will be fulfilled by the lead contact of this manuscript, Shelby Gibson (shelbydgibson@gmail.com).

Materials availability

This study did not generate any new unique materials or reagents.

Data and code availability

The original datasets for this manuscript have been deposited at Mendeley Data and are publicly available as of the date of publication. The DOI is listed in the key resources table. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental Model and Subject Details

Plant Materials and Site Description

This study was conducted at three sites located near Ottawa, Ontario, Canada. Plot A was in Lanark, ON, and Plot B was in Pakenham, ON. Each plot was 20ft x 20 ft. Plot C was in Arnprior, ON – this site was too small for a breeding experiment but was used for the video recording. Round 1 of the experiment was conducted at Plot A between July 27th-August 9th, 2020. Round 2 of the experiment was conducted at Plot B September 11-18th, 2020. Experimental rounds were conducted during peak blooming which varied by location based on differences in external factors such as soil, sun, and wind exposure. *N. rustica* seeds were sourced from Urban Harvest (http://uharvest.ca/shop/) and were placed on top of the soil after the last frost in spring 2020 (May 25). Plots A and B were planted with 175 seeds. Seeds were watered until germination. Flowers bloomed late July into late September.

Land Acknowledgement

This research was undertaken on the traditional territory of many First Nations. The sites located in Tkaronto, including the campus of York University, are located on the traditional territory of the Anishinabek Nation, the Haudenosaunee Confederacy, the Huron-Wendat, and the Métis. The current treaty holders in this location are the Mississaugas of the New Credit First Nation, and the land is subject to the Dish with One Spoon Wampum Belt Covenant. The study sites are located on unceded Algonquin territory and are subject to Treaty 27 and Treaty 27 1/4. We acknowledge the generations of caretaking by the many Indigenous peoples who have and still do call these places home.

Method Details

Bagging Experiment

To determine if there was a difference between diurnal and nocturnal pollinators, the bagging experiment included four treatments: open (open both day and night), day open (bag removed during daytime), night open (bag removed during nighttime), and closed (no visitation). Flower buds were bagged using small mesh bags prior to anthesis to ensure no pollination had occurred prior to treatment (Reader, 1977; Kearns & Inouye, 1994; Usui et al. 2005; MacPhail & Kevan, 2007). Bags remained in place until blooming was completed. Bags were made of Organza fabric (estimated 1-mm mesh size) and were 5 cm x 7 cm in size. Sunrise was between 4:39 AM and 4:55 AM during Round 1, and between 5:38 AM and 5:47 AM during Round 2.

Sunset was between 19:24 PM and 19:43 PM during Round 1, and between 18:10 PM and 18:24 PM during Round 2. Bags were placed on the flowers prior to opening and bags were removed during the day to allow diurnal pollinators and removed at night to allow nocturnal pollinators (Figure 2). Bags were placed on the day open and night open treatments within 1 hour of the time of sunrise or sunset, coinciding with the changeover between diurnal and nocturnal pollinator activity (Bertin & Willson, 1979; Young, 2002.). Flowers were randomly chosen to be included. The number of flowers on each branch was recorded every day throughout the experiment, and the number of pods developed was counted upon collection (Jennersten & Morse, 1991). Tobacco fruits were harvested once mature, approximately 21 days post-flowering (Rao & Rangaswamy, 1972; Sime & Baldwin, 2003). Reproductive success of the plants was determined by fruit set (number of fruits/number of flowers), fruit weight, and seediness (Usui et al. 2005; MacPhail & Kevan, 2007). Seediness was estimated by weighing all seeds from all pods on one plant, dividing this by the total number of pods to get an average mass of seeds per pod (g), and counting the number of seeds within a subset of the sample (0.05g). The number of seeds in the subset of the sample (0.05g) was then factored against the average mass of seeds per pod (g), giving an average seediness per pod. This method was used because the seeds were too small to be counted by a seed counter, and N. rustica produces approximately 250-300 seeds per capsule (Rao and Rangaswamy, 1972).

Insect Visitation Rate and Video Recordings

A time lapse video camera (Edwards et al. 2015) was used to record insect visitation (Kearns & Inouye, 1993). The camera was supported by a tripod and footage was recorded to a memory card. Continuous recording provides a near-complete documentation of floral visitors throughout blooming (Edwards et al. 2015). Floral visitations and behaviour (i.e. visitation rate) were determined by analysing video recordings (Kearns & Inouye, 1993; Edwards, 2015; Steen, 2017). Insects were tallied into the following categories: honey bee, bumble bee, small bee, fly, butterfly/moth, beetle, other, unless they could be identified to species (Kearns & Inouye, 1993; MacPhail & Kevan, 2007). Crepuscular recordings included dawn and dusk when there was some limited natural light available. Insects that landed on the outside of the flower were not included in analysis. Only insects that entered and contacted the reproductive parts of the flower were included. Video recordings occurred around the time of the bagging experiment: July 30-August 9 (Plot A), August 31-September 9 (Plot B), and August 18-August 25 (Plot C). Video recordings were conducted earlier than the bagging experiment to ensure high-quality video, as significant dew began to form on the camera lens as the season progressed making the recordings difficult to interpret. Therefore, it is possible that the floral visitors are different between the bagging experiment at Plot B and the video recording at this location. In addition, bagging and video recordings occurred on the same plant, but not the same flowers, to avoid interference with the video by bags or at changeover time. A collection of voucher specimens was collected randomly from *N. rustica* throughout blooming during the time of video recordings (n=70).

Quantification and Statistical Analysis

Generalized Linear Modelling

Statistical analysis was conducted in RStudio (Version3.6.2) (RStudio Team, 2022) on fruit weight, fruit set, and seediness amongst the various pollination treatments, as well as insect vitiation rate at various times of day. The two rounds of the experiment were combined for pollination analysis to provide the most robust results, however the effect of round on fruit weight, fruit set, and seediness was also assessed. Fruit weight was assessed using a generalized linear model (GLM) fit to a Gaussian distribution with a identity link function. Fruit weight was the continuous response variable while pollination treatment was the fixed effect variable. Fruit set (non-continuous) was assessed using a GLM fit to a binomial distribution with logit link function. Fruit set was the binary response variable while pollination treatment was the fixed effects variable. Seediness was assessed using a GLM fit to a quasipoisson distribution (overdispersed) with a logit link function. Seediness was the continuous response variable while pollination treatment was the fixed effects variable. For fruit weight, fruit set, and seediness, a Tukey post-hoc test was used for pairwise comparisons between treatments (Zar, 1999). This was then repeated using round as the fixed effect variable.

Average visitation rate was calculated as (# of visits/ # of flowers/ # of sites) (Kearns & Inouye, 1993; MacPhail & Kevan, 2007). An average visitation rate was found for each time of day (early morning, late morning, early afternoon, late afternoon, nighttime). The times of day were separated as follows: Round 1: Night (19:24pm-4:55am), Early AM (4:55am-9:00am), Late Morning (9:00am-12:00pm), Early Afternoon (12:01pm-16:00pm), Late Afternoon (16:01pm-19:24pm). Round 2: Night (18:10pm-5:47am), Early AM (5:47am-9:00am), Late Morning (9:00am-12:00pm), Early Afternoon (12:01pm-16:00pm), Late Afternoon (16:01pm-18:01pm). Round 3: Night (19:07pm-5:10am), Early AM (5:10am-9:00am), Late Morning (9:00am-12:00pm), Early Afternoon (12:01pm-16:00pm), Late Afternoon (16:01pm-19:07pm). Insect visitation rate (IVR) was assessed using a GLM fit to a Poisson distribution with log link function. IVR was the continuous response variable while time of day was the fixed effects variable. A Tukey post-hoc test was used for pairwise comparisons between treatments (Zar, 1999). This was then repeated to determine significance of round on IVR.

Key Resources Table

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Deposited data		
Raw and analyzed data	This paper	doi:10.17632/kdc9fgfbrm.1
Experimental models: Organisms/strains		
Nicotiana rustica L.	Urban Harvest	N/A
Software and algorithms		
RStudio	RStudio Team	(Version3.6.2)

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RStudio 2022.07.1+554 "Spotted Wakerobin" Release (7872775ebddc40635780ca1ed238934c3345c5de, 2022-07-22) for Windows

Mozilla/5.0 (Windows NT 10.0; Win64; x64) AppleWebKit/537.36 (KHTML, like Gecko) QtWebEngine/5.12.8 Chrome/69.0.3497.128 Safari/537.36

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Chapter Four: Investigating impacts of fire on insect pollination of Common bearberry (*Arctostaphylos uva-ursi*) in an alvar ecosystem

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Candidate's contribution:
Shelby Gibson and Dr. Colla conceived of the idea for this manuscript. Shelby Gibson, Benjamin Shearer and Dr. Colla contributed to methodology design. Shelby Gibson and Benjamin Shearer selected field sites and conducted field work. Shelby Gibson input data, conducted statistical analysis and led writing the manuscript. Shelby Gibson, Benjamin Shearer, and Dr. Colla contributed to editing the manuscript.

Abstract

Common bearberry (*Arctostaphylos uva-ursi* (L.) Spreng is a plant which has been shown to rely at least in part on insect pollination, however this has not been assessed in North America. A bagging experiment and insect visitation rates were used to investigate the breeding system of Common bearberry. The plant was studied at Burnt Lands Provincial Park which is divided into burned and unburned sections due to a historical fire (1999). Bagging treatments included open, closed, bumble bee, and hand-cross pollinated. Fruit weight, fruit set, and number of seeds per fruit were examined. Fruit set was 0% for the closed treatments, on both burned and unburned sections, indicating no ability to self-pollinate. Bumble bee visits set fruit 60% of the time in the burned and 0% of the time in the unburned sections. Hand-cross pollination set fruit 40% of the time in the burned and 17% of the time in the unburned sections. Insect visitation rate (IVR) was highest on the burned side, and the most frequent visitors were small bees and bumble bees. Further research is needed to determine if burning is a land management technique important to the sustainability of bearberry and its pollinator mutualisms.

Keywords: Insects, pollination, mutualism, wildfire, bearberry, land management, alvar

Introduction

Fire is an important and significant type of disturbance on the landscape (Williams et al. 2010, Bond and van Wilgen, 2012). Western scientific thought has promoted the concept of 'the balance of nature', which in recent history has existed outside of the realm of disturbance by fire (Bond and van Wilgen, 2012). Prior to European colonization, however, fire was an important land management technique used by Indigenous peoples on Turtle Island (Kimmerer and Kanawkha Lake, 2001). With colonization came the concept of fire as hazardous and destructive (Kimmerer and Kanawha Lake, 2001). Fire, however, can have a very positive and necessary impact on an ecosystem, including the stimulation of new growth by enhanced flowering (Bond and van Wilgen, 2012), and positive effects on wild bee communities (Grundel et al. 2010; Galbraith et al. 2019). Fire is a tool that has been used by many Indigenous people to maintain a mosaic of habitat patches at various levels of postfire succession (Kimmerer and Kanawha Lake, 2001, Taylor and Catling, 2011). Doing so increased food security and productivity of food and medicine plants (Kimmerer and Kanawha Lake, 2001). Human use of fire as a land management technique is part of the relationship between humans and non-human beings (Kimmerer and Kanawha Lake, 2001).

The majority of flowering plant species benefit from or rely on insects for reproduction through pollination (Ollerton et al. 2011). For many plant species, it remains unclear to what degree insect pollination is required (Kearns et al. 1998, Padulosi et al. 2004, MacPhail et al. 2018, Gibson and Colla, 2023). Pollination mutualisms can be impacted by various disturbances, such as wildfire, however the degree of change is unknown (LoPresti et al. 2018). Developing a better understanding of how insects and plants interact, especially as it relates to disturbance, is crucial to informing land management and conservation planning.

Common bearberry (*Arctostaphylos uva-ursi;* hereafter, bearberry) is an evergreen shrub in the *Ericaceae* family that grows along sand dunes, beaches, prairies, and rocky hill sides throughout Canada (Small and Catling, 1999, MacKinnon et al. 2009, Dickinson and Royer, 2014). Flowers, which appear in late May to early June, are small (4-6mm), urn-shaped, grow in clusters, and produce bright red berries in August/September (MacKinnon et al. 2009, Dickinson and Royer, 2014). In Canada, *A. uva-ursi* is listed as vulnerable/apparently secure/secure in Newfoundland and imperiled/vulnerable in Labrador (MacKinnon et al. 2009, NatureServe, 2020), and status unknown in the rest of Canada. In the United States, bearberry is Endangered in Illinois and Iowa, rare in Indiana, presumed extirpated in Ohio, and extirpated in Pennsylvania (NRCS, 2019). Bearberry is known to recover well after fire disturbances (Crane, 1991); however, it is unknown how fire events impact the reproduction of bearberry.

Bearberry is an ecologically and culturally significant plant in North America. The Algonquin word Kinnikinnick, meaning tobacco mixture, is used to describe bearberry as the plant is often used in ceremonies in place or in combination with tobacco and other herbs (Small and Catling, 1999, MacKinnon et al. 2009). The berries are eaten by various Indigenous peoples, cooked either with sugar or fat (lard, salmon oil, bear fat) (Hellson, 1974, Marles et al. 2000, MacKinnon et al. 2009). Roots of the plant are used to treat coughs, slow menstrual flow and avoid miscarriages (Marles et al. 2000). The plant is also used in teas and extracts to treat kidney and bladder problems (MacKinnon et al. 2009).

Bearberry has been documented to be able to self-pollinate, but also benefits from pollination by hummingbirds, moths, bumble bees (Small and Catling, 1999), and thrips (Garcia-Fayos and Goldarazena, 2008). Garcia-Fayos and Goldarazena (2008) found that pollination by thrips accounted for 20% of pollination to bearberry. The mating system of a plant can vary between populations, for example, in the levels of outcrossing (Whitehead et al. 2018). Therefore it is important to study this key characteristic of a plant species within different populations. Bearberry blooms very early in the spring and therefore pollinator availability might be limited, since early spring flowering is a high-risk option for plants that require insect-mediated pollination (Schemske et al. 1978, Thomson, 2010).

Maintaining the ecosystem at a specific successional state is important for promoting the health of certain species. Insect sampling conducted in 2008 at Burnt Lands Provincial Park (BLPP) compared the unburned and burned section of the site (Taylor and Catling, 2011). Burnt Lands Provincial Park is a protected area in eastern Ontario, Canada managed by Ontario Parks and the Nature Conservancy of Canada (Ontario Parks, 2016). A historical wildfire took place in 1999, which was stopped by a road, and therefore a burned and unburned section of the park exist (Ontario Parks, 2016). The burned section was found to have a higher bee and butterfly diversity (species richness, number of individuals, Brillouin's Biodiversity Index) (Taylor and Catling, 2011). No bees were captured on the unburned section during that study (Taylor and Catling, 2011). The most abundant bee species on the burned section was *Augochlorella aurata* (Taylor and Catling, 2011), a sweat bee in the family Halictidae. Taylor and Catling (2011) also found higher vascular plant diversity and higher frequency of insect-pollinated plants providing nectar and pollen, including *A. uva-ursi*. The burned section also had higher prevalence of nesting locations for bees (Taylor and Catling, 2011). Taylor and Catling (2011) suggest that the suppression of fire in natural ecosystems where fire plays a crucial role may be correlated to pollinator declines.

Here we investigate whether fire influences the pollination of bearberry in a burned and unburned alvar ecosystem. This study aims to determine (1) if bearberry can set fruit without insect pollination and (2) how fire impacts pollination of bearberry. We hypothesize that, since insect abundance and diversity is higher on the burned side, that fire is beneficial to the breeding system of bearberry. We predict that the reproductive success (fruit set, fruit weight, seeds per fruit) in the burned section will be higher than that on the unburned section of BLPP.

Methods

Study Area

Bearberry was studied at Burnt Lands Provincial Park (BLPP) in eastern Ontario. BLPP is an alvar ecosystem characterised by shallow soils, limestone bedrock, herbaceous plant species, and mixed/coniferous forest located in eastern Ontario (45°15′40″N 76°09′00″W) (Ontario, 2020). The 522-hectare park is classified as an Area of Natural and Scientific Interest (ANSI) and is managed through a lease-agreement by The Nature Conservancy of Canada (NCC) and Ontario Parks (Ontario, 2020). BLPP also falls under the Algonquins of Pikwakanagan land claim (Ontario, 2020). On June 23, 1999, a fire burned through 152 hectares of BLPP, creating a burned and unburned portion of the land (Catling, 2009) (Figure 1). Past research suggests that fire may be a required part of a custodial management strategy for BLPP (Catling and Brownell, 1998, Catling and Sinclair, 2000, Ontario Parks, 2001). Jack Pine, planted in 1966, has been identified as a management concern as it is encroaching on alvar vegetation communities, leading to shading, competition, and modified soil pH (Ontario Parks, 2001). The role of fire as a management tool is currently under consideration (Ontario Parks, 2001, 2016).

Bearberry was found to cover approximately 4% (unburned) and 18% (burned) of sampled alvar woodland in BLPP (Catling, 2009). Bearberry regenerates after fire through sprouting at the root stem and germination of seeds stored in the soil, as fire encourages germination of stored seeds (Crane, 1991).

This experiment was planned for early spring 2020, however due to the global COVID-19 pandemic access to BLPP was limited and permission to enter was not granted until May. At this point blooming had already occurred on the burned section. Therefore only the unburned section is included in the 2020 pilot year of this study. In 2023 both the burned and unburned sections were accessed.

Bagging Experiment

In 2020, a total of ten 1 m^2 quadrats were set up on May 20, by following a 500 m transect and laying quadrats out on sections of bearberry. In 2023, a total of fifteen 1 m^2 quadrats were set up on April 6th on the burned section, and fifteen on April 27th on the unburned section for a total of 30 quadrats. Quadrats were placed at least 15 meters apart to avoid comparing clones (Usui et al. 2005). In 2020, two treatments were included in each quadrat (with up to four replicates): open (no bag – visitation allowed) and closed (bagged using small mesh bags – no visitation). Bags were placed on plants prior to blooming on May 21, 2020. Each bag was placed over a cluster of flowers. In 2023, four treatments were included in each quadrat: (1) open, (2) closed, (3) bumble bee pollinated, and (4) hand-cross pollinated. Flower buds were bagged prior to anthesis to ensure no pollination had occurred prior to the start of the experiment (Reader, 1977, Kearns and Inouye, 1994, Usui et al. 2005, MacPhail and Kevan, 2007). Bumble bee pollination was conducted by catching a bumble bee foraging on bearberry and transferring it to inside the mesh bag on the unpollinated plants. Bumble bees were in the bag until they visited the bearberry flower cluster three times. This was determined by watching the bumble bee's activity and counting the number of visits to the flowers. Hand-cross pollination was done using a fine paintbrush to administer pollen from a fresh, unpollinated flower from a different plant at least 15 m away(to avoid self pollination within a clone) to the treatment unpollinated flower. Bags remained on the plants until after the reproductive parts of the flowers had fallen off (end of June). The fruit were left to develop and were collected when bright red and ripe (late August 2020/23). Fruit weight (g), fruit set (%), and number of seeds per fruit were recorded for all treatments on the unburned section in 2020 and both burned and unburned sections in 2023.

Insect Visitation Rate & Observations

Insect visitation rates (IVR) were recorded in 10-minute sessions for a total of 1 hour per visit to each section (1 hour burned, 1 hour unburned). Observations were conducted in the remaining time between experimental set-up and visitation rates at approximately 1 to 2 hours per site visit. A total of 3 hours was spent on the unburned section in 2020. A total of 9 hours was spent on each of the burned and unburned sections during the 2023 season, for a total of 18 hours spent collecting IVR during blooming. Insects were recorded to morphospecies: honeybee, bumblebee, small bee, fly, butterfly, beetle, other.

Statistical Analysis

Statistical analysis was performed using R-4.3.1. For 2020, a one-way ANOVA was used to analyze fruit set across treatments on the unburned section. For 2023, a two-way ANOVA with an interaction effect was used to examine each dependent variable (fruit weight, fruit set, and seeds per fruit) in relation to the independent variables of Section (burned or unburned) and Treatment (open, closed, bumble bee, hand-cross) and an interaction between Section and Treatment. All models were checked for the assumption of homoscedasticity. A Tukey post-hoc test was used for pairwise comparison between treatments. A t-test was conducted to compare the average IVR on the burned and unburned sections per pollinator group.

Results

Bagging Experiment

2020 - A total of 41 sprigs of bearberry were included in the experiment. 19 sprigs were open-pollinated, and 21 bags were closed prior to anthesis (no pollination). In the open-pollinated treatment 9/19 (47%) of the sprigs produced seed pods, while 0/21 (0%) of the closed treatments produced seeds (Figure 2). Fruit set ranged from 0-83%. The results of a one-way ANOVA show a statistical difference between the open and closed treatments (F(1, 38) = 7.895, p = 0.008). Fruit weight ranged from 0-0.23g in the open pollinated treatment, while in the closed treatments produced no fruit or seeds. Seeds per fruit ranged from 0-8 in the open pollinated group.

2023 – Result of a two-way ANOVA showed fruit set (%) was not significantly different between Sections but was significantly different between Treatments (F=2.6375, df=4, p=0.03774) (Figure 3). There was no interaction effect between Treatment and Section. Post hoc testing showed Cross pollination had a significantly higher fruit set than Closed (p = 0.0362). On the burned section 60% of bumble bee treatments, 53% of open, 40% of hand-cross and 0% of closed treatments set fruit (Figure 3). On the unburned section 0% of bumble bee treatments, 10% of open, 17% of hand-cross, and 0% of closed treatments set fruit (Figure 3).

In the burned section, fruit weight ranged from 0.08g-0.38g. In the burned section, average fruit weight (g) was 0.21g (SD 0.06) in hand-cross, 0.19g (SD 0.06) in open and 0.18g (SD 0.07) in bumble bee treatments (Figure 4). Fruit weight ranged from 0.11-0.33g in the unburned section. Average fruit weight was 0.2g (SD 0.08) in hand-cross and 0.17g (SD 0.04) in open treatments in the unburned section (Figure 4). Fruit weight was not significantly different between Treatment or Section (p>0.05) or an interaction between Treatment and Section (Figure 4). There was no fruit weight for bumble bees on the unburned section due to a lack of fruit set by bumble bees in this section.

Average seeds per fruit was 7 (SD 1.05) for burned bumble bee, 7 (SD 1.07) hand-cross burned treatments, 6 (SD 0.60) hand-cross unburned treatments, and 6 (SD 0.94) burned open and 6 (SD 0.43) for unburned open treatments (Figure 5). Seeds per fruit was not significantly different between Treatment or Section (p>0.05) or an interaction between Treatment and Section (Figure 5). The number of seeds per fruit was not significantly different between the open, bumble bee, and hand-cross pollination treatment on the burned section. Average seediness (seeds per fruit) was 6 for hand-cross and 6 for open treatments and therefore not significantly different. Average fruit set was 8% (fruits/flowers) and total success rate was 25% (proportion of treatments that produced fruit/total sample size).

Insect Visitation Rate & Observations

2020 – In 2020 the average IVR at the unburned section was 0.02 visits per minute. There was a total of 9 visits: small bees (5), bumble bees (3), and fly (1). A total of 3 hours of observation were conducted for the purposes of the IVR during 2020 sampling on the unburned section.

2023 – A two sample t-test was performed to compare IVR at the burned section and the unburned section. There was not a significant difference in IVR between the burned and unburned section (p>0.05). There were 94 total visits and 65 total visits at the burned and unburned section, respectively. The number of visits differed by visitor type (p=0.0001, F=30.87, df= 6), but not between burned and unburned sections ($p>0.05$). Small bees (non-bumble bee or honey bee) made 50 (53%) and 43 (66%) visits on the burned and unburned section, respectively (Figure 6). Small bee visits were significantly higher than all other visitors ($p<0.05$) (Figure 6). Bumble bees made up 26 (28%) and 14 (22%) of visits, significantly higher than all other visitors except flies ($p<0.05$) (Figure 6) (Figure 7). Honeybees and beetles were the least recorded visitors (Figure 6).

Discussion

The availability of wild pollinators appears to be critical for pollination of bearberry, however the impact of fire is less clear. Based on our results, bearberry was not able to set fruit without pollination and therefore has low or no ability to autonomously self-pollinate. In the burned section, bumble bee pollination effected fruit set in 60% of treatments while in the unburned section bumble bee pollination did not affect any fruit set (0%). This difference in fruit set may be attributed to the fact that the bumble bees used to pollinate were collected while foraging on bearberry. Since fruit set is increased with an increased pollen load (Ter-Avanesian, 1978, Bertin, 1990, Dogterom et al. 2000), the bumble bees in the unburned section may not have had a bearberry pollen load large enough to contribute to fruit set. Based on observation of the scattered growth and flowering of bearberry on the unburned section, the bees may not have had access to as much bearberry pollen as did the bees on the burned section where flowers were abundant and flowering consistent. Bumble bees display floral constancy, where they will avoid switching flower types, if possible, which increases efficiency on one flower type (Waser, 1986, Wilson and Stine, 1995, Raine and Chittka, 2005). Higher floral abundance following fire has been correlated with increased bumble bee work body size, number of colonies from which workers hail, and higher abundance of workers and queens (Mola et al. 2020).

Hand-cross pollinated plants on the unburned section still set fruit, however not at the same rate as on the burned section. This could be due to overall plant or pollen health from the plants that were used for pollinating the hand-cross treatment plants. Seed production and fruit set are known to be affected by the source and quantity of pollen deposited (Ter-Avanesian, 1978, Bertin, 1990, Dogterom et al. 2000). In the closely related species highbush blueberry (*Vaccinium corymbosum* L.), low yield is attributed in part to pollination by the same or closely related individuals (Hancock and Siefker, 1982; Czesnik, Bounous, and Gioffre, 1989; Free, 1993. Dogterom et al. 2000). Dogterom et al. (2000) found that fruit characteristics of highbush blueberry, including percentage fruit set, fruit weight, and number of seeds, increased with large pollen loads from a mixed source (outcrossed).

Given the inconclusive results, there may be other environmental factors affecting the health of common bearberry at BLPP. Soil nutrient availability is a common factor that influences plant reproductive success including flower and fruit production and seedling germination (Simms, 1987, Moulton and Gough, 2011). In times of low nutrient availability plants use resources in maintaining vegetative growth as opposed to using energy for reproduction (Simms, 1987, Moulton and Gough, 2011). While fire may increase soil nutrient availability in the form of ash (Simms, 1987), it is unclear for this study whether this was an important factor. Light is another environmental factor that could be impacting the growth and development of common bearberry at BLPP. In other *Vaccinium* species, light has been a significant factor in seedling germination (Nin et al. 2017). Increased exposure to light increases germination (Nin et al. 2017). Other *Vaccinium* species have also shown increased plant fresh weight in high light conditions (Scalzo et al. 2016). Future research should examine the impact of environmental factors on common bearberry in an attempt to unravel the potential impacts a future fire could have on plant growth and reproduction.

Bumble bee presence alone achieved successful pollination of bearberry on par with the open pollinator community and hand-cross pollination. Bumble bee queens have been found to be successful and important pollinators of other early-flowering plant species (Thomson, 2010). The simultaneous early flowering of bearberry on the burned section coincides with the phenology of bumble bee queens seeking and establishing nests sites early in the season (April/May). Bearberry, therefore, may be providing an important nutrition source for queen bumble bees as they are in the critical life cycle stage of initiating a colony, which should be assessed in further bumblebee conservation research. Anecdotally, the blooming of bearberry on the burned section was also more consistent as opposed to the unburned section where multiple phases of blooming seemed to be appearing (S. Gibson personal observation). Increased flowering synchronicity after fire is associated with increased reproductive output in some species (Richardson and Wagenius, 2022), including *Vaccinium* (Rodriquez and Kouki, 2015). Changes to plant phenology and floral density in relation to fire increases habitat suitability for foraging bumble bees (Mola and Williams, 2018).

A more consistent bloom may also be advantageous to the insect fauna utilizing the resource nutritionally as it is easier to forage on the larger patches (Wilson and Stine, 1995). The importance of successional habitat in providing foraging and nesting resources for bees has previously been documented (Fahrig, 2003, Grixti and Packer, 2006, Taylor and Catling, 2011). Bumble bees show preference for dense patches of flowers when foraging and fire events on the landscape has been shown to increase patch quality for bumble bees (Mola and Williams, 2018).

Lastly, in many categories, pollination was only slightly more successful on the burned side (i.e. not statistically different). This may be due in part to the fact that the historical fire occurred in 1999. Because time since fire is a factor that influences the reproductive outcomes of plants, it is possible that there would be a stronger pattern with a new fire event. Previous research recommends the use (cautiously) of fire in the BLPP alvar system due to its benefits to biodiversity (Catling and Brownell, 1998, Catling, 2009). This study increases our understanding of the pollinator mutualisms important to common bearberry and builds a baseline for future research questions examining impacts to overall health of common bearberry.

Figures

Figure 1. Photo displaying characteristic landscapes in the (a) Burned Section and (b) Unburned Section of Burnt Lands Provincial Park in eastern Ontario, Canada.

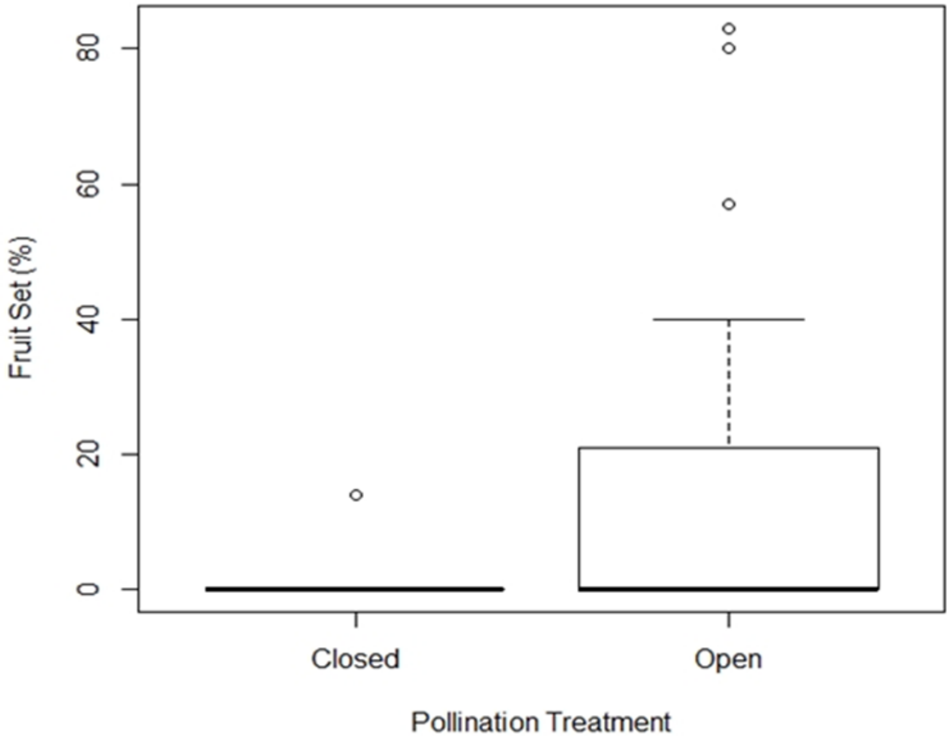

Figure 2. Boxplot displaying fruit set (%) by closed and open pollination treatments during May and June 2020 at the unburned section of Burnt Lands Provincial Park in Eastern Ontario, Canada. Error bars represent standard deviation.

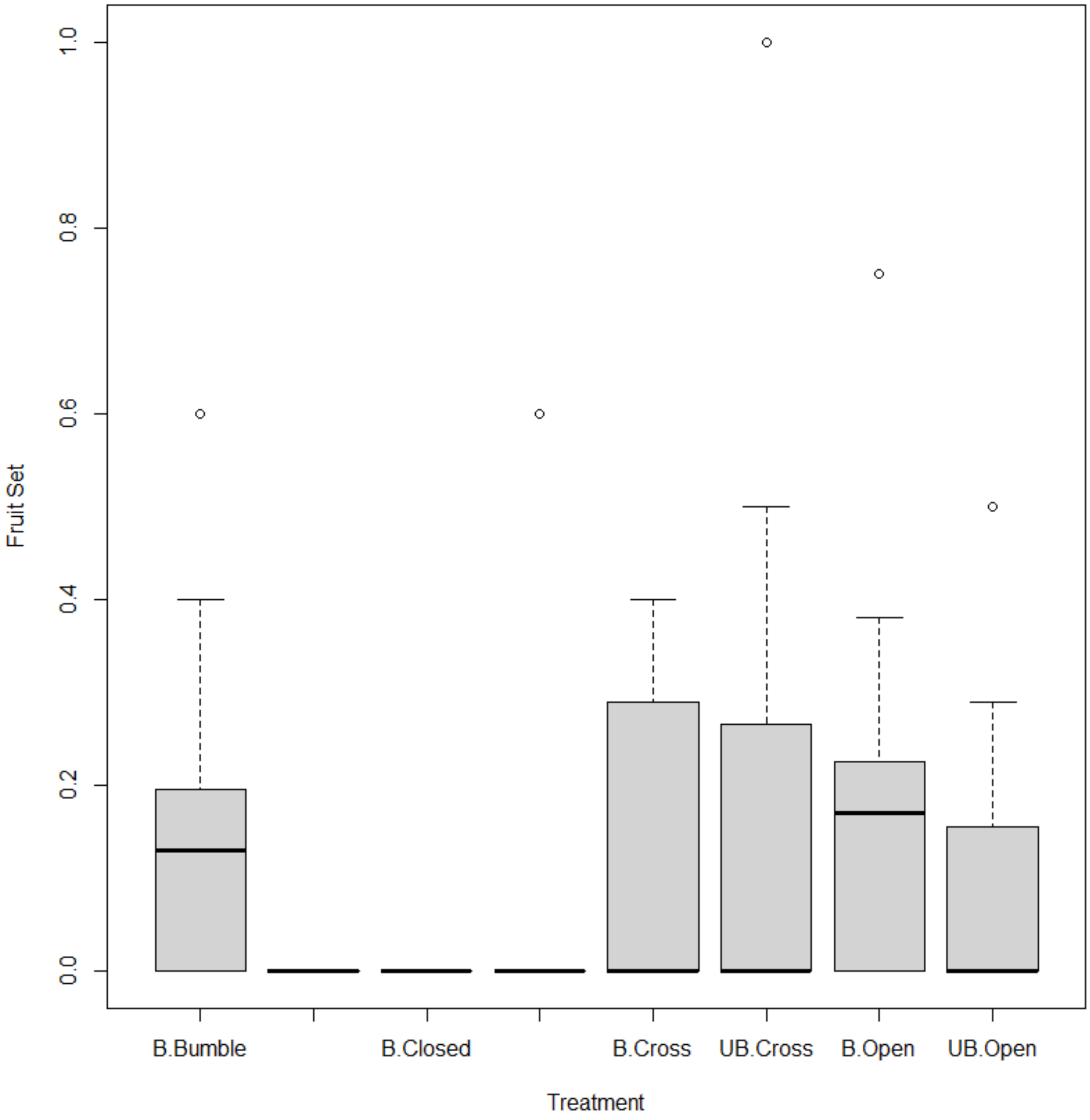

Figure 3. Fruit set (%) of each pollination treatment of the bagging experiment on common bearberry (*Arctostaphylos uva-ursi*) in Burnt Lands Provincial Park. Error bars represent standard deviation.

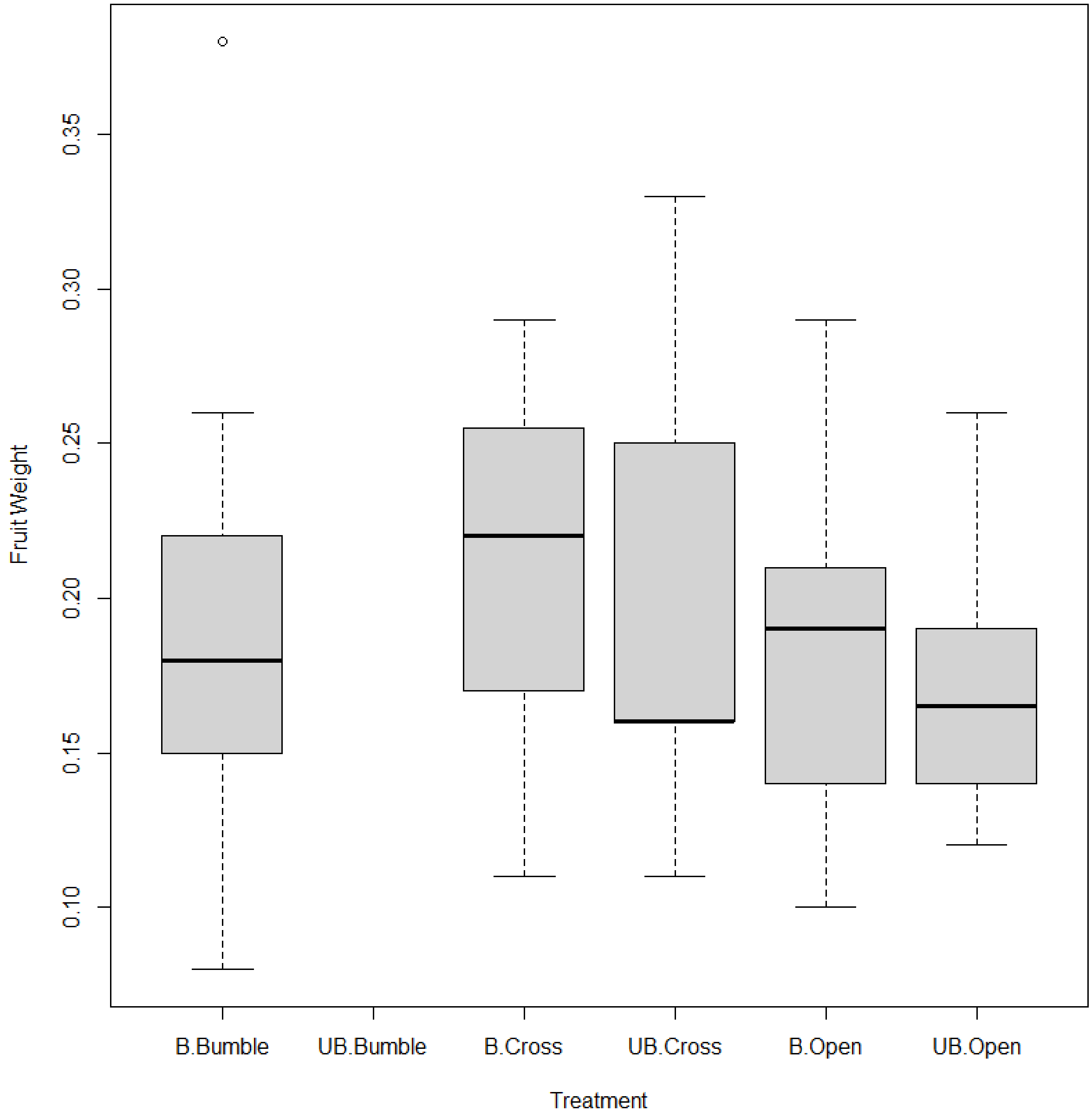

Figure 4. Average fruit weight (g) of each pollination treatment of the bagging experiment on common bearberry (*Arctostaphylos uva-ursi*) in Burned and Unburned Sections of Burnt Lands Provincial Park. Error bars represent standard deviation.

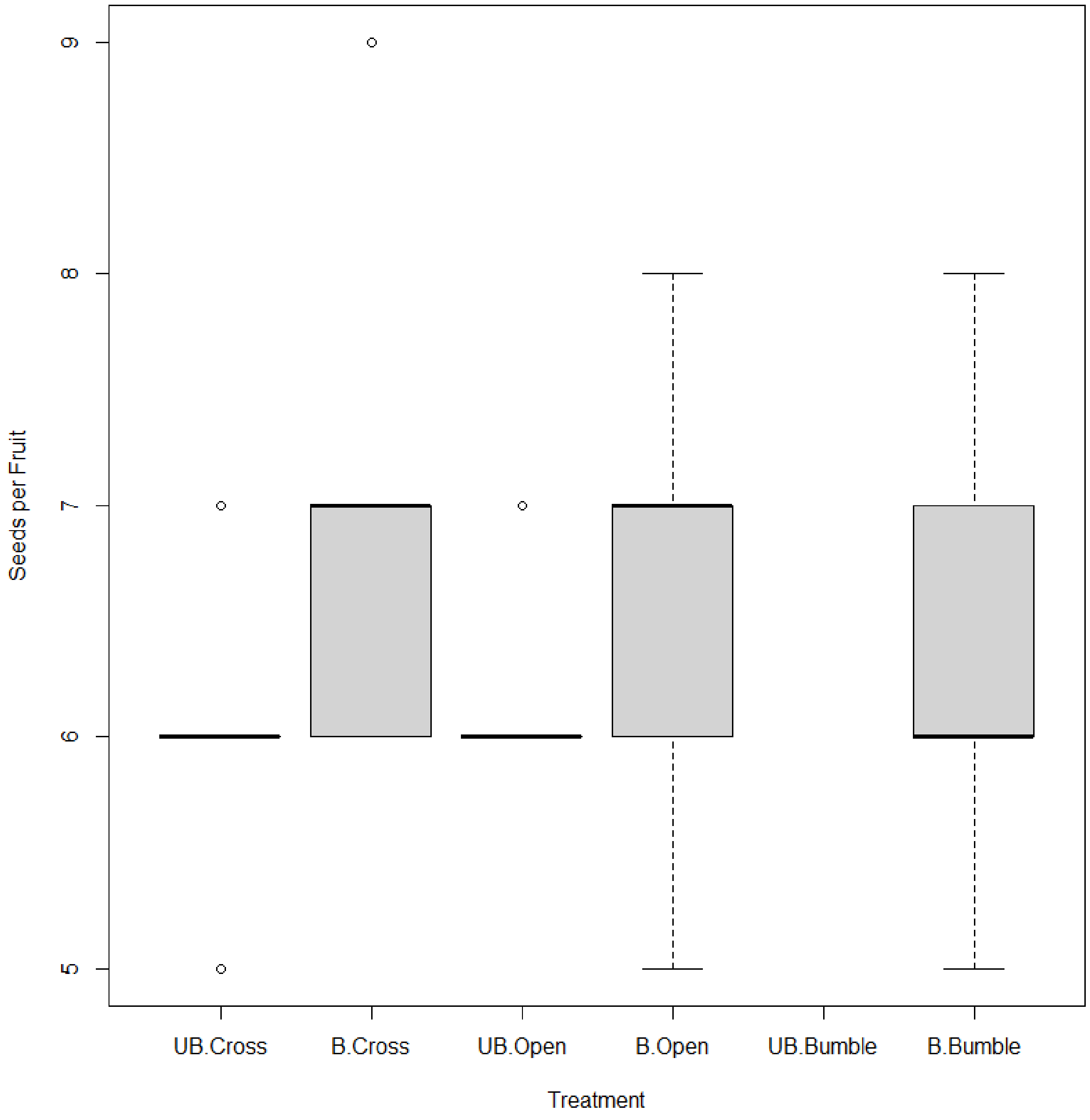

Figure 5. Average seeds per fruitfor each pollination treatment of the bagging experiment on common bearberry (*Arctostaphylos uva-ursi*) in Burned and Unburned Sections of Burnt Lands Provincial Park. Error bars represent standard deviation.

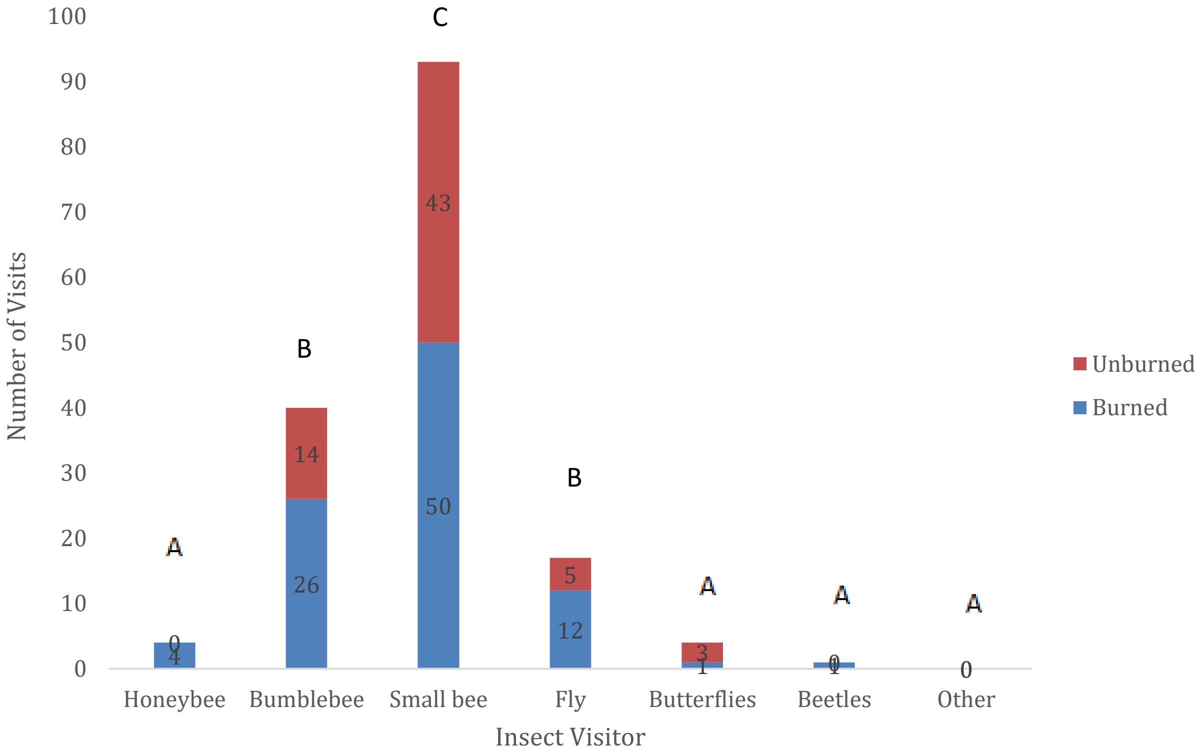

Figure 6. Number of visits of each category of insect visitor recorded on common bearberry (*Arctostaphylos uva-ursi*) in Burned and Unburned sections of Burnt Lands Provincial Park in early Spring 2023. Sample sizes for Burned and Unburned sections are located on the bars. Bars with the same letter show totals for number of visits that are significantly different from each other (Tukey's HSD test, $p<0.05$).

Figure 7. Bumble bee on common bearberry (*Arctostaphylos uva-ursi*) at Burnt Lands Provincial Park in Eastern Ontario, Canada.

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Chapter Five: Summary and future directions for pollination of culturally significant plants

Shelby D. Gibson

Summary of PhD Research

The objective of my PhD research was to determine the role of wild insect pollinators to reproduction in culturally significant food and medicine plants, with a focus on the Great Lakes Region. To do this, I conducted a systematic literature review (Chapter One), determined the plant-pollinator network in a Three Sisters Garden system (Chapter Two), investigated the role of diurnal and nocturnal pollinators to Hopi tobacco (*Nicotiana rustica*) (Chapter Three), and investigated the role of wild pollinators to Common Bearberry (*Arctostaphylos uva-ursi*) in an alvar ecosystem (Chapter Four). Given that this research occurred during a multi-year global pandemic, where a chapter was impacted by the pandemic, I will mention that here.

With the literature review, I aimed to determine what is known about the pollination and breeding systems of culturally significant plants in the Great Lakes Region. I used a list of 215 medicine plants culturally significant to the Anishinaabe people. The review focused on herbaceous plants as they are most commonly used and therefore excluded all other plants (e.g. woody-stemmed shrubs, vines). Two databases, Scopus and Web of Science, were searched in the review. The following search terms were used: (“scientific name” OR “common name” AND “breeding system” OR “pollinat*”). A total of 525 papers were retained for further analysis. All papers were skimmed for data on breeding systems and pollinator/insect visitors. Breeding systems of 108 (50%) plants have not been studied, while 107 (50%) have been studied. Of the 107 plants studied, self compatibility was recorded in 57 (52%) of plant species, while self-incompatibility was recorded in 36 (34%) plant species. A total of 79% of the plants were found to either require or benefit from outcrossing (xenogamous). A total of 27 (33%) xenogamous plants were strictly outcrossing. In terms of insect visitors, the most commonly recorded order was Hymenoptera, with Apidae the most recorded family. This review highlighted the importance of studying plants from a variety of different perspectives, and not focusing largely on economically or agriculturally important plant species (e.g. plants of cultural significance). The results also showed that pollination by wild bees appeared to be an important factor in maintaining healthy plant populations through cross-pollination. I therefore decided to focus on the interaction between wild pollinators and culturally significant plants throughout the following chapters.

In the second chapter, I focus on the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes Region. The aim of this study was to map the network in the garden system and compare the bee diversity of the system to that of the surrounding natural areas. Traditional planting methods from Six Nations Our Sustenance (2020) were followed. Several medicine plants were added to the garden. Sweep net sampling was conducted within the garden four days per week, at three garden plots, between the hours of 8 AM and noon, and 1 PM and 5 PM. Pan trap samples were collected by setting out pan traps (white, yellow, blue) on sampling days, and collecting them 24 hours later. Important visitors in this system included the common eastern bumble bee, *Bombus impatiens* Cresson (Hymenoptera: Apidae), and the hoary squash bee, *Xenoglossa pruinosa* (Say) (Hymenoptera: Apidae), as determined by their abundances and pollinator service index (PSI) values. The diversity of the wild bee community was higher than that of the garden community. While *X. pruinosa* was one of the most dominant visitors to the garden system, only two specimens were collected in the natural areas. This research highlights and reinforces the relationship between *X. pruinosa* and it's cultivated host squash, *Cucurbita pepo*. In addition, *N. rustica* supported the highest diversity of bee species and second highest abundance of bee visits. *Augochlorella aurata* was a significant visitor to *N. rustica*, and also represented 16% of the records in the pan trap samples.

These results highlight that *N. rustica* may provide important forage resources for wild bee species, including *A. aurata.* Field work was conducted in 2019 and 2020. The 2019 season was a pilot study which included sampling in the Three Sisters Garden in eastern Ontario and also at three sites in Toronto (Appendix A). It was intended that for the 2020 field season that I would lead a citizen science project which would collect plant-pollinator interactions in Three Sisters Garden locations across various locations in Ontario. When the pandemic hit, we had had to cancel this citizen science project and therefore lost sites across Ontario. I was able to pivot and access three private properties in order to plant my own plots of the Three Sisters Garden. I planted three plots and decided to add in pan-trap sampling in order to provide something to compare the garden diversity against. Overall, this limited the value of the 2019 pilot sampling for two reasons: 1) insect sampling was largely done via using images to record visitation and therefore was limited to genus-level identified, and 2) there were no longer sites at various locations in Ontario, only eastern Ontario, limiting the value of the 2019 sampling at the Toronto locations. The pandemic had a significant impact on Chapter 2. During peer-review revisions, I decided to remove the 2019 sampling altogether based on feedback from a reviewer that the value of the 2019 sampling is somewhat limited. This chapter, however, still provides an important baseline plant-pollinator network to a culturally significant food and medicine garden in the Great Lakes Region, building a foundation for future research in this system.

My third chapter examined the role of insect visitation and pollination to *N. rustica.* The flowers of *N. rustica* open in the morning and are receptive to pollen until the following day. The aim of my study was to determine the role of diurnal and nocturnal pollinators to reproductive success. We examined seed weight, seed set, and seediness using floral bagging techniques. We also conducted video recording which provided temporally a near-complete record of insect visitation. Results of our study showed *N. rustica* benefited mostly from open (open day and night) and day open (closed at night) pollination. Fruit weight increased with diurnal pollinators, while fruit set and seediness were unaffected. The most abundant visitors documented during the video recording were small bees.

Finally, my fourth chapter was a field study of *A. uva-ursi* in an alvar ecosystem. A search of the literature showed a very limited amount of study and, consequently understanding, on the pollination of *A. uva-ursi.* I conducted a field study at Burnt Lands Provincial Park in eastern Ontario, where a historical fire created two separate unburned and burned sections of the park. Since fire is a land management technique currently being researched and reviewed for alvar ecosystems, we decided to examine the effect of fire on pollination of *A. uva-ursi*. We investigated the breeding system of *A. uva-ursi* using a bagging experiment and flower visitor observation. Bagging treatments included open, closed, bumble bee, and hand-cross pollinated. Fruit weight, fruit set, and number of seeds per fruit were examined. Fruit set was 0% for the closed treatments, on both burned and unburned sections, indicating *A. uva-ursi* is not autogamous. Bumble bee visits set fruit 60% of the time in the burned and 0% of the time in the unburned sections. Hand-cross pollination set fruit 40% of the time in the burned and 17% of the time in the unburned sections. Insect visitation rate (IVR) was highest on the burned side, and the most frequent visitors were small bees and bumble bees. The pandemic had a significant impact on Chapter 4. I had planned a full breeding system study of common bearberry at Burnt Lands Provincial Park which was intended to begin in April 2020. When the pandemic hit, Ontario Parks closed their properties, and I was not granted access until May 20, 2020. This meant that I had missed the start of blooming, and therefore had an extremely limited ability to conduct a breeding system experiment. The flowers were peak blooming throughout most parts of the park by the time I received access. I decided to attempt to collect some data by conducting a limited floral bagging experiment using only open and closed treatments on common bearberry. I conducted this on the limited, late-blooming plants that I could find. Therefore, I only have partial results for 2020 bearberry sampling.

Future Work

As shown by the literature review (Chapter 1), many plants are lacking baseline ecological data including breeding system and pollination data. This dissertation attempts to begin to fill in the details of the relationship between insect pollinators and culturally significant food and medicine plants in the Great Lakes Region. The overall goal is to further understand the ways in which wild pollinators support human well-being, and to better be able to inform conservation practices. The identified loss of pollinators (Colla et al. 2012; Potts et al., 2010; Tylianakis et al. 2013; Rodger et al. 2021; Zattara and Aizen, 2021) will have obvious consequences, however there is also growing evidence, that the loss of pollinators will have not so pronounced consequences. Since the reproductive success of wild flowering plants is at risk due to pollinator declines (Aslan et al. 2013; Biesmeijer et al. 2006; Thomann et al., 2013; Scheper et al. 2014; Rodger et al., 2021), a loss of wild insects is linked to human well-being (Potts et al. 2016; Stout and Dicks, 2022). This research attempts to fill in baseline data, and there remains significant room for future work in this area.

The literature review investigated plants that were listed as being of cultural significance within various sources. There was no consideration, however, of the level of cultural significance on a species-by-species basis. Other forms of knowledge were not incorporated as part of the literature review. The level of cultural significance has been a factor examined in previous studies (e.g., Petelka et al. 2022) and may be a factor that could be incorporated with the results of our literature review to determine a priority list of plants for future research. Plants of the highest level of importance on a cultural basis, and plants that are also strictly outcrossing, would be important to prioritize within the context of pollinator declines.

One of the limitations of the Three Sisters Garden study is lack of comparison to a monocultural system, such as a squash field. Further understanding how growing plants in a polyculture system has potential implications for pollinator conservation in the context of sustainable agriculture. There is also the potential to use the baseline data from the TSG network to determine how the network would respond to various environmental disturbances. One example would be determining the impact of the removal of *X. pruinosa* within the system, which would likely be replaced by the less efficient, yet more abundant, *B. impatiens*.

Research on *N. rustica* could be continued by conducting pollinator effectiveness studies to further determine the extent to which specific pollinator species contribute to reproductive success in the plant. Small wild bees including *A. aurata* and *L. perpunctatum* were significant visitors to *N. rustica* and both require bare sandy soil patches for nesting. Future work examining how to cultivate and maintain this ecological relationship between Halictid bees and *N. rustica* by providing suitable habitat requirements would be beneficial.

Overall it is clear that while honey bees may be used to ensure adequate pollination of some plant species, particularly in large-scale agricultural settings, there are strong relationships between culturally significant food and medicine plants and wild pollinators. These ecological relationships are important to understand in order to better understand how land management techniques can be used to support wild pollinators, and therefore preserve human well-being and cultural diversity through supporting pollination mutualisms.

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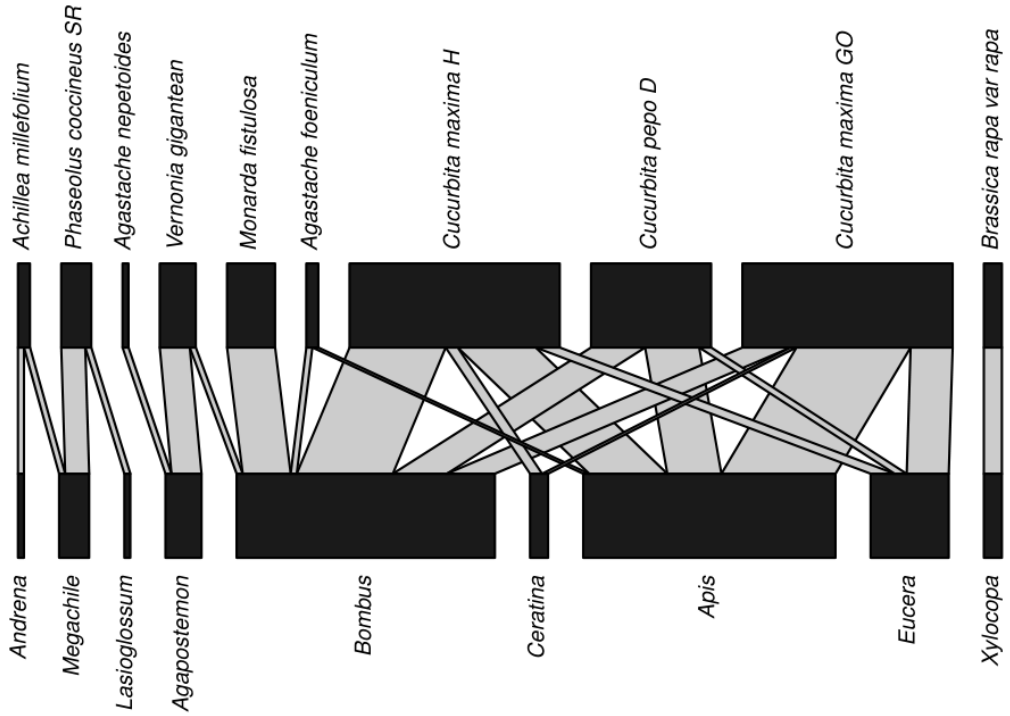
Appendices

Appendix A: Chapter 2 2019 preliminary results



2019 Eastern Ontario Sites Network Level Statistics

```
              connectance                 web asymmetry
               0.4166667                    0.2000000
           links per species          number of compartments
               1.0000000                    1.0000000
       compartment diversity              cluster coefficient
                   NA                   0.2500000
               nestedness                        NODF
              26.1021618                   42.8571429
         weighted nestedness                  weighted NODF
               0.4810058                  38.0952381
 interaction strength asymmetry         specialisation asymmetry
              -0.1543693                    0.4476841
            linkage density            weighted connectance
               1.7034157                    0.1703416
             Fisher alpha               Shannon diversity
               2.3258735                    1.1863191
        interaction evenness      Alatalo interaction evenness
               0.3732848                    0.4838874
                   H2            number.of.species.HL
               0.3585830                    6.0000000
        number.of.species.LL mean.number.of.shared.partners.HL
               4.0000000                    0.6000000
mean.number.of.shared.partners.LL           cluster.coefficient.HL
               1.1666667                    0.5650888
        cluster.coefficient.LL   weighted.cluster.coefficient.HL
               0.5838264                    0.0000000
 weighted.cluster.coefficient.LL                niche.overlap.HL
```

```
        0.0000000                   0.3690731
      niche.overlap.LL            togetherness.HL
        0.4011992                   0.1166667
      togetherness.LL                C.score.HL
        0.3125000                   0.7000000
         C.score.LL               V.ratio.HL
        0.2500000                   1.3750000
         V.ratio.LL            discrepancy.HL
        0.7500000                   3.0000000
       discrepancy.LL          extinction.slope.HL
        2.0000000                   2.9456285
    extinction.slope.LL              robustness.HL
        1.6722238                   0.7510863
       robustness.LL     functional.complementarity.HL
        0.6394444                 244.2888386
functional.complementarity.LL           partner.diversity.HL
      240.1133322                   0.5755254
    partner.diversity.LL              generality.HL
        0.4243703                   1.8775237
      vulnerability.LL
        1.5293076
```

2019 Eastern Ontario Sites Species Level Statistics

```
$`higher level`
   degree normalised.degree species.strength interaction.push.pull nestedrank
V1      1             0.25      0.023622047          -0.9763780        0.4
V2      4             1.00      1.855560081           0.2138900        0.0
V3      1             0.25      0.111111111          -0.8888889        0.6
V4      2             0.50      1.751832745           0.3759164        0.2
V5      1             0.25      0.250000000          -0.7500000        0.8
V6      1             0.25      0.007874016          -0.9921260        1.0
         PDI resource.range species.specificity.index         PSI
V1 1.0000000      1.0000000               1.0000000 0.023622047
V2 0.5000000      0.0000000               0.2643230 0.428009473
V3 1.0000000      1.0000000               1.0000000 0.111111111
V4 0.9262537      0.6666667               0.7774476 0.884746603
V5 1.0000000      1.0000000               1.0000000 0.250000000
V6 1.0000000      1.0000000               1.0000000 0.007874016
   node.specialisation.index.NSI betweenness weighted.betweenness closeness
V1                   1.4           0                0.0 0.1739130
V2                   1.0           1                0.7 0.2173913
V3                   1.8           0                0.0 0.1304348
V4                   1.4           0                0.3 0.1739130
V5                   1.8           0                0.0 0.1304348
V6                   1.4           0                0.0 0.1739130
   weighted.closeness Fisher.alpha partner.diversity effective.partners
V1       0.017800028          NA          0.000000           1.000000
V2       0.054793661          NA          1.278780           3.592254
V3       0.006796567          NA          0.000000           1.000000
V4       0.075988788          NA          0.473147           1.605037
V5       0.007060886          NA          0.000000           1.000000
```

```
V6    0.006929782        NA      0.000000       1.000000
  proportional.generality proportional.similarity        d
V1              0.4667559              0.75147929 0.03707578
V2              1.6767060              0.63692308 0.26169508
V3              0.4667559              0.05325444 0.54642037
V4              0.7491607              0.92307692 0.39487876
V5              0.4667559              0.02366864 0.71382312
V6              0.4667559              0.75147929 0.00000000

$`lower level`
  degree normalised.degree species.strength interaction.push.pull nestedrank
1      4         0.6666667        3.2188406             0.5547101  0.0000000
2      2         0.3333333        0.3411594            -0.3294203  0.3333333
3      2         0.3333333        1.1200000             0.0600000  1.0000000
4      2         0.3333333        1.3200000             0.1600000  0.6666667
        PDI species.specificity.index resource.range PSI
1 0.9752212                 0.8707465            0.4   1
2 0.9680000                 0.8453552            0.8   1
3 0.9333333                 0.7416198            0.8   1
4 0.9750000                 0.8734775            0.8   1
  node.specialisation.index.NSI betweenness weighted.betweenness closeness
1                             1           0                    1      0.25
2                             1           0                    0      0.25
3                             1           0                    0      0.25
4                             1           0                    0      0.25
  weighted.closeness Fisher.alpha partner.diversity effective.partners
1         0.27068662    0.7855867         0.4306712           1.538290
2         0.18565941    0.4875208         0.4011899           1.493601
3         0.05633803    1.5918159         0.5623351           1.754765
```

```
4      0.15023474   0.7972169      0.3488321       1.417411
  proportional.similarity proportional.generality         d
1           0.9189768             0.8351679 0.121905419
2           0.9544991             0.8109054 0.007339104
3           0.1538462             0.9526968 0.567434402
4           0.1538462             0.7695405 0.649423349
```

2019 Toronto Sites Network Level Statistics

```
connectance                web asymmetry
                     0.24444444                     -0.05263158
              links per species           number of compartments
                     1.15789474                      3.00000000
          compartment diversity              cluster coefficient
                     2.40734649                      0.20000000
                     nestedness                             NODF
                    33.09435872                     25.92592593
            weighted nestedness                    weighted NODF
                     0.08135138                     19.03292181
 interaction strength asymmetry          specialisation asymmetry
                    -0.03484174                      0.09015632
                linkage density             weighted connectance
                     2.94563138                      0.15503323
                   Fisher alpha                Shannon diversity
                     8.00498099                      2.66560513
           interaction evenness     Alatalo interaction evenness
                     0.59238175                      0.77308928
                             H2             number.of.species.HL
                     0.40832570                      9.00000000
           number.of.species.LL mean.number.of.shared.partners.HL
                    10.00000000                       0.52777778
mean.number.of.shared.partners.LL           cluster.coefficient.HL
                     0.60000000                      0.42564103
         cluster.coefficient.LL   weighted.cluster.coefficient.HL
                     0.36277303                      0.43408042
  weighted.cluster.coefficient.LL                niche.overlap.HL
                     0.68052738                      0.17042708
```

```
           niche.overlap.LL              togetherness.HL
                 0.22698646                   0.11458333
            togetherness.LL                   C.score.HL
                 0.16000000                   0.76157407
                 C.score.LL                   V.ratio.HL
                 0.65740741                   1.44927536
                 V.ratio.LL               discrepancy.HL
                 0.80555556                   9.00000000
             discrepancy.LL           extinction.slope.HL
                10.00000000                   2.05794181
        extinction.slope.LL                robustness.HL
                 2.09180338                   0.66923862
              robustness.LL functional.complementarity.HL
                 0.67221329                  83.83021489
functional.complementarity.LL          partner.diversity.HL
                80.26121707                   1.12947813
       partner.diversity.LL                generality.HL
                 0.89736892                   3.30322660
           vulnerability.LL
                 2.58803615
```

2019 Toronto Sites Species Level

```
$`higher level`
  degree normalised.degree species.strength interaction.push.pull nestedrank
V1      2             0.2       1.83333333            0.4166667      0.375
V2      1             0.1       0.50000000           -0.5000000      0.875
V3      4             0.4       1.86428571            0.2160714      0.125
V4      6             0.6       2.80238095            0.3003968      0.000
V5      2             0.2       0.08571429           -0.4571429      0.625
V6      3             0.3       0.41428571           -0.1952381      0.250
V7      1             0.1       0.20000000           -0.8000000      1.000
V8      2             0.2       1.30000000            0.1500000      0.500
V9      1             0.1       1.00000000            0.0000000      0.750
         PDI resource.range species.specificity.index        PSI
V1 0.9777778      0.8888889                 0.8314794 0.86111111
V2 1.0000000      1.0000000                 1.0000000 0.50000000
V3 0.8654971      0.6666667                 0.5238095 0.46887755
V4 0.8125000      0.4444444                 0.4117294 0.50836102
V5 0.9444444      0.8888889                 0.7114582 0.04761905
V6 0.9047619      0.7777778                 0.5852673 0.15824176
V7 1.0000000      1.0000000                 1.0000000 0.20000000
V8 0.9722222      0.8888889                 0.8027730 0.74000000
V9 1.0000000      1.0000000                 1.0000000 1.00000000
  node.specialisation.index.NSI betweenness weighted.betweenness  closeness
V1                         1.75        0.00                 0.00 0.11363636
V2                         1.50        0.00                 0.00 0.06818182
V3                         1.25        0.00                 0.00 0.15909091
V4                         1.00        0.75                 0.75 0.18181818
V5                         1.25        0.00                 0.00 0.15909091
V6                         1.25        0.00                 0.00 0.15909091
```

```
V7              1.50     0.00           0.00 0.06818182
V8              1.00     0.25           0.25 0.09090909
V9               NaN     0.00             NA 0.00000000
   weighted.closeness Fisher.alpha partner.diversity effective.partners
V1       0.06020045 1.050523e+00        0.4505612           1.569193
V2               NA 1.342178e+08        0.0000000           1.000000
V3       0.04918478 1.087021e+00        1.1409148           3.129630
V4       0.04926864 1.895940e+00        1.4959093           4.463393
V5       0.02325581 2.622302e+00        0.6365142           1.889882
V6       0.04105656 1.222546e+00        0.9839614           2.675032
V7               NA 1.342178e+08        0.0000000           1.000000
V8               NA 1.235493e+00        0.5004024           1.649385
V9               NA 5.252543e-01        0.0000000           1.000000
   proportional.generality proportional.similarity         d
V1               0.2677571              0.05982906 0.9448312
V2               0.1706337              0.01709402 0.8050410
V3               0.5340203              0.78632479 0.2390105
V4               0.7616052              0.76485788 0.2009912
V5               0.3224775              0.59829060 0.0817973
V6               0.4564506              0.75213675 0.1053562
V7               0.1706337              0.04273504 0.5473192
V8               0.2814406              0.05982906 0.8902200
V9               0.1706337              0.02564103 1.0000000


$`lower level`
   degree normalised.degree species.strength interaction.push.pull nestedrank
1       2         0.2222222       1.20000000            0.10000000  0.5555556
2       2         0.2222222       0.04706534           -0.47646733  0.6666667
3       1         0.1111111       0.16666667           -0.83333333  1.0000000
```

```
4      1       0.1111111      1.00000000          0.00000000  0.8888889
5      4       0.4444444      1.51022234          0.12755558  0.0000000
6      4       0.4444444      1.65597581          0.16399395  0.1111111
7      3       0.3333333      0.57743419         -0.14085527  0.2222222
8      1       0.1111111      0.18604651         -0.81395349  0.7777778
9      2       0.2222222      1.80000000          0.40000000  0.4444444
10     2       0.2222222      0.85658915         -0.07170543  0.3333333
        PDI species.specificity.index resource.range PSI
1  0.8750000              0.6614378        0.875   1
2  0.8750000              0.6614378        0.875   1
3  1.0000000              1.0000000        1.000   1
4  1.0000000              1.0000000        1.000   1
5  0.8947368              0.5578750        0.625   1
6  0.8515625              0.5326101        0.625   1
7  0.8472222              0.5847008        0.750   1
8  1.0000000              1.0000000        1.000   1
9  0.9687500              0.8000000        0.875   1
10 0.9750000              0.8291562        0.875   1
   node.specialisation.index.NSI betweenness weighted.betweenness  closeness
1                   1.000000          0             0.000 0.02564103
2                   1.166667          0             0.000 0.14102564
3                   1.833333          0             0.000 0.08974359
4                        NaN          0             0.000 0.00000000
5                   1.166667          0             0.000 0.14102564
6                   1.166667          0             0.375 0.14102564
7                   1.166667          0             0.000 0.14102564
8                   1.166667          0             0.000 0.14102564
9                   1.000000          0             0.000 0.02564103
10                  1.000000          1             0.625 0.15384615
```

```
   weighted.closeness Fisher.alpha partner.diversity effective.partners
1                  NA           NA         0.6931472           2.000000
2         0.025123655           NA         0.6931472           2.000000
3         0.007736944           NA         0.0000000           1.000000
4                  NA           NA         0.0000000           1.000000
5         0.149773343           NA         1.0924554           2.981586
6         0.176988885           NA         1.1371414           3.117843
7         0.123541524           NA         0.9489154           2.582907
8         0.089585666           NA         0.0000000           1.000000
9                  NA           NA         0.5004024           1.649385
10        0.016366612           NA         0.4505612           1.569193
   proportional.similarity proportional.generality          d
1               0.05128205               0.4304260 0.78537593
2               0.72649573               0.4304260 0.00000000
3               0.05128205               0.2152130 0.52362028
4               0.02564103               0.2152130 1.00000000
5               0.72429792               0.6416761 0.18226452
6               0.86324786               0.6710004 0.11889810
7               0.82649573               0.5558751 0.08406671
8               0.36752137               0.2152130 0.33598338
9               0.05128205               0.3549691 0.93965056
10              0.21794872               0.3377107 0.71921188
```

Appendix B: Chapter 2 network by site

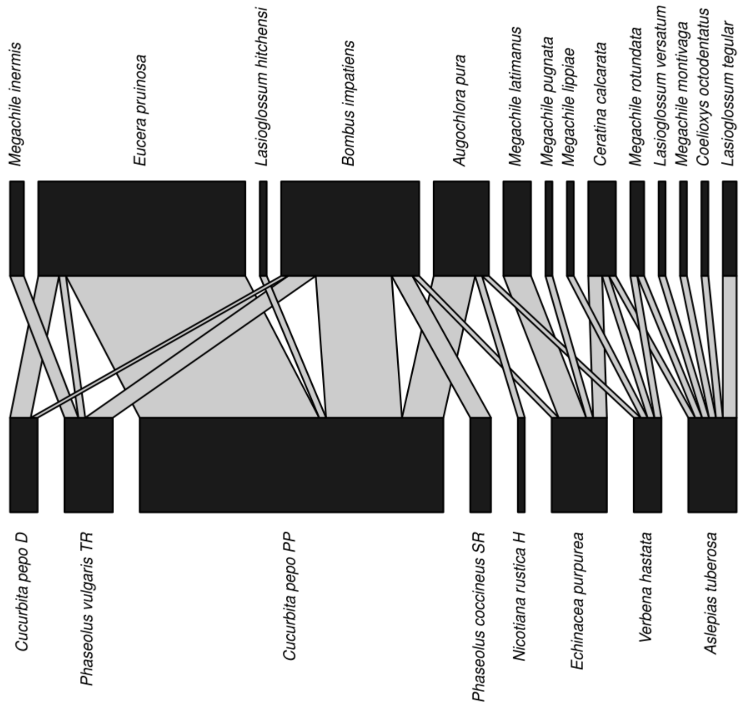


Figure 2. Interaction network displaying plant (species level) and pollinator (genus level) interactions (n = 286) observed in one Three Sisters garden plot (A) in eastern ON between July and August 2020. Interaction line width is proportional to abundance.

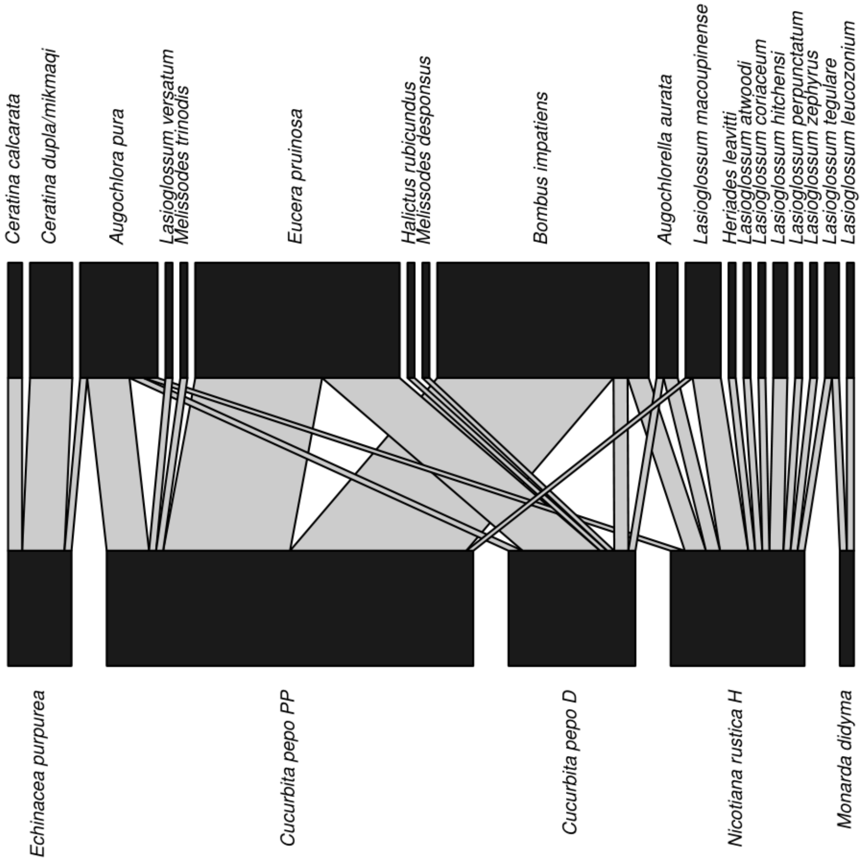


Figure 3. Interaction network displaying plant (species level) and pollinator (genus level) interactions (n = 286) observed in one Three Sisters garden plot (B) in eastern ON between July and August 2020. Interaction line width is proportional to abundance.

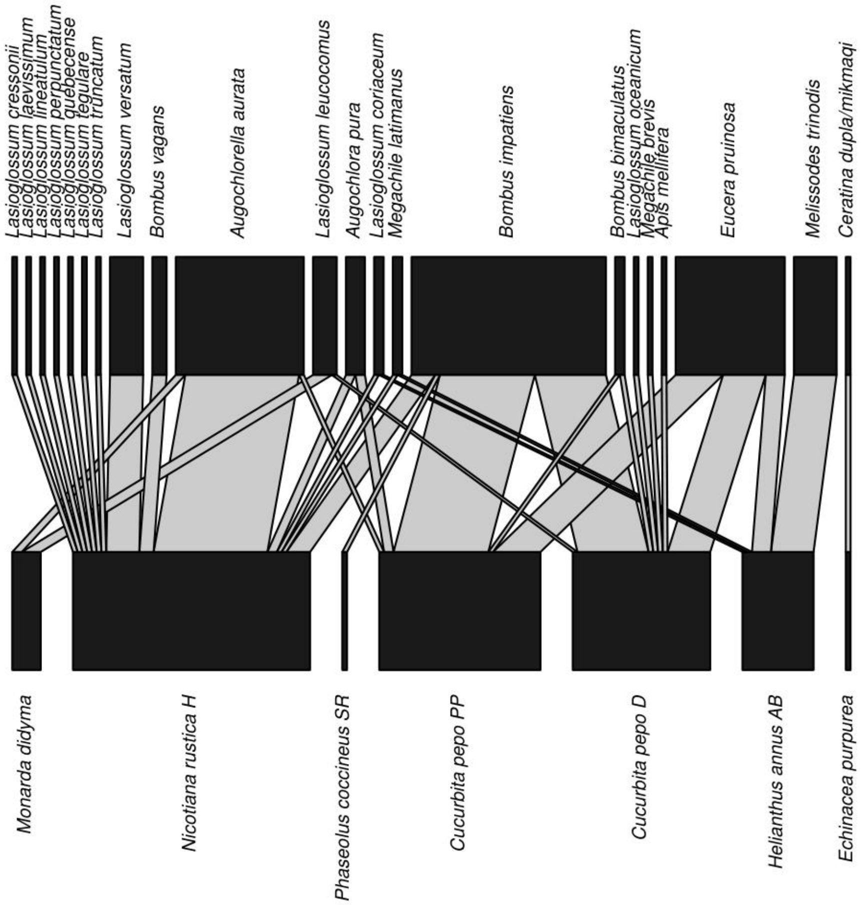


Figure 4. Interaction network displaying plant (species level) and pollinator (genus level) interactions (n = 286) observed in one Three Sisters garden plot (C) in eastern ON between July and August 2020. Interaction line width is proportional to abundance.

Appendix C: Chapter 2 Network statistics for 2020 results

2020 Species Level

```
$`higher level`
                          degree normalised.degree
Apis mellifera                 1               0.1
Augochlora pura                5               0.5
Augochlorella aurata           4               0.4
Bombus bimaculatus             2               0.2
Bombus impatiens               6               0.6
Bombus vagans                  1               0.1
Ceratina calcarata             3               0.3
Ceratine dupla/mikmaqi         1               0.1
Coelioxys octodentatus         1               0.1
Xenoglossa pruinosa            4               0.4
Halictus rubicundus            1               0.1
Heriades leavitti              1               0.1
Lasioglossum atwoodi           1               0.1
Lasioglossum coriaceum         2               0.2
Lasioglossum cressonii         1               0.1
Lasioglossum hitchensi         2               0.2
Lasioglossum laevissimum       1               0.1
Lasioglossum leucocomus        2               0.2
Lasioglossum leucozonium       1               0.1
Lasioglossum lineatulum        1               0.1
Lasioglossum macoupinense      2               0.2
Lasioglossum oceanicum         1               0.1
Lasioglossum perpunctatum      1               0.1
Lasioglossum quebecense        1               0.1
Lasioglossum tegulare          3               0.3
```

```
Lasioglossum truncatum         1               0.1
Lasioglossum versatum          3               0.3
Lasioglossum zephyrus          1               0.1
Megachile brevis               1               0.1
Megachile inermis              1               0.1
Megachile latimanus            3               0.3
Megachile lippiae              1               0.1
Megachile montivaga            1               0.1
Megachile pugnata              1               0.1
Megachile rotundata            2               0.2
Melissodes desponsus           1               0.1
Melissodes trinodis            2               0.2
                          species.strength interaction.push.pull
Apis mellifera                  0.01960784           -0.98039216
Augochlora pura                 0.52747123           -0.09450575
Augochlorella aurata            0.65429345           -0.08642664
Bombus bimaculatus              0.02748186           -0.48625907
Bombus impatiens                2.50657143            0.25109524
Bombus vagans                   0.04347826           -0.95652174
Ceratina calcarata              0.61507937           -0.12830688
Ceratine dupla/mikmaqi          0.38888889           -0.61111111
Coelioxys octodentatus          0.14285714           -0.85714286
Xenoglossa pruinosa             1.27782704            0.06945676
Halictus rubicundus             0.01960784           -0.98039216
Heriades leavitti               0.01449275           -0.98550725
Lasioglossum atwoodi            0.01449275           -0.98550725
Lasioglossum coriaceum          0.09565217           -0.45217391
Lasioglossum cressonii          0.01449275           -0.98550725
Lasioglossum hitchensi          0.03685952           -0.48157024
Lasioglossum laevissimum        0.01449275           -0.98550725
Lasioglossum leucocomus         0.51960784           -0.24019608
Lasioglossum leucozonium        0.12500000           -0.87500000
```

```
Lasioglossum lineatulum         0.01449275           -0.98550725
Lasioglossum macoupinense       0.06584503           -0.46707748
Lasioglossum oceanicum          0.01960784           -0.98039216
Lasioglossum perpunctatum       0.02898551           -0.97101449
Lasioglossum quebecense         0.01449275           -0.98550725
Lasioglossum tegulare           0.43969979           -0.18676674
Lasioglossum truncatum          0.01449275           -0.98550725
Lasioglossum versatum           0.25218043           -0.24927319
Lasioglossum zephyrus           0.01449275           -0.98550725
Megachile brevis                0.01960784           -0.98039216
Megachile inermis               0.28571429           -0.71428571
Megachile latimanus             0.30338164           -0.23220612
Megachile lippiae               0.25000000           -0.75000000
Megachile montivaga             0.14285714           -0.85714286
Megachile pugnata               0.05555556           -0.94444444
Megachile rotundata             0.39285714           -0.30357143
Melissodes desponsus            0.01960784           -0.98039216
Melissodes trinodis             0.60787402           -0.19606299
                          nestedrank       PDI resource.range
Apis mellifera            0.52777778 1.0000000      1.0000000
Augochlora pura           0.02777778 0.9285714      0.5555556
Augochlorella aurata      0.08333333 0.9829060      0.6666667
Bombus bimaculatus        0.36111111 0.8888889      0.8888889
Bombus impatiens          0.00000000 0.9300412      0.4444444
Bombus vagans             0.44444444 1.0000000      1.0000000
Ceratina calcarata        0.13888889 0.9444444      0.7777778
Ceratine dupla/mikmaqi    0.41666667 1.0000000      1.0000000
Coelioxys octodentatus    0.55555556 1.0000000      1.0000000
Xenoglossa pruinosa       0.05555556 0.9412998      0.6666667
Halictus rubicundus       0.58333333 1.0000000      1.0000000
Heriades leavitti         0.61111111 1.0000000      1.0000000
Lasioglossum atwoodi      0.63888889 1.0000000      1.0000000
```

```
Lasioglossum coriaceum    0.30555556 0.9444444      0.8888889
Lasioglossum cressonii    0.66666667 1.0000000      1.0000000
Lasioglossum hitchensi    0.33333333 0.9444444      0.8888889
Lasioglossum laevissimum  0.69444444 1.0000000      1.0000000
Lasioglossum leucocomus   0.25000000 0.9722222      0.8888889
Lasioglossum leucozonium  0.72222222 1.0000000      1.0000000
Lasioglossum lineatulum   0.75000000 1.0000000      1.0000000
Lasioglossum macoupinense 0.27777778 0.9722222      0.8888889
Lasioglossum oceanicum    0.77777778 1.0000000      1.0000000
Lasioglossum perpunctatum 0.47222222 1.0000000      1.0000000
Lasioglossum quebecense   0.80555556 1.0000000      1.0000000
Lasioglossum tegulare     0.19444444 0.8333333      0.7777778
Lasioglossum truncatum    0.83333333 1.0000000      1.0000000
Lasioglossum versatum     0.11111111 0.9682540      0.7777778
Lasioglossum zephyrus     0.86111111 1.0000000      1.0000000
Megachile brevis          0.88888889 1.0000000      1.0000000
Megachile inermis         0.50000000 1.0000000      1.0000000
Megachile latimanus       0.16666667 0.9444444      0.7777778
Megachile lippiae         0.91666667 1.0000000      1.0000000
Megachile montivaga       0.94444444 1.0000000      1.0000000
Megachile pugnata         0.97222222 1.0000000      1.0000000
Megachile rotundata       0.38888889 0.8888889      0.8888889
Melissodes desponsus      1.00000000 1.0000000      1.0000000
Melissodes trinodis       0.22222222 0.9876543      0.8888889
```

```
                          species.specificity.index        PSI
Apis mellifera                            1.0000000 0.01960784
Augochlora pura                           0.6047144 0.09954839
Augochlorella aurata                      0.8549060 0.34415278
Bombus bimaculatus                        0.6666667 0.01374093
Bombus impatiens                          0.6045910 0.41323848
Bombus vagans                             1.0000000 0.04347826
Ceratina calcarata                        0.6666667 0.21362434
Ceratine dupla/mikmaqi                    1.0000000 0.38888889
Coelioxys octodentatus                    1.0000000 0.14285714
Xenoglossa pruinosa                       0.6760629 0.41605164
Halictus rubicundus                       1.0000000 0.01960784
Heriades leavitti                         1.0000000 0.01449275
Lasioglossum atwoodi                      1.0000000 0.01449275
Lasioglossum coriaceum                    0.7114582 0.04154589
Lasioglossum cressonii                    1.0000000 0.01449275
Lasioglossum hitchensi                    0.7114582 0.02194834
Lasioglossum laevissimum                  1.0000000 0.01449275
Lasioglossum leucocomus                   0.8027730 0.40392157
Lasioglossum leucozonium                  1.0000000 0.12500000
Lasioglossum lineatulum                   1.0000000 0.01449275
Lasioglossum macoupinense                 0.8027730 0.04795161
Lasioglossum oceanicum                    1.0000000 0.01960784
Lasioglossum perpunctatum                 1.0000000 0.02898551
Lasioglossum quebecense                   1.0000000 0.01449275
Lasioglossum tegulare                     0.5374838 0.15087992
Lasioglossum truncatum                    1.0000000 0.01449275
Lasioglossum versatum                     0.7671228 0.09565290
Lasioglossum zephyrus                     1.0000000 0.01449275
Megachile brevis                          1.0000000 0.01960784
Megachile inermis                         1.0000000 0.28571429
Megachile latimanus                       0.6666667 0.16167472
```

```
Megachile lippiae                         1.0000000 0.25000000
Megachile montivaga                       1.0000000 0.14285714
Megachile pugnata                         1.0000000 0.05555556
Megachile rotundata                       0.6666667 0.19642857
Melissodes desponsus                      1.0000000 0.01960784
Melissodes trinodis                       0.8944272 0.54078740
                          node.specialisation.index.NSI
Apis mellifera                                 1.777778
Augochlora pura                                1.111111
Augochlorella aurata                           1.222222
Bombus bimaculatus                             1.611111
Bombus impatiens                               1.138889
Bombus vagans                                  1.500000
Ceratina calcarata                             1.694444
Ceratine dupla/mikmaqi                         1.888889
Coelioxys octodentatus                         2.027778
Xenoglossa pruinosa                            1.527778
Halictus rubicundus                            1.777778
Heriades leavitti                              1.500000
Lasioglossum atwoodi                           1.500000
Lasioglossum coriaceum                         1.444444
Lasioglossum cressonii                         1.500000
Lasioglossum hitchensi                         1.416667
Lasioglossum laevissimum                       1.500000
Lasioglossum leucocomus                        1.666667
Lasioglossum leucozonium                       2.027778
Lasioglossum lineatulum                        1.500000
Lasioglossum macoupinense                      1.416667
Lasioglossum oceanicum                         1.777778
Lasioglossum perpunctatum                      1.500000
Lasioglossum quebecense                        1.500000
Lasioglossum tegulare                          1.333333
```

```
Lasioglossum truncatum                         1.500000
Lasioglossum versatum                          1.305556
Lasioglossum zephyrus                          1.500000
Megachile brevis                               1.777778
Megachile inermis                              2.083333
Megachile latimanus                            1.361111
Megachile lippiae                              1.972222
Megachile montivaga                            2.027778
Megachile pugnata                              1.888889
Megachile rotundata                            1.833333
Melissodes desponsus                           1.777778
Melissodes trinodis                            1.722222
                           betweenness weighted.betweenness
Apis mellifera            0.0000000000         0.0000000000
Augochlora pura           0.2515453765         0.0911877395
Augochlorella aurata      0.1189491439         0.3218390805
Bombus bimaculatus        0.0135954136         0.0000000000
Bombus impatiens          0.2114143364         0.3057471264
Bombus vagans             0.0000000000         0.0000000000
Ceratina calcarata        0.0370734371         0.0321839080
Ceratine dupla/mikmaqi    0.0000000000         0.0000000000
Coelioxys octodentatus    0.0000000000         0.0000000000
Xenoglossa pruinosa       0.0436117936         0.0398467433
Halictus rubicundus       0.0000000000         0.0000000000
Heriades leavitti         0.0000000000         0.0000000000
Lasioglossum atwoodi      0.0000000000         0.0000000000
Lasioglossum coriaceum    0.0067226317         0.0000000000
Lasioglossum cressonii    0.0000000000         0.0000000000
Lasioglossum hitchensi    0.0117828868         0.0000000000
Lasioglossum laevissimum  0.0000000000         0.0000000000
Lasioglossum leucocomus   0.0185464685         0.0268199234
Lasioglossum leucozonium  0.0000000000         0.0000000000
```

```
Lasioglossum lineatulum   0.0000000000         0.0000000000
Lasioglossum macoupinense 0.0117828868         0.0000000000
Lasioglossum oceanicum    0.0000000000         0.0000000000
Lasioglossum perpunctatum 0.0000000000         0.0000000000
Lasioglossum quebecense   0.0000000000         0.0000000000
Lasioglossum tegulare     0.1176631177         0.0727969349
Lasioglossum truncatum    0.0000000000         0.0000000000
Lasioglossum versatum     0.1042480792         0.0429118774
Lasioglossum zephyrus     0.0000000000         0.0000000000
Megachile brevis          0.0000000000         0.0000000000
Megachile inermis         0.0000000000         0.0000000000
Megachile latimanus       0.0445877696         0.0659003831
Megachile lippiae         0.0000000000         0.0000000000
Megachile montivaga       0.0000000000         0.0000000000
Megachile pugnata         0.0000000000         0.0000000000
Megachile rotundata       0.0078624079         0.0007662835
Melissodes desponsus      0.0000000000         0.0000000000
Melissodes trinodis       0.0006142506         0.0000000000
                           closeness weighted.closeness
Apis mellifera            0.02419068        0.004573860
Augochlora pura           0.03628602        0.028263613
Augochlorella aurata      0.03415155        0.033616806
Bombus bimaculatus        0.02668090        0.008117399
Bombus impatiens          0.03575240        0.035440216
Bombus vagans             0.02881537        0.011455950
Ceratina calcarata        0.02508004        0.015034354
Ceratine dupla/mikmaqi    0.02170046        0.019837010
Coelioxys octodentatus    0.02081110        0.004398905
Xenoglossa pruinosa       0.02828175        0.035814671
Halictus rubicundus       0.02419068        0.004573860
Heriades leavitti         0.02881537        0.004613931
Lasioglossum atwoodi      0.02881537        0.004613931
```

```
Lasioglossum coriaceum    0.02988260        0.008616513
Lasioglossum cressonii    0.02881537        0.004613931
Lasioglossum hitchensi    0.03041622        0.010968157
Lasioglossum laevissimum  0.02881537        0.004613931
Lasioglossum leucocomus   0.02561366        0.015033748
Lasioglossum leucozonium  0.02009961        0.004528787
Lasioglossum lineatulum   0.02881537        0.004613931
Lasioglossum macoupinense 0.03041622        0.015317640
Lasioglossum oceanicum    0.02419068        0.004573860
Lasioglossum perpunctatum 0.02881537        0.008357578
Lasioglossum quebecense   0.02881537        0.004613931
Lasioglossum tegulare     0.03201708        0.011494395
Lasioglossum truncatum    0.02881537        0.004613931
Lasioglossum versatum     0.03255069        0.020710663
Lasioglossum zephyrus     0.02881537        0.004613931
Megachile brevis          0.02419068        0.004573860
Megachile inermis         0.01938812        0.008102739
Megachile latimanus       0.03148346        0.015980581
Megachile lippiae         0.02045535        0.004581559
Megachile montivaga       0.02081110        0.004398905
Megachile pugnata         0.02170046        0.004611719
Megachile rotundata       0.02276770        0.006445477
Melissodes desponsus      0.02419068        0.004573860
Melissodes trinodis       0.02454642        0.021667959
                          Fisher.alpha partner.diversity
Apis mellifera                      NA         0.0000000
Augochlora pura                     NA         1.1189600
Augochlorella aurata                NA         0.5313039
Bombus bimaculatus                  NA         0.6931472
Bombus impatiens                    NA         1.1575237
Bombus vagans                       NA         0.0000000
Ceratina calcarata                  NA         0.8675632
```

```
Ceratine dupla/mikmaqi              NA         0.0000000
Coelioxys octodentatus              NA         0.0000000
Xenoglossa pruinosa                 NA         0.8378193
Halictus rubicundus                 NA         0.0000000
Heriades leavitti                   NA         0.0000000
Lasioglossum atwoodi                NA         0.0000000
Lasioglossum coriaceum              NA         0.6365142
Lasioglossum cressonii              NA         0.0000000
Lasioglossum hitchensi              NA         0.6365142
Lasioglossum laevissimum            NA         0.0000000
Lasioglossum leucocomus             NA         0.5004024
Lasioglossum leucozonium            NA         0.0000000
Lasioglossum lineatulum             NA         0.0000000
Lasioglossum macoupinense           NA         0.5004024
Lasioglossum oceanicum              NA         0.0000000
Lasioglossum perpunctatum           NA         0.0000000
Lasioglossum quebecense             NA         0.0000000
Lasioglossum tegulare               NA         1.0549202
Lasioglossum truncatum              NA         0.0000000
Lasioglossum versatum               NA         0.6837389
Lasioglossum zephyrus               NA         0.0000000
Megachile brevis                    NA         0.0000000
Megachile inermis                   NA         0.0000000
Megachile latimanus                 NA         0.8675632
Megachile lippiae                   NA         0.0000000
Megachile montivaga                 NA         0.0000000
Megachile pugnata                   NA         0.0000000
Megachile rotundata                 NA         0.6931472
Melissodes desponsus                NA         0.0000000
Melissodes trinodis                 NA         0.3250830
                          effective.partners
Apis mellifera                      1.000000
```

Augochlora pura	3.061668
Augochlorella aurata	1.701149
Bombus bimaculatus	2.000000
Bombus impatiens	3.182044
Bombus vagans	1.000000
Ceratina calcarata	2.381102
Ceratine dupla/mikmaqi	1.000000
Coelioxys octodentatus	1.000000
Xenoglossa pruinosa	2.311321
Halictus rubicundus	1.000000
Heriades leavitti	1.000000
Lasioglossum atwoodi	1.000000
Lasioglossum coriaceum	1.889882
Lasioglossum cressonii	1.000000
Lasioglossum hitchensi	1.889882
Lasioglossum laevissimum	1.000000
Lasioglossum leucocomus	1.649385
Lasioglossum leucozonium	1.000000
Lasioglossum lineatulum	1.000000
Lasioglossum macoupinense	1.649385
Lasioglossum oceanicum	1.000000
Lasioglossum perpunctatum	1.000000
Lasioglossum quebecense	1.000000
Lasioglossum tegulare	2.871746
Lasioglossum truncatum	1.000000
Lasioglossum versatum	1.981272
Lasioglossum zephyrus	1.000000
Megachile brevis	1.000000
Megachile inermis	1.000000
Megachile latimanus	2.381102
Megachile lippiae	1.000000
Megachile montivaga	1.000000

```
Megachile pugnata                   1.000000
Megachile rotundata                 2.000000
Melissodes desponsus                1.000000
Melissodes trinodis                 1.384145
                          proportional.generality
Apis mellifera                          0.1851556
Augochlora pura                         0.5668852
Augochlorella aurata                    0.3149773
Bombus bimaculatus                      0.3703113
Bombus impatiens                        0.5891734
Bombus vagans                           0.1851556
Ceratina calcarata                      0.4408744
Ceratine dupla/mikmaqi                  0.1851556
Coelioxys octodentatus                  0.1851556
Xenoglossa pruinosa                     0.4279542
Halictus rubicundus                     0.1851556
Heriades leavitti                       0.1851556
Lasioglossum atwoodi                    0.1851556
Lasioglossum coriaceum                  0.3499223
Lasioglossum cressonii                  0.1851556
Lasioglossum hitchensi                  0.3499223
Lasioglossum laevissimum                0.1851556
Lasioglossum leucocomus                 0.3053929
Lasioglossum leucozonium                0.1851556
Lasioglossum lineatulum                 0.1851556
Lasioglossum macoupinense               0.3053929
Lasioglossum oceanicum                  0.1851556
Lasioglossum perpunctatum               0.1851556
Lasioglossum quebecense                 0.1851556
Lasioglossum tegulare                   0.5317200
Lasioglossum truncatum                  0.1851556
Lasioglossum versatum                   0.3668436
```

```
Lasioglossum zephyrus                   0.1851556
Megachile brevis                        0.1851556
Megachile inermis                       0.1851556
Megachile latimanus                     0.4408744
Megachile lippiae                       0.1851556
Megachile montivaga                     0.1851556
Megachile pugnata                       0.1851556
Megachile rotundata                     0.3703113
Melissodes desponsus                    0.1851556
Melissodes trinodis                     0.2562824
                          proportional.similarity          d
Apis mellifera                         0.16451613 0.18834150
Augochlora pura                        0.77040673 0.06727051
Augochlorella aurata                   0.31505376 0.46514732
Bombus bimaculatus                     0.57419355 0.03329710
Bombus impatiens                       0.70058651 0.22285680
Bombus vagans                          0.22258065 0.27698721
Ceratina calcarata                     0.09354839 0.58065302
Ceratine dupla/mikmaqi                 0.05806452 0.73703221
Coelioxys octodentatus                 0.02258065 0.59829996
Xenoglossa pruinosa                    0.63492632 0.33873438
Halictus rubicundus                    0.16451613 0.18834150
Heriades leavitti                      0.22258065 0.12594076
Lasioglossum atwoodi                   0.22258065 0.12594076
Lasioglossum coriaceum                 0.27096774 0.24751121
Lasioglossum cressonii                 0.22258065 0.12594076
Lasioglossum hitchensi                 0.55591398 0.08332080
Lasioglossum laevissimum               0.22258065 0.12594076
Lasioglossum leucocomus                0.19032258 0.65445895
Lasioglossum leucozonium               0.02580645 0.57073468
Lasioglossum lineatulum                0.22258065 0.12594076
Lasioglossum macoupinense              0.42258065 0.16323646
```

```
Lasioglossum oceanicum                0.16451613 0.18834150
Lasioglossum perpunctatum             0.22258065 0.21990641
Lasioglossum quebecense               0.22258065 0.12594076
Lasioglossum tegulare                 0.27096774 0.39869646
Lasioglossum truncatum                0.22258065 0.12594076
Lasioglossum versatum                 0.35627240 0.25083206
Lasioglossum zephyrus                 0.22258065 0.12594076
Megachile brevis                      0.16451613 0.18834150
Megachile inermis                     0.02258065 0.72400915
Megachile latimanus                   0.27311828 0.41872113
Megachile lippiae                     0.01290323 0.71382312
Megachile montivaga                   0.02258065 0.59829996
Megachile pugnata                     0.05806452 0.40333193
Megachile rotundata                   0.03548387 0.63294814
Melissodes desponsus                  0.16451613 0.18834150
Melissodes trinodis                   0.14838710 0.71379730

$`lower level`
                       degree normalised.degree species.strength
Asclepias tuberosa          6        0.16216216       3.17777778
Cucurbita pepo D           11        0.29729730       6.30878593
Cucurbita pepo PP           9        0.24324324       3.15443078
Echinacea purpurea          6        0.16216216       3.38817523
Helianthus annus AB         4        0.10810811       1.44938272
Monarda didyma              4        0.10810811       2.06666667
Nicotiana rustica H        19        0.51351351      14.64138120
Phaseolus coccineus SR      1        0.02702703       0.04545455
Phaseolus vulgaris T        3        0.08108108       1.05780022
Verbena hastata             4        0.10810811       1.71014493
                       interaction.push.pull nestedrank       PDI
Asclepias tuberosa                0.36296296  0.4444444 0.9305556
Cucurbita pepo D                  0.48261690  0.1111111 0.9661836
```

```
Cucurbita pepo PP             0.23938120  0.2222222 0.9624486
Echinacea purpurea            0.39802921  0.3333333 0.9563492
Helianthus annus AB           0.11234568  0.5555556 0.9814815
Monarda didyma                0.26666667  0.6666667 0.9722222
Nicotiana rustica H           0.71796743  0.0000000 0.9540598
Phaseolus coccineus SR       -0.95454545  1.0000000 1.0000000
Phaseolus vulgaris T          0.01926674  0.8888889 0.9791667
Verbena hastata               0.17753623  0.7777778 0.9166667
                      species.specificity.index resource.range
Asclepias tuberosa                    0.4012452      0.8611111
Cucurbita pepo D                      0.5603789      0.7222222
Cucurbita pepo PP                     0.5915273      0.7777778
Echinacea purpurea                    0.4885521      0.8611111
Helianthus annus AB                   0.6514940      0.9166667
Monarda didyma                        0.5705443      0.9166667
Nicotiana rustica H                   0.3943343      0.5000000
Phaseolus coccineus SR                1.0000000      1.0000000
Phaseolus vulgaris T                  0.6424161      0.9444444
Verbena hastata                       0.4787136      0.9166667
                              PSI node.specialisation.index.NSI
Asclepias tuberosa      3.17777778                     1.444444
Cucurbita pepo D        6.30878593                     1.111111
Cucurbita pepo PP       3.15443078                     1.000000
Echinacea purpurea      3.38817523                     1.111111
Helianthus annus AB     1.44938272                     1.444444
Monarda didyma          2.06666667                     1.555556
Nicotiana rustica H    14.64138120                     1.000000
Phaseolus coccineus SR  0.04545455                     1.444444
Phaseolus vulgaris T    1.05780022                     1.333333
Verbena hastata         1.71014493                     1.444444
                      betweenness weighted.betweenness
Asclepias tuberosa      0.03846154           0.00000000
```

```
Cucurbita pepo D        0.18846154           0.08695652
Cucurbita pepo PP       0.28076923           0.50000000
Echinacea purpurea      0.16538462           0.07608696
Helianthus annus AB     0.00000000           0.00000000
Monarda didyma          0.01538462           0.00000000
Nicotiana rustica H     0.28076923           0.33695652
Phaseolus coccineus SR  0.00000000           0.00000000
Phaseolus vulgaris T    0.01538462           0.00000000
Verbena hastata         0.01538462           0.00000000
                        closeness weighted.closeness Fisher.alpha
Asclepias tuberosa     0.09090909         0.02040395 1.994888e+01
Cucurbita pepo D       0.11038961         0.13152527 4.310498e+00
Cucurbita pepo PP      0.11688312         0.20532890 2.212838e+00
Echinacea purpurea     0.11038961         0.03747237 3.151573e+00
Helianthus annus AB    0.09090909         0.06738449 1.784783e+00
Monarda didyma         0.08441558         0.03480001 3.183619e+00
Nicotiana rustica H    0.11688312         0.15965262 8.662454e+00
Phaseolus coccineus SR 0.09090909         0.02717653 4.279594e-01
Phaseolus vulgaris T   0.09740260         0.03200407 1.988816e+00
Verbena hastata        0.09090909         0.01154552 2.147484e+09
                       partner.diversity effective.partners
Asclepias tuberosa             1.7478681           5.742347
Cucurbita pepo D               1.4704658           4.351261
Cucurbita pepo PP              1.2002705           3.321015
Echinacea purpurea             1.5174981           4.560800
Helianthus annus AB            1.0200370           2.773297
Monarda didyma                 1.2130076           3.363586
Nicotiana rustica H            2.2864741           9.840181
Phaseolus coccineus SR         0.0000000           1.000000
Phaseolus vulgaris T           0.9556999           2.600490
Verbena hastata                1.3862944           4.000000
                       proportional.similarity
```

```
Asclepias tuberosa                   0.07741935
Cucurbita pepo D                     0.64269450
Cucurbita pepo PP                    0.66517653
Echinacea purpurea                   0.17562724
Helianthus annus AB                  0.32258065
Monarda didyma                       0.13225806
Nicotiana rustica H                  0.40776064
Phaseolus coccineus SR               0.28387097
Phaseolus vulgaris T                 0.43317972
Verbena hastata                      0.10322581
                       proportional.generality         d
Asclepias tuberosa                   0.53329257 0.7294045
Cucurbita pepo D                     0.40410222 0.2166874
Cucurbita pepo PP                    0.30842310 0.3711756
Echinacea purpurea                   0.42356212 0.7682471
Helianthus annus AB                  0.25755648 0.6163535
Monarda didyma                       0.31237664 0.7018825
Nicotiana rustica H                  0.91385894 0.6235825
Phaseolus coccineus SR               0.09287013 0.1975736
Phaseolus vulgaris T                 0.24150785 0.3039131
Verbena hastata                      0.37148053 0.6352397
```

2020 Network Level

```
            connectance
                      0.18108108
                  web asymmetry
                      0.57446809
              links per species
                      1.42553191
         number of compartments
                      1.00000000
          compartment diversity
                              NA
            cluster coefficient
                      0.10000000
                   modularity Q
                      0.43651405
                     nestedness
                     19.73690952
                            NODF
                     27.84270270
            weighted nestedness
                      0.47614328
                  weighted NODF
                     17.95259202
interaction strength asymmetry
                      0.13634435
       specialisation asymmetry
                     -0.17287860
                linkage density
                      3.68191267
           weighted connectance
                      0.07833857
                   Fisher alpha
```

```
                      26.28532259
                Shannon diversity
                       3.17683120
             interaction evenness
                       0.53721647
     Alatalo interaction evenness
                       0.48630349
                               H2
                       0.51705249
             number.of.species.HL
                      37.00000000
             number.of.species.LL
                      10.00000000
mean.number.of.shared.partners.HL
                       0.46996997
mean.number.of.shared.partners.LL
                       1.24444444
           cluster.coefficient.HL
                       0.40548387
           cluster.coefficient.LL
                       0.28753269
  weighted.cluster.coefficient.HL
                       0.56978576
  weighted.cluster.coefficient.LL
                       0.19268916
                 niche.overlap.HL
                       0.24334688
                 niche.overlap.LL
                       0.17613445
                  togetherness.HL
                       0.12066066
                  togetherness.LL
```

```
                   0.07849253
                   C.score.HL
                   0.61292125
                   C.score.LL
                   0.59634749
                   V.ratio.HL
                   1.19484882
                   V.ratio.LL
                   4.97148676
               discrepancy.HL
                  36.00000000
               discrepancy.LL
                  35.00000000
          extinction.slope.HL
                   4.95501790
          extinction.slope.LL
                   1.44736166
                robustness.HL
                   0.78969940
                robustness.LL
                   0.49538094
functional.complementarity.HL
                 218.53585906
functional.complementarity.LL
                 240.71585397
         partner.diversity.HL
                   0.80027801
         partner.diversity.LL
                   1.49027275
                generality.HL
                   2.35847646
             vulnerability.LL
```

```
5.00534888
```

Appendix D: Chapter 4 ANOVA Statistics

BOTH SIDES FRUIT SET

SUMMARY

```
   Section           Treatment      Fruit.Set   
 Length:120         Min.   :1.00   Min.   :  0.0  
 Class :character   1st Qu.:1.75   1st Qu.:  0.0  
 Mode  :character   Median :2.50   Median :  0.0  
                    Mean   :2.50   Mean   :  9.2  
                    3rd Qu.:3.25   3rd Qu.: 14.0  
                    Max.   :4.00   Max.   :100.0  
```

Two way ANOVA with interaction

```
Anova Table (Type II tests)

Response: Fruit_set
                Sum Sq  Df F value  Pr(>F)  
Site             258.1   1  0.9130 0.34141  
Treatment       2982.7   4  2.6375 0.03774 *
Site:Treatment  1956.6   4  1.7301 0.14844  
Residuals      31099.7 110                  
```

Post hoc testing

```
contrast estimate   SE  df t.ratio p.value

 B - UB     3.43 3.25 110   1.054  0.2943
```

Results are averaged over the levels of: Treatment

```
contrast        estimate   SE  df t.ratio p.value
 Bumble - Closed    5.200 4.34 110   1.198  0.7527
 Bumble - Cross    -7.367 4.34 110  -1.697  0.4402
 Bumble - Open     -6.112 5.21 110  -1.174  0.7660
 Bumble - Open     -5.514 5.44 110  -1.013  0.8488
 Closed - Cross   -12.567 4.34 110  -2.895  0.0362
 Closed - Open    -11.312 5.21 110  -2.173  0.1976
 Closed - Open    -10.714 5.44 110  -1.969  0.2882
 Cross - Open       1.254 5.21 110   0.241  0.9992
 Cross - Open       1.852 5.44 110   0.340  0.9971
 Open - Open        0.598 6.15 110   0.097  1.0000

Results are averaged over the levels of: Site 
P value adjustment: tukey method for comparing a family of 5 estimates 
```

BOTH SIDES FRUIT WEIGHT

```
            Df  Sum Sq  Mean Sq F value Pr(>F)
Treatment    1 0.00307 0.003066   0.814  0.371
Section      1 0.00092 0.000924   0.245  0.622
Residuals   57 0.21480 0.003768
```

Two way ANOVA with interaction

```
Anova Table (Type II tests)

Response: Fruit.weight
                 Sum Sq Df F value Pr(>F)
Site           0.002216  1  0.5764 0.4510
Treatment      0.005277  2  0.6864 0.5076
Site:Treatment 0.000002  1  0.0006 0.9802
Residuals      0.211420 55
```

SEEDS PER FRUIT

Two way ANOVA with interaction

```
Anova Table (Type II tests)

Response: Seeds
               Sum Sq Df F value Pr(>F)
Site            1.708  1  2.3807 0.1286
Treatment       0.259  2  0.1804 0.8354
Site:Treatment  0.783  1  1.0918 0.3006
Residuals      39.452 55
```

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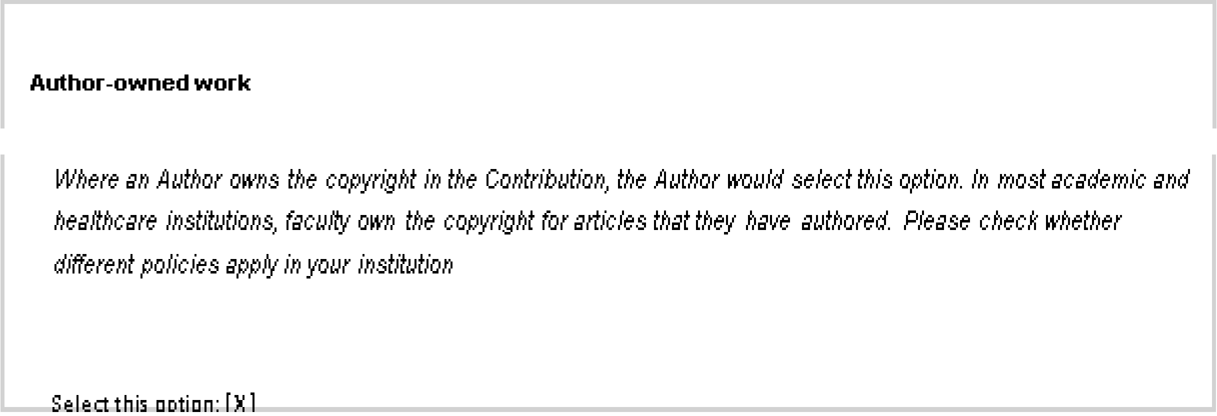

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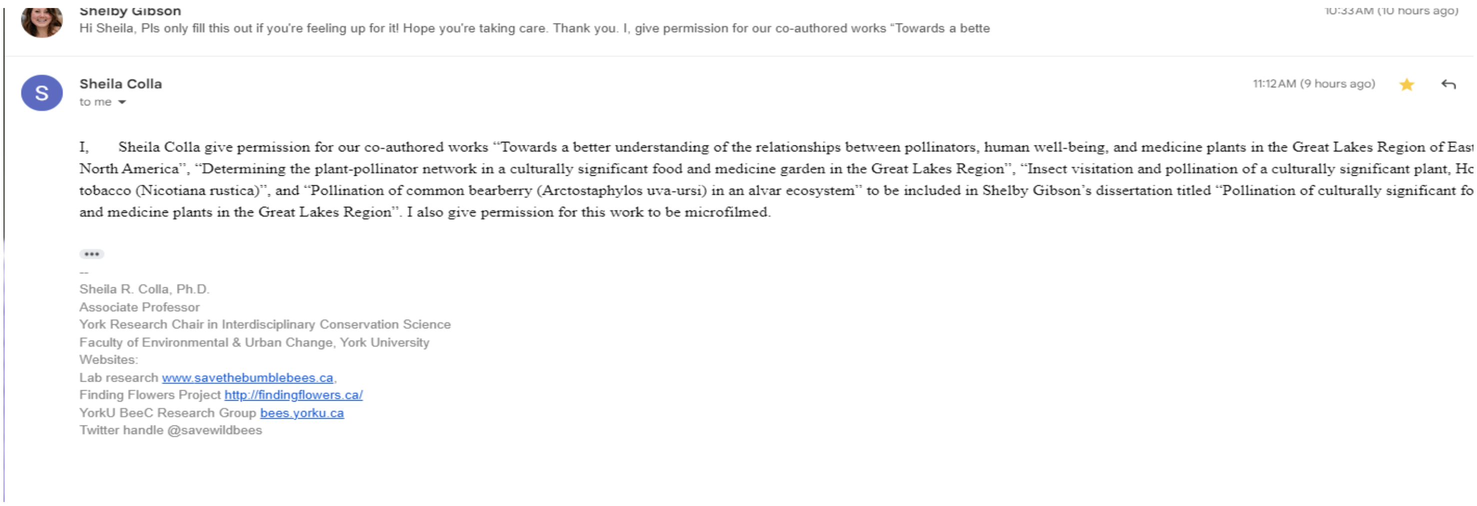

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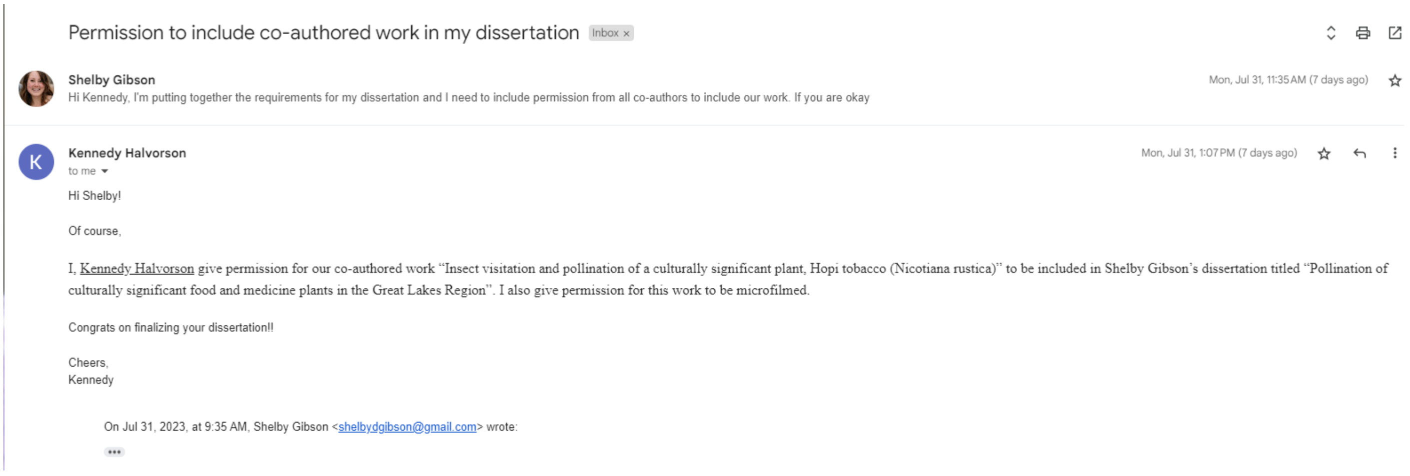


Permission to include co-authored work in my dissertation

Shelby Gibson Mon, Jul 31, 11:35 AM (7 days ago)

Hi Kennedy, I'm putting together the requirements for my dissertation and I need to include permission from all co-authors to include our work. If you are okay

Kennedy Halvorson Mon, Jul 31, 1:07 PM (7 days ago)

to me

Hi Shelby!

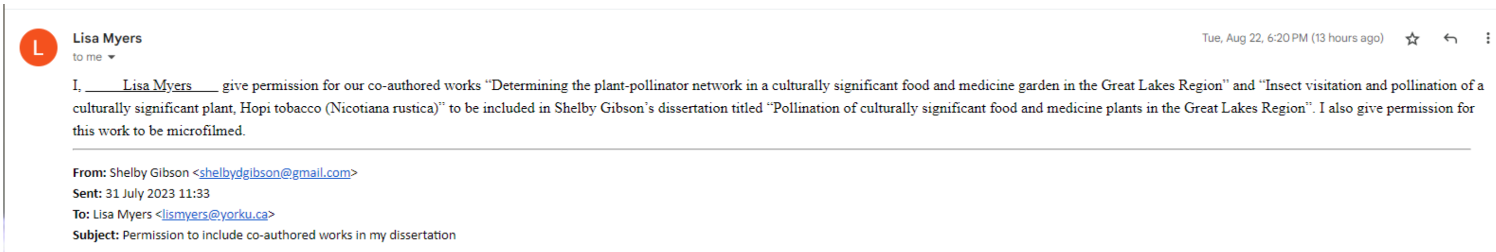
Of course,

I, Kennedy Halvorson give permission for our co-authored work "Insect visitation and pollination of a culturally significant plant, Hopi tobacco (Nicotiana rustica)" to be included in Shelby Gibson's dissertation titled "Pollination of culturally significant food and medicine plants in the Great Lakes Region". I also give permission for this work to be microfilmed.

Congrats on finalizing your dissertation!!

Cheers,
Kennedy

On Jul 31, 2023, at 9:35 AM, Shelby Gibson <shelbydgibson@gmail.com> wrote:

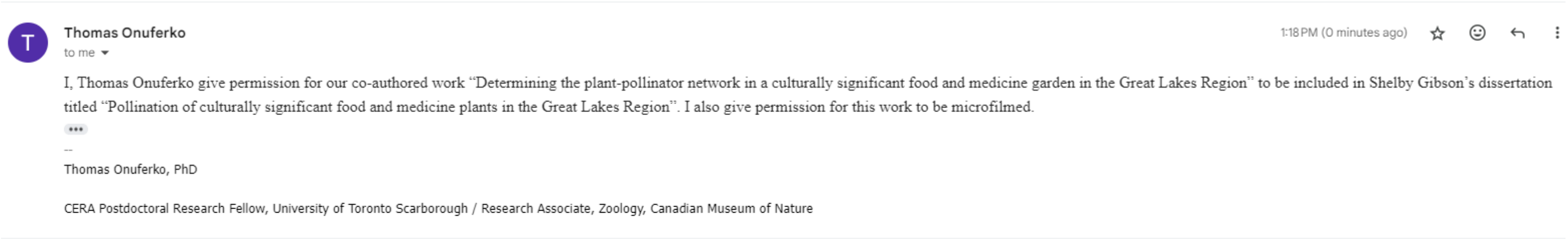


Lisa Myers Tue, Aug 22, 6:20 PM (13 hours ago)

to me

I, Lisa Myers give permission for our co-authored works "Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes Region" and "Insect visitation and pollination of a culturally significant plant, Hopi tobacco (Nicotiana rustica)" to be included in Shelby Gibson's dissertation titled "Pollination of culturally significant food and medicine plants in the Great Lakes Region". I also give permission for this work to be microfilmed.

From: Shelby Gibson <shelbydgibson@gmail.com>
Sent: 31 July 2023 11:33
To: Lisa Myers <lismyers@yorku.ca>
Subject: Permission to include co-authored works in my dissertation



Thomas Onuferko

to me

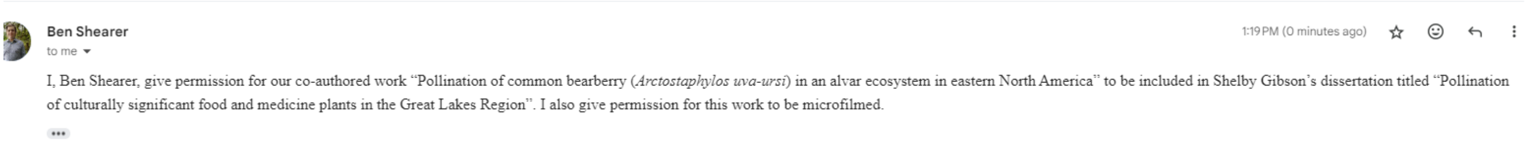
1:18 PM (0 minutes ago)

I, Thomas Onuferko give permission for our co-authored work “Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes Region” to be included in Shelby Gibson’s dissertation titled “Pollination of culturally significant food and medicine plants in the Great Lakes Region”. I also give permission for this work to be microfilmed.

--

Thomas Onuferko, PhD

CERA Postdoctoral Research Fellow, University of Toronto Scarborough / Research Associate, Zoology, Canadian Museum of Nature



Ben Shearer

to me

1:19 PM (0 minutes ago)

I, Ben Shearer, give permission for our co-authored work “Pollination of common bearberry (*Arctostaphylos uva-ursi*) in an alvar ecosystem in eastern North America” to be included in Shelby Gibson’s dissertation titled “Pollination of culturally significant food and medicine plants in the Great Lakes Region”. I also give permission for this work to be microfilmed.

Appendix I: Candidates' contribution to co-authored work

Statement of Shelby Gibson's contribution to co-authored works included in the dissertation titled "Plant-pollinator relationships of culturally significant food and medicine plants in the Great Lakes Region".

Chapter 1: Towards a better understanding of the relationships between pollinators, human well-being, and medicine plants in the Great Lakes Region of Eastern North America

Shelby. D. Gibson, Sheila R. Colla

Published in the journal Plants, People, Planet as: Gibson, S. D., & Colla, S. R. (2023). Towards a better understanding of the relationships between pollinators, human well-being, and medicine plants in the Great Lakes Region of Eastern North America. Plants, People, Planet, 1–10. https://doi.org/10.1002/ppp3.10398.

Candidate's contribution:

Shelby Gibson and Dr. Colla conceived of the idea to conduct a systematic review of the breeding systems and pollinators of culturally significant medicine plants in the Great Lakes Region. Shelby Gibson determined the search terms and conducted the review using Scopus and Web of Science. Shelby Gibson conducted a preliminary assessment of all articles, downloaded relevant articles, and read all retained articles. Shelby Gibson led the writing of the manuscript. Shelby Gibson and Sheila Colla both contributed to the editing of the manuscript.

Chapter 2: Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes Region

Shelby D. Gibson, Tom Onuferko, Lisa Myers & Sheila R. Colla

This chapter will be submitted to the peer-reviewed journal PeerJ.

<u>Candidate's contribution:</u>

Shelby Gibson, Lisa Myers, and Dr. Colla conceived of the ideas and methodology for this manuscript. Shelby Gibson selected the survey sites, prepared garden spaces, organized growing plants for all sites, managed a field assistant, conducted field work, input data, cleaned data, and analyzed the data. Shelby Gibson led the writing of the manuscript. Shelby Gibson and Dr. Colla edited the manuscript. Tom Onuferko provided expert insect identification to the species level for all insects collected during field work.

Chapter 3: Insect visitation and pollination of a culturally significant plant, Hopi tobacco (*Nicotiana rustica*)

Shelby D. Gibson, Kennedy S. Halvorson, Lisa Myers, & Sheila R. Colla

Published in the journal iScience as: Gibson, S.D., Halvorson, K.S., Myers, L., Colla, S.R. (2022). Insect visitation and pollination of a culturally significant plant, Hopi tobacco (*Nicotiana rustica*). doi: https://doi.org/10.1016/j.isci.2022.105613.

Candidate's contribution:

Shelby Gibson, Kennedy Halvorson, Lisa Myers and Dr. Colla conceived of the idea and methodology for the manuscript. Shelby Gibson selected the survey sites, organized growing the plants, managed a field assistant, conducted field work, input data, and conducted statistical analysis. Shelby Gibson led the writing of the manuscript. Kennedy Halvorson, Lisa Myers, and Dr. Colla contributed to editing the manuscript.

Chapter 4: Pollination and insect visitation of common bearberry (Arctostaphylos uva-ursi) in an alvar ecosystem in eastern North America

Shelby D. Gibson, Benjamin P. Shearer, and Sheila R. Colla

This chapter will be submitted to the peer-reviewed journal PeerJ.

Candidate's contribution:

Shelby Gibson and Dr. Colla conceived of the idea for this manuscript. Shelby Gibson, Benjamin Shearer and Dr. Colla contributed to methodology design. Shelby Gibson and Benjamin Shearer selected field sites and conducted field work. Shelby Gibson input data, conducted statistical analysis and led writing the manuscript. Shelby Gibson, Benjamin Shearer, and Dr. Colla contributed to editing the manuscript.

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PhD Candidate
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May 29, 2024

Dr. Sheila Colla
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Dr. Gordon Fitch
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