

Pollinators and Pollination of Haskap (*Lonicera caerulea* L.) in Southern
Nova Scotia

by

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Dedication Page

I dedicate this thesis to my wife, Taylor Olmstead who has been my only family member who has supported me through my university career, and has pushed me to excel in academics and other aspects of life.

Table of Contents

| | |
|---|------|
| List of Tables | v |
| List of Figures | vi |
| Abstract | viii |
| List of Abbreviations and Symbols Used | ix |
| Acknowledgments..... | x |
| Chapter 1: Introduction..... | 1 |
| 1.1 Pollination is Important for Agriculture | 1 |
| 1.2 Haskap..... | 1 |
| 1.3 The European honey bee (<i>Apis mellifera</i>) | 4 |
| 1.4 Non- <i>Apis</i> Pollinators..... | 5 |
| 1.5 Pollinator Community Structure Assessments..... | 7 |
| 1.6 Pollinator Effectiveness and Efficiency..... | 8 |
| 1.6.1 Single Visit Pollen Deposition..... | 8 |
| 1.6.3 Pollinator Efficiency | 9 |
| 1.7 Solitary Bee Nest Boxes | 9 |
| 1.8 Objectives and Hypotheses | 12 |
| Chapter 2: Materials and Methods..... | 15 |
| 2.1 Pollinator Surveys..... | 15 |
| 2.1.1 Study Sites..... | 15 |
| 2.1.2 Pan Trapping for Pollinators | 16 |
| 2.1.3 Transect Walks for Pollinators..... | 18 |
| 2.2 Effectiveness and Efficiency of Potential Pollinators..... | 19 |
| 2.2.1 Floral Visit Pollen Deposition..... | 19 |
| 2.2.2 Pollinator Flower Visits per Minute..... | 21 |
| 2.2.3 Pollinator Tendency to Visit Both Flowers in the Double Inflorescence | 21 |
| 2.2.4 Pollen Trapping..... | 22 |
| 2.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees..... | 24 |
| 2.3.1 Nest Tube Style | 24 |
| 2.3.2 Hatching Out Bees | 29 |

| | |
|---|-----------|
| 2.4 Data Analysis | 30 |
| Chapter 3: Results | 33 |
| 3.1 Pollinator Surveys | 33 |
| 3.2 Effectiveness and Efficiency of Potential Pollinators..... | 41 |
| 3.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees..... | 46 |
| Chapter 4: Discussion | 50 |
| 4.1 Pollinator Surveys..... | 50 |
| 4.2 Effectiveness and Efficiency of Potential Pollinators..... | 57 |
| 4.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees..... | 63 |
| 4.4 Summary and Recommendations | 67 |
| References | 69 |
| Appendix I: Daily weather data from weather station in Kejimkujik Nova Scotia during May 2016 | 76 |
| Appendix II: Daily weather data from weather station in Kejimkujik Nova Scotia during May 2017 | 77 |

List of Tables

| | |
|---|----|
| Table 1. Haskap orchards used for pollinator surveys in Nova Scotia, 2016 and 2017..... | 16 |
| Table 2. Haskap orchards used for placement of trap nests in Nova Scotia, 2016 and 2017..... | 25 |
| Table 3. Wild and managed pollinators captured in pan traps in southern Nova Scotia haskap orchards, 2016 and 2017..... | 34 |
| Table 4: Mean number pollinators captured per day during three bloom periods in haskap orchards in southern Nova Scotia, 2016..... | 36 |
| Table 5: Analysis of variance <i>P</i> -values that show effect of sampling period (pre-bloom, bloom, post bloom), location (edge, 60 m inside field) and the interaction on pollinator pan trap collections per day for 2017 in haskap orchards in southern Nova Scotia..... | 37 |
| Table 6: Mean number of pollinator captures per day during three bloom periods in haskap orchards in southern Nova Scotia, 2017..... | 38 |
| Table 7. Pollen collection from six samples collected by honey bees during haskap (<i>Lonicera</i>) bloom 17 May 2017..... | 45 |

List of Figures

| | |
|--|----|
| <p>Figure 1. Haskap plant, flowers and fruit. (A) Example of haskap shrubs in an orchard. Different sized shrubs are different cultivars which is required for cross pollination. (B) Example of a double flower inflorescence. (C) Haskap fruit produced by successful pollination. (D) Example of a haskap berry where bracteoles have not fused around the ovaries, showing the two distinct berries that make up the single compound fruit.....</p> | 3 |
| <p>Figure 2. Map illustration showing how pan traps were placed in haskap orchards in 2016 (A) and 2017 (B). (A) 3 transects containing 12 pan traps spaced 3m apart alternating colors of blue, yellow and white. Transects were spaced 25 m (5 rows) apart and were 36 m long. (B) 2 blocks of 9 pan traps placed 3 m apart. Blocks were 9 m long, and 9 m wide. 1 block on the field edge and one block 60 m inside the field. Pan trap colors were randomly assigned a location within each block.....</p> | 18 |
| <p>Figure 3. Examples of pollinators collected in haskap fields in Nova Scotia, 2016 and 2017. (A) honey bee, (B) bumble bee, (C) solitary bee, (D) hover fly.....</p> | 19 |
| <p>Figure 4. Example of an entrance mounted pollen trap used to collect pollen from returning honey bee foragers during haskap bloom.....</p> | 23 |
| <p>Figure 5. Dimensions of the trap nest design used in 2017. Trap nests were constructed out of 10 cm diameter PVC pipe (A) and were cut to be 23 cm long (B).....</p> | 27 |
| <p>Figure 6. Nest tube configurations that were tested in haskap orchards for 2017. (A) 6 mm spaced, (B) 6 mm snug, (C) 8 mm spaced, (D) 8 mm snug.....</p> | 28 |
| <p>Figure 7. Mean daily number of different pollinator groups counted during 30 min transect walks during bloom across three haskap fields in southern Nova Scotia, 2016 & 2017. Bars with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD. Error bars show SEM.....</p> | 39 |
| <p>Figure 8. Mean number of bumble bees (A) and honey bees (B) counted on 30 min transect walks during bloom in haskap fields in southern Nova Scotia, 2016 and 2017. Error bars show SEM.....</p> | 40 |

Figure 9. Comparison of single visit pollen deposition on haskap stigmas for bumble bees, honey bees, and control (virgin stigmas). Boxplots show interquartile range, median, data range, and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD. 41

Figure 10. Comparison of visits per minute of haskap flowers for bumble bees and honey bees. Boxplots show interquartile range, median, data range, and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD. 42

Figure 11. Comparison of the percentage of the time that bumble bees and honey bees visit both haskap flowers in the double flower inflorescence. Boxplots show interquartile range median data range and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD..... 43

Figure 12. Mean pollen composition (%) of the sample collected in pollen traps placed on the entrance of honey bee colonies. Haskap pollen (*Lonicera*) comprised of approximately 1.3% of the samples..... 44

Figure 13. Mean nest tube occupancy over time by solitary bees in nest boxes surrounding haskap orchards in southern Nova Scotia, 2017. Error bars for each date show SEM 47

Figure 14. Comparison of number of capped off nest tubes per haskap orchard in southern Nova Scotia. Nest tubes were categorized based on spacing apart from one another and the diameter of the nest tube. Two different size nest tubes (6mm and 8mm) were used and two different spacings (snug and spaced) were tested. Boxplots show interquartile range median data range and outliers..... 48

Figure 15. Total number of emerged bees and wasps from capped nest tubes in an environmental chamber from haskap orchards in southern Nova Scotia..... 49

Abstract

Haskap is an emerging crop in Nova Scotia that relies on cross-pollination for fruit production. There are concerns regarding what native pollinators are available to supplement managed honey bees, and what pollinators are best-suited for haskap in NS due to the early blooming period. In spring of 2016 and 2017, pollinator availability, effectiveness and efficiency were measured by pan trapping, transect walking, single visit pollen deposition, flower visits per minute, and tendency to visit both flowers in the double inflorescence. The usefulness of solitary bee nesting cavities with 6 mm and 8 mm nest tubes arranged snug and spaced were also evaluated. My results indicate that although there are 10 genera of bees that are found in haskap orchards during haskap bloom, only honey bees and bumble bees appear to be important pollinators of the crop. My results also show that honey bees can be effective pollinators of haskap, but bumble bees appear to be the most efficient pollinators. I also demonstrated that solitary bees predominantly nested in 6 mm nest tubes compared to 8 mm nest tubes, and there was no effect of spacing. I also noticed high levels of parasitism in the nest tubes. We recommend that honey bees and commercially managed bumble bees should be used to supplement pollination services from wild bumble bees to ensure maximum pollination success. The use of solitary bee nest boxes is not recommended.

Keywords: haskap, pollination, honey bees, bumble bees, solitary bee

List of Abbreviations and Symbols Used

| | |
|------------|---|
| NS: | Nova Scotia |
| ha: | hectare (1 ha = 10,000m ²) |
| cm: | centimeter (1 cm = 1 x 10 ⁻²) |
| mm: | millimeter |
| et al: | and others |
| mL: | milliliter |
| L: | liter |
| °C: | degree Celcius |
| m: | meter |
| SVPD: | single visit pollen deposition |
| s: | seconds |
| h: | hour |
| g: | gram |
| F: | F-value |
| <i>P</i> : | P-value |
| sd: | standard deviation |
| n: | sample size |
| ™: | trademarked |
| α: | level of significance |
| GLM: | General Linear Model |
| SEM: | standard error of the mean |
| km/h | kilometers per hour |
| LSD | Least Significant Difference |
| df | degrees of freedom |

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Chapter 1: Introduction

1.1 Pollination is Important for Agriculture

Pollination is an ecosystem service as well as a production practice that is extensively used and depended on by many farmers around the world for the production of crops (Gallai et al., 2009). Pollination services are provided by wild, free-living animals as well as commercially-managed bees (Kremen et al., 2007). Insects have been estimated to supply pollination services worth up to US \$215 billion per year (Gallai et al., 2009). Social and solitary bees, wasps, flies, beetles, butterflies, and moths comprise the vast majority of the world's pollinators (Vanbergen et al., 2013). Bees are the main driving force behind the successful pollination of the vast majority of agricultural crops and wild plants (Potts et al., 2010) and are estimated to be responsible for 35% of global food production (Klein et al., 2007). Of the many food crops that require pollination to produce fruit, haskap, an emerging fruit crop in Canada, relies on insect mediated cross-pollination to produce a viable berry crop.

1.2 Haskap

The genus *Lonicera* (f. Caprifoliaceae) includes around 180 species including shrubs with climbing or non-climbing stems. A few species, such as *Lonicera caerulea* L., which is commonly referred to as blue honeysuckle, blue-berried honeysuckle, or more commonly in Canada as haskap, produce edible fruits which ripen very early in the growing season (Božek, 2012). Haskap was mentioned as a horticultural plant for the first time in 1894 (Skupień et al., 2009) but it is a relatively new fruit crop to Canada (Bors, 2009).

The haskap berry is oval and elongated in shape and dark navy blue to purple in color (Jin et al., 2006) (Figure 1). It has a flavor described as a combination of blueberries and raspberries (Skupień et al., 2009). Haskap has become popular in Canada in recent years due to the health attributes of its fruit, including its high content of ascorbic acid and bioactive flavonoids, and outstanding frost resistance of plants and flowers (Bors, 2008; Hummer, 2012; Plekhanova, 2000). Haskap shrubs are very frost hardy; plants are not damaged even if the temperature drops below -40°C (Božek, 2012) and haskap flowers can tolerate spring temperatures as low as -8°C (Plekhanova, 2000). Haskap is suited primarily for high latitudes and colder climates (Hummer, 2006). In Canada, Borealis, Indigo Gem, and Tundra are the most common varieties planted, which were bred and selected at the University of Saskatchewan for their superior fruit characteristics and resistance to disease (Bors, 2007; Rupasinghe et al., 2012).

Haskap has a characteristic feature of a double flower inflorescence typical of other species in the genus *Lonicera* (Frier et al., 2016a) (Figure 1). The ovaries of each flower are enclosed by bracteoles and the haskap berry forms from the combined structures, creating a single compound fruit from the pollination of two flowers (Frier et al., 2016a) (Figure 1). If only one flower of the inflorescence is pollinated, fruit set still occurs but fruit set is lower, and the resulting fruit is typically smaller with fewer seeds (Frier et al., 2016a). Due to being self-incompatible, two unrelated varieties of haskap (different cultivars) need to be in close proximity for cross-pollination to occur (Bors, 2008, Božek, 2012). The movement of pollen between haskap cultivars is predominantly dependent on insect visitors (Božek, 2012). Haskap flowers have been found to have early stigma receptivity, high nectar production, long floral longevity and anther

dehiscence (4-5 days) (Frier et al., 2016b). These floral traits maximize opportunities for pollination by being attractive for a range of insect pollinators.



Figure 1. Haskap plant, flowers and fruit. (A) Example of haskap shrubs in an orchard. Different sized shrubs are different cultivars which is required for cross pollination. (B) Example of a double flower inflorescence. (C) Haskap fruit produced by successful pollination. (D) Example of a haskap berry where bracteoles have not fused around the ovaries, showing the two distinct berries that make up the single compound fruit.

Haskap bloom typically begins in late April to early May in southwest Nova Scotia (NS), which corresponds with the period of high pollen demand by bee colonies for production of brood and spring build up (Bozek, 2007). In NS, haskap bloom typically occurs for about 3 weeks (Cynthia Swinimer, Personal Communication, Lahave

Natural Farms). Honey bees, bumble bees, and occasionally solitary bees can be visitors of haskap flowers (Bozek, 2007; Frier et al., 2016a). Since pollination of the plant is insect-mediated, the use of commercially-managed and native pollinators in haskap production is considered imperative to successful fruit set and harvest of haskap berries. Ensuring successful pollination is a concern of many haskap growers in the region due to the early bloom period. There are concerns of what pollinators are available early in the season, and how the weather early spring impacts the use of commercially managed bees.

1.3 The European honey bee (*Apis mellifera*)

The European honey bee (*Apis mellifera*; Hymenoptera: Apidae) is the most common managed pollinator in North America (Southwick & Southwick, 1992) and is capable of increasing yield in 96% of animal-pollinated crops (Aguilar et al., 2006). The honey bee dominates crop pollination worldwide and are often the most important pollinator of agricultural and horticultural crops (Abrol, 2012; Potts et al., 2010). When wild bees do not visit agricultural fields, or their populations are inadequate to visit all flowers, managed honey bees are often the best solution for farmers to ensure crop pollination. Therefore, over time farmers have become accustomed to incorporating honey bees into their pollination programs (Klein et al., 2007). The body size and proboscis length of honey bees enables them to forage on many types of flowers and their wide host range permits them to pollinate many types of crops (Abrol, 2012). Honey bees are versatile, economical, mobile, have large numbers of individuals in colonies, and are generally convenient compared with the management of several wild bees; attributes that make them a good species to use in commercial pollination (Klein et al., 2007). Despite their value in crop pollination, the European honey bee is only one of more than 20,000

species of bees worldwide (Michener, 2000) and many other bee species are well-or better-suited than honey bees to pollinate certain crops (Garibaldi et al., 2013). As a result, there has been increased interest in evaluating the effectiveness of non-*Apis* pollinators in commercial cropping systems.

1.4 Non-*Apis* Pollinators

The contributions of wild bee populations to crop pollination is often ignored and probably underestimated (Corbet et al., 1991). Fruit set increases significantly with wild insect visitation in many crop systems and can be twice that produced with visitation by honey bees only (Garibaldi et al., 2013). Native pollinators are often more efficient pollinators than honey bees and contribute substantially to pollination of many crops, including coffee (*Coffea arabica*) (Klein et al., 2003), tomato (*Lycopersicon esculentum*) (Greenleaf & Kremen 2006), canola (*Brassica napus*) (Morandin & Winston 2005), raspberries and blackberries (*Rubus idaeus* and *Rubus occidentalis*) (Cane, 2005), cherry (*Prunus avium*) (Bosch & Kemp, 1999; Holzschuh et al., 2012), lowbush blueberry (*Vaccinium angustifolium*) (Javorek et al., 2002), as well as haskap (*Lonicera caerulea*) in western Canada (Frier et al., 2016a). In some agricultural situations, native bee communities could provide full pollination services, even for crops with heavy pollination requirements, without the intervention of managed pollinators (Kremen et al., 2002; Winfree et al., 2007). Unfortunately, for cropping systems in many regions, the contribution of non-*Apis* pollinators to pollination is unknown, and therefore honey bees are often used by default, regardless of whether or not they are the best pollinators for the job (Frier et al., 2016a).

In Saskatchewan, Frier et al. (2016a) determined that *Bombus* queens are highly effective haskap pollinators when compared to managed honey bees and *Osmia lignaria*. In NS, *Bombus* queens and *Andrena* have been found to be more efficient pollinators than honey bees in lowbush blueberry, and bees in the genera *Bombus*, *Andrena*, and *Halictus* successfully pollinate lowbush blueberry flowers a higher percentage of the time than honey bees (Javorek et al., 2002). *Bombus* and *Andrena* deposit larger amounts of pollen per single visit in wild blueberries than honey bees (Javorek et al., 2002). Stubbs & Drummond (2001) found that commercially managed bumble bees were a suitable alternative to honey bees for pollination of lowbush blueberry. Bumble bees are good pollinators of many crops because they are large, have many hairs to move pollen, and “buzz pollinate” by vibrating the anthers of flowers resulting in release of large amounts of pollen (Kawai & Kudo, 2009). Bumble bee queens that are available early in the growing season are more cold tolerant than honey bees and solitary bees, and can forage in temperatures as low as zero degrees centigrade (Heinrich, 2004). This may make them important pollinators in haskap in NS, but the performance of pollinators for haskap pollination in this region has not been studied.

A major disadvantage for growers of sole reliance on native pollinators is that they may not always be available or synchronize with the bloom of the crop. In Birch Hills Saskatchewan, three genera of native bees (*Bombus*, *Halictus*, and *Lasioglossum*) and two genera of managed bees (*Apis* and *Osmia*) were observed visiting haskap flowers in May (Frier et al., 2016a). In NS, there have been 26 reported genera of bees comprising 157 species (Sheffield et al., 2003). However, not all of these genera are available for pollination early in the growing season and wild populations can vary from

year to year. In NS, apples typically bloom in the middle of May and are visited by 42 bee species which are almost entirely all within the genera *Andrena*, *Lasioglossum*, and *Bombus*, as well as the commercially managed honey bee (*A. mellifera*) (Sheffield et al., 2003). Lowbush blueberry in NS has at least 78 species of bee visitors, but it blooms slightly later than apple in NS (Sheffield et al., 2003). Cutler et al. (2015) captured 95 species of bees belonging to 13 genera in blueberry fields in NS, where the majority of bees were within the genera *Lasioglossum* and *Andrena*. As the season progresses in NS, more species of bee pollinators become available. Although apple blooms fairly early in the year, haskap blooms even earlier, at a time when there may be fewer pollinators available to provide pollination services. Pollinator community structure assessments have not been done during haskap bloom in NS, and this is a knowledge gap for haskap growers in NS.

1.5 Pollinator Community Structure Assessments

A variety of methods are used to quantify the diversity and abundance of pollinator communities, though pan trapping and transect walks are the most commonly used. Pan traps are colored plastic bowls filled with soapy water that are attractive to bees. They have been used to evaluate the diversity and abundance of pollinators and other flower visiting insects. Pan trapping is inexpensive and, relative to net collecting, requires fewer man-hours, is not dependent on trained collectors, captures higher numbers of species across seasons, and presumably eliminates collector bias (Leong & Thorp, 1999; Wilson et al., 2008). Pan traps are particularly useful for capturing small wild pollinating insects (Brittain et al., 2010) that could be missed by traditional netting due to their small size and relative inconspicuousness. Pan trap color can influence relative abundance of

pollinators captured (Barker et al., 1997; Leong & Thorp, 1999) and therefore it is a good idea to use a variety of colors when pan trapping. Although pan traps are useful to evaluate the availability of pollinators in landscapes, a major limitation of this method is that pan traps do not inform the collector of what insects are actively visiting flowers of the plant of interest.

Direct observation counts along transects are commonly used with pan trapping to determine diversity and abundance of pollinating insects (Brittain et al., 2010; Cane et al., 2000; Kruess & Tschardtke, 2002). Bumble bees and honey bees are better quantified using transect walks than pan traps (Brittain et al., 2010) due to their large size and ability to sometimes escape from the pan traps. Limitations of pollinator transect walks are the time involved, that skilled observers are needed to identify pollinators, and that they are not useful to determine the most efficient and effective pollinators of the crop.

1.6 Pollinator Effectiveness and Efficiency

1.6.1 Single Visit Pollen Deposition

Determining the effectiveness of pollinators is important when trying to compare how valuable certain pollinators are for particular crops. A commonly used indicator for pollinator effectiveness is single visit pollen deposition (SVPD). This involves exposing virgin stigmas to a single visit by a pollinator, and then harvesting the stigmas and counting how many grains of pollen were deposited during the visit (Kearns & Inouye, 1993). This is commonly done in the field by bagging plants prior to bloom to prevent insect visitation, or under controlled conditions in a laboratory or in a flight cage (Kearns & Inouye, 1993). If waiting time is long, flowers can be removed from the plants and can

be presented to the pollinators (Kearns & Inouye, 1993). It is also of interest in pollination studies to collect pollen samples from foraging bees to determine what plants they are foraging on in the field.

1.6.3 Pollinator Efficiency

Foraging behavior of flower visitors is important because it can influence many aspects of the plant-pollinator interactions (Kearns & Inouye, 1993). The rate of floral visits alongside the patterns of pollinator movement between floral visits can affect the energy budgets of pollinators, gene flow, and more importantly, the success of pollination (Kearns & Inouye, 1993). Floral visitation rate is typically measured as pollinator visits/unit of time, and is commonly quantified by direct observation (Kearns & Inouye, 1993). The pattern of pollinator movement is also important because it may determine many important characteristics of the resulting crop yield. This is particularly important for haskap that exhibits a double flower inflorescence. If both flowers in the inflorescence are pollinated, fruit set occurs 86% of the time and the resulting fruit is typically larger and has more seeds. If only one flower of the inflorescence is pollinated, fruit set typically occurs a lower percentage of the time (64%), and the fruit are typically smaller with fewer seeds (Frier et al., 2016a).

1.7 Solitary Bee Nest Boxes

Knowledge of native bee nesting habits is needed to conserve and promote pollination services of native bees in crops and plant communities (Cane et al., 2007). Artificial nesting cavities or trap-nests are useful for surveying cavity-nesting bees (MacIvor, 2017). If an effective and efficient pollinator is numerically inadequate to supply

pollination services, expanding populations by assuring or introducing adequate nesting resources may be a feasible approach to enhance the pollination services supplied by that pollinator (Cane et al., 2007). Cavity nesting bees in the family Megachilidae (particularly *Osmia* and *Megachile*) can be provided with artificial or natural nesting substrates to promote pollination in agriculture from alternative pollinators, which have been implemented with relatively good success (Cane et al., 2007). A number of Megachilidae can be exploited to provide pollination services for specific crops (Sheffield et al., 2008).

Megachilidae are solitary nesting bees. In each nesting cavity, a reproductive female will build brood cells in a linear series from the back of the tunnel to the front. Each cell is provisioned with a nectar-pollen mixture as food for larvae (Gathmann et al., 1994; MacIvor, 2017). Depending on the species, bees will partition each brood cell using materials that are taxon-specific, such as cut pieces of leaves, tree resins, mud, or pebbles (Cane et al., 2007; Gathmann et al., 1994). Bees select cavities that match their body width to ensure the brood cells fit snugly, and to reduce pathways for parasites to access brood deeper in the nest (MacIvor, 2017). *Megachile* and *Osmia* typically prefer tube diameters between 5 and 8 mm (MacIvor, 2017).

Artificial nest boxes have been used to study cavity nesting bees for more than a century (MacIvor, 2017). Many styles and variations of artificial nest boxes for cavity nesting solitary bees are used, however the basic features of all include a sheltered set of a few to several hundred nesting cavities made of porous materials such as wood, cardboard or polystyrene opened at one end and approximately 10–20 cm in length (MacIvor, 2017). Studies that use solitary bee nest boxes are used to study bees in a

natural setting or for building populations to obtain bees for pollination services or for experimental research (MacIvor, 2017). In the Annapolis Valley of NS, Sheffield et al. (2008) tested the influence of nest tube size on solitary bee occupancy using milk cartons to house the nest tubes. Sheffield et al. (2008) found that the most common captured species of solitary bee, *Osmia tersula* Cockerell preferred nest tubes with 5 mm diameter and 15 cm length, but also nested in 3mm diameter tubes. In addition, later season *Megachile* bees preferred tubes larger than 5 mm. McCallum (2017) found relatively good occupancy using 7 mm diameter tubes made out of paper that were 15 cm long and spaced apart. In a three year study, Sheffield et al. (2008) changed the nest tube orientation design from snugly bundled nest tubes for the first year, to equally spaced nest tubes for the following two years because it was believed that the absence of spacing discouraged nesting. There have been no studies that examined the effect of nest tube spacing on solitary bee nest occupancy.

Solitary bee nest boxes have been used in agricultural systems in NS to determine if they are a viable tool to promote pollination services by wild pollinators. Some species of *Osmia* have even been released into apple orchards in NS and showed potential to improve pollination of apple (Sheffield, 2014). Sheffield et al. (2008) found that trap nests can be used to increase and maintain cavity nesting bee populations in NS apple orchards. Sheffield et al. (2008) also noted that *O. tersula* was the most abundant species of solitary bee found in the trap nests and comprised nearly half of all bees captured. McCallum (2017) investigated the potential of solitary bee nesting cavities in lowbush blueberry fields in NS and had good occupancy with 34% occupancy in nest boxes made

out of milk cartons. Since some *Osmia* species are early season nesters, there may be potential to use trap nesting in haskap pollination.

1.8 Objectives and Hypotheses

In Canada, little is known about the haskap pollination system. One study done by Frier et al. (2016a) in Saskatchewan determined that wild bumble bee queens were the most effective and efficient pollinators of haskap, and that honey bees may be used to supplement haskap pollination. However, the bee fauna and climate in the spring is different in NS compared to Saskatchewan, and therefore it is of interest to study the haskap pollination system in NS. Currently in NS, the majority of haskap growers rely on commercially managed honey bee colonies for haskap pollination. It is unclear how effective or efficient honey bees are at pollinating haskap in NS due to climatic conditions during haskap bloom not favoring honey bee pollination. Therefore, wild bees may be important for haskap pollination in NS. It is also unclear what wild pollinators are available to supplement honey bees for haskap pollination.

The purpose of this research was to determine the availability of pollinators for haskap pollination in southern NS, to determine the performance of available pollinators for haskap pollination, and to test methods of attracting early season, solitary bee pollinators to haskap orchards. This involved pan trapping in haskap orchards to determine the availability of potential pollinators, transect walking during haskap bloom to determine the most significant pollinators of haskap, measuring pollinator performance characteristics and pollen collection to determine the effectiveness and efficiency of important pollinators, and finally the placement of solitary bee nest boxes on the edge of

haskap orchards to test the usefulness of certain nest tube configurations in attracting solitary cavity nesting bees and to determine if cavity nesting bees coincide with haskap bloom. Understanding the pollination system of haskap in southern NS can assist growers in selection of suitable pollinators to fit their pollination requirements but will also assist in the decision of what methods to implement to promote pollination services of wild, free-living pollinators.

The objectives and hypotheses of this research were to:

- i. To assess the diversity and abundance of native, early season pollinators in haskap orchards that may provide pollination services and to determine what pollinators are the most influential to haskap pollination in southern NS. I hypothesized that there will be a limited range of pollinators available for haskap pollination due to the early blooming period of the plants, but large pollinators including bumble bees (*Bombus spp.*) and commercially maintained honey bees (*A. mellifera*) will be important pollinators of haskap flowers.
- ii. To measure the effectiveness and efficiency of the most influential pollinators through single visit pollen deposition, pollen collection from honey bee hives, pollinator flower visits per minute, and the tendency of the pollinator to pollinate both flowers. I hypothesized that wild bumble bee queens will be the most effective and efficient pollinators of haskap but commercially maintained honey bees will also contribute to haskap pollination.
- iii. To evaluate the usefulness of solitary bee nesting cavities placed on the edge of haskap orchards to attract native, early season solitary nesting bees and to

assess the influence of nest tube size and spacing on solitary bee nest occupancy. I hypothesized that solitary bee nesting cavities will be useful to attract native early season pollinators. I also hypothesized that the 6 mm spaced nest tubes will have the most solitary bee occupancy, and there will be more capped nest tubes in the spaced design, compared to the snug design.

Chapter 2: Materials and Methods

2.1 Pollinator Surveys

2.1.1 Study Sites

Pollinator surveys were done in 2016 and 2017 in three haskap orchards belonging to Lahave Natural Farms, a haskap growing group from Lunenburg county, NS (Table 1). All three orchards used for this study were located near Blockhouse, NS (44°26'54.2"N 64°25'20.1"W). All three haskap orchards were stocked with commercial honey bee colonies which were placed on the edge of each haskap orchards in a single location. Stocking densities of honey bee hives was roughly the same for all orchards, at approximately 0.4 hives per ha. In 2017, more hives were stocked in Honeyberry Hurst, equaling approximately 0.8 hives per hectare, while stocking density in Silver Hurst and Lohnnes was consistent. Honeyberry Hurst was a more mature orchard at the time of sampling compared to Silver Hurst or Lohnnes. Many haskap plants were 4-5 years old at Honeyberry Hurst compared to plants that were 2-3 years old at Silver Hurst and Lohnnes. In all orchards, Borealis, Tundra and Indigo Gem were the cultivars planted as well as the cultivar Honeybee, which is used as a pollinizer.

Table 1. Haskap orchards used for pollinator surveys in Nova Scotia, 2016 and 2017.

| Field | Area (ha) | Geographic Co-ordinates |
|-------------------------|------------------|--------------------------------|
| Honeyberry Hurst | 6.36 | 44°28'02.9"N 64°32'15.8"W |
| Silver Hurst | 5.32 | 44°28'45.3"N 64°35'08.3"W |
| Lohnnes | 1.97 | 44°29'24.4"N 64°35'55.0"W |

2.1.2 Pan Trapping for Pollinators

In 2016 and 2017, pan traps were used to study the haskap pollinator community at each field. Plastic bowls (355 ml; Solo Cup Company, Urbana, Illinois, U.S.A.) were filled half to two-thirds full with water and two drops of unscented dish soap was added per bowl to help break the surface tension of the water so that insects trapped in the water would not escape. Blue, yellow, and white bowls were used for sampling to collect a range of pollinators.

In 2016, pan traps and were placed along 3 transects in each of the three field sites. The pan traps were placed 3 m apart set up in a straight line running parallel to the crop rows at ground level (Figure 2). The transects were placed between crop rows, but within close proximity to haskap bushes. Pan traps were placed in areas that had the least amount of human disturbance (roughly the center of each field) and transects were placed five crop rows apart. Pan traps were set out between 8:00 a.m. and 4:00 p.m. on sampling days. Sampling was typically only done on days that were sunny, with temperatures more

than 12⁰C, and low wind. However, because haskap bloom occurred during a short window of time, and since the crop blooms early in the season, these sampling requirements were not met on all sampling days. Thirty pan traps per transect were used in two sampling periods pre-bloom. Because the collection during the pre-bloom period showed that a lot of bumble bee queens were being killed in pan trap collections, it was decided that only 12 pan traps per transect would be used instead of 30 traps for the consecutive bloom and post bloom sampling periods. Five collections were done during bloom and three were done post bloom. In 2016, pan trap sampling spanned from 21 April to 21 June. Bees were stored in ethanol, and returned to the laboratory for counting and identification. Bees captured were identified to genus using the bee genera of eastern Canada key (Packer et al., 2007) and Discover Life online key (Discover Life, 2017). Pollinating flies were identified to family.

The pan trap sampling procedure was modified in 2017 to look at field edge effects. In 2017, two blocks of pan traps were placed in each of the three field sites. Each block consisted of three transects of pan traps that each contained three pan traps, one of each pan trap color. The pan traps were placed approximately 3 m away from each other within each transect and the transects were placed 3 m apart from each other, such that each block was a 9 m x 9 m block of nine pan traps (Figure 2). Pan trap color was randomly assigned in transects during each sampling period. In each of the three fields, one block of pan traps was placed on the west edge of the field, in attempt to keep the pan traps away from solitary nest boxes that were deployed on the north edge of the field (see below). A second block of nine pan traps was placed near the center of each field, approximately 60 m from the block situated on the western edge of each field. Sampling

collection times and conditions were as described above, with two collections done pre-bloom, five collections done during bloom, and three done post bloom. In 2017, pan trap sampling spanned 21 April to 8 June. Bees were identified as described above.

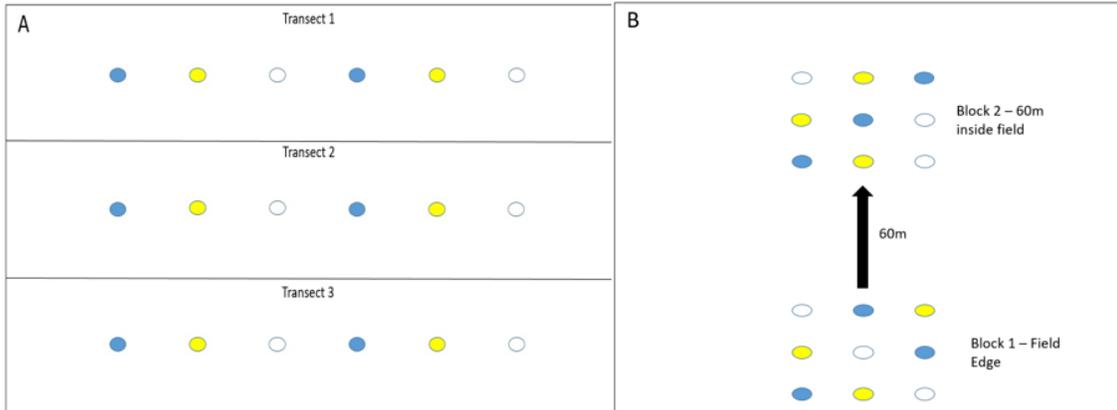


Figure 2. Map illustration showing how pan traps were placed in haskap orchards in 2016 (A) and 2017 (B). (A) 3 transects containing 12 pan traps spaced 3 m apart alternating colors of blue, yellow and white. Transects were spaced 25 m (5 rows) apart and were 36 m long. (B) 2 blocks of 9 pan traps placed 3 m apart. Blocks were 9 m long, and 9 m wide. 1 block on the field edge and one block 60 m inside the field. Pan trap colors were randomly assigned a location within each block.

2.1.3 Transect Walks for Pollinators

Transect walks were done in 2016 and 2017 to determine the most common pollinators in the haskap fields. This method typically groups pollinators into higher taxa levels since species identification can be difficult. Transect walks were done the same days during bloom as pan trap sampling. One 30 m transect along a haskap row per field was observed for 30 minutes at each of the three haskap orchards locations described above (Table 1), in the afternoon when most bees were active and flying (personal observation) and were done on the same day for all orchards. Transects were set up away from areas of high human activity in the fields and sampled as quietly and as inconspicuously as possible to obtain a representative sample. One pass was used per transect, and one

observer counted bees along transects to maintain consistency. Pollinators observed were classified as bumble bees, honey bees, solitary bees, or pollinating flies (Figure 3).

Pollinators were only counted if they were actively foraging on haskap flowers.



Figure 3. Examples of pollinators collected in haskap fields in Nova Scotia, 2016 and 2017. (A) honey bee, (B) bumble bee, (C) solitary bee, (D) hover fly.

2.2 Effectiveness and Efficiency of Potential Pollinators

2.2.1 Floral Visit Pollen Deposition

Counts of SVPD from visits to virgin flowers were used to measure pollinator effectiveness of haskap. In spring 2016, pairs of haskap flower buds were bagged using pollinator exclusion bags in the Honeyberry Hurst farm before they opened, such that pollination could not occur unless bags were removed. Pollinator exclusion bags were

made of 1 mm white nylon mesh fabric (Fabricville, Montreal Quebec) designed to exclude pollinators from accessing open flowers. Bags were 10 cm wide and 15 cm long. Before haskap flower buds opened, exclusion bags were slipped over a pair of haskap flowers and fastened to the branches using twist ties. Although pollen may have been able to be transferred by wind to the stigmas, this amount was taken into account by adding a control no pollination treatment (bags always closed) to the experiment. After flowers opened within a bag, the bag was removed and the flower was presented to a bee. This was done by removing the virgin flower, finding a bee in the haskap field, near the bagged flower and presenting the flower to the bee for pollination. Once a flower was pollinated by a bee, the stigma was carefully removed, with care to minimize dislodging pollen from the stigma, and mounted on a microscope slide in basic fuchsin gel (modified from Kearns & Inouye, 1993). Honey bees and bumble bees were used for SVPD measures because they were much more abundant in the haskap orchards than solitary bees or flies, and it was impossible to present a flower to a solitary bee or fly without them flying away. When mounting the stigmas in fuchsin gel, approximately 1 cm³ block of fuchsin gel prepared in the lab was placed on a microscope slide. The removed stigma was then carefully placed on top of the cube of fuchsin gel. Using a pocket lighter, the microscope slide was slowly and carefully heated so that the gel melted into a liquid form without burning. Once the fuchsin gel was melted, a coverslip was carefully placed on top of the melted gel and haskap stigma. The microscope slide was labelled and stored in a horizontal position until the fuchsin gel hardened. Microscope slides were returned to the lab and observed under a digital dissection microscope at 5.6X magnification. The number of pollen grains deposited was counted on the microscope slide and was used to

infer the single visit pollen deposition rates for honey bees and bumble bees. I counted the pollen grains myself with the help two summer students who verified that my counting was accurate to ensure consistency.

This experiment was repeated in 2017 at the Honeyberry Hurst orchard, but instead of removing flowers and presenting them to bees, the bag was removed and the flower remained on the plant for the pollinator to come visit. Unfortunately, this method was not effective and yielded no substantial data from non-*Apis* bees. Only data on honey bees were collected using this method in 2017.

2.2.2 Pollinator Flower Visits per Minute

Flower visits per minute were used as an indicator of pollinator efficiency. In 2016 and 2017 honey bees and bumble bees were observed and followed during haskap bloom on the Honeyberry Hurst Farm to determine the number of flowers visited in 60 seconds. If the pollinators went out of sight of the observer for any period of time during the 60 seconds, the pollinator was not followed anymore and the data were not used in analyses. Data from 2016 and 2017 were grouped together for statistical analysis, where the observation year was not used as a factor of interest since year would not effect the foraging efficiency of pollinators.

2.2.3 Pollinator Tendency to Visit Both Flowers in the Double Inflorescence

Although fruit set is still possible if only one of the flowers in the double flower inflorescence is pollinated, previous research has found that fruit set is higher if both flowers are pollinated (Frier et al., 2016a). It is therefore of interest to determine if the key pollinators of haskap in southern NS have a tendency to visit one or both flowers of

the inflorescence. In the spring of 2017, honey bees and bumble bees were observed and followed during haskap bloom at the Honeyberry Hurst Farm to determine if they had a tendency to visit both flowers of the inflorescence or if they had a tendency to visit only one flower of the inflorescence. To do this, pollinators were followed until they visited 10 different inflorescences, and each time it was recorded how often the pollinator visited both flowers in the inflorescence. If a bee went out of sight of the observer before 10 inflorescences were visited, the data were not used in the analysis. The number of times that each pollinator visited both flowers was divided by 10 and multiplied by 100 to get a percentage value used for statistical analysis.

2.2.4 Pollen Trapping

On 17 May 2017, three pollen traps (Figure 4) were placed on honey bee hives in Honeyberry Hurst Farm and Lohnnes Farm during haskap bloom. On the day that the pollen traps were placed on the hives, it was a sunny with daily high temperature of 19.6 °C and 30 km/h maximum winds. Pollen trapping was done only one day in order to limit the impact of pollen trapping on the hives, and was only done on hives deemed to be strong by previous visual inspection. Plastic front-mount pollen traps (Country Fields Beekeeping Supplies, Nova Scotia) remained on hives for approximately 24 h to gather pollen. The collected pollen was stored on ice, returned to the laboratory, and placed in a freezer at -20 °C until it was sent away for morphometric analysis.



Figure 4. Example of an entrance mounted pollen trap used to collect pollen from returning honey bee foragers during haskap bloom.

Pollen samples collected in 2017 were analyzed morphometrically and identified by Johanne Parent at the Université de Montréal in Rimouski, Quebec. To do so, frozen pollen samples were set at room temperature to thaw, after which 2 g of pollen was weighed into a 50 mL centrifuge tube. Distilled water was added to bring the volume within the tube to approximately 40 mL. The tube was capped and shaken on a vortex mixer to completely mix the sample. Depending on the time of dissolution of the pollen loads, the tube was set out for a few hours, agitating the sample from time to time. For samples with less than 2 g, the entire sample was weighted and an appropriate dilution ratio was used (Johanne Parent, Personal Communication, Laboratoire BSL).

When the dissolution was complete, the sample was agitated using a vortex mixer for 60 s. A small drop of the solution was put on a slide using a glass pipette (Barth et al., 2010). The slide was put on a warm histology plate (not over 65°C), and a small cube of

glycerin jelly stained with basic fuchsin was added to the preparation, which was then stirred delicately with a mounted needle until the cube was completely melted and the preparation is homogenous. A cover glass was put over the preparation and a drop of melted paraffin was added to seal the slide. Clear nail polish was used to seal the cover slip onto the slide (Johanne Parent, Personal Communication, Laboratoire BSL)

When dried, the slide was turned upside down and a vertical line was drawn passing through the center of the drop. Pollen grain identification began near the center line then towards one side of the slide at 1000X magnification. Once the slide was scanned all the way to the right, the slide was moved up by one microscope field of view, then the slide was scanned for pollen grains as the field of view moved left on the slide until the center line was reached. Again the slide was adjusted and moved up by one microscope field of view and the slide was scanned moving to the right again creating an “S” pattern that was followed continuously until 500 pollen grains (or palynomorphs) were identified. From this, percentages were calculated for each pollen form identified (Johanne Parent, Personal Communication, Laboratoire BSL).

2.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees

2.3.1 Nest Tube Style

The usefulness of solitary bee nest cavities placed on the edge of haskap orchards to attract native, early season solitary nesting bees was assessed in 2016 and 2017. In 2017, a second objective with the nest box design was to evaluate the influence of nest tube size and nest tube spacing on solitary bee nest occupancy. In 2016, bee nest boxes were placed on the edge of three haskap orchards located in Lunenburg County, NS

(Honeyberry Hurst, Silver Hurst and Lohnnes) (Table 2). In 2017, bee nest boxes were placed on the edge of seven haskap orchards located in Lunenburg County, NS (Table 2).

Table 2. Haskap orchards used for placement of trap nests in Nova Scotia, 2016 and 2017.

| Field | Field Owner | Area (ha) | Geographic Co-ordinates |
|---------------------------|----------------------|----------------------|--------------------------------|
| Honeyberry Hurst | Lahave Natural Farms | 6.36 | 44°28'02.9"N 64°32'15.8"W |
| Silver Hurst | Lahave Natural Farms | 5.32 | 44°28'45.3"N 64°35'08.3"W |
| Lohnnes | Lahave Natural Farms | 1.97 | 44°29'24.4"N 64°35'55.0"W |
| Barrs Corner | Richard Joudrey | 1.77 | 44°33'43.3"N 64°40'32.7"W |
| Lonetree | Haskapa | 18.08 | 44°28'10.4"N 64°39'24.9"W |
| Lahave River Berry | Chris Berry | 0.21 | 44°19'08.5"N 64°23'41.3"W |
| Mahone Bay | Brian O'Kane | 1.48 | 44°27'12.6"N 64°22'21.7"W |

In 2016, nest boxes were constructed following a design used by Robyn McCallum (Robyn McCallum, Personal Communication). The bee nest boxes were constructed by inserting 7 mm paper nest tubes into clean, white painted 2-L milk cartons. The paper nest tubes were created by rolling newspaper over a piece of 21 x 10 cm white paper. The paper nest tubes were made 7 mm in diameter by rolling them

around a 0.7 cm diameter wooden dowel rod, and taping them together using a piece of Scotch™ tape (3M, St. Paul, Minnesota). The tubes were cut to 15 cm long. Sixteen of the tubes were inserted equidistant apart into a piece of 2.5 cm purple Styrofoam cut 9.5 x 9.5 cm square that was inserted into the front of the milk carton. Spray foam insulation (Home Hardware, St. Jacobs, Ontario) was sprayed around the nest tubes to provide structure. Fifteen nest boxes per field (three clusters of five nest boxes) were placed in each of the three haskap orchards. At each field, nest boxes were placed approximately 1 m apart within a cluster, and clusters of nest boxes were placed approximately 50 m apart.. The nest boxes were placed on the north side of each field along the tree line. The opening of each nest box faced south and the nest tubes were tilted down slightly to allow water to run off. The nest boxes were mounted on wooden stakes and placed approximately 1 m off the ground. Tangle foot® (The Ortho Group, Marysville Ohio) was added near the bottom of the stake to inhibit any insects that may chew the nest tubes or tear open the capped nest tubes. Nest boxes were placed in the field on 21 April 2016 and checked once every 2-3 weeks for capped nest tubes. During the nesting season, long grass was cut around the nest boxes once a month. The nest boxes remained in each of the haskap orchards until 27 September 2016 when the total number of capped tubes was recorded and the boxes were brought back to the lab.

Due to poor occupancy in 2016, possibly due to design issues favoring moisture retention in the nest boxes, in 2017 the nest boxes were created differently. Each nest box was constructed using a 10 cm diameter piece of white PVC pipe that was cut 23 cm long (Figure 5). A sewer cap was used to close off one end of the PVC tube. A 10 cm diameter piece of 2.5 cm purple Styrofoam™ was inserted into the front of each nest box

containing 12 nesting tubes. Spray foam insulation (Home Hardware, St. Jacobs, Ontario) was sprayed around the nest tubes to provide stability.

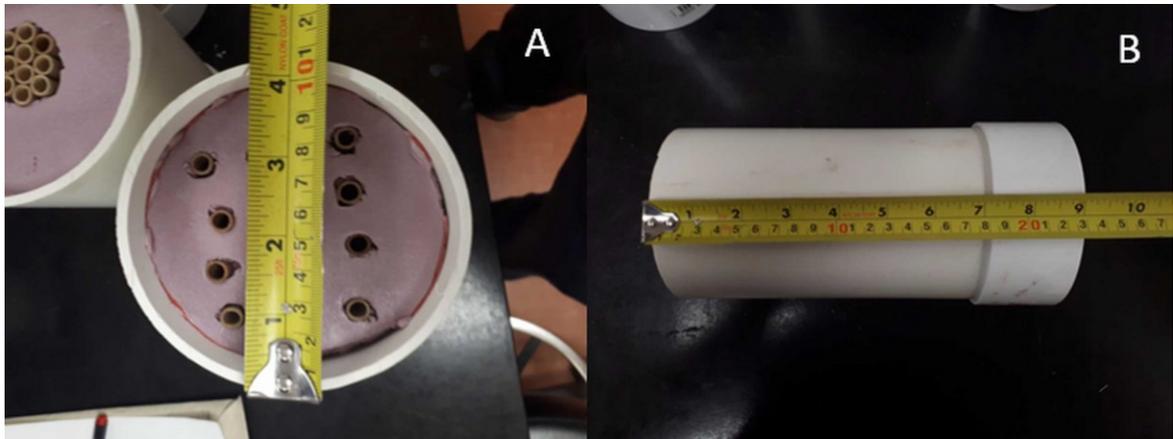


Figure 5. Dimensions of the trap nest design used in 2017. Trap nests were constructed out of 10 cm diameter PVC pipe (A) and were cut to be 23 cm long (B).

The nest tubes used for the solitary bee nest boxes were cardboard tubes that were purchased from Crown Bees (Woodinville, WA). Two different diameter cardboard tubes (6 mm and 8 mm) were purchased to determine the influence of nest tube size on solitary bee occupancy. The influence of nest tube spacing was also studied with two distinctive arrangements: nest tubes that were bundled together with no spacing (snug), and nest tubes spread equidistant at 2.5 cm apart (spaced). This resulted in four different style trap nest styles (Figure 6).

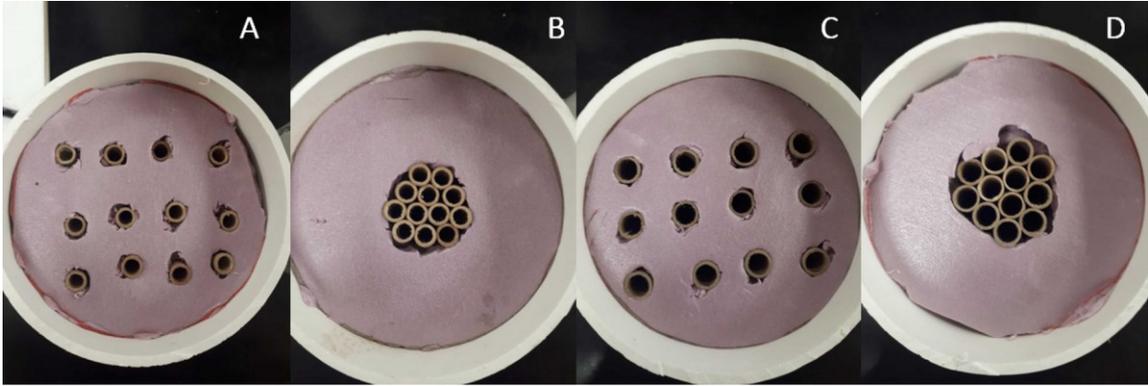


Figure 6. Nest tube configurations that were tested in haskap orchards for 2017. (A) 6 mm spaced, (B) 6 mm snug, (C) 8 mm spaced, (D) 8 mm snug.

Twelve nest boxes per field (three clusters of four nest boxes) were placed in each of the seven haskap orchards. At each field, nest boxes were placed approximately 1 m apart from each other within a cluster, and clusters of nest boxes were placed approximately 50 m from each other. Within each of the clusters, one of the four different types of nest boxes was randomly assigned to a location. The nest boxes were placed on the north side of each field along the tree line. The opening of each nest box faced south and the nest tubes were tilted down slightly to allow water to run off. The nest boxes were mounted on wooden stakes and placed approximately 1 m off the ground. Tangle foot® (The Ortho Group, Marysville Ohio) was added near the bottom of the stake to capture any insects that may chew the nest tubes or tear open the capped nest tubes.

Nest boxes were placed in the field on 13 April 2017 and checked once every 2-3 weeks for capped nest tubes. During the nesting season, long grass was cut around the nest boxes once a month. The nest boxes remained in each of the haskap orchards until 13 September 2017, at which time the total number of capped nest tubes of each type was recorded. Once the capped nest tubes were returned to the laboratory, they were stored in 4°C cold storage. A subgroup of the nest tubes were dissected to attempt to identify

haskap pollen in the nest tubes, but the solitary bees had already reached the pupal stage of development and therefore all of the pollen provision in the tubes had been consumed so none was available for analysis. The original design of this experiment was a 2 x 2 factorial arrangement of nest tube size (6 mm, 8 mm) and nest tube spacing (snug, spaced). However, there was very little occupancy in the 8 mm nest tubes for both spacing arrangements and therefore data from the 8 mm nest tubes were not statistically analyzed.

2.3.2 Hatching Out Bees

Nest boxes were collected from the haskap orchards 13 September 2017. Nest boxes that contained one or more capped tubes were stored in 4⁰ C cold storage (MacIvor & Packer, 2015) until 5 March 2018. The cardboard nest tubes were then removed from the nest boxes, labelled, and placed in separate inflated plastic bag in a growth chamber (McCallum, 2017). Only nest tubes with capped cells that were not damaged by presumably predation or parasitism were used for the experiment. After dissecting a subset of the nest tubes and omitting any damaged nest tubes, this left 50 nest tubes that were used for incubation in the environmental growth chamber. The temperature of the growth chamber was initially set at 8 °C on 5 March 2018, then warmed with daily increments of 4 °C up to 24 °C by 9 March 2018. After 10 days at 24 °C, the temperature was increased to 30 °C for the remainder of the experiment (Robyn McCallum, Personal Communication) until 2 April 2018. Relative humidity was maintained at 60% throughout the hatching period (MacIvor & Packer, 2015; McCallum, 2017). Bees and wasps that emerged were placed in the freezer and later pinned and identified to family for wasps, and genus or species for bees.

2.4 Data Analysis

Pan trapping occurred on multiple dates during pre-bloom, bloom and post bloom across multiple fields for both 2016 and 2017. For 2016, all collections were standardized to a per day measure for each collection site (haskap orchard) for each sampling period. In 2017, with the addition of the factor pan trap location (0 m or 60 m from edge), I standardized all collections to a per day measure for each collection site, for each sampling location and for each sampling period. For both years, each pollinator genus or family was used as a separate response variable. In 2016 sampling period was used as a fixed factor for each response variable, and the mean capture per day per haskap orchard was used for replication. In 2017 sampling period, sampling location, and the interaction between sampling period and sampling location were used as fixed factors for each response variable using orchard for replication. Only pollinators that were captured with mean captures per day of at least one individual were used for analysis due to some pollinators being captured infrequently during sampling. For each response variable, analysis of variance was used to examine the main effects and interaction effect where appropriate using proc GLM in SAS v. 9.4 (SAS Institute Inc., 2014). Assumptions of normality of error terms and constant variance of residuals were verified using Minitab 18 statistical software (Minitab, 2018). Multiple means comparison where appropriate were done using Fishers Least Significant Difference (LSD) at $\alpha = 0.05$.

Data analysis for transect walks was done using Proc MIXED in SAS v. 9.4 (SAS Institute Inc. 2014). Assumptions of normality of error terms and constant variance of residuals were verified using Minitab 18 statistical software (Minitab 2018). Transect walk count was used as the response variable, and data were square root transformed to

normalize the residuals and give constant variance. Pollinator category, year, and the interaction between pollinator category and year were used as fixed factors, and haskap orchard was a blocking factor and was modeled as a random factor. Multiple means comparison was done using Fishers LSD at $\alpha = 0.05$

Data analysis for SVPD was done using Proc GLM in SAS v. 9.4 (SAS Institute Inc. 2014). Assumptions of normality of error terms and constant variance of residuals were verified using Minitab 18 statistical software (Minitab 2018). Pollinator (honey bee, bumble bee, control) was used as a fixed factor in the model, and the number of grains of pollen deposited was used as the response variable. Multiple means comparison was done using Fishers LSD at $\alpha = 0.05$.

Data analysis for flower visits was done using Proc GLM in SAS v. 9.4 (SAS Institute Inc. 2014). Assumptions of normality of error terms and constant variance of residuals were verified using Minitab 18 statistical software (Minitab 2018). Pollinator (honey bee, bumble bee) was used as a fixed factor in the model, and the number of flowers visited per minute was used as the response variable. Multiple means comparison was done using Fishers LSD at $\alpha = 0.05$.

Data analysis for tendency to visit both flowers was done using Proc GLM in SAS v. 9.4 (SAS Institute Inc. 2014). Assumptions of normality of error terms and constant variance of residuals were verified using Minitab 18 statistical software (Minitab 2018). Pollinator (honey bee, bumble bee) was used as a fixed factor in the model, and the percent of the time that both flowers were visited was used as the response variable. Multiple means comparison was done using Fishers LSD at $\alpha = 0.05$.

Data analysis for preference for nest tube spacing was done using Proc GLM in SAS v. 9.4 (SAS Institute Inc. 2014). Although this experiment was originally designed as a factorial experiment, due to very limited occupancy in the 8 mm nest tubes, only the 6 mm nest tubes were used for analysis. Therefore, for overall nesting preference, spacing was the only fixed factor used for analysis. The number of capped nest tubes was used as the response variable. Although three groups of nest boxes were set out per field, I determined the total number of capped nest tubes per field for each nest tube style, and used field as a replicate. To determine if there was a particular preference for nest tube spacing at different observation times, I added observation date into the analysis and looked at nest tube spacing, date, and the interaction between spacing and date. Assumptions of normality of error terms and constant variance of residuals were verified for both analyses using Minitab 18 statistical software (Minitab 2018).

Chapter 3: Results

3.1 Pollinator Surveys

A total of 630 bees were captured in pan traps between 2016 and 2017 (Table 3). In 2016, 362 bees belonging to 9 genera were captured. In 2017, a total of 268 bees belonging to 10 genera were captured. The most common captured pollinators in general were bees in the genera *Andrena*. In 2016 and 2017, 41.7% and 34.4%, respectively, of all bees collected before, during, and after bloom were bees in the genera *Andrena*. The four most common pollinators, *Andrena*, *Apis*, *Bombus*, and *Lasioglossum* made up the majority of pollinators captured during bloom, comprising 87.0% of all pollinators captured during bloom in 2016 and 75.7% of all pollinators captured during bloom in 2017. Two families of pollinating flies, Syrphidae and Bombyliidae, were also captured in pan traps, but were captured in low numbers in both 2016 and 2017. In 2016, 0, 12 and 12 Syrphidae were captured pre-bloom, during bloom and post bloom respectively and 0, 5 and 0 Bombyliidae were captured pre-bloom, during bloom and post bloom respectively. In 2017, 0, 12 and 12 Syrphidae were captured pre-bloom, during bloom and post bloom respectively and 0, 14 and 0 Bombyliidae were captured pre-bloom, during bloom and post bloom respectively.

Generally, the majority of the taxa were collected during bloom, but not always. For instance, in 2016 the largest number of *Bombus* queens was captured pre-bloom compared to during bloom or post bloom (Table 3). Also noteworthy, with the exception of *Lasioglossum* and the single *Sphecodes* captured, most Halictidae (*Agapostemon*, *Augochlorella*, and *Halictus*) were captured post bloom. *Osmia*, which are well known as

early season pollinators, were not captured in high numbers in pan traps at any period of sampling.

Table 3. Wild and managed pollinators captured in pan traps from three haskap orchards in southern Nova Scotia, 2016 and 2017.

| Family | Genus | Bloom Status | Number of Specimens Collected (% of Overall Capture per Year) | | Total Number of Specimens Collected (% of Overall Capture for 2016 and 2017 Combined) |
|------------|----------------------|--------------|---|-----------|---|
| | | | 2016 | 2017 | |
| Andrenidae | <i>Andrena</i> | Pre-Bloom | 38 (10.5) | 5 (1.9) | 43 (6.8) |
| | | Bloom | 105 (29.0) | 68 (25.4) | 173 (27.5) |
| | | Post Bloom | 8 (2.2) | 19 (7.1) | 27 (4.3) |
| Apidae | <i>Apis</i> | Pre-Bloom | 5 (1.4) | 2 (0.7) | 7 (1.1) |
| | | Bloom | 29 (8.0) | 8 (3.0) | 37 (5.9) |
| | | Post Bloom | 10 (2.8) | 6 (2.2) | 16 (2.5) |
| | <i>Bombus</i> | Pre-Bloom | 21 (5.8) | 2 (0.7) | 23 (3.6) |
| | | Bloom | 16 (4.4) | 9 (3.4) | 25 (4.0) |
| | | Post Bloom | 1 (0.3) | 1 (0.4) | 2 (0.3) |
| | <i>Nomada</i> | Pre-Bloom | 1 (0.3) | 4 (1.5) | 5 (0.8) |
| | | Bloom | 5 (1.4) | 13 (4.9) | 18 (2.9) |
| | | Post Bloom | 1 (0.3) | 0 (0) | 1 (0.2) |
| Halictidae | <i>Agapostemon</i> | Pre-Bloom | 0 (0) | 0 (0) | 0 (0) |
| | | Bloom | 0 (0) | 0 (0) | 0 (0) |
| | | Post Bloom | 5 (1.4) | 1 (0.4) | 6 (0.9) |
| | <i>Augochlorella</i> | Pre-Bloom | 1 (0.3) | 1 (0.4) | 2 (0.3) |
| | | Bloom | 0 (0) | 10 (3.7) | 10 (1.6) |
| | | Post Bloom | 10 (2.8) | 14 (5.2) | 24 (3.8) |
| | <i>Halictus</i> | Pre-Bloom | 0 (0) | 1 (0.4) | 1 (0.2) |
| | | Bloom | 3 (0.8) | 4 (1.5) | 7 (1.1) |
| | | Post Bloom | 20 (5.5) | 6 (2.2) | 26 (4.1) |

| Family | Genus | Bloom Status | Number of Specimens Collected (% of Overall Capture per Year) | | Total Number of Specimens Collected (% of Overall Capture for 2016 and 2017 Combined) | |
|----------------|---------------------|--------------|--|------------|---|---------|
| | | | 2016 | 2017 | | |
| | <i>Lasioglossum</i> | Pre-Bloom | 17 (4.7) | 18 (6.7) | 35 (5.6) | |
| | | Bloom | 35 (9.7) | 37 (13.8) | 72 (11.4) | |
| | | Post Bloom | 30 (8.3) | 33 (12.3) | 63 (10) | |
| | <i>Sphecodes</i> | Pre-Bloom | 0 (0) | 0 (0) | 0 (0) | |
| | | Bloom | 0 (0) | 1 (0.4) | 1 (0.2) | |
| | | Post Bloom | 0 (0) | 0 (0) | 0 (0) | |
| | Megachilidae | <i>Osmia</i> | Pre-Bloom | 0 (0) | 2 (0.7) | 2 (0.3) |
| | | | Bloom | 0 (0) | 3 (1.1) | 3 (0.5) |
| | | | Post Bloom | 1 (0.3) | 0 (0) | 1 (0.2) |
| Totals | | Pre-bloom | 83 (22.9) | 35 (13.0) | 118 (18.7) | |
| | | Bloom | 193 (53.3) | 153 (57.1) | 346 (55.0) | |
| | | Post Bloom | 86 (23.8) | 80 (29.9) | 166 (26.3) | |
| Overall | | | 362 (57.4) | 268 (42.6) | 630 (100) | |

In 2016, the four most common genera of pollinators, *Andrena*, *Apis*, *Bombus*, and *Lasioglossum* had mean per day captures of 1-7 individuals for all bloom periods (Table 4). One exception was *Bombus*, which were not captured very often post bloom (Table 4). *Andrena*, *Apis*, *Bombus* and *Lasioglossum* were captured during all sampling periods. All other bee genera, and the two families of pollinating flies (Syrphidae and Bombyliidae) were captured infrequently during sampling and were not considered for analysis.

There was a significant difference between the captures per day for *Andrena* in 2016 during the sampling periods. *Andrena* captures per day (mean \pm SEM) pre-bloom

(6.33 ± 1.86) and bloom (7.00 ± 0.95) were significantly higher than post bloom (0.66 ± 0.33) (Table 4). There was no significant difference in mean captures per day of *Apis*, *Bombus* or *Lasioglossum* pre-bloom, during bloom or post bloom (Table 4).

Table 4: Mean number of pollinators captured per day during three bloom periods in haskap orchards in southern Nova Scotia, 2016.

| Pollinator | Mean Number of Pollinators Captured per Day (mean \pm SEM) | | | F-value | df | P- value |
|---------------------|---|-------------------|--------------------|---------|-----|--------------|
| | Pre-Bloom | Bloom | Post Bloom | | | |
| <i>Apis</i> | 0.83 ± 0.60^a | 1.93 ± 0.47^a | 1.11 ± 0.47^a | 1.57 | 2,6 | 0.28 |
| <i>Bombus</i> | 3.50 ± 1.61^a | 1.06 ± 0.41^a | 0.11 ± 0.11^a | 3.32 | 2,6 | 0.11 |
| <i>Andrena</i> | 6.33 ± 1.86^a | 7.00 ± 0.95^a | 0.66 ± 0.33^b | 8.18 | 2,6 | 0.019 |
| <i>Lasioglossum</i> | 2.86 ± 1.30^a | 2.33 ± 2.33^a | 3.33 ± 0.193^a | 0.11 | 2,6 | 0.90 |

^a Means within rows with the same letter are not significantly different according to Fishers LSD at P = 0.05

In 2017, the addition of pan trap location was added to the analysis. There was no significant interaction between sampling period and pan trap location for any of the pollinators, nor was there a significant effect of location for any of the pollinators (Table 5). Bee captures per day on the field edge did not differ from those 60 m into the field for *Apis* (0.31 ± 0.09 bees at 0 m; 0.16 ± 0.08 bees at 60 m), *Bombus* (0.20 ± 0.10 bees at 0 m; 0.14 ± 0.06 bees at 60 m), *Andrena* (1.57 ± 0.35 bees at 0 m; 0.98 ± 0.36 bees at 60 m), or *Lasioglossum* (1.48 ± 0.35 bees at 0 m; 1.57 ± 0.30 bees at 60 m). There was a significant effect of sampling period for *Andrena* only in 2017 (Table 5).

Table 5: Analysis of variance *P*-values that show effect of sampling period (pre-bloom, bloom, post bloom), location (edge, 60 m inside field) and the interaction on pollinator pan trap collections per day for 2017 in haskap orchards in southern Nova Scotia.

| Pollinator | Source of Variation | | |
|---------------------|---------------------|----------|-----------------|
| | Period | Location | Period*Location |
| <i>Apis</i> | 0.75 | 0.25 | 0.59 |
| <i>Bombus</i> | 0.29 | 0.63 | 0.93 |
| <i>Andrena</i> | 0.006 | 0.13 | 0.53 |
| <i>Lasioglossum</i> | 0.59 | 0.85 | 0.35 |

Fewer individuals were captured per day in 2017 compared to 2016 (Table 6). Similar to 2016, the four most common pollinators were *Andrena*, *Apis*, *Bombus*, and *Lasioglossum*. These pollinators had mean daily captures of less than 1 individual, but as high as 2 individuals for *Andrena* (Table 6). These pollinators were captured during all sampling periods similar to 2016. Also similar to 2016, per day captures of *Bombus* post bloom were very small.

In 2017, there was a significant difference in captures per day for *Andrena* between sampling periods. More *Andrena* were captured during bloom (2.27 ± 0.30) compared to either pre-bloom (0.5 ± 0.32) or post bloom (1.06 ± 0.36) (Table 6). There was no significant difference in mean captures per day of *Apis*, *Bombus* or *Lasioglossum* pre-bloom, during bloom or post bloom (Table 6).

Table 6: Mean number of pollinators captured per day during three bloom periods in haskap orchards in southern Nova Scotia, 2017.

| Pollinator | Mean Number of Pollinators Captured per Day (mean \pm SE) | | | F-value | df | P-value |
|---------------------|---|------------------|------------------|---------|-------|--------------|
| | Pre-Bloom | Bloom | Post Bloom | | | |
| <i>Apis</i> | 0.17 \pm 0.11a ^a | 0.27 \pm 0.12a | 0.27 \pm 0.10a | 0.29 | 2, 12 | 0.75 |
| <i>Bombus</i> | 0.17 \pm 0.10a | 0.30 \pm 0.11a | 0.05 \pm 0.05a | 1.39 | 2, 12 | 0.29 |
| <i>Andrena</i> | 0.5 \pm 0.32b | 2.27 \pm 0.30a | 1.06 \pm 0.36b | 8.01 | 2, 12 | 0.006 |
| <i>Lasioglossum</i> | 1.5 \pm 0.36a | 1.23 \pm 0.48a | 1.84 \pm 0.31a | 0.56 | 2, 12 | 0.59 |

^a Means within rows with the same letter are not significantly different according to Fishers LSD at $P = 0.05$

In both 2016 and 2017, transect walks were completed along the same transects in each field and therefore comparison between years could be made. There was a significant difference across taxa in the mean number of pollinators observed on transect walks during bloom in 2016 and 2017 ($F_{3, 112} = 7.22, P = 0.0002$) (Figure 7). Honey bees and bumble bees were most commonly observed, whereas solitary bees and flies were not often observed visiting haskap flowers. There were only half as many honey bees observed in 2016 as in 2017, but the number of bumble bees observed was relatively consistent in both years.

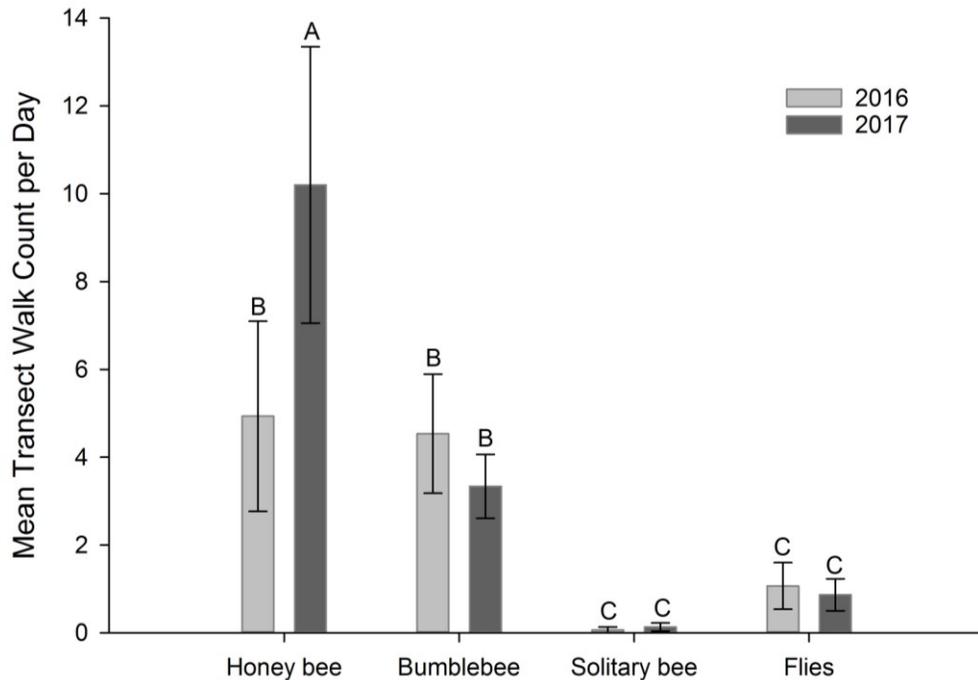


Figure 7. Mean daily number of different pollinator groups counted during 30 min transect walks during bloom across three haskap fields in southern Nova Scotia, 2016 & 2017. Bars with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD. Error bars show SEM.

Although there was no significant difference in the mean number of bumble bee queens counted on transect walks per day in 2016 and 2017, anecdotally there seemed to be fewer bumble bee queens visiting haskap flowers in 2017 than in 2016 during early and mid-bloom. In 2016, the abundance of bumble bee queens counted visiting the haskap flowers increased sharply after the first week of May, whereas in 2017, this increase didn't occur until the second week of May (Figure 8). As a result, in 2017 wild bumble bee queens did not appear to be as abundant until late haskap bloom. The abundance of honey bees in 2016 and 2017 varied between 2016 and 2017 (Figure 8). Generally there were more honey bees observed in 2017 compared to 2016, however there was no trend in the abundance of honey bees observed visiting flowers throughout

the month of May. Similar to bumble bees, there was considerable variability of honey bee abundance between haskap orchards shown by the large error bars (Figure 8).

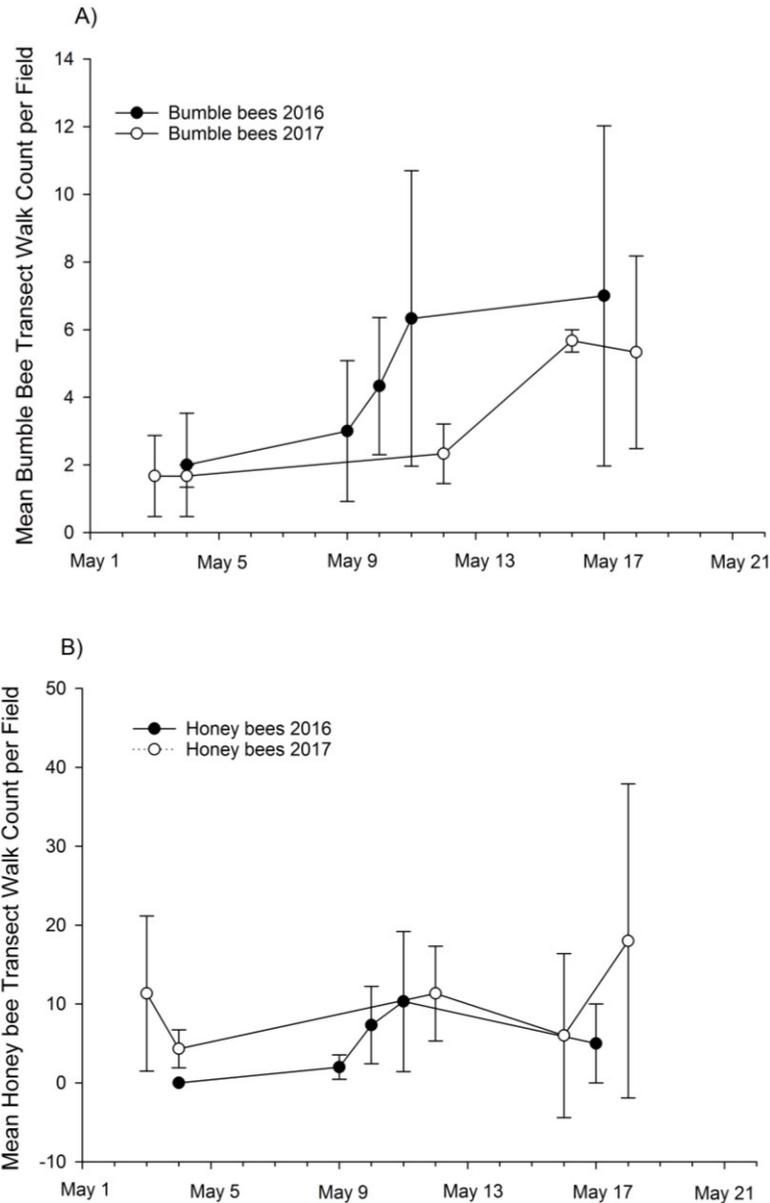


Figure 8. Mean number of bumble bees (A) and honey bees (B) counted on 30 min transect walks during bloom in haskap fields in southern Nova Scotia, 2016 and 2017. Error bars show SEM.

3.2 Effectiveness and Efficiency of Potential Pollinators

There was no significant difference between the average number of pollen grains deposited by honey bees or bumble bees but pollen deposition was greater in both of these treatments than in the control ($F_{2,62} = 8.31, P = 0.0006$) (Figure 9). Honey bees deposited an average of 111.4 pollen grains per stigma ($sd = 71.2, range = 0-335, n = 30$) and bumble bees deposited an average of 95.3 pollen grains per stigma ($sd = 64.5, range = 0-240, n = 27$), compared to only 7.50 pollen grains per stigma in control flowers ($sd = 11.40, range = 0-29, n = 8$) (Figure 9).

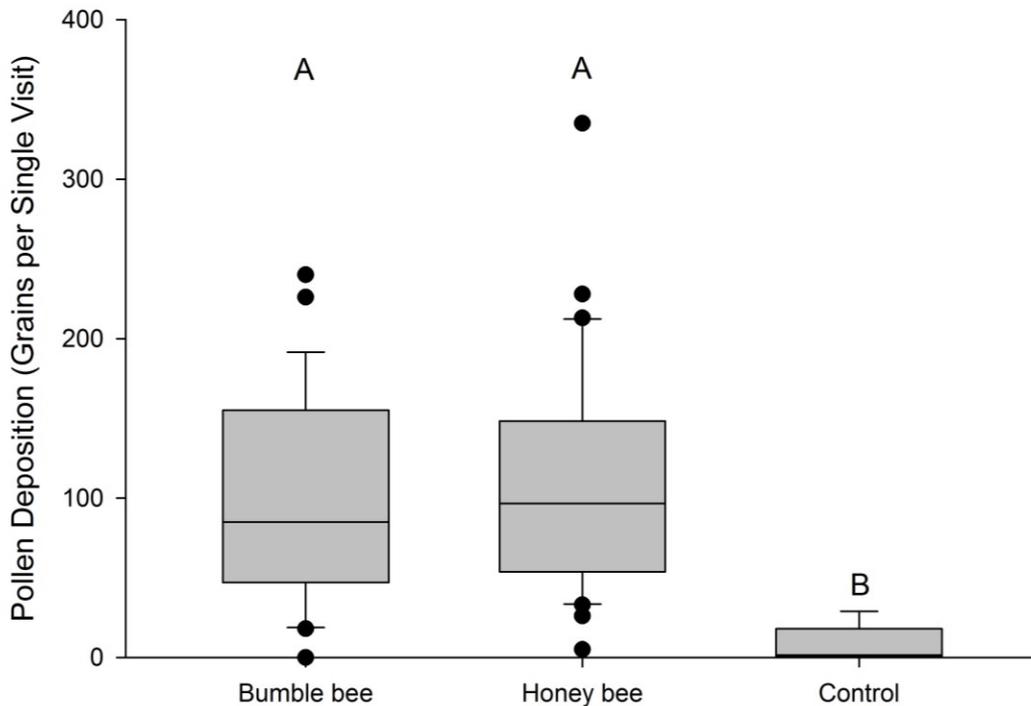


Figure 9. Comparison of single visit pollen deposition on haskap stigmas for bumble bees, honey bees, and control (virgin stigmas). Boxplots show interquartile range, median, data range, and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD.

Bumble bees visited significantly more haskap flowers per minute than honey bees ($F_{1,54} = 127.2, P < 0.001$) (Figure 10). On average, bumble bees visited approximately 27 flowers per minute ($n = 30$) and honey bees visited approximately 9 flowers per minute ($n = 26$): a three fold difference, meaning it took bumble bees approximately 2.2 seconds to move from flower to flower, whereas honey bees took on average 6.7 seconds to move from flower to flower. There was greater variability among bumble bees than honey bees in the number of flower visits per minute (Figure 10).

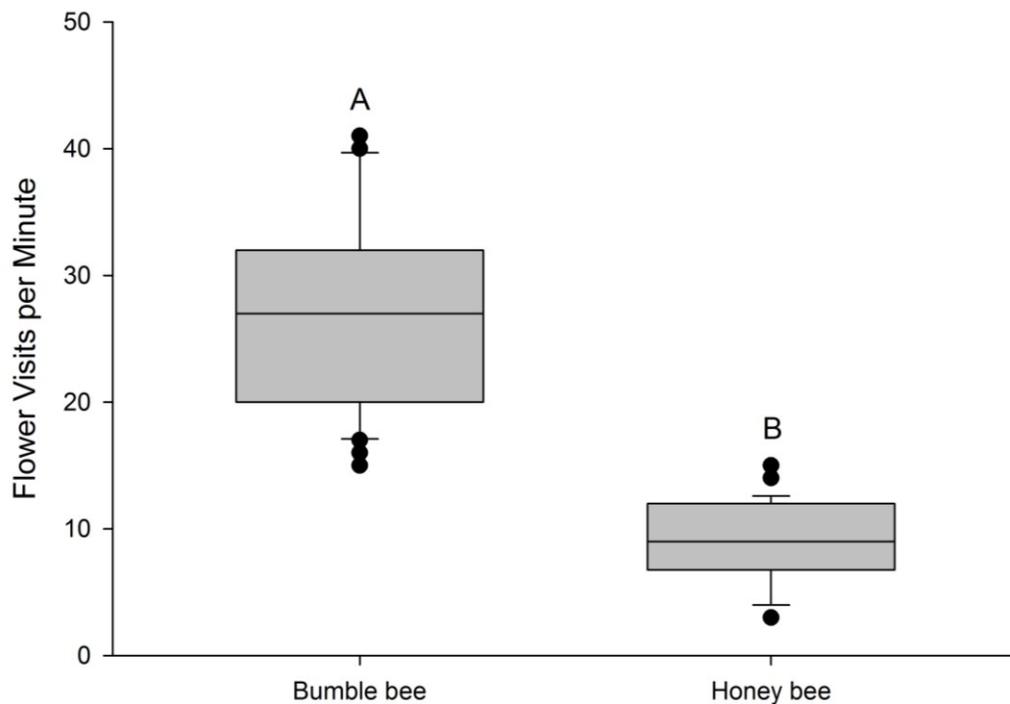


Figure 10. Comparison of visits per minute of haskap flowers for bumble bees and honey bees. Boxplots show interquartile range, median, data range, and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD.

Bumble bees tended to visit both flowers on an inflorescence more often than honey bees ($F_{1,38} = 86.83, P = < 0.0001$) (Figure 11). Bumble bees visited both flowers

an average of 77% of the time (n=20), while honey bees visited both flowers only 35% of the time (n = 20) (Figure 11).

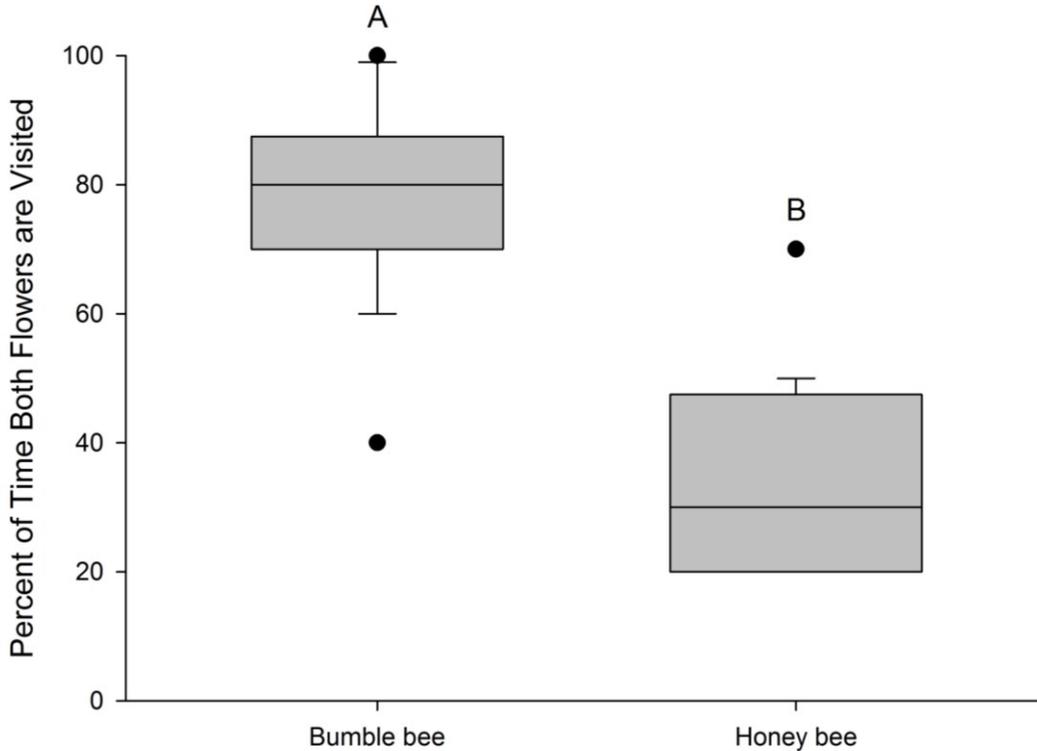


Figure 11. Comparison of the percentage of the time that bumble bees and honey bees visit both haskap flowers in the double flower inflorescence. Boxplots show interquartile range median data range and outliers. Boxes with different letter groupings differ significantly ($\alpha = 0.05$) using Fishers LSD.

Haskap pollen was found in five of six (83.3%) pollen samples collected from pollen traps placed on honey bee hives (Table 7). The pollen samples collected generally were composed of four to seven types of pollen, and only a few pollen grains were unidentifiable (Table 7). Although haskap pollen was present in the majority of the pollen samples collected, haskap pollen made up a relatively small percentage (1.3%) of the total amount of pollen collected (Figure 12). Sugar maple (*Acer saccharum*) made up

45% of all pollen collected. The second most abundant pollen collected was dandelion (*Taraxacum*) (23%) (Figure 12).

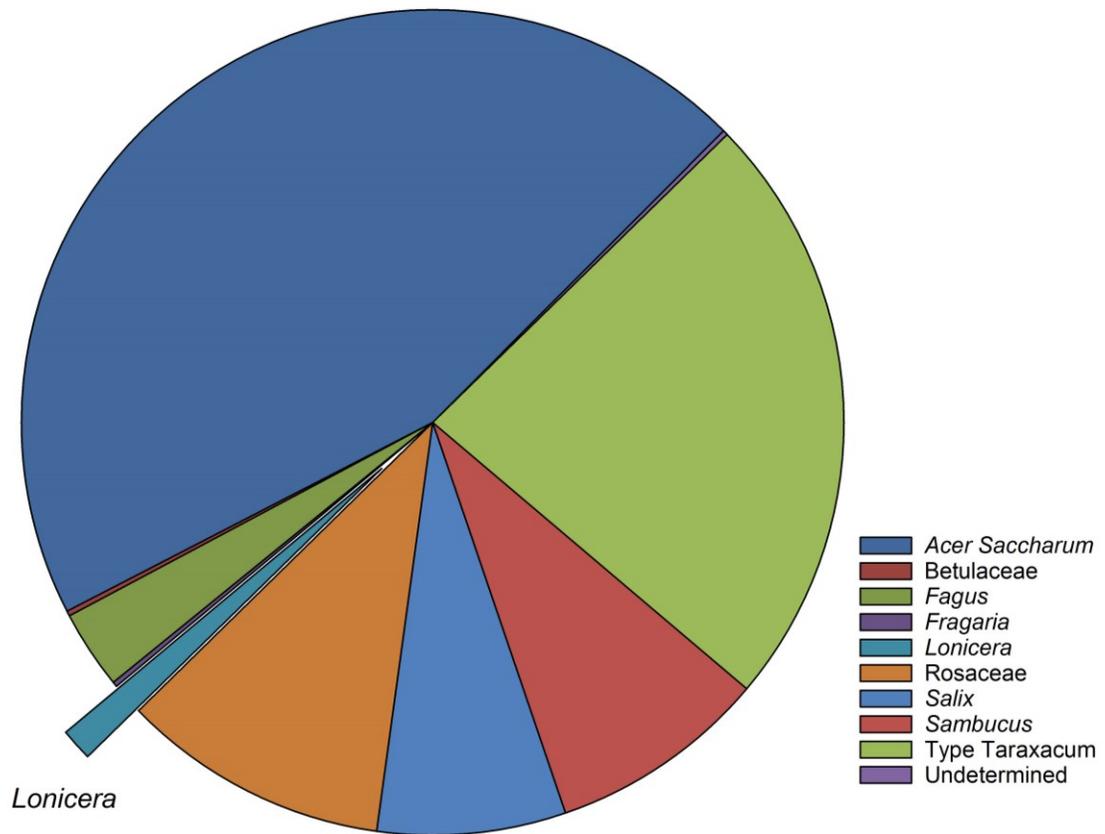


Figure 12. Mean pollen composition (%) of the sample collected in pollen traps placed on the entrance of honey bee colonies. Haskap pollen (*Lonicera*) comprised approximately 1.3% of the samples.

Table 7. Pollen collection from six samples collected by honey bees during haskap (*Lonicera*) bloom 17 May 2017.

| Sample | Identification | Composition of Sample (%) |
|--------------|-------------------------------|---------------------------|
| Honeyberry 1 | <i>Taraxacum</i> ^a | 37.2 |
| | Rosaceae ^b | 22.0 |
| | <i>Acer saccharum</i> | 18.8 |
| | <i>Sambucus</i> | 17.2 |
| | <i>Fagus</i> | 4.2 |
| | Betulaceae | 0.2 |
| | <i>Lonicera</i> | 0.2 |
| | Undetermined | 0.2 |
| Honeyberry 2 | <i>Acer saccharum</i> | 78.6 |
| | <i>Taraxacum</i> ^a | 10.6 |
| | Rosaceae ^b | 7.0 |
| | <i>Fagus</i> | 3.8 |
| Honeyberry 3 | <i>Acer saccharum</i> | 54.2 |
| | <i>Taraxacum</i> ^a | 23.4 |
| | Rosaceae ^b | 20.6 |
| | <i>Lonicera</i> | 1.2 |
| | <i>Fagus</i> | 0.4 |
| | <i>Salix</i> | 0.2 |
| Lohnnes 1 | <i>Acer saccharum</i> | 64.8 |
| | <i>Salix</i> | 14.8 |
| | <i>Taraxacum</i> ^a | 12.6 |
| | Rosaceae ^b | 5.2 |
| | <i>Lonicera</i> | 2.6 |
| Lohnnes 2 | <i>Acer saccharum</i> | 54.2 |
| | <i>Taraxacum</i> ^a | 23.4 |
| | Rosaceae ^b | 20.6 |
| | <i>Lonicera</i> | 1.2 |
| | <i>Fagus</i> | 0.4 |
| Lohnnes 3 | <i>Acer saccharum</i> | 65.0 |
| | <i>Taraxacum</i> ^a | 24.4 |
| | Rosaceae ^b | 7.0 |
| | <i>Lonicera</i> | 1.8 |
| | <i>Fagus</i> | 1.2 |
| | Betulaceae | 0.2 |
| | <i>Sambucus</i> | 0.2 |
| | Undetermined | 0.2 |

^a *Taraxacum* is a large genus of flowers in the aster family, but pollen is most likely dandelion

^b Rosaceae in these samples are fruit tree types

3.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees

Solitary cavity nesting bees occupied nest tubes in all three of the fields in 2016. The first capped nest tubes were noticed 20 June 2016. Total occupancy of capped nest tubes in 2016 was very poor, with only 16 of the available 720 nest tubes (2.2%) were occupied. The nest boxes designed out of milk cartons seemed to hold a lot of moisture, and as a result, the paper nest tubes always appeared damp. This caused a design change in 2017.

Solitary cavity nesting bees occupied nest tubes in six of the seven fields in 2017. In one field there was no solitary bee occupancy, and this field was omitted from the analysis. Total occupancy of capped nest tubes was 13.5% of all tubes available. Occupancy in 6 mm tubes was 25.7% and 8 mm tubes was 1.4%. Excluding the field with no nesting, 26 (72%) nest boxes containing 6 mm tubes had at least one capped nest tube, with up to 10 capped nest tubes (83.3% occupancy in the nest box) being recorded. Only three (8.3%) of the nest boxes with 8 mm tubes contained one or more capped nest tubes.

The first capped nest tubes were observed on 22 June 2017, approximately one month after haskap bloom, and capping was observed up to 13 September 2017. The majority of nesting occurred between 6 July 2017 and 2 August 2017 (Figure 13).

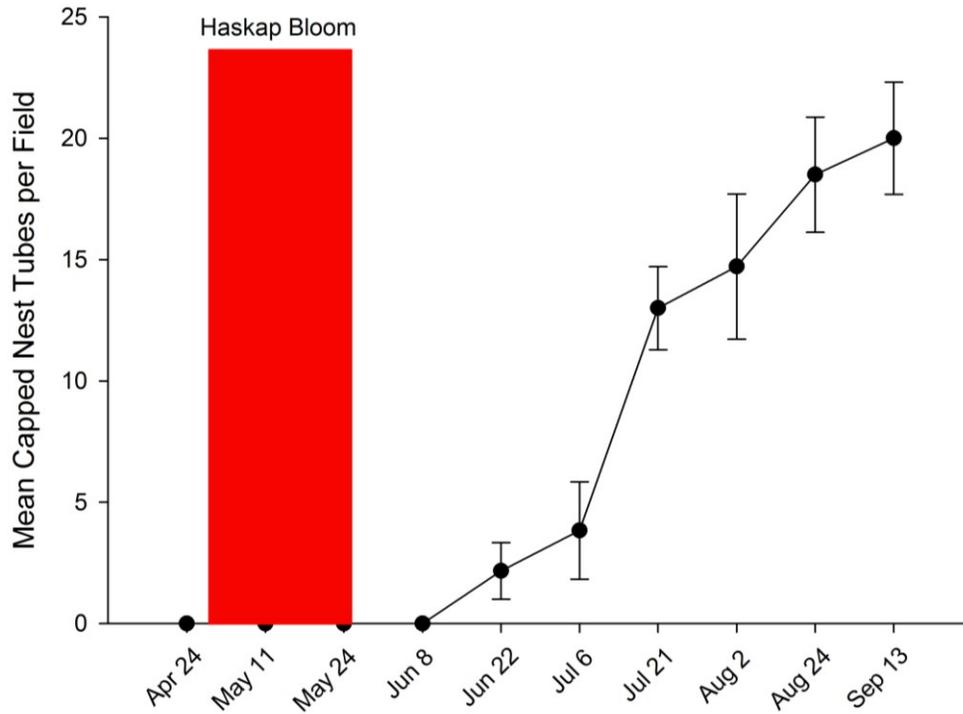


Figure 13. Mean nest tube occupancy over time by solitary bees in nest boxes surrounding haskap orchards in southern Nova Scotia, 2017. Error bars for each date show SEM.

There was an obvious nesting preference for the 6 mm nest tubes compared to 8 mm nest tubes (Figure 14). Since there was very little nesting in 8 mm nest tubes, only data from 6 mm nest tubes were analyzed further. By the end of the season, there was no significant difference in the number (mean \pm SEM) of capped nest tubes in the 6 mm snug style (9.00 ± 2.41) or the 6 mm spaced style (9.50 ± 1.52) ($F_{1,10} = 0.03$, $P = 0.86$) per field (Figure 14). There was also interest in determining if there were more capped nest tubes of a particular style at different observation periods throughout the season. There was no significant interaction between date and spacing ($F_{5,60} = 0.05$, $P = 0.99$) nor was there a significant effect of spacing ($F_{1,60} = 1.47$, $P = 0.23$). Date was significant

($F_{5,60} = 10.95, P < 0.001$), which makes sense because more capped nest tubes were observed over time (Figure 13).

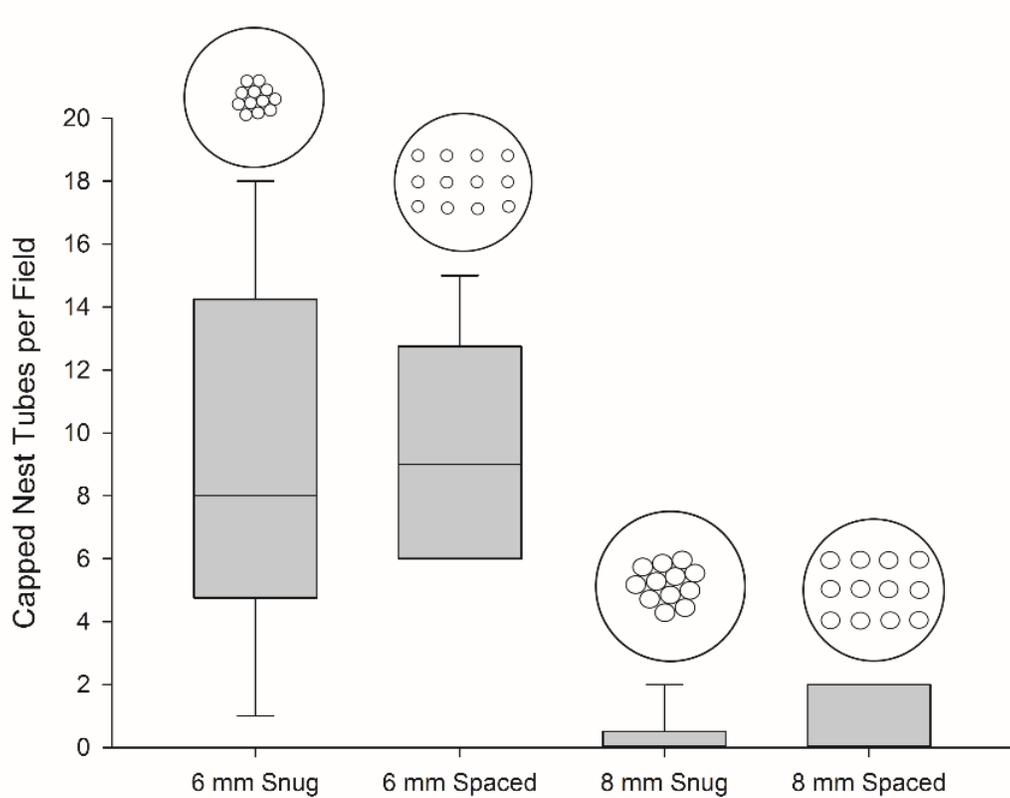


Figure 14. Comparison of number of capped off nest tubes per haskap orchard in southern Nova Scotia. Nest tubes were categorized based on spacing apart from one another and the diameter of the nest tube. Two different size nest tubes (6 mm and 8 mm) were used and two different spacings (snug and spaced) were tested. Boxplots show interquartile range median data range and outliers.

After the period of time in the environmental chamber, a total of 156 insects emerged from the 50 nest tubes that were placed in the chamber. Bees or wasps hatched out from 82% (41 of the 50 nest tubes) that were placed in the environmental chamber: 51 *O. tersula*, 27 leaf cutter bees (*Megachile* spp.), 75 Sapygidae wasps, 2 Chrysidid

wasps and 1 ichneumonid wasp emerged from the tubes. The wasps were the first insects to emerge, beginning on 22 March 2018, 17 days after being placed in the environmental chamber. Wasp emergence was completed by 25 March 2018. The first bees that hatched out were *O. tersula* which began hatching in 26 March 2018. By the 29 March, all bees had emerged (Figure 15). In total, 78 bees and 78 wasps emerged, meaning 50% of all tubes contained kleptoparasitic wasps, with the exception of the ichneumon wasp.

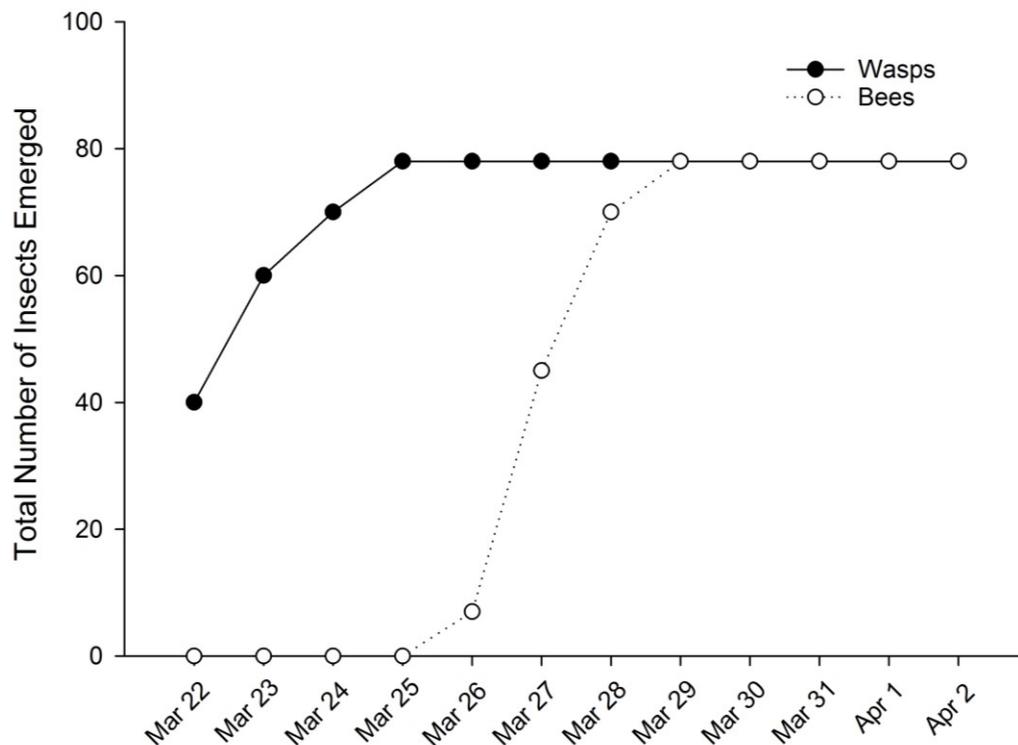


Figure 15. Total number of emerged bees and wasps from capped nest tubes in an environmental chamber after collection from haskap orchards in southern Nova Scotia.

Chapter 4: Discussion

4.1 Pollinator Surveys

Availability of pollinators during pre-bloom, bloom, and post bloom was documented in haskap fields in southern NS in 2016 and 2017. More bees were collected in 2016 compared to 2017, which makes sense because more pan traps were placed out per field in 2016 compared to 2017. In 2017, one individual of one additional genus of bee (*Sphecodes*) was captured during bloom. Despite the difference in layout of pan traps between 2016 and 2017, the same four genera, *Andrena*, *Apis*, *Bombus*, and *Lasioglossum* comprised the majority of pollinators captured, and most of these pollinators were captured during haskap bloom (Table 3). One notable exception to this trend was that in 2016 more *Bombus* queens were captured during the pre-bloom pan trapping period than during bloom, even though pan traps were placed out for 3 extra days during bloom. I believe this happened because during the pre-bloom sampling period in 2016, 30 pan traps per transect were used. When we noticed how many *Bombus* queens were being captured in the pan traps, we decided to decrease the number of pan traps in a transect from 30 to 12. For most genera, there were more pollinators captured in 2016 compared to 2017, although genera *Nomada*, *Augochlorella*, and *Osmia* did not follow this trend.

In Saskatchewan, Frier et al. (2016a) observed 5 genera of bees visiting haskap flowers compared to the 9-10 genera that I captured in pan traps (Table 3). Frier et al. (2016a) noticed bees from the genera *Apis*, *Bombus*, *Halictus*, *Lasioglossum*, and *Osmia*, and flies from family Syrphidae. In my pan trapping, I did not capture many *Osmia*, but

Frier et al. (2016a) released *O. lignaria* into haskap orchards to evaluate their performance as haskap pollinators and this could have inflated their estimates of *Osmia*. Although pan trapping in my study was done earlier in the season relative to other pollinator studies in NS, others have found similar results. Forty-two species of bees may visit apple in NS, and the vast majority of those species belong to the genera *Andrena*, *Lasioglossum* and *Bombus* (queens) (Sheffield et al., 2003). These are the same three genera of wild bees that comprised the vast majority of pollinators captured in pan traps during haskap bloom. In lowbush blueberry fields, Cutler et al. (2015) collected 95 species of bees from 13 genera. Similarly, Cutler et al. (2015) found that the majority of bees they captured were *Andrena* and *Lasioglossum*. In my study, I captured all of the same genera as Cutler et al. (2015), but post bloom I also captured 5 *Agapostemon* individuals in 2016, and 1 *Agapostemon* individual in 2017, which Cutler et al. (2015) did not collect. I also captured the same genera as McCallum (2017), however more genera and more bees overall were captured by McCallum (2017) than in my study. Similar to my findings, McCallum (2017) found that the most prominent genera captured in her study were *Lasioglossum*, *Bombus*, and *Calliopsis*, however fairly high numbers of *Halictus* were captured as well. In contrast to my results, McCallum (2017) did not catch many bees from the genus *Andrena*. This difference is likely due to the fact that McCallum (2017) completed her research in a wild blueberry agroecosystem, which blooms a few weeks later in NS than haskap.

Bees from genus *Nomada*, as well as flies in the families Syrphidae and Bombyliidae, were collected from pan traps in my study. *Nomada* species are “cuckoo bees” or parasitic bees that do not forage for pollen and therefore do not often provide

important pollination services (Cane, 1983). With the exception of a few crops, Syrphidae and Bombyliidae do not provide pollination services to the same extent as bees, often visiting flowers less frequently and being less effective pollinators than bees (Rader et al., 2016).

For the four most common bee genera, there was no significant difference in captures between sampling periods for 2016 or 2017 (Tables 4 & 6 respectively). This is important because for most of the common genera, if they are as abundant in haskap orchards pre-bloom and post bloom as they are during bloom, it suggests that these bees are available to provide pollination services if the haskap bloom is early or is delayed. For both years, only *Andrena* showed any significant difference in captures per day between bloom periods. In 2016, more *Andrena* were captured pre-bloom and during bloom compared to post bloom ($P = 0.019$). In 2017 more *Andrena* were captured during bloom compared to pre-bloom or post bloom ($P = 0.006$). This similar trend of fewer bees being captured post bloom is shared with *Bombus*, although the difference was not significant. Bees are dependent on available flowers for food and areas with diverse periods of flowering plants have higher bee diversity (Sheffield et al., 2003). Due to mowing that frequently happens in haskap orchards under organic production, there is a limited amount of forage available in haskap orchards post bloom, and this may explain why fewer bees were captured post bloom. Since honey bees are left in the orchards all season long, it makes sense that collections would not differ between bloom periods. It is interesting that captures of *Lasioglossum* did not reduce post bloom compared to *Andrena* and *Bombus*. This may be explained in part by the limited foraging range of small bees like *Lasioglossum* (Wright et al., 2015). Interestingly, in both years of study, *Andrena*

were captured in high numbers in pan traps during bloom, however these solitary bees were hardly ever seen visiting haskap flowers (Figure 7). We did notice many *Andrena* visiting dandelions in the haskap orchards during bloom, which were very abundant due to the organic production of this crop. Since the pan traps were placed at ground level, there is potential that many of these *Andrena* were captured because they were primarily foraging on dandelion. If the pan traps were elevated closer to crop level, captures of bee genera may have been different. In highbush blueberry, a greater diversity of bees were captured when the pan traps are placed mid canopy compared to ground level or above the canopy (Tuell & Isaacs, 2009).

When pan traps were placed on the edge of the haskap orchard vs. 60 m inside the orchard in 2017, the per day captures of each genera did not differ significantly between locations (Table 5). Contrary to my findings, Cutler et al. (2015) found that overall abundance of pollinators was greater on the edge of lowbush blueberry fields in NS, but generally diversity did not change the further into blueberry fields they collected. However, the difference in abundance found by Cutler et al. (2015) was only noted generally over 100 m from the forest edge. At distances around 50 m from the forest edge, there appeared to be no differences in captures of most bee genera. Chacoff & Aizen (2006) also noticed a trend where there were fewer individual bees captured inside grapefruit plantations than on the edge, but these trends did not begin until the distance was over 100 m from the edge of the field. It is possible that I did not notice a significant difference in captures of bee genera on the edge or 60 m inside the haskap orchard because the distance may not have been great enough to make a difference. Therefore, the

general small size of haskap orchards in NS appears not to be limiting the availability of pollinators in the center of the orchard.

In 2016 and 2017, transect walks were completed during haskap bloom in three haskap orchards to determine the most common pollinators of haskap in southern NS. In both years, honey bees and bumble bees were by far the most common pollinators observed foraging on haskap flowers, with pollinating flies and solitary bees only occasionally observed (Figure 7). These results suggest that honey bees and bumble bees are the most common pollinators of haskap. Even with high numbers of these solitary bees being captured in pan traps during bloom, they were almost never seen visiting haskap flowers. We did notice many *Andrena* and *Lasioglossum* bees, as well as other genera of solitary bees, visiting dandelion in and around the haskap orchards, as well as willows, apples, and cherry blossoms on the edge of the orchards. It therefore seems that these other floral resources are favored by solitary bees over haskap. This is similar to what Frier et al. (2016a) noticed with *O. lignaria* that they released in haskap orchards, where the bees were more interested in other floral sources than haskap. Another reason that solitary bees may not have been noticed was that haskap bushes are quite large with a fair number of large flowers on them. Due to the small size and inconspicuous nature of many solitary bees, there is a possibility, despite our best efforts, that small solitary bees could have been undetected. Quantifying honey bees and bumble bees using transect walks is much easier due to the large size and “clumsiness” of the pollinators, making them easier to pick out on a haskap flower. Nevertheless, due the large size of haskap flowers and the position of the stigma being located so far out of the flower (Figure 1), small solitary bees are not likely ideal pollinators of haskap. This is because many small,

solitary bees would be able to access a nectar or pollen reward from the flower, and may not contact with the stigma during the flower visit.

We noticed that in 2017 there were far more honey bees than bumble bees visiting haskap flowers, whereas the results for each species were fairly similar in 2016 (Figure 7). This is most likely due to an increase in the number of honey bee colonies that were placed at the Honeyberry Hurst orchard. The stocking density for Silver Hurst and Lohnnes orchard stayed approximately the same in 2016 and 2017. It is possible that performing transect walks during favorable foraging conditions influenced how many honey bees that we observed. If transect walk counting was done during unfavorable foraging conditions for honey bees, there would likely be more bumble bees counted than honey bees. Interestingly, although more honey bees were counted visiting haskap flowers in 2017 compared to 2016, this trend was not noticed using pan traps, where the trend was actually opposite. In Saskatchewan, Frier et al. (2016a)'s findings support mine and show that honey bees were the most common visitor of haskap during fair weather conditions.

Due to the large numbers of honey bees in the haskap orchards, work done by Frier et al. (2016a) as well as my own support the notion that during good weather conditions, honey bees provide a strong pollination force that can exceed that of native pollinators if honey bee stocking density is high enough. This was especially true in 2017 when the bumble bee abundance foraging on haskap flowers was lower than in 2016 because there appeared to be a delay in when the bumble bees began foraging on haskap flowers (Figure 8). This delay could potentially be explained by the amount of precipitation in 2017 compared to 2016. Generally, the mean daily temperature for 2016

and 2017 during bloom in the month of May was very similar (Appendix I and II respectively). In 2016 the average daily temperature was 11.5 °C compared to 11.1 °C in 2017. The main difference between the two years was the amount of precipitation. In 2016, 87.4 mm of rain fell during the month of May (Appendix I), whereas in 2017, 172.9 mm of rain fell during the month of May (Appendix II). This may explain the difference in the number of bumble bees that we observed on transect walks between these years. Honey bees appeared to be unaffected by the precipitation. This is likely due to the fact that the colony stashes resources in the hives so they can continue to grow under days of poor foraging conditions. Furthermore, the beekeeper fed the honey bee colonies during unfavorable foraging conditions to aid in colony buildup. As a result, honey bee populations are less likely affected by precipitation. The delay of bumble bee abundance was approximately one week compared to 2016, even though haskap began blooming within a day or two of each other in 2016 and 2017. It appears that honey bees should be an integral part of any haskap growers pollination plans because they provide an insurance when native pollinators are either not available for pollination, or are not present in high enough numbers to supply sufficient pollination. In addition, haskap orchards are mowed so often, there isn't much additional food for native pollinators in haskap orchards once haskap is finished blooming. Although we did not place pollen traps on hives after the haskap bloom, anecdotally I did notice an absence of flowers in the orchards that otherwise would have been present if the orchards were not mowed. This presents challenges for keeping native pollinators in the area for the next growing season.

4.2 Effectiveness and Efficiency of Potential Pollinators

Pollinator effectiveness and efficiency was studied to determine what the most suitable pollinators are for haskap in NS. Pollinator effectiveness was based on SVPD and pollinator efficiency was based on flower visits per minute and tendency of pollinators to visit both flowers. Honey bees and bumble bees were by far the most abundant taxa to visit haskap flowers (Figure 7) and were therefore the two pollinators used for these experiments. On the basis of SVPD, both honey bees and bumble bees deposit roughly the same amount of pollen per single visit (95 grains bumble bees and 110 grains for honey bees, (Figure 9) although there is a large variation in the amount of pollen deposited by either taxa. In Saskatchewan, Frier et al. (2016a) studied the performance of three taxa, honey bees, bumble bees, and blue orchard mason bees (*O. lignaria*) as haskap pollinators. They found no significant difference between the amounts of pollen deposited on a single visit due to a large variation in deposition. The average pollen deposition reported by Frier et al. (2016a) ranged from 85 grains for honey bees to 99 grains for bumble bees. Frier et al. (2016a) found in the control group, the mean pollen grains found on the stigma was 72 grains with a range of 0-296 grains. In my study, unvisited flowers had an average of 8 grains of pollen with a range of 0-29 grains. To determine pollen deposition for the control group, Frier et al. (2016a) presented a branch of flowers to a pollinator for SVPD, but used unvisited flowers from the same branch for controls to account for pollen that contacted the stigma by means other than a bee visit. In my study, I used previously bagged flowers that were removed from the plant and walked around for about a minute. The idea was to attempt to mimic the same conditions when we presented the flower to a bee. It is likely that Frier et al. (2016a) had such high pollen

deposition due to repeatedly walking the same branch with flowers on it for a while before removing the stigma. Furthermore, due to the aggressiveness of pollination of other flowers on the haskap branch, it is possible that pollen could have been released and deposited onto the stigma of the same flower, due to the downward orientation of the flower.

In lowbush blueberries in NS, Javorek et al. (2002) studied the performance of a few pollinator taxa (including honey bees and bumble bees) for the pollination of lowbush blueberries. They determined that honey bees deposit significantly fewer grains of pollen than bumble bee queens and workers (11.7, 50.6, and 34.3 grains per single visit, respectively). The differences in pollen deposition in Javorek et al. (2002) was linked to the foraging task of the bee, whether it was a pollen forager or nectar forager where honey bees were considered nectar foragers and bumble bees were considered pollen foragers. Another reason for differences in pollen deposition in Javorek et al. (2002) is due to the ability of bumble bees to buzz pollinate, where honey bees cannot. The lowbush blueberry flower is well adapted to buzz pollination (Kearns & Inouye, 1997), making bumble bees generally more effective pollinators of this plant. Although honey bees can be effective pollinators of lowbush blueberry, the blueberry flower has a more 'specialist' pollination strategy favoring insects that can buzz pollinate. Differing from blueberry, haskap has a more 'generalist' pollination strategy having early stigma receptivity, early and long lived nectar production that is replaced once removed, a long period of anthesis, and does not require buzz pollination (Frier et al., 2016*b*). All of these traits suggest haskap takes on a generalist pollination strategy, which is important due to the early blooming period, a time when there are not many pollinators around (Frier et al.,

2016*b*). The trend of pollen deposition varies from crop to crop. For example, bumble bees deposit more pollen per single visit than honey bees on apple flowers (Thomson & Goodell, 2001) and cranberries (Stubbs & Drummond, 1996) but honey bees and bumble bees deposit similar amounts of pollen on almond flowers (Thomson & Goodell, 2001).

Foraging behavior of bees is a characteristic that influences the end result of pollination success. To determine the most efficient pollinators of haskap, flower visits per minute and the tendency of honey bees and bumble bees to visit both flowers was studied to examine the foraging behavior of these bees. Bumble bees were found to visit three times as many flowers per minute than honey bees (Figure 10). In Saskatchewan, Frier et al. (2016*a*) found that honey bees stayed at a haskap flower for on average 15 seconds, where bumble bees stayed at the flower for an average of 6 seconds. These results are congruent with my findings in haskap. In wild blueberry, Javorek et al. (2002) found that bumble bee queens would pollinate blueberry flowers over six times as fast as honey bees. Although this is the same trend that Frier et al. (2016*a*) and I observed, it was not near the same magnitude as Javorek et al. (2002) reported. One possible reason for this is that, in a blueberry system, many honey bees may have switched over to become nectar foragers, but because haskap blooms so early, many of the honey bee workers would still be pollen foragers and may be faster at collecting pollen than nectar. It is also likely that due to the close proximity and uniformity of lowbush blueberry flowers, the bumble bees could move from flower to flower very efficiently. The trend that bumble bees visit more flowers per minute than honey bees has been documented in other crops as well. For example, bumble bees visit more flowers per minute than honey bees in raspberry (Willmer et al., 1994), cucumber and watermelon (Stanghellini et al., 2002),

and highbush blueberry and cranberry (Stubbs & Drummond, 1996).

For the tendency of pollinators to visit both flowers, bumble bees visited both flowers of the double flower inflorescence twice as often as honey bees (Figure 11). Bumble bees visited both flowers 77% of the time, where honey bees visited both flowers only 35% of the time. Frier et al. (2016a) found that honey bees visit both flowers of the inflorescence 35% of the time, but bumble bees visited both flowers only 48% of the time. This is an important result because not only are bumble bees faster pollinators, but they also visit both flowers of the inflorescence more often which results in a higher percent fruit set, and typically larger fruit with more seeds (Frier et al., 2016a). These results suggest that bumble bees may be more favorable pollinators of haskap compared to honey bees, but typically honey bees are present in higher numbers. One potential downfall with bumble bees visiting both flowers in the inflorescence more often is that because haskap requires cross-pollination for successful fruit set, if the bumble bees did not previously pick up pollen from a different, compatible haskap cultivar, successful pollination may not occur as often as if only one flower was visited at a time. However, number of pollinators is also important when considering pollination success. In 2017 there were about three times as many honey bees counted on haskap flowers during transect walks than bumble bees (Figure 7). As a result, there were likely multiple visits by different honey bees to the same flower or pair of flowers, which would increase the chances of honey bees moving the correct pollen, from the pollinizer, to pollinate the haskap flower.

Pollen traps were placed on honey bee colonies in haskap orchards during haskap bloom to determine the proportion of haskap pollen that was being brought back into the

hives. Although haskap pollen was found in five of six samples, haskap pollen comprised a small proportion of total pollen collected (Table 7, Figure 12). By far the most abundant source of pollen brought into the hives was sugar maple, followed by dandelion and other fruit trees. Haskap only comprised 1.3% of all of the pollen collected, even though we noticed honey bees actively foraging on the haskap flowers the day that the pollen traps were deployed. It is possible that on the particular day of sampling, many of the honey bees may have been out looking for nectar instead of pollen and not collected a lot of pollen. Although Frier et al. (2016b) determined that haskap provides nectar for long periods during anthesis, there is no information available reporting the composition of haskap pollen for protein, fat, or amino acids. As a result, haskap pollen may not be nutritious for honey bees, and therefore may be undesirable for pollen foraging honey bees.

Honey bees, as well as other bees require a number of elements to satisfy their nutritional requirements. Pollen typically supplies most of the protein, minerals, lipids and vitamins (Somerville, 2001), however not all pollen is equally beneficial to colony health. Pollen can vary in the protein content, amino acid profile and lipid content and therefore the quality of can vary between plant species. As a result, bees collect a range of pollens to satisfy the nutritional requirements of the colony and maintain colony function (Somerville, 2001). Pollen quality has been directly related to bee health and tolerance to certain diseases, where pollen quality is more important than pollen diversity. (Di Pasquale et al., 2013). In my study, honey bees collected almost half of their pollen from sugar maple (*Acer Saccharum*), and another large portion was dandelion (*Taraxacum*). Maples (*Acer spp.*) have a high protein content of over 20% and be a good

quality pollen (Liolios et al., 2015). Dandelion also has good quality pollen for honey bees having a protein content of approximately 18%. Many of the other pollens that the bees collected including *Salix*, and Rosaceae have good quality pollen (Somerville, 2001). Although the honey bees were not bringing back large amounts of *Lonicera* pollen in my study, they were bringing back large amounts of high quality pollen during the haskap bloom period for brood production.

If this experiment was repeated, pollen collection would be done over multiple days instead of just one day. Around all of the hives, there were a large number of sugar maple trees in bloom which would explain the high percentage of sugar maple in the pollen sample. There was also a lot of dandelion blooming around the haskap orchards during bloom which would explain the high percentage of dandelion pollen being collected. The very limited amount of haskap pollen may be somewhat troublesome because it suggests that haskap may be much less attractive to honey bees than other pollen sources. However, my results from transect walks and SVPD show that honey bees are actively foraging on haskap plants, moving pollen around, and deposit similar amounts of pollen per single visit as bumble bees. Since haskap blooms the same time as many other attractive pollen sources for honey bees, it may be difficult to keep honey bees from pollinating non-target crops. Anecdotal observations suggest that pollination by honey bees is quite successful in many of the more established orchards and therefore it may not be an issue that honey bees tend to prefer non-target flowers. One potential way to increase the number of honey bees visiting haskap flowers is to only bring honey bee hives into the haskap orchards at 10-15% bloom. In the particular haskap orchards that I did my research in, the hives are left in the haskap orchards all season long, and

overwintered in the orchards. It has been shown that in blueberry fields, reorienting hives or moving bees into orchards at 10-15% bloom is advantageous because the honey bees are more crop specific and bring back larger amounts of pollen from the target plants (Wardell, 1982).

4.3 Artificial Nest Boxes to Attract Cavity Nesting Solitary Bees

To determine the usefulness of solitary bee nesting cavities placed on the edge of haskap orchards to attract native early season solitary nesting bees and to assess the influence of nest tube size and spacing on solitary bee nest occupancy, solitary bee nest boxes were placed out in haskap fields and tracked throughout the season. The total number of tubes that were capped was not very high, but the majority of the bees used the 6 mm nest tubes (27.5% of all tubes capped) rather than 8 mm nest tubes (1.4% of all tubes capped) (Figure 14). McCallum (2017) ran a similar experiment using milk cartons and 7 mm diameter paper tubes and had 34% of 512 possible nesting tubes occupied. Although these results are similar and we both placed out similar amounts of nest tubes, McCallum (2017) placed her nest boxes out in 12 different blueberry fields. For my research, nest boxes were put out in 7 haskap orchards. As a result, in my experiment, I had more potential nesting sites placed out per field compared to McCallum (2017) and we still had similar results. Using a milk carton design, Torchio (1985) reported 33% occupancy of *O.lignaria* in set out nest tubes. Jenkins & Matthews (2004) reported 35% occupancy in a study done in Georgia and South Carolina. It appears that my trapping was comparable because occupancy in the 6 mm nest tubes was similar to other reports using different style nest boxes and nesting substrate.

In a three year study in NS, Sheffield et al. (2008) showed, that bees from the genus *Osmia*, which are early season pollinators that would be most suitable for haskap pollination, preferred nest tubes with a diameter of 5 mm, and to a lesser extent 3 mm. Sheffield et al. (2008) also found that most *Megachile* species preferred the larger, 9 mm diameter nesting tubes, however certain *Megachile* species preferred 7 mm diameter nest tubes. This would suggest that 6 mm nest tubes would be more appropriate to use in nest boxes set out in haskap orchards since the goal would be to attract *Osmia* for an early season pollinator.

Capped nest tubes were first observed on 22 June 2017, approximately one month after the haskap bloom, and capping was observed up to 13 September 2017, the majority of which occurred between 6 July 2018 and 2 August 2017 (Figure 13). Similar to my findings, McCallum (2017) noted that the first capped nest tube was observed 25 June 2015 and the last capped nesting tube was observed on 30 July 2015 where the majority of nest capping was completed by mid-July. These results suggest that although *Osmia* are early season pollinators, haskap may bloom too early in the season for native *Osmia* to visit the flowers. In Wisconsin, most *Osmia* make and provision their nests with pollen during June and July (Medler, 1967) which is a similar period that I observed. Furthermore, it only takes approximately 20 days for *Osmia* females to finish laying in a nest tube and to cap it off (Torchio, 1984). As a result, *Osmia* were not likely foraging in or around the haskap orchards where my nest boxes were placed until after haskap bloom in 2017.

Although there was a strong preference for cavity nesting bees to use the 6 mm diameter nest tubes, there was no significant difference with respect to spacing (Figure

14). This is a notable result because it is much easier to make nest boxes with the nest tubes clumped together compared to being spaced apart. There was no significant interaction between date and spacing with respect to capped off nest tubes, indicating that there was no preference for spacing overall, or at different times throughout the season. If there was, it may suggest that the solitary cavity nesting bees may prefer a certain style, and once those nest tubes get used up they may start to use the other style. However, this was not the case. We did notice that there was a significant effect of date ($P < 0.001$), which makes sense because the bees would have been making use of more nest boxes over time (Figure 13). There is very little research that looks at the effect of nest tube spacing on the use of solitary bee nesting cavities. Sheffield et al. (2008) tried out a few different styles of nest boxes and suggested that the absence of spacing was believed to discourage nesting, however there was no research to back up the anecdotal claim.

The original plan of my research with artificial nesting cavities was to dissect some of the nest tubes to collect the pollen provision to determine if there was any haskap pollen being brought in by the cavity nesting bees. If there was no haskap pollen being brought in, it would suggest that solitary cavity nesting bees may not be good pollinators of haskap and support the results found by Frier et al. (2016a). Unfortunately, by the time the nest boxes were collected from the field and brought to the lab for dissection, the pollen provision had already been consumed by the developing bee and therefore I could not determine if the solitary cavity nesting bees were collecting haskap pollen or not. We did not notice many solitary bees foraging on haskap flowers in 2016 or 2017, but we did notice them foraging on other flowers surrounding the haskap orchard at the time of haskap bloom similar to Frier et al. (2016a). The main species of solitary bee that we

captured in our nesting cavities was *O. tersula*, which comprised 65% of the bees that hatched from the incubation experiment, and 33% of all of the insects that hatched out during the experiment. McCallum (2017) and Sheffield et al. (2008) also found *O. tersula* to be the main species of solitary bee that made use of artificial nesting cavities. Since both of these studies were also done in NS agroecosystems, it suggests that regionally *O. tersula* is the most abundant, early season cavity nesting bee in NS.

Although solitary bee nesting cavities seem useful at attracting cavity nesting bees in haskap orchards, they may not be useful tools to be implemented to enhance haskap pollination. Since these early season solitary bees seem to favor other pollen sources that are available at the time and due to the small size of the bee in respect to the morphology of the haskap flower, these pollinators may not be suitable to provide significant pollination services in haskap orchards. A bee that is too small or too large for a flower will transfer few, if any, pollen grains when it visits the flower, and therefore should not be considered an effective pollinator (Kearns & Inouye, 1997). In flowers where the stigma is generally positioned away from the anthers, like haskap, effective pollinators must be of an appropriate size and be able to work the blossom correctly (Webb & Lloyd, 1986). Because many *Osmia* spp. are generally small in size and the stigma of haskap flowers protrudes outside of the flower, they probably would not do an adequate job pollinating the flower. However, Frier et al. (2016a) found that *Osmia lignaria*, a commercially reared species, did deposit more pollen per single visit than honey bees, but noticed that *Osmia lignaria* rarely visited haskap, a trend that I noticed with all solitary bees from my transect walk results.

Substantial levels of predation in the field based off of a number of capped tubes that damaged or excavated in the field was noticed in this study. In addition, after placing capped tubes into an incubation chamber and hatching them out, 50% of the nest tubes that appeared to be fine, had kleptoparasitic wasps that emerged (Figure 15), primarily belonging to the family Sapygidae. Others have found similar troubling rates of parasitism (Drummond & Stubbs, 1997; McCallum, 2017; Sheffield et al.,2008) in trap nest studies. High rates of parasitism suggest that congregating solitary bee nest boxes may actually be harmful for the native bee population (MacIvor & Packer 2015). The high rates of parasitism coupled with the early bloom period of haskap suggests that the use of solitary bee nesting cavities may not be a useful tool to use in haskap pollination.

4.4 Summary and Recommendations

Pollination is an ecosystem service and a production practice that is valued in nature and for agriculture. Honey bees are the most valued commercial pollinators. Native pollinators are often better pollinators of certain crops, although their pollination biology is generally not understood in most crop systems. Haskap is a new crop being grown in Nova Scotia, but the pollinators, and the efficacy of those pollinators in the region, were generally not understood. My thesis research surveyed the native and managed pollinator community in and around haskap orchards in southern Nova Scotia, determined the key pollinators of haskap in these orchards, examined the efficiency and the effectiveness of the most important pollinators identified, and evaluated the usefulness of solitary bee nesting cavities to attract native, early season solitary nesting bees. Although there are 10 genera of bees and 2 families of pollinating flies that are present in haskap orchards in southern Nova Scotia, the majority of the pollinators present in the orchards belong to

only 4 genera. Of these 4 common genera, only two, honey bees and bumble bees, are frequent visitors of haskap flowers in southern Nova Scotia. Honey bees and bumble bees were equally effective at depositing pollen on haskap stigmas, but bumble bees were more efficient pollinators and visited both flowers a higher percentage of the time. Solitary bee nest cavities were useful to attract early season cavity nesting bees in haskap orchards, but these pollinators were rarely seen visiting haskap flowers and the nest tubes had very high levels of parasitism.

Based off these results, I would recommend that a combination of honey bees and bumble bees be used in the commercial production of haskap in Nova Scotia. Although bumble bees are more efficient pollinators of haskap, honey bees provide a pollination insurance when populations of native bumble bees are not synchronized with bloom, or if the populations are not sufficient to supply adequate pollination services. The use of commercially produced bumble bee quads may be an effective alternative or addition to honey bees. I would not recommend the use of solitary bee nest boxes to increase the pollination of early season cavity nesting bees in haskap. The small size of these pollinators, infrequent visitation to haskap flowers, accompanied by the high parasitism rates observed, suggest that solitary bee nesting cavities are not very suitable in haskap pollination. I also recommend haskap growers to plant pollinator friendly plants that do not coincide with haskap bloom to supply native pollinators with a food source after haskap bloom.

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Appendix I: Daily weather data from weather station in Kejimikujik Nova Scotia during May 2016

| Day | Max Temp (°C) | Min Temp (°C) | Mean Temp (°C) | Total Precip (mm) |
|------|---------------|---------------|----------------|-------------------|
| 1 | 17.4 | -2.3 | 7.6 | 0 |
| 2 | 12.9 | 4.7 | 8.8 | 8.1 |
| 3 | 9.3 | 4.6 | 7 | 0.6 |
| 4 | 12.5 | 2.8 | 7.7 | 2.1 |
| 5 | 10 | 3.9 | 7 | 22.7 |
| 6 | 10.9 | 2.8 | 6.9 | 6 |
| 7 | 17.7 | 2.9 | 10.3 | 0.2 |
| 8 | 21.5 | 4.7 | 13.1 | 0.2 |
| 9 | 11.2 | 3.2 | 7.2 | 0 |
| 10 | 14.8 | 4.7 | 9.8 | 0 |
| 11 | 15.3 | 2.3 | 8.8 | 0 |
| 12 | 20.1 | 0.9 | 10.5 | 0 |
| 13 | 19.7 | 4.8 | 12.3 | 8.3 |
| 14 | 21 | 9.1 | 15.1 | 1.8 |
| 15 | 15.8 | 6.4 | 11.1 | 2.8 |
| 16 | 7.7 | 3.7 | 5.7 | 0.4 |
| 17 | 14.4 | 4.6 | 9.5 | 0 |
| 18 | 19.6 | 3.6 | 11.6 | 0 |
| 19 | 19.9 | 1.6 | 10.8 | 0.6 |
| 20 | 20.2 | 8.1 | 14.2 | 0 |
| 21 | 25.9 | 7.7 | 16.8 | 0 |
| 22 | 15.6 | 9.6 | 12.6 | 15.8 |
| 23 | 14.9 | 10.6 | 12.8 | 4.4 |
| 24 | 20.6 | 9.8 | 15.2 | 0 |
| 25 | 20.7 | 12.2 | 16.5 | 0 |
| 26 | 20.2 | 6.7 | 13.5 | 0 |
| 27 | 17.3 | 6.9 | 12.1 | 0 |
| 28 | 26.3 | 9.5 | 17.9 | 5.4 |
| 29 | 16.9 | 8.3 | 12.6 | 0 |
| 30 | 18.2 | 9 | 13.6 | 7.8 |
| 31 | 23.4 | 11.5 | 17.5 | 0.2 |
| Mean | 17.2 | 5.8 | 11.5 | 87.4 (sum) |

Appendix II: Daily weather data from weather station in Kejimikujik Nova Scotia during May 2017

| Day | Max Temp (°C) | Min Temp (°C) | Mean Temp (°C) | Total Precip (mm) |
|------|---------------|---------------|----------------|-------------------|
| 1 | 6.7 | 2.3 | 4.5 | 14.2 |
| 2 | 16.1 | 3.8 | 10 | 8.1 |
| 3 | 16 | 6.1 | 11.1 | 0.5 |
| 4 | 15.9 | 3.6 | 9.8 | 0 |
| 5 | 15 | 3.4 | 9.2 | 4 |
| 6 | 17.2 | 11.3 | 14.3 | 33.8 |
| 7 | 21.2 | 13.3 | 17.3 | 24.2 |
| 8 | 15.9 | 4 | 10 | 0.9 |
| 9 | 8.9 | 3.8 | 6.4 | 15.7 |
| 10 | 10.6 | 3.8 | 7.2 | 0 |
| 11 | 12.7 | 5.2 | 9 | 0.5 |
| 12 | 17.6 | 2.3 | 10 | 0 |
| 13 | 20.3 | 0.9 | 10.6 | 0 |
| 14 | 11.8 | 3.6 | 7.7 | 16.1 |
| 15 | 9.3 | 6.3 | 7.8 | 2.4 |
| 16 | 18.9 | 4.7 | 11.8 | 0 |
| 17 | 23.7 | 5.8 | 14.8 | 0 |
| 18 | 31.8 | 10.5 | 21.2 | 0 |
| 19 | 28.7 | 10.5 | 19.6 | 0 |
| 20 | 15.2 | 3.1 | 9.2 | 0 |
| 21 | 18.3 | 1.4 | 9.9 | 0 |
| 22 | 19.1 | 1.2 | 10.2 | 3.6 |
| 23 | 13.2 | 6.3 | 9.8 | 9.6 |
| 24 | 19.4 | 7 | 13.2 | 0 |
| 25 | 18.3 | 7.2 | 12.8 | 2.1 |
| 26 | 12.8 | 6.6 | 9.7 | 32.4 |
| 27 | 10.1 | 7.2 | 8.7 | 0 |
| 28 | 16.8 | 7 | 11.9 | 0.2 |
| 29 | 17.7 | 5.7 | 11.7 | 0.2 |
| 30 | 19.2 | 3.5 | 11.4 | 0 |
| 31 | 20.2 | 3 | 11.6 | 4.4 |
| Mean | 16.7 | 5.3 | 11.1 | 172.9 (sum) |